Universidad de Valladolid

ESCUELA DE INGENIERÍAS INDUSTRIALES

DEPARTAMENTO DE INGENIERÍA QUÍMICA Y TECNOLOGÍA DEL MEDIO AMBIENTE

TESIS DOCTORAL:

# MICROAEROBIC REMOVAL OF HYDROGEN SULPHIDE FROM BIOGAS

Presentada por Iris Ramos Castaño para optar al grado de doctor por la Universidad de Valladolid

Dirigida por:

Dr.- Ing. María Fdz-Polanco Íñiguez de la Torre

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Memoria para optar al grado de Doctor,

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Certifica que:

IRIS RAMOS CASTAÑO ha realizado bajo su dirección el trabajo *"Microaerobic removal of hydrogen sulphide from biogas"*, en el Departamento de Ingeniería Química y Tecnología del Medio Ambiente de la Escuela de Ingenierías Industriales de la Universidad de Valladolid. Considerando que dicho trabajo reúne los requisitos para ser presentado como Tesis Doctoral expresa su conformidad con dicha presentación.

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Fdo. María Fdz-Polanco Íñiguez de la Torre

Reunido el tribunal que ha juzgado la Tesis Doctoral titulada *"Microaerobic removal of hydrogen sulphide from biogas"* presentada por la licenciada Iris Ramos Castaño y en cumplimiento con lo establecido por el Real Decreto 99/2011 de 28 de enero de 2011 acuerda conceder por_____ la calificación de _____.

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PRESIDENTE SECRETARIO

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A Rodrigo…

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# ABSTRACT / RESUMEN

Biogas production through anaerobic digestion represents one of the most important routes in order to fulfil the national and international regulations aiming for environment preservation and efficient utilisation of the natural resources. For profitable and safe use of its energetic potential, the biogas must satisfy the quality standards of the appliance.

Biogas quality is crucial in both its methane content and purity. Hydrogen sulphide is one of the most common pollutants in biogas. Several process-level (implemented in the reactor) and end-of-pipe strategies (applied in another unit) exist for its control, based on physical-chemical and biological phenomena. In contrast to physical-chemical methods, the biological technologies are environmentally friendly and economical. The removal of hydrogen sulphide directly in the reactor by imposing oxygen-limiting/microaerobic conditions is the most attractive biological solution due to its simplicity of implementation and operation, and the fact that the biogas is produced and desulphurised in a single unit. By contrast, the operation of the alternative biological methods is difficult and complex. However, the possible costs arising from elemental sulphur accumulation in the gas space during microaerobic digestion could hinder the widespread application of this method of hydrogen sulphide control. A potentially ideal external process for biogas desulphurisation would integrate the simplicity of the process of hydrogen sulphide removal in microaerobic digesters.

The general objective of this thesis is to control the hydrogen sulphide content in the biogas produced during digestion by microaerobic processes. For this purpose, two different strategies are investigated: a process-level approach, which involves imposing microaerobic conditions directly in the reactor, and an end-of-pipe solution, implying the usage of an additional unit where the conditions present in the headspace of microaerobic reactors are reproduced. The thesis is structured as a compendium of eight research articles.

In order to achieve the aims of this thesis, two lab-pilot reactors and one industrial-pilot reactor are operated. At both scales, mixed sludge from a municipal wastewater treatment plant is treated under mesophilic conditions. The experiments aimed to develop a new end-of-pipe biotechnology for biogas desulphurisation based on the findings obtained from operation of lab-pilot microaerobic reactors are carried out at lab-pilot scale. Digestate from lab-pilot reactors treating municipal sewage sludge is used as the reaction media.

Benefits of oxygen on the digestion process and the biogas quality can be reached simultaneously, for which the micro-oxygenation level must be precisely adjusted in order to achieve and maintain minimum concentration of both hydrogen sulphide and oxygen in the biogas. For this purpose, the production and the sulphide content of biogas can be used. Under variable organic loading rate and steady sulphur loading rate, biogas production is an efficient regulating parameter of the oxygen supply. However, under variable sulphur loading rate, hydrogen sulphide concentration must be the basis for the development of a reliable and precise control strategy.

Elemental sulphur is the main by-product from sulphide oxidation in microaerobic reactors. When the moisture availability on the different surfaces of the gas space is sufficient, high amounts of this compound are deposited there, which can lead to increasing oxygen demand over time. As a result, the intervals of time at which the reactor must be cleaned can be reduced. Sulphide-oxidising bacteria grow all over the headspace. The composition, species richness and the size of this microbial community depended on the location (more specifically, on the moisture level) and the operation

time. Although the hydrogen sulphide removal from biogas predominantly occurs in the gas space, the efficiency of the process is rapidly recovered after cleaning.

Conversely, when the surfaces of the gas space suffer from dryness, elemental sulphur hardly accumulates there. The desulphurisation performance and the oxygen demand of the reactor are low relatively high (respectively) at the early stage of the microaerobic operation. Nevertheless, hydrogen sulphide can eventually be efficiently removed from the biogas under different configurations. The biogas recirculation raises the oxygen transfer rate to the liquid phase, which can increase the microbial richness and evenness and, in the log-term, cause an important shift in the biodiversity and structure of the bacterial and the archaeal communities.

The conditions of biogas desulphurisation present in microaerobic reactors are successfully reproduced inside an external chamber called a “microaerobic desulphurisation unit”. Microaerobic digestate is an efficient and durable reaction media. This system is robust against fluctuations in operating parameters, such as biogas residence time and mass loading rate and inlet concentration of hydrogen sulphide. Although neither nutrients nor water are added, it presents a high bacterial diversity. The microaerobic desulphurisation unit can be operated at 20ºC, and achieve almost the same removal efficiencies than microaerobic reactors. Nonetheless, relatively high temperatures at the start-up could be the key to achieving successful operation. Elemental sulphur can be the main by-product, since the system performs successfully at oxygen/hydrogen sulphide (v/v) supplied ratios of up 1.0.

La producción de biogás mediante digestión anaerobia es una de las vías más importantes para cumplir la legislación nacional e internacional que vela por la preservación del medio ambiente y utilización eficiente de los recursos naturales. Para un aprovechamiento seguro y rentable de su potencial energético, el biogás debe satisfacer los estándares de calidad del equipo de utilización.

La calidad del biogás es crucial tanto en su contenido en metano como en su pureza. El sulfuro de hidrógeno es uno de los contaminantes más comunes en el biogás. Existen varias estrategias de control que se aplican nivel de proceso (en el reactor) y en una etapa final (en otros dispositivos), basadas en fenómenos físico-químicos y biológicos. A diferencia de los métodos físico-químicos, las tecnologías biológicas son respetuosas con el medioambientalmente y económicas. La eliminación de sulfuro de hidrógeno directamente en el reactor aplicando condiciones limitantes de oxígeno/microaerobias es la solución biológica más atractiva debido a su simplicidad de implementación y operación, y a que el biogás se produce y trata en un único equipo. Por el contrario, la operación de los métodos biológicos alternativos es difícil y compleja. Sin embargo, los posibles costes que surgen de la acumulación de azufre elemental en la cabeza del reactor durante la digestión microaerobia podrían obstaculizar la aplicación generalizada de este método de control del sulfuro de hidrógeno. Un potencial proceso externo ideal para eliminar de sulfuro de hidrógeno del biogás integraría la simplicidad del proceso de eliminación en digestores microaerobios.

El objetivo general de esta tesis es controlar el contenido del sulfuro de hidrógeno del biogás producido durante la digestión mediante procesos microaerobios. Para este propósito, se investigan dos estrategias diferentes: una estrategia a nivel de proceso, que implica la implementación de condiciones microaerobias directamente en el reactor, y un estrategia al final del proceso, que implica el uso de una unidad adicional donde se reproducen las condiciones presentes en la cabeza de reactores microaerobios. La tesis se estructura como un compendio de ocho artículos de investigación.

Para alcanzar los objetivos de esta tesis, se operan dos reactores piloto-laboratorio y uno piloto-industrial. A ambas escalas, se trata fango mixto procedente de una planta de tratamiento de aguas residuales urbanas bajo condiciones mesófilas. Los experimentos para desarrollar una nueva tecnología externa de eliminación de sulfuro de hidrógeno del biogás en base a los hallazgos realizados durante la operación de reactores piloto-laboratorio se desarrollan a escala piloto-laboratorio. Se utiliza fango digerido de reactores piloto-laboratorio que tratan lodos de depuradora urbana como medio de reacción.

Los beneficios del oxígeno sobre el proceso de digestión y la calidad del biogás pueden alcanzarse simultáneamente, para lo cual el nivel de micro-oxigenación debe ser ajustado de forma precisa para alcanzar y mantener una mínima concentración de sulfuro de hidrógeno y de oxígeno en el biogás. Con este fin, pueden usarse la producción de biogás y el contenido de sulfuro de hidrógeno del biogás. En condiciones de carga orgánica variable y carga de azufre estable, la producción de biogás es un eficiente parámetro de regulación del suministro de oxígeno. Sin embargo, bajo condiciones de carga de azufre variable, la concentración de sulfuro de hidrógeno debe ser la base para el desarrollo de una estrategia de control fiable y precisa.

Azufre elemental es el principal subproducto de la oxidación de sulfuro en reactores microaerobios. Cuando la humedad disponible en las distintas superficies de la cabeza del reactor es suficiente,

grandes cantidades de este compuesto se depositan allí, lo que con el tiempo puede conducir a una creciente de demanda de oxígeno. Como consecuencia, los intervalos de tiempo a los cuales el digestor debe limpiarse pueden acortarse. Las bacterias sulfuro-oxidantes crecen en la cabeza del reactor, y la composición, riqueza de especies y el tamaño de la comunidad bacteriana dependen de la localización (más específicamente, del nivel de humedad) y del tiempo de operación. Aunque la eliminación del sulfuro de hidrógeno del biogás ocurre principalmente en la cabeza del reactor, la eficiencia del proceso se recupera rápidamente tras la limpieza.

En cambio, cuando las superficies de la cabeza del reactor sufren sequedad, el azufre elemental apenas se adhiere. El rendimiento de eliminación de sulfuro de hidrógeno del biogas y la demanda de oxígeno del reactor es bajas y relativamente alta (respectivamente) al principio de la operación en condiciones microaerobias. Sin embargo, con el tiempo, el sulfuro de hidrógeno puede ser eficientemente eliminado del biogás bajo distintas configuraciones. La recirculación de biogás aumenta la tasa de transferencia del oxígeno a la fase líquida, lo que puede incrementar la riqueza y abundancia microbiana y, a largo plazo, causar un cambio importante en la biodiversidad y estructura de las comunidades de bacterias y arqueas.

Las condiciones de desulfurización del biogás presentes en reactores microaerobios pueden ser reproducidas con éxito dentro de una cámara externa llamada “unidad desulfurización microaerobia”. El fango digerido es un medio de reacción eficiente y duradero. Este sistema es robusto frente a fluctuaciones en parametros de operación tales como el tiempo de residencia del biogás, y la carga y concentración de entrada de sulfuro de hidrógeno. Aunque no se añaden nutrientes ni agua, éste presenta una alta diversidad bacteriana. La unidad microaerobia de desulfurización microaerobia puede operarse a 20ºC, y alcanzar casi las mismas eficiencias de eliminación que los reactores microaerobios. Sin embargo, temperaturas relativamente altas en el arranque podrían ser la clave para conseguir el éxito de la operación. Azufre elemental puede ser el principal subproducto, ya que el sistema rinde satisfactoriamente a ratios de oxígeno frente a sulfuro de hidrógeno suministrado (v/v) de hasta 1.0.

# ABBREVIATIONS

| | |
|---|---|
| AD | anaerobic digestion |
| BRT | biogas residence time |
| CHP | combined heat and power |
| COD | chemical oxygen demand |
| CSTR | continuous stirred tank reactor |
| DGGE | denaturing gradient gel electrophoresis |
| GC | gas chromatography |
| HPLC | high-liquid performance chromatography |
| HRT | hydraulic retention time |
| IR | infrared |
| MLR | mass loading rate |
| NCBI | National Centre for Biotechnology Information |
| ORP | oxidation-reduction potential |
| PA | partial alkalinity |
| PCR | polymerase chain reaction |
| RE | removal efficiency |
| RDP | ribosomal database project |
| SRB | sulphate-reducing bacteria |
| SOB | sulphide-oxidising bacteria |
| TA | total alkalinity |
| TCD | thermal conductivity detector |
| TS | total solids |
| UV | ultraviolet |
| VFA | volatile fatty acid |
| VS | volatile solids |
| WWTP | wastewater treatment plant |

# INTRODUCTION

## BIOGAS PRODUCTION AND UTILISATION

Most of the world's overall energy supply is derived from fossil fuels. With steadily increasing oil and natural gas prices and improved legal framework conditions, biogas production constitutes a great opportunity (Murphy et al., 2011). Biogas is a renewable energy source that can be used for the replacement of fossil fuels in several applications: heat, steam, electricity, cooling, chemical and protein production, as fuel for vehicles and fuel cells, and for injection into natural gas grids (Holm-Nielsen et al., 2009). Though substantially inferior to other common fuels such as compressed natural gas, which produces approximately 8,600kcal/$m^3$, it has a good calorific value (around 5,000kcal/$m^3$) (Abbasi et al., 2012).

The production of biogas through anaerobic digestion (AD) is considered as one of the most energy-efficient and environmentally beneficial technology for bioenergy generation (Weiland, 2010). Therefore, AD is gaining increasing attention worldwide (Jagadabhi et al. 2010). Besides of biogas production, this technology is a very attractive alternative for operators of wastewater treatment plants (WWTPs) due to many other reasons (Ward et al., 2008).

Biogas is a mixture of gases whose composition depends on the type of material to be digested, as well as on the operational conditions in the reactor (Noyola et al., 2006). It is generally composed of methane and carbon dioxide in a ratio of 3:1, and other minor constituents. Hydrogen sulphide is one of the most common trace compounds in biogas (Maestre et al., 2010; Rasi et al., 2007), where its concentration can range from 0.10 to extremely high values of 2.00%v/v (1,000-20,000ppmv) (Fortuny et al., 2011).

Hydrogen sulphide is a by-product obtained from fermentation of organic sulphur-containing compounds (such as proteins) by acidogenic bacteria, and reduction of anionic forms of sulphur (such as sulphate and thiosulphate) contained in the feedstocks by sulphate-reducing bacteria (SRB) (Stams et al., 2003). High concentrations of sulphate are typical in wastewaters from the food, fermentation, pulp/paper, edible oil, tannery, photography, and chemical industry (Cirne et al., 2008; Zhou et al., 2007). Therefore, this compound can also be present in municipal sewage sludge due to collection of industrial streams rich in this anion, or to natural content in water supply (Noyola et al., 2006). Likewise, feedstocks from urban WWTPs and agri-food facilities can present high concentrations of proteins (Chen et al., 2008; Peu et al., 2012).

The rate of sulphide generation depends on several variables, such as pH, temperature, hydraulic retention time, and oxidation-reduction potential (ORP) (Firer et al., 2008). At pH characteristic of methanogenic systems, sulphide exists as bisulphide ($HS^-$) and hydrogen sulphide ($H_2S$) at 50% (Eq. 1 and 2). The undissociated form is partially released to gas space according to a distribution coefficient ($K_H$) (Eq. 3) (Rittmann and McCarty, 2001).

$$H_2S \leftrightarrow H^+ + HS^- \qquad pK_1 = 7.04 \qquad (1)$$

$$HS^- \leftrightarrow H^+ + S^{2-} \qquad pK_2 = 12.9 \qquad (2)$$

$$[H_2S]_d = K_H\,[H_2S]g \qquad K_H = 13\ \text{at/mol}\ (35^oC) \qquad (3)$$

Dissolved sulphide impairs the organic matter removal (it provides COD to the effluent), leads to accumulation of inert material in the sludge (metal sulphides), is toxic for microorganisms, and promotes growth of filamentous sulphide-oxidising bacteria (SOB) that can cause bulking sludge in the aerobic post treatment system (Cirne et al., 2008).

Gaseous sulphide is considered the most characteristic bad odor in the environment of anaerobic reactors and wastewater facilities in general (Noyola et al., 2006). Besides reducing the methane yield and the biogas quality, it causes contamination and toxicity to humans (Speece, 2008), and corrosion to many types of steel, thereby reducing the lifetime of pipework and installations for the utilisation of biogas (Deublein and Steinhauser, 2008). As a result, the presence of hydrogen sulphide can represent an important limitation for the biogas use. With traditional boilers and internal combustion engines, the recommendations are that hydrogen sulphide content should not be more than 1,000 ppmv, which in some cases means than biogas can be used without any treatment (Rasi et al., 2011). However, for trouble-free operation of combined heat and power (CHP) stations, manufacturers usually set standards below 100 or 300ppmv, depending on the equipment concerned (Peu et al., 2012). Nevertheless, short peaks can occasionally be accepted (Deublein and Steinhauser, 2008). The limit values are even stricter for the rest of the biogas applications (Rasi et al., 2011).

## HYDROGEN SULPHIDE CONTROL

Hydrogen sulphide can be controlled according three different strategies (Peu et al., 2012):

a) at the source, by controlling the feedstock
b) at the end, by desulphurising the biogas in a later treatment unit
c) at process level, directly inside the anaerobic digester

The first solution (a) is not realistic, and it is in fact the latter end-of-pipe treatment (b) which is the most consolidated strategy in practice (Cirne et al., 2008). For this purpose, a number of technologies exist, based on physical-chemical and biological principles (Lin et al., 2013). Combinations of physical-chemical and biological methods are frequently used too (Deublein and Steinhauser, 2008).

Physical-chemical processes are the most commonly applied for biogas desulphurisation. Among them, activated carbon and water scrubbing are two of the most popular techniques (Persson et al., 2007). Physical-chemical technologies are expensive due to high energy, chemical and disposal costs, and they cause detrimental impact on the environmental due to secondary pollutants production (Syed et al., 2006). Therefore, end-of-pipe biotechnologies are gaining tremendous popularity due to their cost-effectiveness and environment friendliness (Mudliar et al., 2010). Bioreactors offer high removal efficiencies (REs), even higher than the physical-chemical methods (Kobayashi et al., 2012), under relatively low temperatures and atmospheric pressure (Vergara-Fernández et al., 2007) and with limited or none chemical consumption (Díaz et al., 2010b). Furthermore, they result in harmless and odorless by-products. Solid elemental sulphur is a possible one, which can be returned to a production process (to a sulphuric acid plant) or utilised in another field (in agriculture as fertiliser) (Kleinjan, 2005).

The biotechnologies for hydrogen sulphide control consist of the utilisation of SOB to oxidise sulphide, and are usually based on the aerobic methods (Fernández et al., 2013). They are different in

the phase of the microorganisms, attached or suspended, and the phase of the liquid, flowing or stationary (Potivichayanon et al., 2006). The three most conventional configurations of bioprocesses for biological desulphurisation are: biofilters, bioscrubbers, and biotrickling filters. The desirable characteristics of a bioreactor are: simple configuration, no hydraulic problems, minimum volume, low micro-aeration/micro-oxygenation costs, high capability to transform sulphide into elemental sulphur, easy recovery of the generated elemental sulphur, no or minimum nutrient requirements, and robustness to fluctuations in operational conditions (Abatzoglou and Boivin, 2009; Lohwacharin and Annachhatre, 2010; Syed et al., 2006).

Despite its several advantages, there are many fewer full-scale bioscrubbers in operation than biofilters and bioscrubbers. This is probably related to the excessive biomass growth therein and the consequent high amounts of sludge needing for disposal, and the fact that two process units (absorption column and bioreactor) are required instead of one (Burgess et al., 2001). Moreover, the solubility of hydrogen sulphide limits the applicability of bioscrubbing for its removal (Ramírez et al., 2009). This technique has been reported to be useful for pollutants with a non-dimensional Henry's coefficient (*H*) lower than 0.01, while for hydrogen sulphide, *H*=0.92 (at 25ºC) (Kennes et al., 2009; Mudliar et al., 2010). On the other hand, the main drawbacks in biofilters and biotrickling filters are difficult control of the operational parameters and clogging (Montebello et al., 2012; Rodríguez et al., 2013). This latter problem intensifies under low oxygen availability, when elemental sulphur is the main by-product (Fernández et al., 2013; Fortuny et al., 2008).

The process-level strategies for hydrogen sulphide removal from biogas (strategy (c)) are also based on both chemical and biological methods. Most of the chemical-based techniques are impracticable or ineffective (Cirne et al., 2008). In general, they involve high technicality and costs, due to the prices of the reactants (Peu et al., 2012). Nevertheless, one of the most common methods for biogas desulphurisation at process level in sewage water treatment plants consists of dosing iron chloride to precipite sulphide (Díaz, 2011). Hydrogen sulphide concentrations in biogas below 150ppmv can be achieved (Appels et al., 2008).

The implementation of microanoxic or microaerobic conditions during digestion by air/oxygen or nitrate/nitrite injection is a very simple process-level strategy for hydrogen sulphide control. This method is possible due to the presence of SOB in the feedstocks (Weiland, 2010). In contrast to air/oxygen addition, nitrate/nitrite addition has been hardly evaluated, and the results are fairly equivocal (Cirne et al., 2008). According to Díaz et al. (2010a), applying microanoxic conditions in the reactor can be an ineffective solution for biogas desulphurisation due to heterotrophic denitrification can prevail over the mechanism of sulphide removal (chemolithoautotrophic denitrification).

Besides simple, the introduction of air/oxygen to bioreactors is an economical method for hydrogen sulphide control (Abatzoglou and Boivin, 2009; Díaz, 2011). It neither requires harmful reagent utilisation nor produces secondary wastes needing for disposal. Moreover, as the rest of biological systems, this method results in harmless and odorless (even valuable) by-products. Importantly, although the mixtures of methane-oxygen formed inside microaerobic reactors are far from being explosive, safety measures need to be taken into consideration to prevent the formation of explosive mixtures in case of a failure of the dosing equipment (Speece, 2008).

## MICROAEROBIC DIGESTERS

### Mechanisms and reactions involved

The basic mechanisms and reactions involved in the biogas desulphurisation in microaerobic reactors are all the same as in the other biological methods. After absorption (Eq. 1), species of aerobic SOB such as *Halothiobacillus*, *Sulfurimonas* and *Thiofaba* (Rodríguez et al., 2012; Kobayashi et al., 2012) consume sulphide according to Eq. 4 and 5 (Rodríguez et al., 2013; Lohwacharin and Annachhatre, 2010). The ratio between the available electron acceptor and electron donor, namely, the oxygen/sulphide ratio, is the key parameter determining the sulphate/elemental sulphur produced ratio (Fortuny et al., 2008). At this point, it should be noted that the biologically produced sulphur is often called biosulphur in order to highlight its different (positive) properties in relation to the sulphur obtained from other sources (Kleinjan, 2005).

$2HS^- + O_2 \rightarrow 2S^0 + 2OH^-$ $\Delta G^0$= -210.8kJ/mol (4)

$2HS^- + 4O_2 \rightarrow 2SO_4^{2-} + 2H^+$ $\Delta G^0$= -796.5kJ/mol (5)

$2HS^- + 2O_2 \rightarrow S_2O_3^{2-} + H_2O$ $\Delta G^0$= -387.3kJ/mol (6)

$S^0 + H_2O + 3/2\ O_2 \rightarrow SO_4^{2-} + 2H^+$ $\Delta G^0$= -587.1kJ/mol (7)

$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2SO_4^{2-} + 2H^+$ $\Delta G^0$= -818.3kJ/mol (8)

In case of highly loaded bioreactors, chemical sulphide oxidation to thiosulphate becomes relatively important due to the limitation in biological activity (Eq. 6) (Lohwacharin and Annachhatre, 2010). Any metal ion present in the reactor can function as a catalyst of this reaction (Kleinjan, 2005). SOB can effectively compete with the chemical oxidation mechanisms at low oxygen and sulphide concentrations (Robertson and Kuenen, 2006). Elemental sulphur and thiosulphate can be also biologically oxidised to sulphate according to Eq. (7) and (8), respectively (Fortuny et al., 2011; Tang et al., 2009). Although sulphide oxidation proceeds through several intermediates, elemental sulphur, sulphate and thiosulphate have been reported to be the stable by-products (Duan et al., 2005).

### Oxygen effects on digestion

Traditionally the introduction of oxygen in anaerobic reactors has a negative perception due to this agent is toxic for strictly anaerobic microorganism (acetogens and methanogens). However, these microbial groups have several deterrence mechanisms to tolerate microaerobic conditions with no or minor inhibitory effects (Botheju and Bakke, 2011).

The potential of oxygen to remove sulphide from the liquid and the gas phase is its most popular benefit of micro-aeration/micro-oxygenation during digestion, since has been investigated by several authors (Díaz et al., 2010a, 2010b; Duangmanee, 2009; Fdz-Polanco et al., 2009; Janssen et al., 1998; Jenicek et al., 2008, 2010, 2011; Khanal and Huang, 2003a, 2003b; van der Zee et al., 2007; Zhou et al., 2007). Under microaerobic conditions, sulphide removal has been proved to effectively compete for oxygen versus other processes, and to be faster than the re-reduction of oxidised sulphur compounds (van der Zee et al. 2007; Fdz-Polanco et al., 2009). Fdz-Polanco et al. (2009), Díaz et al.

(2010b), and Zhou et al. (2007) confirmed the feasibility of removing dissolved and gaseous sulphide concurrently by applying microaerobic conditions during digestion of different feedstocks. In addition, micro-aeration alleviated a severe sulphide inhibition in the study of Zhou et al. (2007), which resulted in a great improvement in the digestion performance and productivity both.

Recently, a number of studies have reported other important benefits of oxygen on the digestion process. Several researchers have achieved higher hydrolysis rates by applying oxygen-limiting conditions during digestion, or even subjecting the waste to an aerobic or microaerobic pre-treatment, without causing toxicity towards anaerobic microorganisms (Hasegawa et al., 2000; Johansen and Bakke, 2006; Zhu et al., 2009; Jagadabhi et al., 2010). The basis of this effect resides in the increased cell growth rates, synthesis and activity of the hydrolytic enzymes (Charles et al., 2009: Zhu et al., 2009), and the augmentation of microbial species diversity (Jenicek et al., 2010). As a result, higher methane yield and/or and improved effluent quality can be achieved (Lim and Wang, 2013; Botheju et al., 2010; Jenicek et al., 2008, 2010, 2011). Nonetheless, taking into account the findings of Charles et al. (2009) and Ye et al. (2006), the benefits of oxygen on digestion would not be recognisable under stationary conditions, but only under unbalanced circumstances.

Conversely, oxygen supply to reactors can lead to lower methane yield due to the fact that facultative biomass consumes easy-to-degrade components of the feedstock, such as volatile fatty acids, which would otherwise have been used by methanogens to produce biogas (Charles et al., 2009; Johanssen and Bakke, 2006). Similarly, the presence of methanotrophes in microaerobic reactors, which are strictly aerobic bacteria capable of oxidising methane (Madigan et al., 2003), can induce a negative effect on the methane productivity (Zitomer and Shrout, 1998). This highlights the importance of a precise adjustment of the air/oxygen flow rate or the micro-aeration/micro-oxygenation regime during (or prior to) digestion, which has been already suggested by Zhu et al. (2009) and Lim and Wang (2013). In fact, Botheju et al. (2010) revealed the possibility of the existence of an optimum micro-oxygenation level corresponding to maximum methane yield in a specified digestion system.

The impact of oxygen on the microbial communities has been investigated by some authors in order to achieve better understanding and predictability of microaerobic reactors, which in turn can be the basis to improve the performance of these systems. For this purpose, Jenicek et al. (2011) evaluated the specific activity of various microbial groups under both anaerobic and microaerobic conditions. At the beginning of the experiment, the methanogenic activity of the microaerobic biomass was slightly lower; however, in the long-term, under increasing dissolved sulphide concentration, it surpassed that of the anaerobic biomass. In contrast to the anaerobic sludge, the methanogenic activity of the microaerobic sludge was found to be independent on sulphide concentration. Additionally, Jenicek et al. (2011) also reported a slight and a several increase in the levels of sulphate-reducing and sulphide-oxidising activity (respectively) under microaerobic conditions.

Shifts in the microbial communities due to micro-aeration/micro-oxygenation have been also monitored by molecular techniques. The results of Tang et al. (2004) indicated that the presence of oxygen in municipal solid waste (MSW) reactor did not cause a dramatic shift in the structure of the microbial community. Nonetheless, they reported a dramatic rise in the ratio of hydrogenotrophic to acetoclastic methanogens, and indicated that SRB were not repressed under microaerobic conditions. This latter finding was consistent with the results obtained by Jenicel et al (2011). Similarly, Zhou et

al. (2007) observed that the rod-shaped methanogens almost disappeared and were replaced by cocci-shaped after imposing microaerobic conditions.

### Oxidative reactants and doses

The findings of Díaz et al. (2010a) and Jenicek et al. (2010) suggest that, though expensive, oxygen is the most profitable oxidative reactant when applying microaerobic conditions, as micro-aeration dilutes further the biogas due to the presence of nitrogen. Due to mass transfer limitations, some oxygen can also remain in the biogas (Díaz et al., 2010a, 2010b). This can considerably reduce the energetic efficiency of some applications, such as engines, or even prevent the biogas use, thereby giving rise to the need for nitrogen removal (such as vehicles). Nevertheless, the economic analysis carried out by Díaz (2011) showed that, despite the losses of energetic efficiency in the combustion engine resulting from biogas dilution by nitrogen, the most favorable microaerobic scenario was the introduction of air due to lower operational costs. However, unless the biogas is used for CHP or boilers, pure oxygen is recommended because nitrogen removal is difficult and expensive (Petersson and Wellinger, 2009). Therefore, for the most restrictive applications, the use of an oxygen concentrator could be an attractive alternative.

Despite the multiple definitions or the term “micro-aeration”, “micro-oxygenation” and “microaerobic” included in literature, none of them indicate a range of air/ oxygen flow rates. The micro-aeration/micro-oxygenation level to reactors should be adjusted according to the objective/s of the microaerobic treatment. Nonetheless, in any case, lack or surplus of nitrogen and/or oxygen in biogas should be avoided in order to preserve the biogas methane content. Both nitrogen and oxygen are expensive to remove (Pettersson and Wellinger, 2009). Moreover, the higher the accuracy in the micro-aeration/micro-oxygenation adjustment, the lower the sulphate/elemental sulphur produced ratio. ORP has been reported as an accurate regulation parameter of oxygen dosing in order to eliminate sulphide toxicity (Khanal and Huang, 2003a; 2003b), maximise elemental sulphur recovery (Janssen et al., 1998), and even desulphurise biogas (Duangmanee, 2009). However, its response to micro-oxygenation can be insufficient (Díaz et al., 1010a). Hence, Díaz et al. (2011) proposed to regulate the oxygen flow rate according to the biogas production, as they found a linear correlation between the ratio of the oxygen supply to the biogas flow rate, and the biogas sulphide content. Similarly, Jenicek et al. (2008), Tang et al. (2004) and Kobayashi et al. (2012) adjusted the air flow rate at approximately 10, 7.5 and 5%v/v of the biogas production (respectively). According to Pérez et al. (2012), at full-scale, micro-aeration is regulated manually by plant operators.

### Configurations

Microaerobic conditions can be implemented using different air/oxygen dosing points and mixing methods (Fig. 1):

a) Air/oxygen injection into the headspace and mechanical mixing and/or liquid recirculation and/or biogas recirculation
b) Air/oxygen injection into the feed stream and mechanical mixing and/or liquid recirculation and/or biogas recirculation

c) Air/oxygen injection into the liquid recirculation and mechanical mixing and/or biogas recirculation
d) Air/oxygen injection into the biogas recirculation and mechanical mixing and/or liquid recirculation
e) Air/oxygen injection into the liquid phase and mechanical mixing and/or liquid recirculation and/or biogas recirculation

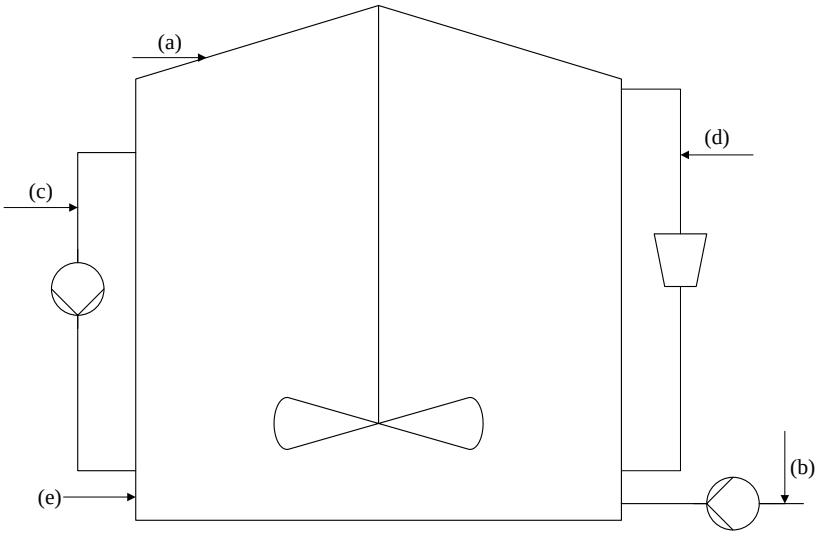


**Fig. 1**. Alternative configurations of microaerobic reactors.

On full-scale, due to the "oxygenophobia" of plant operators, the lack of full-scale (and even pilot-scale) studies, and the fact that air is costless, microaerobic conditions are generally applied by injecting air in the gas space, and the reactor's content is mixed mechanically (Abatzoglou and Boivin, 2009; Pérez et al., 2012; Weiland 2010). Thus, the oxygen transfer to the liquid phase is minimised. According to Abatzoglou and Boivin (2009), Pérez et al. (2012), and Weiland (2010), in Europe, this technique is applied in agricultural and on-farm reactors. However, Kobayashi et al (2012) are the only reference with regard to this. They reported the results obtained during operation of a full-scale reactor treating dairy cow manure under the aforementioned microaerobic configuration. Elemental sulphur was found to accumulate on the reactor walls, on the ceiling, and on the plastic net and the stainless catwalk that were installed in the gas space of the reactor in order to provide additional surface area for SOB. Nonetheless, according to Weiland (2010), sulphide oxidation also occurs at the liquid interface, and the installation of specific supports made of wood and fabric in the headspace is a frequent practice. Additionally, Kobayashi et al. (2012) indicated that the levels of sulphide-oxidising activity at the different locations in the headspace depended on the water and nutrients availability and, as a result, they are expected to be higher at the areas nearest the liquid phase.

When air/oxygen is injected into the liquid phase, or when it is introduced to the gas space and biogas recirculacion is implemented as the mixing method, the oxygen transfer rate to the liquid phase increases (Díaz et al., 2010b). As highlighted, this can be a more attractive option, since other benefits of oxygen on the process can be achieved. However, under such conditions, the air/oxygen

demand of the reactors has been reported to increase due to oxygen is partially consumed in other oxidatives processes (Díaz et al., 2010b; Jenicek et al., 2008). As a result, elemental sulphur has been reported to leave the reactor with the effluent, and/or to accumulate therein (Cirne et al., 2008; Jenicek et al., 2011). Jenicek et al. (2008; 2010) are the only reference of full-scale microaerobic digestion with air injection into the liquid phase. They reported the results obtained during operation of a full-scale microaerobic reactor treating a mixture activated sludge from a municipal WWTP and bone flour (50:1). Its content was mixed by a paddle-wheel stirrer and sludge recirculation, and micro-aeration was introduced to the recirculation stream.

Both Kobayashi et al. (2012) and Jenicek et al. (2008; 2010) achieved high efficiencies of biogas desulphurisation, which is consistent with the results obtained by Díaz et al. (2010b) on lab-pilot-scale. They reported similar performances of hydrogen sulphide removal under various configurations (different oxygen dosing points and recirculation methods both), which was attributed to the fact that the biogas desulphurisation took place in the gas space independently of the oxygen transfer rate to the liquid phase. Furthermore, Díaz et al., (2010b) found that, when biogas recirculation was applied as the mixing method, the oxygen consumption in undesired processes rose. In the meantime, gaseous and dissolved sulphide was removed simultaneously, which was indeed consistent with the previous results (Fdz-Polanco et al., 2009). Accordingly, Díaz et al., (2010b) indicated that biogas recirculation could be an interesting alternative in reactors suffering from sulphide inhibition; however, in reactors aiming only for biogas recirculation, the configuration ensuring the minimum costs would consist of air/oxygen injection into the headspace and sludge recirculation.

Besides on the similar REs achieved under the various configurations tested, the hypothesis proposed by Díaz et al. (2010b) that the hydrogen sulphide removal from biogas occurred in the headspace was supported by microbial analysis. Although oxygen was introduced from the bottom of the reactor, SOB were not identified in the samples retrieved from the liquid phase. Conversely, genera belonging to this microbial group were found in the sulphur-rich biomass attached to the gas space. These results were consistent with those obtained by Rodríguez et al. (2012).

## Economic feasibility

The economic feasibility of applying microaerobic conditions in order to remove hydrogen sulphide in an already built and a new WWTP were both evaluated by Díaz (2011). They considered four different scenarios: three microaerobic scenarios involving the implementation of microaerobic conditions by pure oxygen (from a cryogenic tank), concentrated oxygen, and air, and the existing scenario consisting in ferric chloride dosing. Díaz et al. (2011) concluded that the profitability of implementing microaerobic conditions during digestion in an already built WWTP depended on the required intervals of time at which the headspace of the reactor must be cleaned. The cost arising from cleaning intervals shorter than 10 years could be prohibitive. Conversely, in newly constructed WWTPs, they indicated that, even with cleaning intervals of slightly less than 3 years, the microaerobic treatment of biogas would be a more profitable method for hydrogen sulphide control than ferric chloride addition.

## References

Abatzoglou, N., Boivin, S., 2009. A review of gas purification processes. Biofuels Bioprod. Biorefin. 3, 42-71.

Abbasi, T., Tauseef, S.M., Abbasi, S.A., 2012. Anaerobic digestion for global warming control and energy generation - An overview. Renew. Sustain. Energy Rev. 16, 3228-3242.

Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy and Combustion Sci. 34, 755-781.

Botheju, D., Lie, B., Bakke, R., 2010. Oxygen effects in anaerobic digestion – II. Model., Identif. Control 31, 55-65.

Botheju, D., Bakke, R., 2011. Oxygen effects in anaerobic digestion - A review. Open Waste Manag. J. 4, 1-19.

Burgess, J. E., Parsons, S. A., Stuetz, R. M., 2001. Developments in odour control and waste gas treatment biotechnology: a review. Biotechnol. Adv. 19, 35-63.

Charles, W., Walker, L., Cord-Ruwisch, R., 2009. Effect of pre-aeration and inoculum on the start-up of batch thermophilic anaerobic digestion of municipal solid waste. Bioresour. Technol. 100, 2329-2335.

Chen, Y., Cheng, J. J., Creamer, K. S., 2008. Inhibition of anaerobic digestion process: A review. Bioresour. Technol. 99, 4044-4064.

Cirne, D. G., van der Zee, F. P., Fdz-Polanco, M., Fdz-Polanco, F., 2008. Control of sulphide during anaerobic treatment of S-containing wastewaters by adding limited amounts of oxygen or nitrate. Rev. Environ. Sci. Biotechnol. 7, 93-105.

Deublein, D., Steinhauser, A., 2008. Biogas from Waste and Renewable Resources: An Introduction. Wiley-VCH, Weinheim.

Díaz, I., Lopes, A. C., Pérez, S. I., Fdz-Polanco, M., 2010a. Performance evaluation of oxygen, air and nitrate for the microaerobic removal of hydrogen sulphide in biogas from sludge digestion. Bioresour. Technol. 101, 7724-7730.

Díaz, I., Pérez, S. I., Ferrero, E. M., Fdz-Polanco, M., 2010b. Effect of oxygen dosing point and mixing on the microaerobic removal of hydrogen sulphide in sludge digesters. Bioresour. Technol. 102, 3768-3775.

Díaz, I., Lopes, A. C., Pérez, S. I., Fdz-Polanco, M., 2011. Determination of the optimal rate for the microaerobic treatment of several $H_2S$ concentrations in biogas from sludge digesters, Water Sci. Technol. 64, 233-238.

Díaz, I., 2011. $H_2S$ removal from biogas. From anaerobic to microaerobic. PhD Thesis. University of Valladolid, Valladolid, España.

Duan, H., Yan, R., Koe, L.C., 2005. Investigation on the mechanism of $H_2S$ removal by biological activated carbon in a horizontal biotrickling filter. Appl. Microbiol. Biotechnol. 69, 350-357.

Duangmanee, T., 2009. Micro-aeration for hydrogen sulfide removal from biogas. PhD Thesis. Iowa State University, Ames, Iowa.

Fdz-Polanco, M., Díaz, I., Pérez, S. I., Lopes, A. C., Fdz-Polanco, F., 2009. Hydrogen sulphide removal in the anaerobic digestion of sludge by micro-aerobic processes: pilot plant experience. Water Sci. Technol. 60, 3045-3050.

Fernández, M., Ramírez, M., Pérez, R. M., Gómez, J. M., Cantero, D., 2013. Hydrogen sulphide removal from biogas by an anoxic biotrickling filter packed with Pall rings. Chem. Eng. J. 225, 456-463.

Firer, D., Friedler, E., Lahav, O., 2008. Control of sulfide in sewer systems by dosage of iron salts: Comparison between theoretical and experimental results, and practical implications. Sci. Total Environ. 392, 145-156.

Fortuny, M., Baeza, J.A., Gamisans, X., Casas, C., Lafuente, J., Deshusses, M.A., Gabriel, D., 2008. Biological sweetening of energy gases mimics in biotrickling filters. Chemosphere 71, 10-7.

Fortuny, M., Gamisans, X., Deshusses, M. A., Lafuente, J., Casas, C., Gabriel, D., 2011. Operational aspects of the desulfurization process of energy gases mimics in biotrickling filters. Water Res. 45, 5665-5674.

Hasegawa, S., Shiota, N., Katsura, K., Akashi, A., 2000. Solubilization of organic sludge by thermophilic aerobic bacteria as a pretreatment for anaerobic digestion. Water Sci. Technol. 41, 163-169.

Holm-Nielsen, J. B., Al Seadi, T., Oleskowicz-Popiel, P., 2009. The future of anaerobic digestion and biogas utilization. Bioresour. Technol. 100, 5478-5484.

Jagadabhi, P. S., Kaparaju, P., Rintala, J., 2010. Effect of micro-aeration and leachate replacement on COD solubilisation and VFA production during mono-digestion of grass-silage in one-stage leach-bed reactors. Bioresour. Technol. 101, 2818-2824.

Janssen, A. J. H., Meijer, S., Bontsema, J., Lettinga, J., 1998. Application of the redox potential for controlling a sulfide oxidizing bioreactor. Biotechnol. Bioeng. 60, 147-155.

Jenicek, P., Keclik, F., Maca, J., Bindzar, J., 2008. Use of microaerobic conditions for the improvement of anaerobic digestion of solid wastes. Water Sci. Technol. 58, 1491-1496.

Jenicek, P., Koubova, J., Bindzar, J., Zabranska, J., 2010. Advantages of anaerobic digestion of sludge in microaerobic conditions. Water Sci. Technol. 62, 427-434.

Jenicek, P., Celis, C.A., Koubova, J., Pokorna, D., 2011. Comparison of microbial activity in anaerobic and microaerobic digesters. Water Sci. Technol. 63, 2244-2249.

Johansen, J. E., Bakke, R., 2006. Enhancing hydrolysis with microaeration. Water Sci. Technol. 53, 43-50.

Kennes, C., Rene, E. R., Veiga, M. C., 2009. Bioprocesses for air pollution control. J. Chem. Technol. Biotechnol. 84, 1419-1436.

Khanal, S. K., Huang, J. C., 2003a. Anaerobic treatment of high sulfate wastewater with oxygenation to control sulfide toxicity. J. Environ. Eng. 129, 1104-1111.

Khanal, S. K., Huang, J. C., 2003b. ORP-based oxigenation for sulfide control in anaerobic treatment of high sulphate wastewater. Water Res. 37, 2053-2062.

Kleinjan, W., 2005. Biologically produced sulfur particles and polysulfide ions: effects on a biotechnological process for the removal of hydrogen sulfide from gas streams. PhD Thesis. Wageningen Universiteit, Wageningen, The Netherlands.

Kobayashi, T., Li, Y.-Y., Kubota, K., Harada, H., Maeda, T., Yu, H.-Q., 2012. Characterization of sulfide-oxidizing microbial mats developed inside a full-scale anaerobic digester employing biological desulfurization. Appl. Microbiol. Biotechnol. 93, 847-857.

Lim, J. W., Wang, J.-Y., 2013. Enhanced hydrolysis and methane yield by applying microaeration pretreatment to the anaerobic co-digestion of brown water and food waste. Waste Manag. 33, 813-819.

Lin, W.-C., Chen, Y.-P., Tseng, C.-P., 2013. Pilot-scale chemical-biological system for efficient $H_2S$ removal from biogas. Bioresour. Technol. 135, 283-291.

Lohwacharin, J., Annachhatre, A. P., 2010. Biological sulfide oxidation in an airlift bioreactor. Bioresour. Technol. 101, 2114-2120.

Madigan, M. T., Martinko, J. M., Parker, 2003. Brock Biología de los microorganismos (Brock Biology of microorganisms). 10th ed. Pearson Prentice Hall, Madrid.

Maestre, J. P., Rovira, R., Álvarez-Hornos, F. J., Fortuny, M., Lafuente, J., Gamisans, X., Gabriel, D., 2010. Bacterial community analysis of a gas-phase biotrickling filter for biogás mimics desulfurization through the rRNA approach. Chemosphere 80, 872-880.

Montebello, A., M., Fernández, M., Almenglo, F., Ramírez, M., Cantero, D., Baeza, M., Gabriel, D., 2012. Simultaneous methylmercaptan and hydrogen sulfide removal in the desulfurization of biogas in aerobic and anoxic biotrickling filters. Chem. Eng. J. 200-202, 237-246.

Mudliar, S., Giri, B., Padoley, K., Satpute, D., Dixit, R., Bhatt, P., Pandey, R., Juwarkar, A., Vaidya, A., 2010. Bioreactors for treatment of VOCs and odours - A review. J. Environ. Manag. 91, 1039-1054.

Murphy, J., Braun, R., Weiland, P., Wellinger, A., 2011. Biogas from crop digestion. IEA Bioenergy, Task 37 - Energy from biogas.

Noyola, A., Morgan-Sagastume, J. M., López-Hernández, J. E., 2006. Treatment of biogas produced in anaerobic reactors for domestic wastewater: odor control and energy/resource recovery. Rev. Environ. Sci. Biotechnol. 5, 93-114.

Pérez, A., Li. T., de Arespacochaga, N., Bouchy, L., 2012. Full-scale experiences of oxygen injection to reduce $H_2S$ content in anaerobic digestion. World Congress of Water, Climate and Energy. Dublin, Ireland.

Persson, A., Jonsson, O., Wellinger, A., 2007. Biogas upgrading to vehicle fuel standards and grid injection. IEA Bioenergy, Task 37 - Energy from biogas and landfill gas.

Petersson, A., Wellinger, A., 2009. Biogas upgrading technologies - developments and innovations, IEA Bioenergy, Task 37 - Energy from biogas and landfill gas.

Peu, P., Picard, S., Diara, A., Girault, R., Béline, F., Bridoux, G., Dabert, P., 2012. Prediction of hydrogen sulphide production during anaerobic digestion of organic substrates. Bioresour. Technol. 121, 419-424.

Potivichayanon, S., Pokethitiyook, M., Kruatrachue, M., 2006. Hydrogen sulfide removal by a novel fixed-film bioscrubber system. Process Biochem. 41, 708-715.

Ramírez, M., Gómez, J. M., Aroca, G., Cantero, D., 2009. Removal of hydrogen sulfide by immobilized Thiobacillus thioparus in a biotrickling filter packed with polyurethane foam. Bioresour. Technol. 100, 4989-4995.

Rasi, S., Veijanen, A., Rintala, J., 2007. Trace compounds of biogas from different biogas production plants. Energy 32, 1375-1380.

Rasi, S., Läntelä, J., Rintala, J., 2011. Trace compounds affecting biogas energy utilisation – A review. Energy Convers. Manag. 52, 3369-3375.

Rittmann, B. E., McCarty, P. L., 2001. Environmental biotechnology: principles and applications. McGraw-Hill.

Robertson, L. A., Kuenen, J. G., 2006. The Genus Thiobacillus. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer K. H., Stackebrandt, E. (eds). The Prokaryotes. Vol 5. 3rd ed. Springer, New York, 812-827.

Rodríguez, E., Lopes, A., Fdz-Polanco, M., Stams, A. J., García Encina, P. A., 2012. Molecular analysis of the biomass of a fluidized bed reactor treating synthetic vinasse at anaerobic and micro-aerobic conditions. Appl. Microbiol. Biotechnol. 93, 2181-2191.

Rodríguez, G., Dorado, A. D., Fortuny, M., Gabriel, D., Gamisans, X., 2013. Biotrickling filters for biogas sweetening: Oxygen transfer improvement for a reliable operation. Process Saf. Environ. Prot., DOI: http://dx.doi.org/10.1016/j.psep.2013.02.002.

Stams, A. J. M., Oude Elferink, S. J. W. H., Westermann, P., 2003. Metabolic interactions between methanogenic consortia and anaerobic respiring bacteria. Adv. Biochem. Eng./Biotechnol. 81, 31-56.

Speece, R. E., 2008. Anaerobic Biotechnology and Odor/Corrosion Control for Municipalities and Industries, Archae Press, Nashville, Tennessee.

Syed, M., Soreanu, G., Falletta, P., Béland, M., 2006. Removal of hydrogen sulfide from gas streams using biological processes - A review. Canadian Biosyst. Engin. 48, 2.1-2.14.

Tang, Y., Shigematsu, T., Ikbal, Morimura, S., Kida, K., 2004. The effects of micro-aeration on the phylogenetic diversity of microorganisms in a thermophilic anaerobic municipal solid-waste digester. Water Res. 38, 2537-2550.

Tang, K., Baskaran, V., Nemati, M., 2009. Bacteria of the sulphur cycle: An overview of microbiology, biokinetics and their role in petroleum and mining industries. Biochem. Engineer. J. 44, 73-94.

van der Zee, F. P., Villaverde, S., García, P. A., Fdz-Polanco, F., 2007. Sulphide removal by moderate oxygenation of anaerobic sludge environments. Bioresour. Technol. 98, 518-524.

Vergara-Fernández, A., Molina, L. L., Pulido, N. A., b , Aroca, G., 2007. Effects of gas flow rate, inlet concentration and temperature on the biofiltration of toluene vapors. J. Environ. Manag. 84, 115-122.

Ward, A. J., Hobbs, P. J., Holliman, P- J., Jones, D. L., 2008. Optimisation of the anaerobic digestion of agricultural resources. Bioresour. Technol. 99, 7928-7940.

Weiland, P. (2010). Biogas production: current state and perspectives. Appl. Microbiol. Biotechnol. 85, 849-860.

Ye, F., Chen, Y., Feng, X., 2006. Advanced start-up of anaerobic attached film expanded bed reactor by pre-aeration of biofilm carrier. Bioresour. Technol. 96, 115-119.

Zhou, W., Imai, T., Ukita, M., Li, F., Yuasa, A., 2007. Effect of limited aeration on the anaerobic treatment of evaporator condensate from a sulfite pulp mill. Chemosphere, 66, 924-929.

Zhu, M., Lü, F., Hao, L.-P., He, P.-J., Shao, L.-M., 2009. Regulating the hydrolysis of organic wastes by micro-aeration and effluent recirculation. Waste Manag. 29, 2042-2050.

Zitomer, D. H., Shrout, J. D., 1998. Feasibility and benefits of methanogenesis under oxygen-limited conditions. Waste Manag. 18, 107-116.

# OBJECTIVES

The general objective of this thesis is TO CONTROL THE HYDROGEN SULPHIDE CONTENT IN THE BIOGAS PRODUCED DURING DIGESTION BY MICROAEROBIC PROCESSES. For this purpose, **two different strategies** are investigated:

a. Biogas desulphurisation at process level by imposing microaerobic conditions in the reactor
b. End-of-pipe desulphurisation of biogas by reproducing the conditions present in the gas space of microaerobic reactors in an additional treatment unit called microaerobic desulphurisation unit (MDU)

The main aims of the section of this thesis dealing with **process-level control** of hydrogen sulphide (strategy (a)) are:

1. to recognise and describe the possible effects that oxygen can provide during digestion, including on the microbial communities
2. to explore the possible alternatives to accurately regulate the oxygen flow rate in order to achieve a precise control the biogas sulphide content
3. to estimate the optimum oxygen dose of operation under different configurations
4. to locate and characterise the SOB growing in the gas space
5. to study both the impact of headspace cleaning and the operation time on the efficiency of biogas desulphurisation
6. to clarify where the process of biogas desulphurisation predominantly takes place
7. to test the feasibility of the process of hydrogen sulphide removal from biogas during digestion under different configurations

The main objectives of the section of this thesis dealing with **end-of-pipe control** of hydrogen sulphide (strategy (b)) are:

1. to evaluate the feasibility of the process and the effect of various operating parameters in its performance
2. to investigate the basis for design and scale-up

This thesis is organised as a compendium or eight research articles, which are arranged in eight chapters. The six first chapters focus on strategy (a), and Chapter 7 and 8 on strategy (b). Table 1 links the above objectives with the different chapters of the work. In addition, the scale at which the different objectives are implemented is indicated.

| STRATEGY | SPECIFIC OBJECTIVE | SCALE | CHAPTER |
|---|---|---|---|
| (a) Process-level control | 1. to recognise and describe the possible effects that oxygen can provide during digestion, including on the microbial communities | Lab-pilot Industrial-pilot | 1, 6 |
| | 2. to explore the possible alternatives to accurately regulate the oxygen flow rate in order to achieve a precise control the biogas sulphide content | Lab-pilot | 2 |
| | 3. to estimate the optimum oxygen dose of operation under different configurations | Lab-pilot Industrial-pilot | 2, 6 |
| | 4. to locate and characterise the SOB growing in the gas space | Lab-pilot | 3 |
| | 5. to study both the impact of headspace cleaning and the operation time on the efficiency of biogas desulphurisation | Lab-pilot | 3 |
| | 6. to clarify where the process of biogas desulphurisation predominantly takes place | Lab-pilot | 3, 4, 5 |
| | 7. to test the feasibility of the process of hydrogen sulphide removal from biogas under different configurations | Lab-pilot Industrial-pilot | 2, 6 |
| (b) End-of-pipe control | 1. to evaluate the feasibility of the process and the effect of various operating parameters on its performance | Lab-pilot | 7, 8 |
| | 2. to investigate the basis for design and scale-up | Lab-pilot | 8 |

**Table 1**. List of objectives and chapters of the thesis organised into the two possible strategies of hydrogen sulphide control. The scale of the experiments included in each chapter is indicated.

# MATERIALS AND METHODS

This thesis is developed at two different scales: lab-pilot and industrial-pilot scale. Table 1 (included in the "Objectives" section) indicates the scale/s of operation in the different chapters. The characteristics and equipment of the pilot plants are described below. Subsequently, the analytical techniques used for digestion and sulphur-compounds monitoring are indicated.

## PILOT PLANTS

### Lab-pilot reactors

The experiments presented in Chapter 1-5 are carried out in a lab-pilot plant comprising two continuous stirred tank reactors (CSTRs) operated under mesophilic conditions (35±1ºC). Temperature is maintained by an electric resistor surrounding their walls, which are in turn covered with insulation. In Chapter 1-3, they have a working and a total volume of 200 and 250L, respectively. In Chapter 4 and 5, one CSTR is modified; the flat ceiling is replaced by a conical one with a transparent cylindrical piece on top. Thus, the total reactor volume is 266L. The working volume varies between almost 266 and 216L, depending on the experiment. Most of the chapters include a diagram of the digester/s.

Mixed sludge with variable organic load and sulphur load is collected weekly from the WWTP of Villalonquéjar (Burgos) and stored at 4ºC. Feed is supplied to both digesters from continuously stirred tanks at ambient temperature by peristaltic pumps. The specific hydraulic retention time (HRT) maintained in every experiment is indicated in the respective "Materials and Methods" section of the different chapters. Sludge recirculation is applied at 50L/h by peristaltic pumps. Microaerobic conditions are implemented by supplying pure oxygen from a cylinder by mass flow controllers (Bronkhorst EL-FLOW Select). Oxygen is injected into the sludge recirculation or into the gas space, depending on the chapter.

Digestion pressure and temperature are monitored by using a sensor and a probe, respectively. The pressure control of the reactor is performed hydraulically. Biogas is transported by flexible Tygon® tubing to an inverted cylinder equipped with an electrovalve, where the flow rate is measured by water displacement (550±5mL). Biogas is characterised online in terms of methane, carbon dioxide, nitrogen, oxygen, hydrogen sulphide and hydrogen by gas chromatography (GC) in a CP-4900 Micro-GC according to Díaz et al. (2010b). This is equipped with a thermal conductivity detector (TCD) and two modules: CP-Molsieve 5A PLOT (10m×32mm, df=30µm) for oxygen and nitrogen analysis, and CP-PoraPLOT Q (10m×32mm, df=10µm) for methane, carbon dioxide and hydrogen sulphide quantification. Helium is used as the carrier gas. All the aforementioned data are displayed and stored in real-time in a computer. Only in Chapter 5, biogas composition is determined in a CP-3800 GC by manual injection with a 100µL-syringe, as described by Díaz et al. (2010a). This chromatograph is equipped with a TCD and two capillary columns: CP-Molsieve 5A (15m×0.53mm, df 15=µm) for oxygen, nitrogen and methane analysis, and a CP-PoraBOND Q (25m×0.53mm, df 10=µm) for carbon dioxide and hydrogen sulphide quantification. Helium is the carrier gas.

### Industrial-pilot reactor

A CSTR with a working volume of 5$m^3$ and a total volume of 7$m^3$ located at the aforementioned WWTP (Villalonquéjar, Burgos) is operated. Temperature (35±1ºC) is maintained by heating the recirculation stream with a water heat exchanger. Mixed sludge produced on site (namely, the same sludge used as the feed in the lab-pilot plant) is continuously pumped to the digester by a screw pump. HRT is approximately 20d. The reactor's content is recirculated by a peristaltic pump at approximately 25$m^3$/d. Biogas recirculation is implemented by compressor at a rate of 21$m^3$/d. Microaerobic conditions are implemented by an oxygen concentrator (PRECISE 6000). The concentrated oxygen purity is permanently monitored on an ultrasound basis, and introduced intermittently at different points of the reactor. A diagram of the digester and the different configurations implemented is included in Chapter 6.

Digestion pressure and temperature are monitored by sensors and probes, respectively. Biogas production is quantified by a BROOKS thermal mass flow meter, and its composition is determined by a CP-4900 Micro-GC according to Díaz et al. (2010b) and a GA3000 Range Gas Analyser, depending on the operational stage. All this data, along with recirculation rate, are displayed and stored in real-time in a computer. SCADA software is used for monitoring.

### Lab-pilot microaerobic desulphurisation unit

Biogas produced during pilot-scale digestion is desulphurised in an external process unit (MDU). In Chapter 8, a bottle is used as the source of the hydrogen sulphide. Oxygen and hydrogen sulphide both are injected by the means of mass flow controllers (Bronkhorst EL-FLOW Select). The system is kept in a thermostated chamber in order to regulate temperature. The inlet and the outlet biogas stream are characterised by a CP-4900 Micro-GC according to Díaz et al. (2010b), as described above. Digested sludge was used as the reaction media. The biogas flow rate leaving the system was measured by water displacement in an inverted cylinder (550±5 or 120±2mL, in Chapter 7 and 8, respectively).

## CHEMICAL ANALYSIS

### Pilot-scale reactors

Digestion performance is evaluated at least weekly by the conventional parameters for sludge digestion. The feed and the digestate are analysed in terms of total and soluble chemical oxygen demand (TCOD and SCOD) total and volatile solids (TS and VS) by standard methods (APHA, 1998). Total kjeldahl nitrogen (TKN) and ammonium are also measured according to APHA (1998). Specifically, they are quantified by digestion-titration and distillation-titration, respectively. Moreover, nitrate, nitrite and chloride are determined by ion chromatography (APHA, 1998). At industrial-pilot scale, these anions are analysed by ultraviolet (UV)-visible spectrophotometry.

Carbon is analysed occasionally by infrared (IR) spectroscopy in a LECO CS-225. As chemical indicators of the bioreactor state, pH is monitored by a pH-meter with a temperature probe, and the alkalinity measurements (total alkalinity and partial alkalinity, TA and PA, respectively) are based on standard methods (APHA, 1998), and the methodology proposed by Ripley et al. (1986). Volatile fatty acids (VFAs) are determined separately (acetic, propionic, butyric, isobutyric, valeric,

isovaleric and hexanoic acids) by GC. At industrial-pilot scale, VFAs are quantified as acetic acid by titration.

In order to evaluate the impact of oxygen on the sulphur balances, sulphate and thiosulphate are determined by ion chromatography (APHA, 1998) and high-performance liquid chromatography (HPLC), respectively. Thiosulphate is quantified according to the methodology described by van der Zee et al. (2007). In addition, dissolved sulphide concentration is measured by potentiometric titration with a silver/sulphide ion selective electrode (APHA, 1998). Sulphur is quantified occasionally by IR spectroscopy (LECO CS-225).

### Pilot-scale microaerobic desulphurisation unit

The inoculum is characterised in terms of TS and VS according to APHA (1998). The content of carbon and sulphur in the inoculum and sulphur-rich biomass accumulated inside the MDU is determined in a LECO CS-225 by IR spectroscopy. Additionally, sulphate and thiosulphate are analysed by ion chromatography (APHA, 1998) and HPLC (van der Zee et al., 2007), respectively.

## MICROBIAL ANALYSIS

### Sample collection, DNA isolation, and 16S rRNA gene amplification

Samples are stored at -20ºC for further analysis. The V6-V8 regions of the bacterial 16S rRNA genes are amplified by polymerase chain reaction (PCR) using the universal primers 968-F-GC and 1401-R (Sigma- Aldrich, St. Louis, MO, USA) (Nübel et al., 1996). The primers A 109(T)-F and 515-GC-R (Sigma-Aldrich, St. Louis, MO, USA) (Muyzer and Stams, 2008; Großkopf et al. 1998) are used for the PCR amplification procedure of the V2-V3 regions of the archaeal 16S rRNA genes. The PCR mixture (50µL) contains 1µL of each primer (10ng/µL each primer), 25µL of BIOMIX ready-to-use 2×reaction mix (Bioline, Ecogen), PCR reaction buffer and deoxynucleotide triphosphates (dNTPs), 2µL of the extracted DNA, and Milli-Q water. PCR is performed in an iCycler Thermal Cycler (Bio Rad Laboratories, Inc.) applying the thermo-cycling program described by Rodríguez et al. (2012).

### DGGE analysis, sequencing and DNA sequence analysis

Denaturing gradient gel electrophoresis (DGGE) of the bacterial and archaeal amplicons are performed according to Rodríguez et al. (2012). DGGE profiles are compared using the GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). The Shannon-Wiener diversity index of every sample and the pair-wise similarity coefficient were both calculated according to Lebrero et al. (2013). Similarity dendrograms were constructed by using UPGMA clustering with error resampling (500 resampling experiments).

Individual bands were excised from the DGGE gels. Both DNA extraction and purification of PCR products are carried out according to Rodríguez et al. (2012). The taxonomic position of the sequenced DGGE bands is obtained using the ribosomal database project (RDP) classifier tool (Wang et al., 2007). Moreover, the closest matches to every band are obtained from the BLAST search tool at the National Centre for Biotechnology Information (NCBI) website (McGinnis and Madden, 2004). Along with BLAST, DECIPHER is used as the chimera checking tool (Wright et al.,

2012). Sequences are deposited in the GenBank database (NCBI). Alignment (ClustalW) and phylogenetic analysis are performed using the MEGA software (version 6.0). The phylogenetic trees are constructed using the neighbor-joining method (1,000-fold bootstrap analysis).

## REFERENCES

APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th edn. American Public Health Association/American Water Works Association/Water Environmental Federation, Washington DC, USA.

Díaz, I., Lopes, A. C., Pérez, S. I., Fdz-Polanco, M., 2010a. Performance evaluation of oxygen, air and nitrate for the microaerobic removal of hydrogen sulphide in biogas from sludge digestion. Bioresour. Technol. 101, 7724-7730.

Díaz, I., Pérez, S. I., Ferrero, E. M., Fdz-Polanco, M., 2010b. Effect of oxygen dosing point and mixing on the microaerobic removal of hydrogen sulphide in sludge digesters. Bioresour. Technol. 102, 3768-3775.

Großkopf, R., Janssen P. H., Liesack, W., 1998. Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. Appl. Environ. Microbiol. 64, 960-969.

Lebrero, R., Volckaert, D., Pérez, R., Muñoz, R., Van Langenhove, H., 2013. A membrane bioreactor for the simultaneous treatment of acetone, toluene, limonene and hexane at trace level concentrations. Water Res. 47, 2199-2212.

McGinnis, S., Madden, T. L., 2004. BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Res. 32, W20-25.

Muyzer, G., Stams, A. J. M., 2008. The ecology and biotechnology of sulfate-reducing bacteria. Nat. Rev. Microbiol. 6, 441-454.

Nübel, U., Engelen, B., Felske, A., Snaidr, J., Wieshuber, A., Amann, R. I., Ludwig, W., Backhaus, H., 1996. Sequence heterogeneities of genes encoding 16S rRNAs in Paenibacillus polymyxa detected by temperature gradient gel electrophoresis. J. Bacteriol. 178, 5636-5643.

Ripley, L.E., Boyle, W.C., Converse, J.C., 1986. Improved alkalimetric monitoring for anaerobic digestion of high strength wastes. Water Pollut. Control Fed. 58, 406-411.

Rodríguez, E., Lopes, A., Fdz-Polanco, M., Stams, A. J., García Encina, P. A., 2012. Molecular analysis of the biomass of a fluidized bed reactor treating synthetic vinasse at anaerobic and micro-aerobic conditions. Appl. Microbiol. Biotechnol. 93, 2181-2191.

van der Zee, F. P., Villaverde, S., García, P. A., Fdz-Polanco, F., 2007. Sulphide removal by moderate oxygenation of anaerobic sludge environments. Bioresour. Technol. 98, 518-524.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261-5267.

Wright, E.S., Yilmaz, L.S., Noguera, D.R., 2012. DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences. Appl. Environ. Microbiol. 78, 717-725.

# OUTLINE

This thesis is organised into two sections, according with the two strategies of hydrogen sulphide control. The first one focuses on process-level control of hydrogen sulphide, namely, microaerobic reactors, and includes six chapters (Chapter 1-6). The second section focus on end-of-pipe control of hydrogen sulphide, that is, MDUs, and comprises two chapters (Chapter 7 and 8).

In **Chapter 1**, the potential benefits of oxygen on the digestion process are investigated by subjecting a lab-scale reactor to a hydraulic overload. Only a mild imbalance is caused, which is overcome without deterioration in the digestion performance or productivity. In fact, biogas and methane yield both are slightly higher during the period of imbalance. Under anaerobic conditions, hydrogen partial pressure rose, and acetic acid formation became less favourable. Therefore, oxygen seems to form a more stable digestion system, which means increased ability to deal successfully with overloads. In the meantime, micro-oxygenation improves the biogas quality independently of the HRT, due to it minimises the hydrogen sulphide concentration and reduces negligibly the methane content, while maintaining the oxygen surplus around zero. This chapter highlights the importance of precisely adjust the micro-oxygenation level during digestion in order to maximise the benefits of oxygen injection.

**Chapter 2** investigates the suitability of using biogas production and hydrogen sulphide concentration in biogas as the parameters to precisely regulate the oxygen flow rate during microaerobic digestion for the development of efficient control strategies. The micro-oxygenation level is automatically adjusted according to the biogas sulphide content by a feedback Proportional-Integral-Derivative (PID) controller. The target hydrogen sulphide concentration is rapidly achieved (in 5.5h at most), and the oxygen supply reached is considered to be the optimum in the short-medium term, since it keeps high REs (around 100%) and oxygen concentrations in the biogas of approximately 0.09%v/v during the days following the controller application. As an alternative, two different relationship of oxygen flow rate to biogas production (depending on the biogas sulphide content) are used to adjust the micro-oxygenation level under two different configurations: oxygen injection into the gas space and into the sludge recirculation. REs around 99% and biogas oxygen contents of less than 0.08%v/v are achieved. Under steady sulphur load and variable organic load, biogas production can be used to accurately regulate the oxygen supply, independently of the oxygen dosing point. Conversely, this parameter is proved to be an inefficient regulating parameter under variable sulphur load. Therefore, under such circumstances, biogas sulphide content should be used instead.

In **Chapter 3**, the SOB governing the gas space of two lab-pilot scale microaerobic reactors are characterised after 7 and 15 months of operation. The composition, species richness and the size of the sulphide-oxidising population is found to depend on the location, and more specifically, moisture availability. Moreover, the SOB richness seems to increase with time. Sulphur-rich deposits are found all over the headspace. They are separately sampled, and next, the walls, the ceiling and the dip tube are exhaustively cleaned. After restarting micro-oxygenation, the biogas is entirely desulphurised within 6-24h, and the oxygen demand of the reactor that operated for longer decreases substantially. This highlights that cleaning the headspace is needed in order to minimise the micro-oxygenation costs. The ceiling of one reactor is removed 1 month after once again, and all the hydrogen sulphide removed during this period is recovered from its headspace as elemental sulphur.

**Chapter 4 and 5** describe two lab-pilot scale experiments designed to clarify where the hydrogen sulphide removal takes place during microaerobic digestion. Biogas is entirely desulphurised when the headspace volume is higher than 4% of the total reactor volume. However, when the headspace volume is lower than 1% of the reactor volume, the efficiency of hydrogen sulphide removal rapidly drops up to 15%, which is consistent with the decrease in the oxygen consumption. Thus, Chapter 4 and 5 conclude that the biogas desulphurisation occurs predominantly in the gas space.

The findings obtained from lab-pilot scale experiments are tested at industrial-pilot scale in **Chapter 6**. At the early stage of the microaerobic operation, the RE of hydrogen sulphide is low, and the oxygen demand of the reactor is relatively high. They increase and decrease over time (respectively). After approximately one HRT, biogas was efficiently desulphurised by imposing microaerobic conditions under various configurations: different mixing methods and injection points of concentrated oxygen. Although the hydrogen sulphide removal from biogas seems to occur in the headspace, elemental sulphur, which is found to be the main oxidation product, hardly accumulates there. This is related to the low moisture levels maintained on the different surfaces of this area. Oxygen has not a significant impact on the digestion performance. However, the higher oxygen transfer rate to the sludge maintained by biogas recirculation increases the bacterial and archaeal richness and evenness, and causes an important shift in the structure of the microbial communities in the long term.

Taking into account the important limitations in operation of other biological technologies, and based on the findings described in Chapter 3-5, a new external process is presented in **Chapter 7.** The desulphurisation conditions present in microaerobic reactors are reproduced inside an external chamber called a microaerobic desulphurisation unit (MDU). Biogas produced in a lab-pilot scale reactor is treated in a 10L-MDU using 1L of microaerobic digested sludge as the reaction media. The MDU proves to be robust against rapid fluctuations in biogas residence time (BRT), inlet hydrogen sulphide concentration, oxygen/hydrogen sulphide supplied ratio and temperature, although neither nutrients nor water are added, and the digestate is not changed during the 128 days of the experiment. The biogas sulphide content remains around 0.02%v/v, and the average RE is 94%. Importantly, at the lowest oxygen/hydrogen sulphide supplied ratios (around 2.5 (v/v)), the oxygen concentration in the outlet biogas stream is 0.30%v/v on average. The biogas injection point does not affect significantly the system performance. Elemental sulphur is the main by-product, which accumulates in the form of extremely pure multilayered sheets, which settles to the bottom of the system. After the system shutdown, the inoculum presents high bacterial diversity, and three genera of SOB are identified.

In **Chapter 8**, a 1L-MDU is operated under significantly higher inlet concentrations and mass loading rates (MLRs) of hydrogen sulphide, and lower BRTs and oxygen/hydrogen sulphide supplied ratios than in the previous chapter, thus subjecting the system to more demanding and changing operational conditions. Moreover, the effect of temperature and the type of inoculum (anaerobic and microaerobic) on the system performance is investigated. Though higher in the presence of the microaerobic inoculum, REs higher than 94% are achieved under all the conditions set. At inlet hydrogen sulphide concentrations of approximately 0.48%v/v, MLR of 0.7kg/$m^3$/d, BRT of 12min, oxygen/hydrogen sulphide supplied ratio (v/v) of 1.8, and 35ºC, almost equal REs are achieved in a 0.5L-MDU with 0.1L of microaerobic inoculum. Although temperature (20-35ºC) has

not a significant effect on the steady state RE, relatively high temperatures at the start-up period seems to be key to achieving successful operation, in addition to faster start-up. The MDU proves to be sensitive to starvation episodes. Hydrogen sulphide is converted into elemental sulphur at the liquid interface and on the walls of the gas space.

Table 2 lists the four articles that have been already published in JCR journals. Three of them are published in Bioresource Technology, and one in Water Science and Technology. They are included in Chapters 1, 3, 4 and 7 in the specific format of the journal. The year of publication and the authors are also indicated in Table 2. In addition, worthy of noting is that the research article included in Chapter 2 is under review in *Chemical Engineering Journal*.

| **CHAPTER** | **TITLE** | **JOURNAL (YEAR)** | **AUTHORS** |
|---|---|---|---|
| 1 | The potential of oxygen to improve the stability of anaerobic reactors during unbalanced conditions: Results from a pilot-scale digester treating sewage sludge | *Bioresource Technology* (2013) | I. Ramos, M. Fdz-Polanco |
| 3 | The headspace of microaerobic reactors: sulphide-oxidising population and the impact of cleaning on the efficiency of biogas desulphurisation | *Bioresource Technology* (2014) | I. Ramos, R. Pérez, M. Fdz-Polanco |
| 4 | The role of the headspace in hydrogen sulfide removal during microaerobic digestion of sludge | *Water Science and Technology* (2012) | I. Ramos, I. Díaz, M. Fdz-Polanco |
| 7 | Microaerobic desulphurisation unit: A new biological system for the removal of $H_2S$ from biogas | *Bioresource Technology* (2013) | I. Ramos, R. Pérez, M. Fdz-Polanco |

**Table 2**. List of articles published in JCR Journals and their respective chapters. The year of publication and the authors are indicated.

The MDU unit is protected by the following **patent**:

- Title: Sistema microaerobio para controlar la concentración de sulfuro de hidrógeno en reactores de biometanización (“Microaerobic system for controlling the hydrogen sulphide concentration in biomethanisation systems”)
- Authors: Fernando Fdz-Polanco, María Fdz-Polanco, Israel Díaz, Iris Ramos, Sara Isabel Pérez
- Application number: P 201100721
- Application date: June 27$^{th}$, 2011
- Country: Spain

# PROCESS-LEVEL CONTROL

# The potential of oxygen to improve the stability of anaerobic reactors during unbalanced conditions: Results from a pilot-scale digester treating sewage sludge

1

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# The potential of oxygen to improve the stability of anaerobic reactors during unbalanced conditions: Results from a pilot-scale digester treating sewage sludge

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## HIGHLIGHTS

- The benefits that oxygen can provide under unbalanced conditions are studied.
- A microaerobic digester is subjected to a hydraulic overload.
- Micro-oxygenation seems to prevent a severe imbalance.
- The reactor's productivity increases during the imbalance.
- Micro-oxygenation improves the biogas quality independently of the HRT.

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## ABSTRACT

A well-functioning pilot reactor treating sewage sludge at approximately 4.4 NL/$m^3$/d of oxygen supply and 18 d of hydraulic retention time (HRT) was subjected to a hydraulic overload to investigate whether oxygen benefits successful operation in stressful circumstances. Only a mild imbalance was caused, which was overcome without deterioration in the digestion performance. Volatile solids (VS) removal was 45% and 43% at 18 and 14 d of HRT, respectively. Biogas productivity remained around 546 NmL/$g_{VS}$, but it was slightly higher during the period of imbalance. Thereafter, similar performances were achieved. Under anaerobic conditions, VS removal and biogas productivity were respectively 41% and 525 NmL/$g_{VS}$, hydrogen partial pressure rose, and acetic acid formation became less favourable. Oxygen seemed to form a more stable digestion system, which meant increased ability to deal successfully with overloads. Additionally, it improved the biogas quality; methane concentration was negligibly lower, while hydrogen sulphide and oxygen remained around 0.02 and 0.03% v/v, respectively.



## 1. Introduction

Anaerobic digestion is an established technology for the treatment of many wastes from different origins. Recent European policies encourage its application seeking environmental and socio-political targets (Pöschl et al., 2010). However, low methane yield and poor operational stability still hinder its widespread application (Chen et al., 2008).

Several authors have identified hydrolysis as the rate-limiting step in anaerobic digestion of particulate substrates, such as sewage sludge (Appels et al., 2008). Recent studies have demonstrated that it can be enhanced by introducing limited amounts of oxygen (or air) directly into the anaerobic digester (Jenicek et al., 2008) or during a pre-treatment step (Jagadabhi et al., 2010). In fact, Botheju et al. (2010a) managed to raise methane yield by microaeration. Nonetheless, only the hydrolysis of carbohydrates and proteins have reported improvements (Johansen and Bakke, 2006).

According to Zhu et al. (2009), the basis of the hydrolytic effect of oxygen resides in the increased synthesis and activity of extracellular hydrolytic enzymes. Similarly, Botheju and Bakke (2011) pointed to enhanced growth rates of facultative acidogens and the consequent larger release of these enzymes. Cellular growth stimulation is indeed a well-known oxygen benefit that has been exploited to raise the yield of some biochemical conversions (Chen et al., 2003).

It is an accepted issue that strict anaerobes (acetogens and methanogens) have several deterrence mechanisms to tolerate microaerobic conditions with no or minor inhibitory effects

*Abbreviations:* AN14, anaerobic period at 14 d of HRT; MA14, microaerobic period at 14 d of HRT; MA18, microaerobic period at 18 d of HRT; ORR, organic removal rate; PA, partial alkalinity; TA, total alkalinity.

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(Botheju and Bakke, 2011). Nevertheless, the presence of oxygen can induce other negative effects on methane potential, such as excessive oxidation of readily available substrates by fermentative (acidogenic) biomass (Johansen and Bakke, 2006) or methane consumption by aerobic methanotrophs (Zitomer and Shrout, 1998); hence the need for micro-oxygenation optimisation. Zhu et al. (2009) showed that the efficiency of the hydrolysis depends on the micro-aeration level. On the other hand, oxygen can cause positive effects on the digesters' productivity by alleviating sulphide inhibition (Zhou et al., 2006). Furthermore, under certain conditions, simultaneous sulphide removal from both the gas and liquid phase is feasible (Díaz et al., 2010b). Therefore, microaerobic conditions can also be applied to enhance biogas quality.

Obviously, the specific operational conditions that have been applied in every research have determined the overall oxygen impact on digestion; hence the different results reported. Nguyen et al. (2007) achieved higher methane yield by micro-aeration without evidence of improvement in hydrolysis. Conversely, Díaz et al. (2010a) effectively desulphurised biogas from a sewage sludge digester without affecting substrate conversion or methane yield, which is consistent with Ye et al. (2005). They found that, while pre-aeration of the biofilm carriers of three anaerobic attached film expanded reactors did not lead to improved digestion performance under normal circumstances, its resistance and recovery speed from hydraulic and organic overloads, as well as start-up rapidity, rose in comparison with the anaerobic ones. Moreover, they reported significantly lower VFA concentrations in the effluents from the pre-aerated reactors. This is consistent with Botheju et al. (2010b), who pointed out that oxygen could help digesters to confront shock loads by VFA oxidation, and the consequent prevention of pH instabilities. In fact, Zitomer and Shrout (1998) observed faster recovery from a pH drop under microaerobic conditions.

The main objective of this study was to recognise and describe the possible benefits that oxygen can provide under stressful circumstances. Therefore, the performance and stability of a pilot sludge digester treating sewage sludge at non-standard (short) hydraulic retention time (HRT) were evaluated under both microaerobic and anaerobic conditions.

## 2. Methods

### 2.1. Pilot-scale digester

Digestion was carried out in a continuous stirred tank reactor (CSTR) of 250 L total volume and 50 L headspace. It was heated at 35 °C by an electric resistor; temperature was maintained by insulating the walls of the digester. Mixed sludge with a variable organic load was collected weekly from a wastewater treatment plant, and stored at 4 °C. It was pumped to the bioreactor from a continuously stirred tank at ambient temperature. HRT ranged from 14 to 18 d depending on the operational stage (Table 1). As a result, organic loading rate (OLR) fluctuated between 1.4 and 2.9 $kg_{VS}/m^3/d$. Microaerobic conditions were implemented by supplying pure oxygen into the sludge recirculation, which was set at 50 L/h. Pure oxygen has been proved to be more profitable than air when applying microaerobic conditions (Díaz et al., 2010a).

**Table 1**
Operational conditions.

| Period | MA18 | MA14 | AN14 |
|---|---|---|---|
| Duration (d) | 23 | 54 | 35 |
| HRT (d) | 18 | 14 | 14 |
| OLR ($kg_{VS}/m^3/d$) | 1.7 | 2.1 | 1.9 |
| Conditions | Microaerobic | Microaerobic | Anaerobic |
| Oxygen flow rate ($NL/m^3/d$) | 4.4 | 5.8–6.2 | 0 |

### 2.2. Monitoring and experimental analysis

Digestion pressure and temperature were monitored by using a sensor and a probe, respectively. Biogas production was measured volumetrically by water displacement, and its composition in terms of methane, carbon dioxide, nitrogen, oxygen, hydrogen sulphide and hydrogen was determined by gas chromatography (GC) according to Díaz et al. (2010b). All this data was displayed and stored in real-time in a computer.

In order to evaluate digestion performance, the feed and the digestate were analysed in terms of total and soluble chemical oxygen demand (TCOD and SCOD), total and volatile solids (TS and VS) by standard methods (APHA, 1998). Additionally, total Kjeldahl nitrogen (NKT) and ammonium were measured according to APHA (1998).

As chemical indicators of the bioreactor state, pH was monitored by a pH-meter with a temperature probe, and the alkalinity measurements (total alkalinity and partial alkalinity, TA and PA, respectively) were based on standard methods (APHA, 1998), and the methodology proposed by Ripley et al. (1986). Acetic, propionic, butyric, isobutyric, valeric, isovaleric and hexanoic acids were analysed by GC.

In order to keep track of the sulphur inputs and outputs, sulphate and thiosulphate were determined by ion chromatography and high-performance liquid chromatography (HPLC), respectively. This last method was applied according to van der Zee et al. (2007). Additionally, dissolved sulphide concentration was measured by potentiometric titration with a silver/sulphide ion selective electrode (APHA, 1998).

### 2.3. Experimental procedure

The digester operated under 18 d of HRT and microaerobic conditions during the 40 d preceding this study (before MA18). Therefore, data obtained in MA18 was considered the baseline of the stationary state (Table 1). Oxygen supply was interrupted from the 23rd to 25th day, to approximate more accurately the hydrogen sulphide flow rate removed in the adjacent microaerobic stages. Once it was restarted, the HRT was sharply lowered to 14 d by increasing the feeding rate (MA14). That value was set according to Díaz et al. (2011), who reported that the maximum methane production under oxygen-limiting conditions is reached within approximately 14 d. It must be noted that the micro-oxygenation was adjusted at the beginning of both MA18 and MA14 to achieve a high biogas desulphurisation performance and minimum oxygen flow rate leaving the reactor. Thus, the capacity of the digestion system to tolerate shock loads and return to a stationary state (robustness) was evaluated. Finally, the role of oxygen in the performance and stability of the process under short HRT (14 d) and anaerobic conditions was assessed (AN14).

## 3. Results and discussion

### 3.1. Digestion performance

The bioreactor adapted immediately to the shorter HRT and consequent increase in OLR (Table 1), thereby demonstrating a great hydrolytic capability. Higher organic removal rates (ORR) were reached in MA14 (0.92 against 0.75 $kg_{VS}/m^3/d$), and as a result, the biogas production rose from an average of 0.97–1.13 $Nm^3/m^3/d$ (Fig. 1a). Considering the profile of both SCOD (Fig. 1b) and total VFA concentration (Fig. 2a), higher hydrolysis rates were definitely reached.

The ORR decreased in AN14 (0.78 $kg_{VS}/m^3/d$) (Fig. 1a); hence the lower average biogas production in relation to MA14

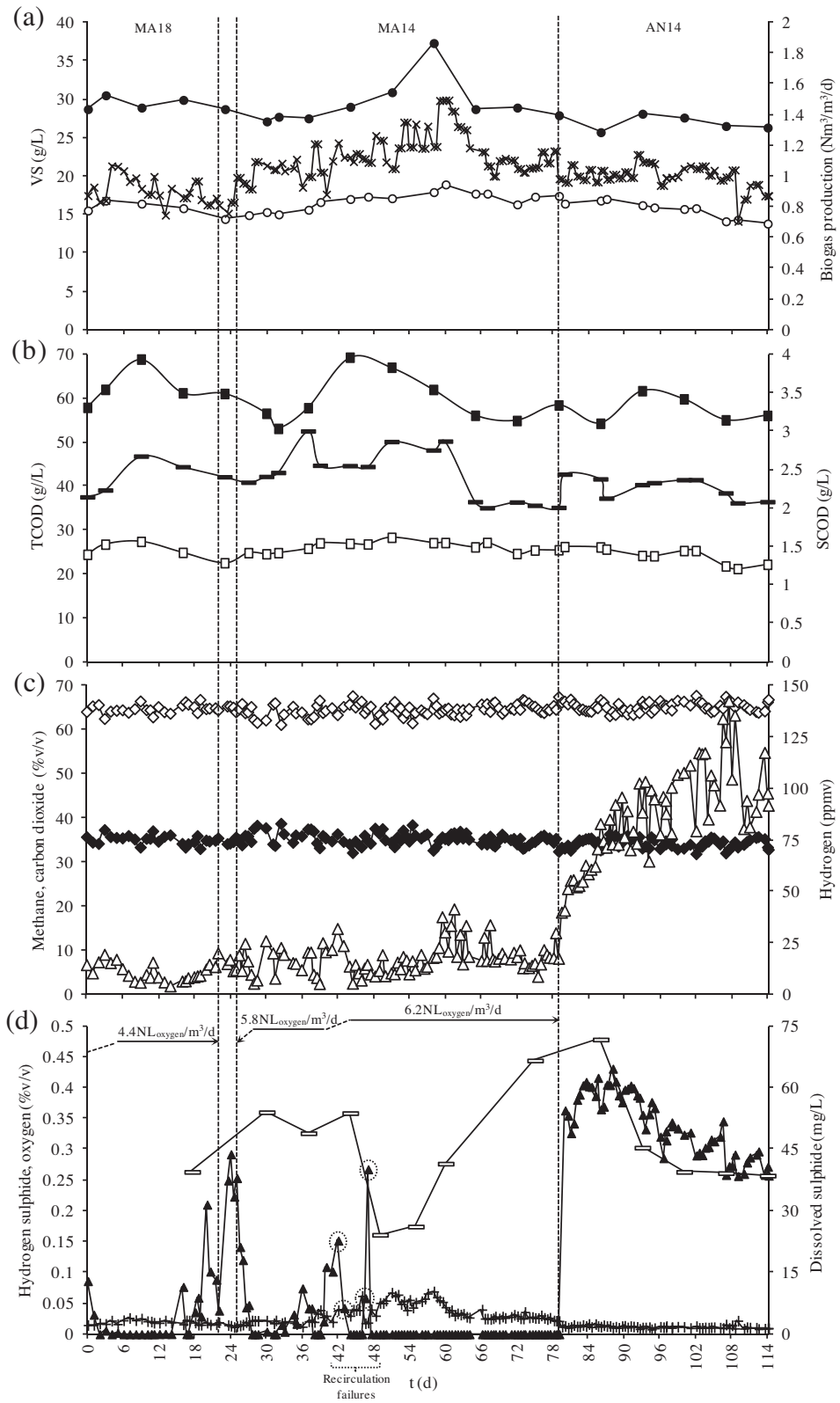


**Fig. 1.** (a) VS in sewage sludge (●) and in digestate (○), and biogas production (×). (b) TCOD in sewage sludge (■), and TCOD (□) and SCOD (▬) in digestate. (c) Methane (◇), carbon dioxide (♦) and hydrogen (Δ) concentration in biogas. (d) Hydrogen sulphide (▲) and oxygen (+) concentration in biogas, dissolved sulphides in the reactor (═), and micro-oxygenation level (above).

(1.00 $Nm^3/m^3/d$). Nevertheless, it was attributed to the reduction in the OLR rather than to the absence of oxygen (Table 1). On the other hand, although this parameter remained fairly stable all through that stage, the digester's VS content was declining (Fig. 1a), which in turn explained the increasing removal of TCOD (Fig. 1b). Considering the findings of Botheju and Bakke (2011), it pointed to lower growth rates of facultative biomass under anaerobic conditions. In fact, it is possible that the increased rates in this

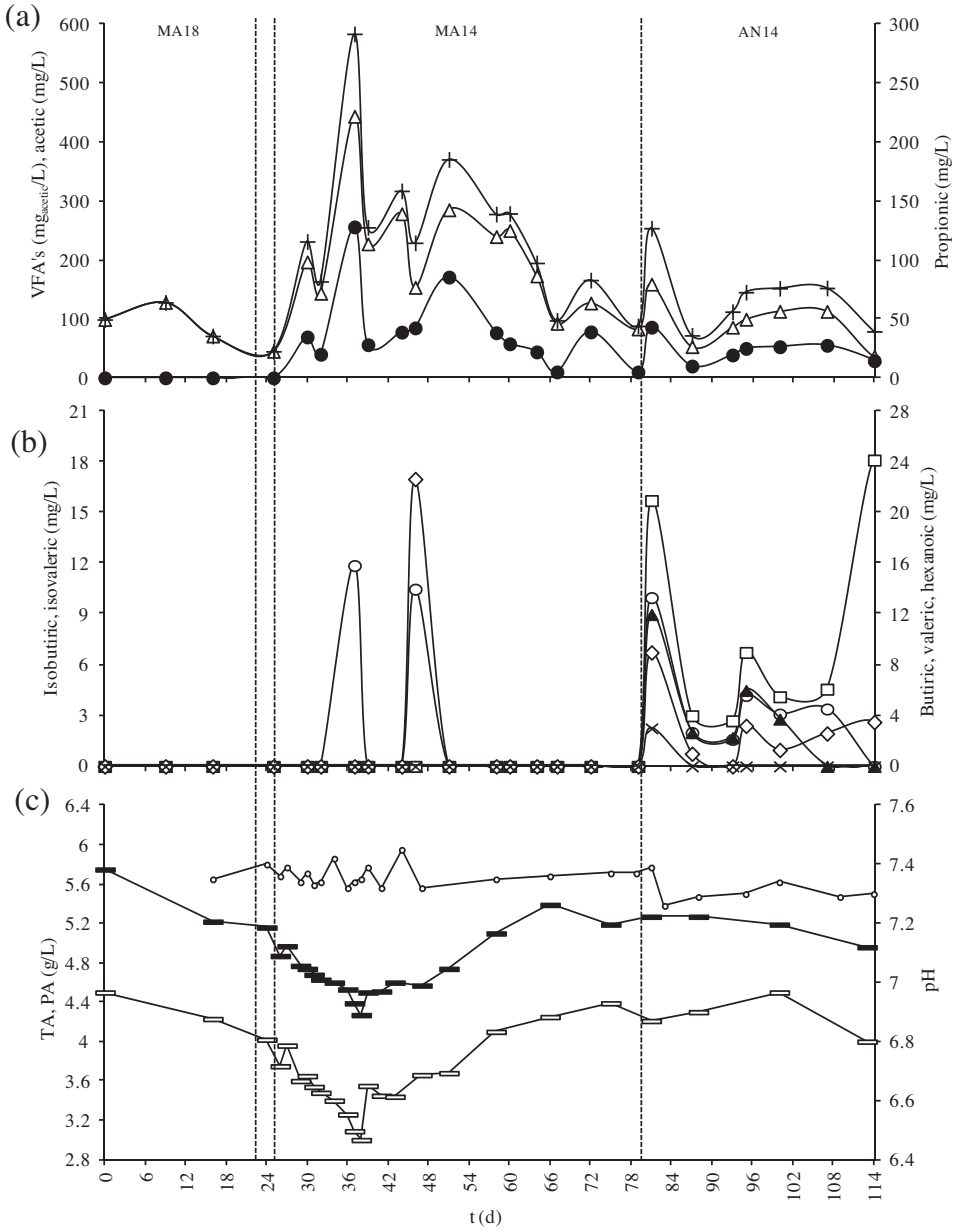


**Fig. 2.** (a) Total VFA concentration (+), and acetic (Δ) and propionic (●) acid. (b) Butyric (□), isobutyric ▲, valeric (◇), isovaleric (○), and hexanoic (×) acid. (c) TA (▬), PA (═), and pH (◦).

bacterial group in the presence of oxygen helped the digester to overcome the shock load without any deterioration in its productivity (see below). Furthermore, it must be taken into account that higher OLRs were confronted in MA14 (Fig. 1a).

The biogas methane content was slightly higher under anaerobic conditions, which suggested that some soluble substrate (including VFA's) could be aerobically oxidised during the microaerobic periods (Table 2). In this regard, it is noteworthy that the ratio of methane to carbon dioxide was especially variable in the first half of MA14 (Fig. 1c). This was attributed to the pH fluctuations, which regulated the solubility of the gases (Fig. 2c). Nonetheless, brief rises in aerobic activity or alkalinity consumption could also explain the momentary increases in carbon dioxide concentration, and the resulting lower biogas methane content.

On the other hand, taking into account that the highest VFA levels were reached in MA14, the lowest average of both biogas productivity and methane yield were expected at this point. However, they were obtained in AN14, while similar average values were reached in MA18 and MA14 (Table 2). Nonetheless, it must be noted that both parameters varied more during the first half of MA14 than in the rest of the stages, and they remained fairly stable from approximately the 50th day. Accordingly, it was shown that

**Table 2**
Digestate and biogas quality, and digestion productivity.

| Period | MA18 | MA14 | AN14 |
|---|---|---|---|
| TCOD (g/L) | 25.6 | 26.0 | 24.4 |
| SCOD (g/L) | 2.5 | 2.4 | 2.2 |
| VS (g/L) | 16.1 | 16.7 | 16.0 |
| Methane (%v/v) | 64.7 | 64.5 | 65.2 |
| Hydrogen sulphide (%v/v) | 0.03 | 0.02 | 0.34 |
| Oxygen (% v/v) | 0.02 | 0.03 | 0.01 |
| Biogas productivity (NmL/g$_{VS}$) | 547 | 546 | 525 |
| Methane yield (NmL/g$_{VS}$) | 354 | 352 | 342 |

oxygen enhanced digestion performance only under stressful conditions, which indeed agreed with Ye et al. (2005). Similarly, previous research has demonstrated that digestion performance is not affected (neither positively nor negatively) by oxygen injection during steady-state operation at 20 d of HRT (Díaz et al., 2010a, 2010b).

### 3.2. Digestion stability

Acetic acid was the only VFA detected during MA18, and its concentration remained fairly stable below 128 $mg_{acetic}/L$ (Fig. 2a). Along with the low biogas hydrogen content (11 ± 4 ppmv) (Fig. 1c), it was indicative of a well-balanced methanogenic system (Schink, 1997). Once the overload was imposed, larger amounts of both VFAs and hydrogen were released due to increased hydrolysis, acidogenesis, and acetogenesis. Almost all the extra hydrogen released was promptly consumed, which was consistent with the research pointing to the hydrogen availability being the factor limiting the hydrogenotrophic methanogens (Demirel and Scherer, 2008). Along with sulphate-reducing bacteria, they are the main hydrogen-utilising microorganisms. Nevertheless, since sulphate was provided only occasionally and in low concentrations and thiosulphate was not fed to the digester, the sulphate-reducing activity was considered to be substrate-limited (Stams et al., 2003). Accordingly, the methanogenic archaea were presumably the responsible for that result.

Hydrogen concentration in biogas started to increase instantly after the micro-oxygenation was stopped; it stabilised at approximately 100 ppmv (Fig. 1c). According to Tang et al. (2004), it could be due to the decreasing population size of hydrogen-consuming methanogens, which indeed consisted with the declining VS concentration. In their study, this microbial group proved to highly tolerate oxygen by considerably increasing its population size under microaerobic conditions. Additionally, it is worth highlighting that the lower pH maintained in AN14 could certainly have resulted from the higher hydrogen levels (Fig. 2c).

Stephenson et al. (1999) found increased hydrogen-scavenging activity under oxygen-limiting conditions, which they attributed to a higher release of growth factors to methanogens by facultative microorganisms. Accordingly, the declining concentration of acidogenic biomass in AN14 (Fig. 1a) could result in decreasing rates of hydrogenotrophic methanogenesis. Furthermore, considering that at higher hydrogen partial pressure this reaction is thermodynamically favoured (Boe, 2006), those substances definitely played a key role in stabilising the process at short HRT (see below). As shown in Fig. 1c, the hydrogen concentration remained at baseline values when the oxygen supply was interrupted at standard HRT (23rd–25th days). On the other hand, the aforementioned hydrogenotrophic effect was reported to disappear at increasing micro-oxygenation levels (Stephenson et al., 1999). Therefore, the micro-oxygenation rates set were probably crucial in achieving this effect.

According to Fig. 2a, an imbalance between the kinetics of production and the consumption of VFAs (basically propionic and acetic) took place in MA14 (Fig. 2a). The propionic acid accumulation agreed with Pind et al. (2002), who found that propionic-degraders were the acetogenic bacteria with the lowest specific growth rates. However, these microorganisms adapted surprisingly rapidly; although acetotrophic methanogens have significantly faster growth rates (Angedilaki et al., 1999), propionic was entirely degraded when acetic acid returned to the baselines. On the other hand, though in low concentrations, valeric and isovaleric were only detected when the highest ratios of propionic to acetic were reached (Fig. 2b). Hence, as Nielsen et al. (2007) indicated, this parameter was found to be a reliable indicator of the status of the process.

While VFAs accumulated, PA was gradually consumed to neutralise additional VFAs; hence the decrease in TA (Fig. 2a and c). It was indeed sufficiently high enough to avoid a pH drop. From the 37th day, the VFA consumption rates started to equal the production ones, leading to a gradual alkalinity regeneration. According to Fig. 2, the system re-stabilised from approximately the 66th day. However, only 2 d after the micro-oxygenation was stopped (around 1HRT before the process re-normalisation), the total VFA concentration doubled, and the contribution of all the VFAs increased, apart from acetic (Fig. 2a and b). Similarly, Botheju et al. (2010b) found that the higher the oxygen load, the lower the concentration of every measured VFA. Although the total VFA concentration remained close to the baseline levels thereafter, its composition continued to be more diverse. Along with acetic and propionic, butyric acid was the most important VFA during AN14. In fact, it accounted for 25% on the last day of the study, while acetic contribution dropped to 55%. Based on the above observations, it indicated the formation of a more unstable digestion system.

The shift in VFA distribution was linked to the higher content of hydrogen in the biogas. According to Boe (2006), acetogenic reactions and the fermentation pathway from simple substrates to acetic acid (that is, the formation of direct substrate to aceticlastic methanogens), carbon dioxide and hydrogen became less thermodynamically favourable in AN14. Since VFA levels re-normalised prior to the micro-oxygenation stop, its diversification did not affect digestion performance. However, the hydraulic overload in the absence of oxygen could have resulted in increasing accumulation of "other" VFAs at the expense of a reduction in acetic acid formation, which in turn could have brought on a much more severe process imbalance, or even the reactor failure. Moreover, insufficient hydrogen-scavenging activity could also have cause a pH drop. Hence, oxygen could sustain both acetoclastic and hydrogenotrophic methanogenesis in MA14, which could certainly prevent pH instabilities and help to maintain digestion performance during the imbalance.

### 3.3. Biogas quality

Obviously, the increased degradation rate of organic compounds (including those containing sulphur, such as proteins) maintained at 14 d of HRT led to a rise in hydrogen sulphide production; hence the higher oxygen demand in order to achieve equivalent desulphurisation efficiencies in MA14 (≈6.0 against 4.4 $NL/m^3/d$). It was indeed confirmed in AN14 (Fig. 1d).

As illustrated in Fig. 1d, the digester's sulphide content determined the hydrogen sulphide concentration in biogas according to the liquid–gas equilibrium; hence the high similarity between the profiles of these variables in AN14 (Fig. 1d). Likewise, negative correlation was found between dissolved sulphide and biogas oxygen content in MA14.

The average removal efficiency of hydrogen sulphide was estimated as 90% in MA20 and MA14 (Table 2). This percentage was consistent with the tight micro-oxygenation levels set, which were insufficient to prevent some of the concentration peaks (Fig. 1d). It should be noted that some of them (circled with a dotted line) arose from clogging problems in the recirculation stream. Hence, the biogas was successfully desulphurised independently of the HRT. Besides, considering both its negligibly lower methane concentration and the minute content of oxygen during the microaerobic periods (Table 2), it could be affirmed that the biogas quality was enhanced in relation to AN14. As a result, considering the above conclusions, benefits of micro-oxygenation on the digestion process and the biogas quality could be achieved simultaneously with oxygen supplies aiming for efficient hydrogen sulphide removal from biogas, thereby maximising the profit from micro-

oxygenation. Therefore, in full-scale, where oxygen concentrators could be employed in order to apply microaerobic conditions, the supply of limited amounts of oxygen could certainly be cost-effective.

As indicated above, most of the oxygen supplied was consumed inside the digester (90–95%) (Fig. 1d). Elemental sulphur was considered to be almost the only desulphurisation product accumulating in the headspace during both MA18 and MA14 (Díaz et al., 2010b). In fact, negligible concentrations of both sulphate and thiosulphate were infrequently found in the effluent. Thus, it was estimated that most of the oxygen supplied (60–70%) was consumed in unidentified processes at both 14 and 18 d of HRT. Among them, dissolved sulphide oxidation did not occur, which agreed with the mixing mode of the digester (Díaz et al., 2010b).

## 4. Conclusion

Oxygen could increase the ability of reactors to handle overloads by forming more stable digestion systems. It was hypothesised that micro-oxygenation prevented a severe imbalance resulting from the hydraulic overload by promoting growth of hydrogenotrophic methanogens, which in turn could favour acetic formation and could help to maintain pH. Furthermore, higher biogas productivity and methane yield were obtained during the period of imbalance, which was related to increased activity rates of acidogens. However, during steady-state operation, oxygen had impact only on the biogas quality. It hardly affected the content of methane and oxygen, while hydrogen sulphide concentration decreased by 90%.

## Acknowledgements

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## References

Angedilaki, I., Ellegaard, L., Ahring, B.K., 1999. A comprehensive model of anaerobic bioconversion of complex substrates to biogas. Biotechnol. Bioeng. 63, 363–372.

APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th edn. American Public Health Association/American Water Works Association/Water Environmental Federation, Washington DC, USA.

Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. 34, 755–781.

Boe, K., 2006. On-line monitoring and control of the biogas process. Ph.D. Theses. Institute of Environment and Resources, Technical University of Denmark, Denmark.

Botheju, D., Bakke, R., 2011. Oxygen effects in anaerobic digestion – a review. The Open Waste Manage. J. 4, 1–19.

Botheju, D., Samarakoon, G., Chen, C. and Bakke, R., 2010a. An experimental study on the effects of oxygen in bio-gasification; Part 1. In: International Conference on Renewable Energies and Power Quality, Granada.

Botheju, D., Samarakoon, G., Chen, C. and Bakke, R., 2010b. An experimental study on the effects of oxygen in bio-gasification; Part 2. In: International Conference on Renewable Energies and Power Quality, Granada.

Chen, X., Xiu, Z., Wang, J., Zhang, D., Xu, P., 2003. Stoichiometric analysis and experimental investigation of glycerol bioconversion to 1,3-propanediol by *Klebsiella pneumoniae* under microaerobic conditions. Enzyme Microb. Technol. 33, 386–394.

Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. Bioresour. Technol. 99, 4044–4064.

Demirel, B., Scherer, P., 2008. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. Rev. Environ. Sci. Biotechnol. 7, 173–190.

Díaz, I., Lopes, A.C., Pérez, S.I., Fdz-Polanco, M., 2010a. Performance evaluation of oxygen, air and nitrate for the microaerobic removal of hydrogen sulphide in biogas from sludge digestion. Bioresour. Technol. 101, 7724–7730.

Díaz, I., Pérez, S.I., Ferrero, E.M., Fdz-Polanco, M., 2010b. Effect of oxygen dosing point and mixing on the microaerobic removal of hydrogen sulphide in sludge digesters. Bioresour. Technol. 102, 3768–3775.

Díaz, I., Donoso-Bravo, A., Fdz-Polanco, M., 2011. Effect of microaerobic conditions on the degradation kinetics of cellulose. Bioresour. Technol. 102, 10139–10142.

Jagadabhi, P.S., Kaparaju, P., Rintala, J., 2010. Effect of micro-aeration and leachate replacement on COD solubilization and VFA production during mono-digestion of grass-silage in one-stage leach-bed reactors. Bioresour. Technol. 101, 2818–2824.

Jenicek, P., Keclik, F., Maca, J., Bindzar, J., 2008. Use of microaerobic conditions for the improvement of anaerobic digestion of solid wastes. Water Sci. Technol. 58, 1491–1496.

Johansen, J.E., Bakke, R., 2006. Enhancing hydrolysis with microaeration. Water Sci. Technol. 53, 43–50.

Nguyen, P.H.L., Kuruparan, P., Visvanathan, C., 2007. Anaerobic digestion of municipal solid waste as a treatment prior to landfill. Bioresour. Technol. 98, 380–387.

Nielsen, H.B., Uellendahl, H., Ahring, B.K., 2007. Regulation and optimization of the biogas process: propionate as a key parameter. Biomass Bioenergy 31, 820–830.

Pind, P.F., Angelidaki, I., Ahring, B.K., 2002. Dynamics of the anaerobic process: effects of volatile fatty acids. Biotechnol. Bioeng. 82, 791–801.

Pöschl, M., Warda, S., Owende, P., 2010. Evaluation of energy efficiency of various biogas production and utilization pathways. Appl. Energy 87, 3305–3321.

Ripley, L.E., Boyle, W.C., Converse, J.C., 1986. Improved alkalimetric monitoring for anaerobic digestion of high strength wastes. J. Water Pollut. Control Fed. 58, 406–411.

Schink, B., 1997. Energetics of syntrophic cooperation in methanogenic degradation. Microbiol. Mol. Biol. Rev. 61, 262–280.

Stams, A.J.M., Oude Elferink, S.J.W.H., Westermann, P., 2003. Metabolic interactions between methanogenic consortia and anaerobic respiring bacteria. Adv. Biochem. Eng./Biotechnol. 81, 31–56.

Stephenson, R.J., Patoine, A., Guiot, S.R., 1999. Effects of oxygenation and upflow liquid velocity on a coupled anaerobic/aerobic reactor system. Water Res. 33, 2855–2863.

Tang, Y., Shigematsu, T., Ikbal, Morimura, S., 2004. The effects of micro-aeration on the phylogenetic diversity of microorganisms in a thermophilic anaerobic municipal solid-waste digester. Water Res. 38, 2537–2550.

van der Zee, F.P., Villaverde, S., García-Encina, P.A., Fdz-Polanco, F., 2007. Sulphide removal by moderate oxygenation of anaerobic sludge environments. Bioresour. Technol. 98, 518–524.

Ye, F., Chen, Y., Feng, X., 2005. Advanced start-up of anaerobic attached film expanded bed reactor by pre-aeration of biofilm carrier. Bioresour. Technol. 96, 115–119.

Zhou, W., Imai, T., Ukita, M., Li, F., Yuasa, A., 2006. Effect of limited aeration on the anaerobic treatment of evaporator condensate from a sulfite pulp mill. Chemosphere 66, 924–929.

Zhu, M., Lü, F., Hao, L.-P., He, P.-J., Shao, L.-M., 2009. Regulating the hydrolysis of organic wastes by micro-aeration and effluent recirculation. Waste Manage. 29, 2042–2050.

Zitomer, D.H., Shrout, J.D., 1998. Feasibility and benefits of methanogenesis under oxygen-limited conditions. Waste Manag. 18, 107–116.

# Microaerobic control of biogas sulphide content during sewage sludge digestion by using biogas production and hydrogen sulphide concentration

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# Microaerobic control of biogas sulphide content during sewage sludge digestion by using biogas production and hydrogen sulphide concentration


## Abstract

This paper presents the potentials of using biogas production and hydrogen sulphide concentration as the parameters to regulate the oxygen supply to microaerobic reactors in order to control the biogas sulphide content, thereby ensuring safe and efficient use of the biogas. Research was carried out in two identical bioreactors of 200L at 35ºC and 19d of hydraulic retention time. The feed consisted of mixed sludge from a municipal wastewater treatment plant with variable organic and sulphur load. The oxygen flow rate was automatically adjusted according to the biogas sulphide content (which ranged from 0.62 to 0.24%v/v) by a feedback Proportional-Integral-Derivative controller. The target hydrogen sulphide concentration (0.01%v/v) was achieved in 4.0-5.5h, and the micro-oxygenation level reached was considered to be the optimum in the short-medium term, since it kept the removal efficiency above 99% and minimised the oxygen concentration in the biogas during the days following the controller application; specifically, the average biogas oxygen content was 0.09%v/v. Subsequently, biogas production was used as the parameter to regulate the oxygen supply. An average sulphide removal efficiency of 99% and oxygen concentrations in the biogas of less than 0.08%v/v were achieved when operating at around 0.33 and 0.50%v/v of biogas sulphide content by supplying approximately 3.5 and 5.0NL of oxygen per $Nm^3$ of biogas, respectively. Biogas production could be employed to develop precise control strategies during microaerobic digestion under variable organic load and steady sulphur load. Under unstable sulphur load, biogas sulphide content should be used instead.

## 1. Introduction

Biogas is a versatile and renewable energy source produced mainly by the anaerobic digestion of sewage sludge [1]. Methane and carbon dioxide are the main constituents, but it also contains significant quantities of undesirable compounds such as hydrogen sulphide, whose concentration can reach 1.0%v/v [2]. It is produced by sulphate-reducing and acidogenic bacteria mainly from sulphate and proteins, respectively [3]. Hydrogen sulphide escapes with the biogas, and has detrimental impacts on society and health, environment, and installations for biogas utilisation. Namely, it causes bad odour and eye damage below 0.01%v/v, and even death when above 0.03%v/v [4]. Manufacturers of combined heat and power (CHP) production units recommend limiting values between 0.01 and 0.03%v/v in order to prevent corrosion in piping systems and equipment. However, short peaks can occasionally be accepted [5]. Therefore, biogas sulphide content has to be controlled in order to prevent damage and fulfil the quality standards required according to the final application of the biogas.

Recently, there has been wide interest in desulphurisation biotechnologies as being an effective and environmentally friendly solution to the large investments and operational costs of the physicochemical processes [6]. Inside them, hydrogen sulphide is removed by sulphide-oxidising bacteria (SOB), which obtain energy by employing sulphide as the electron donor and oxygen as the electron acceptor [7]. The pathway of sulphide biological oxidation inside bioreactors has been suggested as: $H_2S \rightarrow S^0 \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow S_3O_6^{2-} \rightarrow SO_3^{2-} \rightarrow SO_4^{2-}$ [8]. Sulphide can be also chemically oxidised [9]. Among the bioprocesses, many investigators have turned to microaerobic removal, which consists of supplying limited amounts of oxygen (or air) directly into the anaerobic bioreactor. It is possible because SOB are present in numerous substrates treated by anaerobic digestion [2]. Thus, no additional unit (such as a bioscrubber, a biofilter or a biotrickling filter) is needed. Importantly, these (microaerobic) reactors yield just like the anaerobic ones [10], or even further [11].

During digestion under microaerobic conditions, SOB colonise the headspace of the reactor and oxidise hydrogen sulphide by using the oxygen that reaches this area independently of both the oxygen dosing point and mixing method [12]. Díaz et al. [12] demonstrated that the most efficient reactor configuration in order to microaerobically desulphurise biogas involves injecting the oxidant agent into the headspace and implementing liquid recirculation as the mixing method. Thus, the oxygen consumption in undesired processes was minimised. As a result, elemental sulphur accumulates all over the gas space [13]; it must be taken into account that both reactants (air or oxygen) are supplied in limited amounts in order to minimise their concentration in the biogas and the operating costs. With regard to this, it is worth noting that the mixtures of methane-oxygen formed inside microaerobic reactors are far from being explosive, since the limits of methane flammability in air are 5.0-15.0%v/v. Additionally, it should be considered that gases such as carbon dioxide reduce this concentration range [4].

The digester response to the presence of limited amounts of oxygen in terms of biogas composition and, more specifically, in terms of hydrogen sulphide and oxygen concentration, has been proved to be really rapid [14]. Moreover, Jenicek et al. [15] and Díaz et al. [16] highlighted the steady dynamic behaviour of microaerobic reactors treating solid wastes and sewage sludge, respectively, within a wide range of hydrogen sulphide concentrations, and pointed out that microaeration decreases the

heating value of the biogas (that is, methane concentration) due to the presence of nitrogen. Accordingly, though expensive, the most profitable oxidant agent is pure oxygen. Nonetheless, it must be taken into account that not all the oxygen supplied to a digester is generally used therein [16, 17]. With regard to this, it is worth noting that unless the biogas is used for CHP or boilers, the presence of oxygen should be avoided; it is expensive to remove [18]. In Europe, if biogas is to be used as vehicle fuel or injected into fuel cells or natural gas networks, its oxygen content must not exceed concentrations of 1.0 and 3.0%v/v, respectively [5].

In full-scale, since hydrogen sulphide production can vary according to the feeding composition, the micro-oxygenation rate must be periodically regulated in order to avoid lack or surplus of oxygen in biogas while maintaining the biogas quality standards required. For this purpose, the key issue is to find a variable capable of providing a precise control of the oxygen supply; little research efforts have been made within this context. Oxidation-reduction potential (ORP) has been reported as an accurate regulation parameter of oxygen dosing in order to eliminate sulphide toxicity [19], maximise sulphur recovery [20], and even desulphurise biogas [21]. Nonetheless, its response to micro-oxygenation can be insufficient to develop a reliable control of the hydrogen sulphide concentration in biogas during digestion [16]. Therefore, and on the basis that the gaseous sulphide and the biogas production both increase and decrease concurrently with the organic loading rate (OLR) as a result of rises and decreases (respectively) in fermentative activity, Díaz et al. [22] proposed to regulate the oxygen flow rate according to the biogas production. They found a linear correlation between the ratio of the oxygen supply to the biogas flow rate, and the biogas sulphide content. Under steady hydrogen sulphide concentration, biogas production would be used to develop a reliable and consistent control strategy. Otherwise, biogas sulphide content could be an efficient regulating parameter of the micro-oxygenation.

Since the performance of oxygen utilisation inside a bioreactor is expected to vary with time due to increasing elemental sulphur accumulation in the headspace, which could alter the oxygen transfer conditions and affect biological oxidation rates as a result of the change in the growing conditions, a control approach utilising hydrogen sulphide concentration in biogas as the regulating parameter of the oxygen supply would automatically absorb changes in both performance of oxygen utilisation in the digester and biogas production. Proportional-Integral-Derivative (PID) control is the standard automatic controller in industrial settings. Among this type of control systems, the feedback PID controller determines an input variable to the control process by using the measurement of an output variable [23]. Besides being applicable to many real-world control problems, the PID controller is simple, intuitive, efficient, and reliable for processes with steady dynamic behaviour [24]. Therefore, it could be successfully applied to control the biogas sulphide content in microaerobic digesters by using biogas sulphide content as the regulating parameter of the oxygen flow rate.

The aim of this study is to investigate the feasibility of using biogas production and hydrogen sulphide concentration in biogas to regulate the oxygen flow rate and thereby achieving a consistent and efficient control of the hydrogen sulphide concentration during microaerobic digestion.

## 2. Materials and methods

### *2.1. Pilot-scale digesters*

Two identical continuous stirred tank reactors called R1 and R2 with a working volume of 200L and a headspace of 50L were operated under mesophilic conditions and 19±1d of hydraulic retention time (Fig. 1). Temperature was maintained by an electric resistor. The pressure control was performed hydraulically; an electro-valve regulated the biogas outflow.

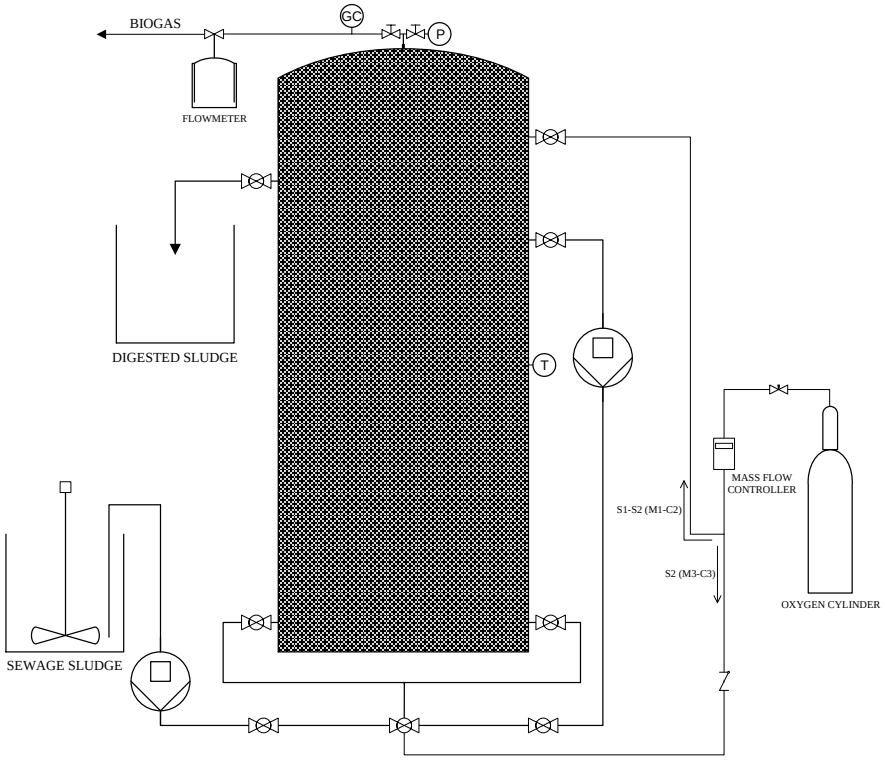


**Fig. 1**. R1 and R2 diagram.

Mixed sludge with variable composition was transported weekly to the pilot plant from a municipal wastewater treatment plant. It was stored at 4ºC, and was fed continuously from two stirred tanks at room temperature into both digesters by peristaltic pumps. Sludge recirculation at a rate of 50L/h ensured mixing. Pure oxygen from a cylinder was injected by means of two mass flow controllers (Bronkhorst EL-FLOW Select) into the headspace or the recirculation stream, depending on the operational stage (Fig. 1).

*2.2. Monitoring and experimental analysis*

Pressure was monitored by a sensor. Temperature was measured by probes. Biogas was quantified by the displacement of a fixed liquid volume, and its composition was determined by a CP-4900 Micro-GC [12].

The feed and the digested sludge were sampled weekly for total and soluble chemical oxygen demand, total solids, volatile solids (VS), volatile fatty acids, total kjeldahl nitrogen, ammonia, sulphide, sulphate and thiosulphate analysis. This last anion was measured by high performance liquid chromatography, according to the procedure described by van der Zee et al. [9]; the rest of the parameters were determined according to standard methods [25].

*2.3. Design of the feedback PID controller*

The object of the feedback PID controller was to control the microaerobic process of biogas desulphurisation. Therefore, it was used to set the oxygen flow rate according to the error (*e*) between the measurement of hydrogen sulphide concentration and the target concentration of hydrogen sulphide according to the Eq. (1)[1]. It was implemented using *macros* of *Microsoft Office Excel*.

$$Oxygen\ \ flow\ rate(t) = Oxygen\ \ flow\ rate(t-T) + g_0 e(t) + g_1 e(t-T) + g_2 e(t-2T) \quad \textbf{(1)}$$

The sampling time of the controller (*T*) was set at 30min. It was fixed according to the well-known dynamic behavior of the digester (which was steady) and the lax control of the biogas sulphide content required in this study; a lower value of *T* was considered unnecessary, whereas a larger value could be insufficient to achieve an efficient control of hydrogen sulphide concentration in biogas due to the controller acting too infrequently. The target biogas sulphide content or setpoint was set at 0.01%v/v, which is the lowest concentration limit recommended in CHP [5].

The control parameters[2] ($K_p$, $T_i$, and $T_d$) were set at 1, 100 and 15min, respectively. They were estimated manually based on the vast experience of the authors in operating the pilot plant, and the individual effects of the three actions of the PID controller on the performance thereof[3]. In order to minimise the surplus of oxygen, and to operate as close as possible to the linear range, $K_p$ must be low, and $T_i$ must be fairly high. Although the steady state error decreases with decreasing $T_i$, since the process to be controlled in this study was not expected to present steady-state error (it could be eliminated), a low value of $T_i$ was considered to be unnecessary; besides, it could have degraded the stability of the controller. On the other hand, due to high values of $T_d$ improving both the stability and the drop time both, and taking into account that the reactor presented a steady dynamic behaviour, $T_d$ was set relatively high.

Regarding the preceding paragraph, it should be mentioned that a wide range of tuning methods exist [27]; however, they were not applicable to this study due to the fact that the model of the process (*oxygen supply=f(biogas sulphide content)*) was not estimated and the relationship between hydrogen sulphide concentration and oxygen supply was obviously inverse (which did not permit the

application of tuning rules based on empirical procedures). As a result, the accuracy range of the linear relationship between the oxygen supply and the biogas sulphide content could not be estimated. However, it was considered to be unnecessary for the adjustment of the control parameters ensuring the achievement of the research objective due to the great experience of the authors in operating the digester. Moreover, it must be highlighted that the linearity premise was the basis for the estimation of the control parameters (see above).

### *2.4. Experimental procedure*

The research was divided into two stages, S1 and S2. In S1, the oxygen flow rate was regulated according to the biogas sulphide content by implementing the PID controller presented above. In S2, biogas production was used as the parameter to regulate the oxygen supply. Both stages were in turn divided into several periods, which were designated with A (anaerobic periods), M (microaerobic periods in which the oxygen flow rate was maintained constant or regulated arbitrarily) and C (microaerobic periods in which the oxygen supply was regulated according to the biogas production or the hydrogen sulphide concentration).

#### *2.4.1. Stage S1*

The first stage of the study (S1) was carried out in R1 (Table 1). The oxygen supply was always injected into the headspace. As shown in Table 1, the controller was tested six times over approximately 168d in order to evaluate its performance in the long term and at different starting concentrations of hydrogen sulphide; hence the pauses introduced between the last three trials. Substantial changes in both the biogas sulphide content (which arose from the variability of the feeding) and the dynamic of the desulphurisation process were not expected in the short term. It is worth noting that the bioreactor was operated most of the time under microaerobic conditions during the pause periods; as a result, the amount of elemental sulphur attached to the headspace presumably increased.

Except for C1, the rest of the C periods (from C2 to C6) were started after a 2-day anaerobic period in which the hydrogen sulphide concentration remained stationary; namely, *oxygen flow rate(t-T)*=0 and $e(t) \approx e(t-T) \approx e(t-2T)$. Conversely, a concentration peak of hydrogen sulphide was induced at the beginning of C1 (that is, $e(t-2T) < e(t-T) < e(t)$) by stopping the oxygen supply shortly before the start of the controller (namely, *oxygen flow rate(t-T)*=0) in order to evaluate the suitability thereof in dealing with concentration peaks.

**Table 1**. Sequence of variations applied to R1 during S1 and digester response. A: anaerobic period, M: microaerobic period in which the oxygen supply was maintained constant, C: microaerobic period in which the oxygen supply was regulated according to the hydrogen sulphide concentration, HS: headspace.

| Trial | Period | Oxygen dosing point | Oxygen supply ($NL/Nm^3_{biogas}$) | VS (g/L) | Biogas production ($Nm^3/m^3/d$) | Hydrogen sulphide (%v/v) | Oxygen (%v/v) | Methane (%v/v) |
|---|---|---|---|---|---|---|---|---|
| 1 | A1[a] | - | 0 | 19 | 0.75 | 0.17 | 0.02 | 65.3 |
| | C1 | HS | 4.8-12.6 | 19 | 0.75 | 0.00 in 4.5h | 0.04 | 65.6 |
| 2 | A2[a] | - | 0.0 | 16 | 0.60 | 0.58 | 0.02 | 67.1 |
| | C2 | HS | 6.1-19.1 | 16 | 0.61 | 0.01 in 4.0h | 0.05 | 67.1 |
| | M2 | HS | 12.6 | 15 | 0.58 | 0.00 | 0.05 | 65.9 |
| 3 | A3[a] | - | 0 | 21 | 0.77 | 0.58 | 0.02 | 61.8 |
| | C3 | HS | 4.4-12.3 | 22 | 0.81 | 0.01 in 4.5h | 0.05 | 62.0 |
| | M3 | HS | 7.9 | 22 | 0.81 | 0.00 | 0.05 | 64.2 |
| 4 | A4[a] | - | 0 | 22 | 0.84 | 0.52 | 0.02 | 62.5 |
| | C4 | HS | 3.8-10.7 | 27 | 0.92 | 0.00 in 4.5h | 0.05 | 62.2 |
| | M4 | HS | 7.3 | 27 | 0.89 | 0.00 | 0.05 | 62.4 |
| 52-d pause | | HS | | | | | | |
| 5 | A5[a] | - | 0 | 23 | 0.61 | 0.58 | 0.02 | 63.8 |
| | C5 | HS | 4.2-15.9 | 23 | 0.59 | 0.00 in 5.0h | 0.15 | 63.4 |
| | M5 | HS | 12.1 | 23 | 0.60 | 0.00 | 0.19 | 63.5 |
| 69-d pause | | HS | | | | | | |
| 6 | A6[a] | - | 0 | 35 | 1.04 | 0.26 | 0.02 | 61.2 |
| | C6 | HS | 1.8-7.3 | 35 | 1.01 | 0.00 in 5.5h | 0.03 | 61.3 |
| | M6 | HS | 5.9 | 34 | 0.94 | 0.00 | 0.12 | 62.0 |

[a] Includes the three measurements for the calculation of the first oxygen supply (see Fig. 2a, b, d, f, h and j).

### *2.4.2. Stage S2*

As shown in Table 2, S2 was divided into three trials, according to the different hydrogen sulphide concentrations in biogas during the A periods, and the oxygen dosing point during the C and M periods. It included a total of eight operational periods. During the first two trials, R1 and R2 were operated in parallel and were subjected to the same variations in the operational conditions in terms of presence or absence of oxygen. During M1, C1 and C2, the oxygen was injected into the headspace (Fig. 1). Thus, only R1 was operated with oxygen supply to the recirculation stream (last trial).

**Table 2**. Sequence of variations applied during S2 and response of the digesters. A: anaerobic period, M: microaerobic period in which the oxygen supply was adjusted arbitrarily, C: microaerobic period in which the oxygen supply was regulated according to the biogas production, HS: headspace, RS: recirculation stream.

| Trial | Period | Reactor | Oxygen dosing point | Oxygen supply ($NL/Nm^3_{biogas}$) | VS (g/L) | Biogas production ($Nm^3/m^3/d$) | Hydrogen sulphide (%v/v) | Oxygen (%v/v) | Methane (%v/v) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | A1 | R1 | - | 0 | 35 | 1.03 | 0.25 | 0.01 | 62.9 |
| | | R2 | - | 0 | 36 | 1.01 | 0.37 | 0.01 | 61.1 |
| | M1 | R1 | HS | 5.7 | 35 | 0.95 | 0.00 | 0.11 | 62.2 |
| | | R2 | HS | 7.9 | 31 | 0.89 | 0.00 | 0.23 | 61.3 |
| | C1 | R1 | HS | 3.9 | 28 | 0.77 | 0.03 | 0.03 | 62.4 |
| | | R2 | HS | 3.9 | 26 | 0.72 | 0.01 | 0.04 | 61.9 |
| 2 | A2 | R1 | - | 0 | 28 | 0.75 | 0.54 | 0.01 | 62.5 |
| | | R2 | - | 0 | 22 | 0.57 | 0.47 | 0.01 | 62.0 |
| | C2 | R1 | HS | 5.0 | 31 | 0.88 | 0.00 | 0.02 | 63.1 |
| | | R2 | HS | 4.9 | 29 | 0.82 | 0.00 | 0.06 | 62.5 |
| 3 | A3 | R1 | - | 0 | 33 | - | 0.39 | 0.01 | 62.5 |
| | M3 | R1 | RS | 9.4 | 33 | 0.95 | 0.00 | 0.03 | 63.7 |
| | C3 | R1 | RS | 3.8 | 32 | 0.90 | 0.02 | 0.02 | 64.5 |

## 3. Results and discussion

### *3.1. Stage S1: control based on biogas sulphide content*

As illustrated in Fig. 2a, b, d, f, h and j, the controller started injecting oxygen at t=1h according to the last three measurements of hydrogen sulphide concentration recorded in the respective A periods (Table 1). Even in C1, when the controller was confronted with a sudden increase in hydrogen sulphide concentration, biogas sulphide content dropped below the setpoint (0.01%v/v) in a time range from 4.0 to 5.5h; it stabilised at zero thereafter. Meantime, the biogas oxygen content rose from 0.02%v/v (minute amounts of oxygen entering the digester are inevitable under anaerobic conditions) to, at most, 0.21%v/v (C5 in Table 1); it remained around 0.05%v/v during most of the C periods. Hence, the controller performance was successful, since the biogas was rapidly desulphurised and accurately adjusted (or equivalently, optimised) the micro-oxygenation level independently of the hydrogen sulphide flow rate (which varied widely, as shown in Table 1).

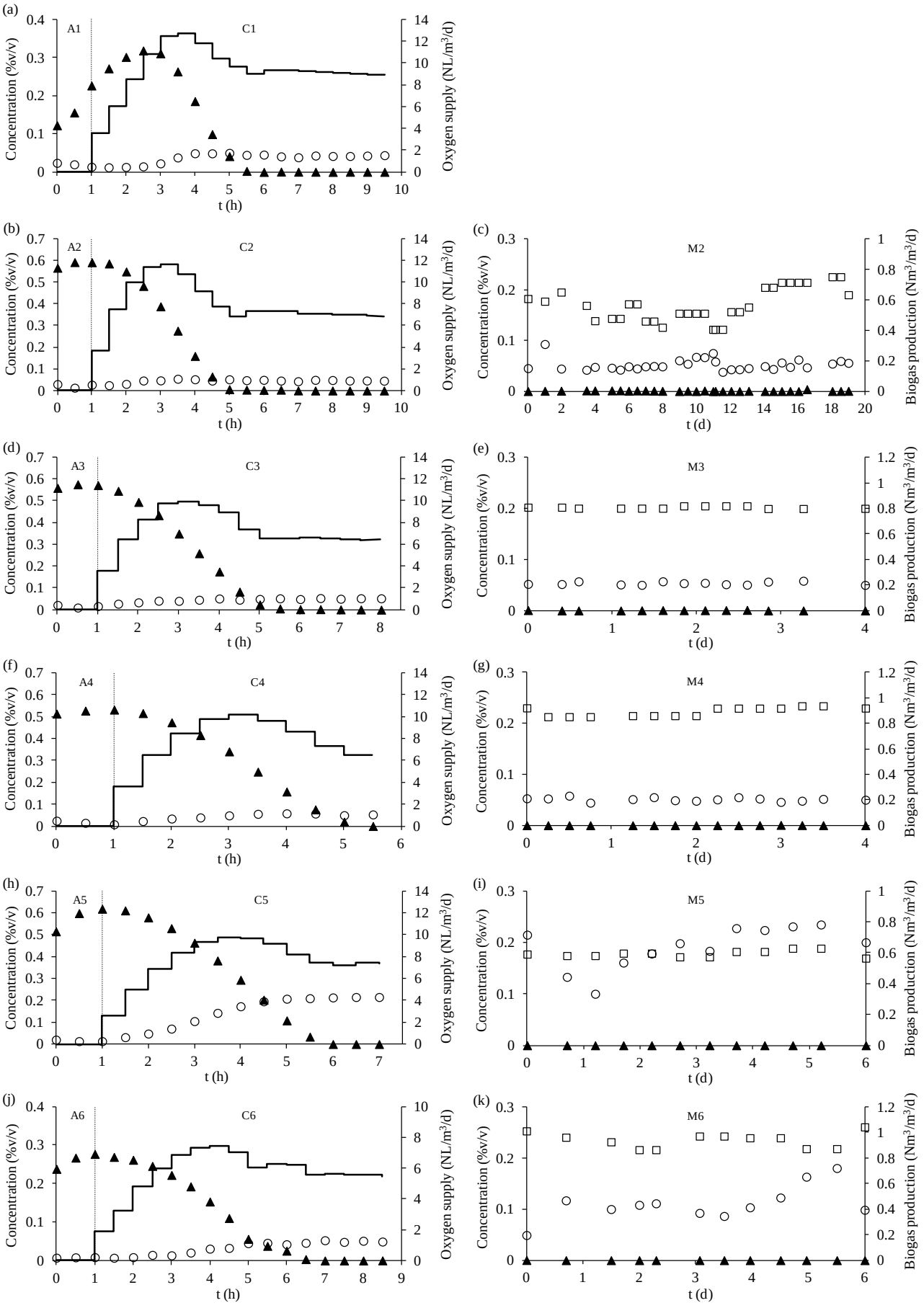


**Fig. 2**. Sulphide (▲) and oxygen (o) content of biogas, oxygen supply (continuous line) and biogas production (□) in trial 1 (a), 2 (b and c), 3 (d and e), 4 (f and g), 5 (h and i) and 6 (j and k) during S1.

Although the hydrogen sulphide production was significantly higher in C3 than in C2, and the starting concentration was 0.58%v/v in both periods, the optimum micro-oxygenation levels adjusted and the resulting biogas oxygen contents were almost equal (Table 1). Therefore, although the performance of oxygen utilisation inside the digester certainly changed, the controller continued providing successful results. Presumably the longer biogas residence time increased the oxygen transfer to the liquid phase during C2. Thus, the oxygen consumption in unidentified processes rose, thereby reducing the amount available to SOB in the headspace, where the process occurred; hence the higher oxygen demand and consequently larger oxygen amount supplied by the controller. At this point, it is worth noting that neither the concentration of dissolved sulphides decreased nor the sulphate or the thiosulphate content rose in S1 (data not shown). This was indeed as expected, according to Díaz et al. [12], and the presumably low oxygen transfer rate to the liquid phase; it should be considered that a limited contact area existed between the liquid media and the oxygen-rich biogas. Besides, the methane concentration was not negatively affected (Table 1).

The controller response (oxygen flow rate) outlined a similar profile in all the C periods (Fig. 2a, b, d, f, h and j); it corresponded to a perfectly predicted PID controller behaviour. Besides, both oxygen supply and hydrogen sulphide concentration (even biogas oxygen content) reached the stationary state simultaneously. As shown in Fig. 2a, b, d, h and j, once the setpoint was achieved, the controller set oxygen flow rates which were negligibly different as a result of the minute variations in biogas sulphide content (namely, due to the steady dynamic behaviour of the reactor). Therefore, the micro-oxygenation level reached once the gaseous sulphide concentration decreased below the setpoint was considered to be the optimum in order to maintain, at least in the short term, high removal efficiencies of hydrogen sulphide and low surpluses of oxygen; hence the shorter duration of C4 in relation to C1, C2, C3, C5 and C6. In C4, the controller was stopped once the setpoint was achieved (Fig. 2f).

As noted, the oxygen supplies reached in C2, C3, C4, C5 and C6 just after achieving the setpoint were considered to be the optimum. Therefore, they were maintained during M2, M3, M4, M5 and M6, respectively (Fig. 2c, e, g, i and k). Although the hydrogen sulphide flow rate presumably fluctuated during M2, M5 and M6, which was deduced from the variations in biogas production and oxygen concentration, the putative optimum oxygen flow rates effectively sufficed to maintain the removal efficiency over 99% after all the C periods (Table 1). Furthermore, they were also low enough to maintain biogas oxygen contents below 0.23%v/v. As a result, the biogas methane content was preserved. Is it worth highlighting that M2 was prolonged for 19d, while M3, M4, M5 and M6 lasted only 4-6d. After the putative optimum oxygen supply was confirmed to be over 1 hydraulic retention time (M2), a 4-day M period was considered to suffice in order to conclude that it could have maintain a successful performance in the short-medium term.

Though unnecessary during this study, due to the successful results obtained from the application of the PID controller, the refinement of some or all the control parameters could become necessary in the longer term in order to maintain the efficiency of the control. For example, the oxygen demand could rise due to the increasing deposition of elemental sulphur in the headspace and the consequent reduction in oxygen transfer rate, thus requiring (for instance) a rise in $K_p$.

*3.2. Stage S2: control based on biogas production*

As illustrated in Fig. 3a and c, R1 and R2 operated under anaerobic conditions (period A1 in Table 2) until the 4$^{th}$ day, and the hydrogen sulphide concentration in biogas was around 0.25 and 0.37%v/v, respectively. Thereafter, oxygen was injected into the headspace of both reactors (M1); the flow rate was set according to the ratio of oxygen supplied to hydrogen sulphide produced adjusted in the last trial of S1, which was approximately 2.0 (v/v). In R2, the micro-oxygenation level was reduced stepwise during M1 in order to minimise further the oxygen concentration in biogas, while maintaining the biogas sulphide content below 0.01%v/v. It was achieved on the 10$^{th}$ day by injecting 3.5NL of oxygen per Nm$^3$ of biogas. As a result, the oxygen supply to both reactors during C1 was readjusted daily (or occasionally every two or three days) based on the biogas flow rate measured over the previous 24h and according to that relationship.

R2 apparently utilised the oxygen more efficiently; as noted, the hydrogen sulphide concentration in R2 during A1 was significantly higher than in R1. Nevertheless, since both digesters were fed with the same sludge and operated under almost the same conditions (slight differences occurred due to the significant variability in feeding), very similar biogas compositions were generally recorded. In fact, considering the profile of both the concentration and the flow rate of oxygen in R2 during M1, as well as the subsequent results (presented below), the hydrogen sulphide concentration in R2 presumably approached that in R1 after the 4$^{th}$ day (Fig. 3c). Unfortunately, sulphur-containing anions were not determined during M1. That presumable delay in the change in the hydrogen sulphide concentration was related to the variability of the sewage sludge, which is reflected in Table 2, Fig. 3b and d.

Until the 17$^{th}$ day, the biogas produced in both digesters was entirely desulphurised (Fig. 3a and c). Moreover, although the oxygen flow rate was significantly reduced (by approximately 62%) due to the significant decrease in the feeding VS content and the resulting lower biogas production, the oxygen concentration remained fairly stable around 0.04 and 0.05%v/v in R1 and R2, respectively, which suggested that the hydrogen sulphide production certainly varied concurrently with the biogas production and the OLR both (Table 2). Accordingly, and considering the above observations, the optimum ratio of oxygen supplied to gaseous sulphide produced was estimated at around 1.4 (v/v) for both digesters.

From the 17$^{th}$ day, some peaks of hydrogen sulphide concentration were detected, while the biogas oxygen content decreased appreciably. This pointed to a rise in the hydrogen sulphide concentration, which was confirmed in A2; it was around 0.54 and 0.47%v/v in R1 and R2, respectively (Table 2). This was at least partially attributed to the increase in the feeding sulphate content and the consequent rise in the sulphate-reducing activity, which was indeed consistent with the rise in the dissolved sulphide concentration observed on the 24$^{th}$ day (Fig. 3b and d). It should be noted that thiosulphate was rarely detected in the feed sludge, and its concentration did not exceed 10mg/L; therefore, it was not depicted in Fig. 3.

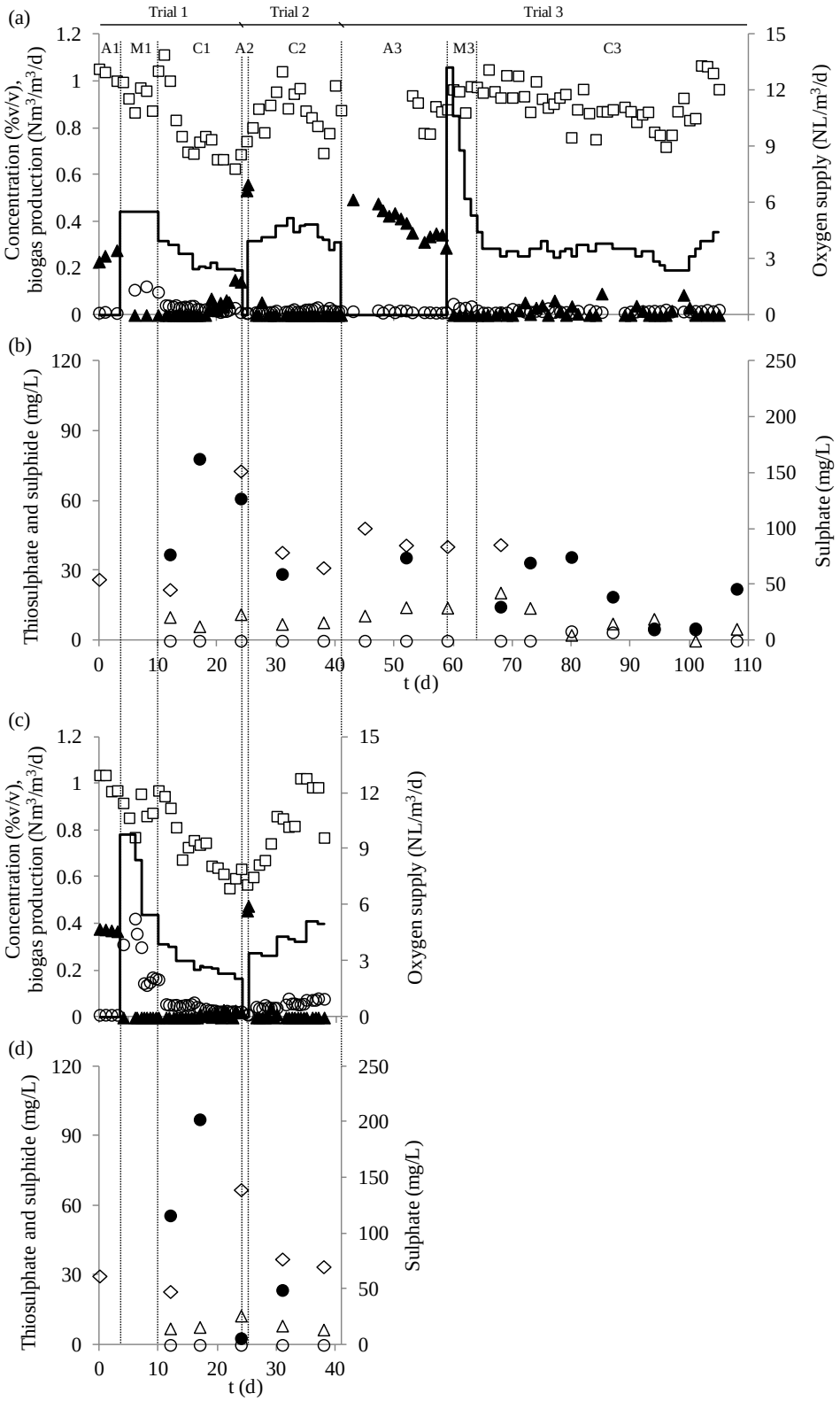


**Fig. 3**. Sulphide (▲) and oxygen (○) content of biogas, biogas production (□), oxygen flow rate (continuous line), sulphate (●) concentration in feed sludge, and sulphate (○), thiosulphate (▲) and sulphide (◊) concentration in R1 (a and b) and R2 (c and d) during S2.

After almost two days in the absence of oxygen (period A2 in Table 2), microaerobic conditions were restored. In C2, the oxygen supply to both digesters was regulated according to a relationship of $5.0NL_{oxygen}/Nm^3_{biogas}$ in order to operate under an oxygen/hydrogen sulphide ratio similar to that maintained until the 17$^{th}$ day. This micro-oxygenation relationship proved to be the optimum, since the average removal efficiency was 100%, and minute amounts of oxygen remained unused in the biogas (Fig. 3a and c). Furthermore, similarly to C1, the oxygen concentration remained fairly stable despite the substantial change in the oxygen supply; it was raised by approximately 65% in order to maintain the aforementioned micro-oxygenation relationship (Table 2). With regard to this, it is worth noting that the excess of oxygen was slightly higher in R2, which was related to the somewhat lower starting concentration of hydrogen sulphide in the biogas (see period A2).

Micro-oxygenation was interrupted for maintenance from the 41$^{st}$ to the 59$^{th}$ day (period A3 in Table 2). Once biogas sulphide content stabilised at approximately 0.33%v/v, the micro-oxygenation was restarted (period M3); it was supplied to the recirculation stream instead of to the gas space. Accordingly, a higher oxygen demand was expected in order to maintain performances similar to those achieved until then; hence the relatively larger initial dose (Fig. 3a). In fact, considering the oxygen concentrations in the biogas resulting from the significantly lower oxygen supplies maintained in the preceding microaerobic periods under very similar starting values of both hydrogen sulphide concentration and biogas production, the oxygen consumption effectively increased. Nevertheless, since the biogas's oxygen content was slightly higher than in those periods, and the removal efficiency was 100%, the micro-oxygenation level was gradually lowered. A relationship of $3.5NL_{oxygen}/Nm^3_{biogas}$ was able to be eventually reached (65$^{th}$ day); it was used to regulate the oxygen flow rate during C3. Hence, the oxygen transfer to the liquid phase was minimised, thereby reaching rates similar to those maintained with oxygen supply to the headspace. Furthermore, as in the previous M and C periods, the methane concentration was not negatively affected.

At this point, it should be noted that Díaz et al. [22] found that the optimum micro-oxygenation relationship at 0.33%v/v of hydrogen sulphide concentration was $6.4NL_{oxygen}/Nm^3_{biogas}$. The higher oxygen demand in comparison with this study was due to they operated with biogas recirculation instead of sludge recirculation, which resulted in an increased $O_2$ transfer to the liquid phase. In fact, in contrast to this research, dissolved sulphide was also removed, which was consistent with the previous findings [12].

In the last control period (C3 in Table 2), the mean removal efficiency was 95% (Fig. 3a); several peaks of hydrogen sulphide concentration were recorded. Some peaks, such as those detected around the 98$^{th}$ day, were ascribed to an insufficient regulation frequency of micro-oxygenation. The others probably resulted from a higher sulphate-reducing activity; a substantial rise in the feeding sulphate content was recorded between the 73$^{rd}$ and the 80$^{th}$ day (Fig. 3b). In the meantime, the oxygen concentration in the biogas did not exceed 0.03%v/v, and remained even more stable than in the previous C periods (C1 and C2), which supported the assumption that the biogas and the hydrogen sulphide production both varied concurrently with the OLR. At this point, it should be highlighted that the oxygen supplies maintained in S2 were significantly tighter than in S1; hence the lower oxygen concentrations and larger amount of peaks of hydrogen sulphide concentration in the biogas produced during S2.

As shown in Fig. 3b and d, similarly low concentrations of thiosulphate were maintained all through S2. This suggested incomplete reduction of the sulphate contained in the feed sludge. Consequently, its presence inside both reactors in the M and C periods could not be attributed to the further oxidation of sulphide. On the other hand, sulphate was detected only inside R1 and in concentrations lower than 10mg/L from the 80$^{th}$ day, which was linked to the change in the oxygen dosing point. Therefore, as in S1, elemental sulphur was presumably the main by-product of the hydrogen sulphide oxidation during S2; it was indeed consistent with the really limited micro-oxygenation levels maintained during the research.

### *3.3. Application proposals*

The parameter to regulate the oxygen supply during microaerobic digestion in order to control the biogas sulphide content must be selected according to the operational conditions of the bioreactor. Under variable organic and sulphur loading rate both, biogas sulphide content could be the basis for the development of a precise and consistent control strategy. A feedback PID control could be employed as the tool to continuously adjust the oxygen supply according to the hydrogen sulphide concentration. On the other hand, biogas production could be the basis for regulate the oxygen flow rate during digestion under variable OLR and steady sulphur load. Although biogas sulphide content could also be used under such conditions, it must be considered that monitoring the biogas flow rate is cheaper due to lower costs of the measuring equipment.

The optimum micro-oxygenation relationship (oxygen supply/biogas production) at a particular hydrogen sulphide concentration is specific for every plant, since it depends on the feedstock, the operational conditions, and even the reactor configuration. A readjustment of the micro-oxygenation is recommended at least daily; obviously, the higher the frequency, the lower the probability of surpassing the concentration limit of hydrogen sulphide and of wasting oxygen, thereby ensuring an optimum biogas quality. Evidently, the readjustment periodicity would depend on the stringency required in the control. Similarly, the PID control could be run in discontinuous mode; after the oxygen flow rate was adjusted, the controller could be kept on standby, while the micro-oxygenation level could be maintained constant. Meantime, hydrogen sulphide concentration in biogas would be determined with the required periodicity; thus, in the event of an increasing concentration or successive zeros, the controller would be restarted in order to re-optimise the oxygen supply.

## 4. Conclusions

The suitability of using biogas production and hydrogen sulphide concentration in biogas in order to regulate the oxygen supply during microaerobic digestion for the development of precise and consistent control strategies was studied. Biogas production could be an efficient regulating parameter under variable OLR and steady sulphur load, independently of the oxygen dosing point. Under non-steady sulphur load, biogas sulphide content should be used instead. A feedback PID controller could be implemented in order to accurately adjust the oxygen flow rate according to the hydrogen sulphide concentration.

## Appendix

*Description of a feedback PID control*

The basic structure of the feedback (or closed loop) PID controller in parallel form is shown in Fig. A.1. It assumes a linear relationship between the input and the output variable to the process (*u* and *y*) within a limited operational range involving small values of both variables and around an equilibrium point [26], and acts (*u*) according to the control error (*e*), which is the difference between the process output or controlled variable (*y*) and the desired process output or setpoint (*r*).

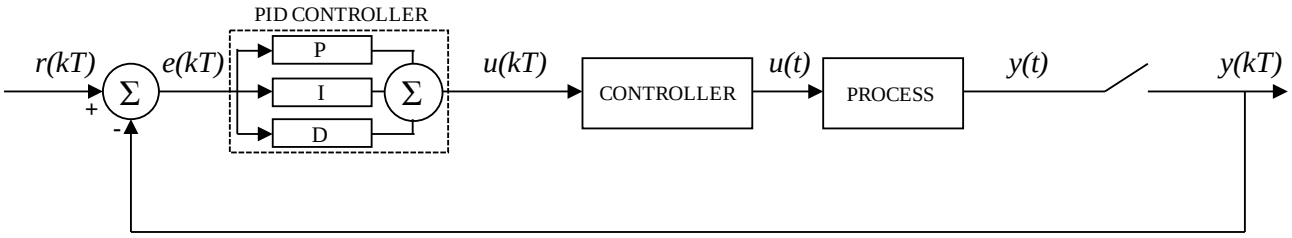


**Fig. A.1**. Feedback PID controller in parallel form.

The algorithm of this version of the PID controller is shown in Eq. (A.1), where $K_p$ (proportional gain), $T_i$ (integral time), and $T_d$ (derivative time) are the control parameters [23]. It combines three types of actions; one of them (P in Fig. A.1) is proportional to *e* (*y*-*r*), another (I) is proportional to the integral of *e*, and the last one (D) is proportional to the derivative of *e*.

$$u(t) = K_p\left(e(t) + \frac{1}{T_i}\int_0^t e(t)dt + T_d\frac{de(t)}{dt}\right) \qquad \textbf{(A.1)}$$

In order to digitally implement this continuous-time control law, Eq. (A. 1) must be discretised by defining a sampling time (T) [23]. So, it can be rewritten as:

$$u(t) = u(t-T) + g_0 e(t) + g_1 e(t-T) + g_2 e(t-2T) \qquad \textbf{(A.2)}$$

where $g_0$, $g_1$ and $g_2$, are the control constants, which depend on the control parameters according to Eq. (A. 3), (A. 4) and (A. 5). Therefore, the PID controller set the oxygen supply at every *T* based on the current control error, the accumulated control error, and the tendency of the control error.

$$g_0 = K_P\left(1 + \frac{T}{T_i} + \frac{T_d}{T}\right) \qquad \textbf{(A.3)}$$

$$g_1 = K_P\left[-1 - \left(\frac{2T_d}{T}\right)\right] \qquad \textbf{(A.4)}$$

$$g_2 = K_P\left(\frac{T_d}{T}\right) \qquad \textbf{(A.5)}$$

The individual effects of the three functionalities of the PID control on three of the most important characteristics of the closed loop response of the controller are shown in Table A.1. “Drop time” reflects the time required to achieve the setpoint, “steady-state error” is the residual error (namely, that which remains after the controlled variable stabilises around the setpoint), and the concept “stability” refers to operation within the linear range (see below). As suggested, the controller must keep the process variables within a range (that is, stable).

**Table A.1**. Effects of independent P, I and D tuning (adapted from [24]).

| | Drop time | Steady-state error | Stability |
|---|---|---|---|
| Increasing $K_p$ | Decrease | Decrease | Degrade |
| Decreasing $T_i$ | Small decrease | Large decrease | Degrade |
| Increasing $T_d$ | Small decrease | Minor change | Improve |

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## References

[1] L. Appels, , J. Baeyens, J. Degrève, R. Dewil, Principles and potential of the anaerobic digestion of waste-activated sludge, Prog. Energy Combust. Sci. 34 (2008) 755-781.

[2] N. Abatzoglou, S. Boivin, A review of gas purification processes, Biofuels Bioprod. Biorefin. 3 (2009) 42-71.

[3] A. J. M. Stams, S. J. W. H. Oude Elferink, P. Westermann, Metabolic interactions between methanogenic consortia and anaerobic respiring bacteria, Adv. Biochem. Eng./Biotechnol. 81 (2003) 31-56.

[4] R. E. Speece, Anaerobic Biotechnology and Odor/Corrosion Control for Municipalities and Industries, Archae Press, Nashville, Tennessee, 2008.

[5] D. Deublein, A. Steinhauser, Biogas from Waste and Renewable Resources: An Introduction, Wiley-VCH, Weinheim, 2008.

[6] Q. Mahmood, P. Zheng, J. Cai, Y. Hayat, M. J. Hassan, D. Wu, B. Hu, Sources of sulfide in waste streams and current biotechnologies for its removal, J. Zheijang Univ. Sci. A 8 (2007) 1126-1140.

[7] K. Tang, V. Baskaran, M. Nemati, Bacteria of the sulphur cycle: An overview of microbiology, biokinetics and their role in petroleum and mining industries, Biochem. Eng. J. 44 (2009) 73-94.

[8] H. Duan, R. Yan, L. C. Koe, Investigation on the mechanism of $H_2S$ removal by biological activated carbon in a horizontal biotrickling filter, Appl. Microbiol. Biotechnol. 69 (2005) 350-357.

[9] F. P. van der Zee, S. Villaverde, P. A. García, F. Fdz-Polanco, Sulphide removal by moderate oxygenation of anaerobic sludge environments, Bioresour. Technol. 98 (2007) 518-524.

[10] M. Fdz-Polanco, I. Díaz, S. I. Pérez, A. C. Lopes, F. Fdz-Polanco, Hydrogen sulphide removal in the anaerobic digestion of sludge by micro-aerobic processes: pilot plant experience, Water Sci. Technol. 60 (2009) 3045-3050.

[11] P. Jenicek, F. Keclik, J. Maca, J. Bindzar, Use of microaerobic conditions for the improvement of anaerobic digestion of solid wastes, Water Sci. Technol. 58 (2008) 1491-1496.

[12] I. Díaz, S. I. Pérez, E. Ferrero, M. Fdz-Polanco, Effect of oxygen dosing point and mixing on the microaerobic removal of hydrogen sulphide in sludge digester, Bioresour. Technol. 102 (2010) 3768-3775.

[13] T. Kobayashi, Y. Li, K. Kubota, H. Harada, T. Maeda, H.-Q. Yu, Characterization of sulfide-oxidizing microbial mats developed inside a full-scale anaerobic digester employing biological desulfurization, Appl. Microbiol. Biotechnol. 93 (2012) 847-857.

[14] I. Ramos, I. Díaz, M. Fdz-Polanco, The role of the headspace in hydrogen sulfide removal during microaerobic digestion of sludge, Water Sci. Technol. 66 (2012) 2258-2264.

[15] P. Jenicek, J. Koubova, J. Bindzar, J. Zabranska, Advantages of anaerobic digestion of sludge in microaerobic conditions, Water Sci. Technol. 62 (2010) 427-434.

[16] I. Díaz, A. C. Lopes, S. I. Pérez, M. Fdz-Polanco, Performance evaluation of oxygen, air and nitrate for the microaerobic removal of hydrogen sulphide in biogas from sludge digestion, Bioresour. Technol. 101 (2010) 7724-7730.

[17] E. Rodríguez, A. Lopes, M. Fdz-Polanco, A. J. M. Stams, P. A. García-Encina, Molecular analysis of the biomass of a fluidized bed reactor treating synthetic vinasse at anaerobic and micro-aerobic conditions, Environ. Biotechnol. 93 (2012) 2181-2191.

[18] A. Petersson, A. Wellinger, Biogas upgrading technologies - developments and innovations, IEA Bioenergy, 2009.

[19] S. K. Khanal, J. C. Huang, ORP-based oxigenation for sulfide control in anaerobic treatment of high-sulphate wastewater, Water Res. 37 (2003) 2053-2062.

[20] A. J. H. Janssen, S. Meijer, J. Bontsema, J. Lettinga, Application of the redox potential for controlling a sulfide oxidizing bioreactor, Biotechnol. Bioeng. 60 (1998) 147-155.

[21] T. Duangmanee, S. K. Khanal, S. Sung, Micro-aeration for sulfide removal in anaerobic treatment of high-solid wastewaters: A pilot scale study, AgSTAR National Conference Sacramento, California, November (2007).

[22] I. Díaz, A. C. Lopes, S. I. Pérez, M. Fdz-Polanco, Determination of the optimal rate for the microaerobic treatment of several $H_2S$ concentrations in biogas from sludge digesters, Water Sci. Technol. 64 (2011) 233-238.

[23] A. Visioli, Practical PID Control, Advances in Industrial Control, Springer-Verlag, London, 2006.

[24] K. H. Ang, G.C.Y. Chong, Y. Li, PID Control System Analysis, Design, and Technology, IEEE Trans. Control Syst. Technol. 13 (2005) 559-576.

[25] L. S. Clesceri, A.E. Greenberg, A. D. Eaton, Standard Methods for the Examination of Water and Wastewater, twentieth ed., American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D,C., 1998.

[26] K. J. Åström, T. Hagglund, The future of PIC control, Control Eng. Pract. 9 (2001) 1163-1175.

[27] K. Ogata, Ingeniería de Control Moderna (Modern Control Engineering), third ed., Prentice-Hall Hispanoamericana, México, 1998.

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# The headspace of microaerobic reactors: sulphide-oxidising population and the impact of cleaning on the efficiency of biogas desulphurisation

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# The headspace of microaerobic reactors: sulphide-oxidising population and the impact of cleaning on the efficiency of biogas desulphurisation

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## Abstract

$O_2$-limiting/microaerobic conditions were applied in order to control the $H_2S$ content of biogas. The $S^0$-rich deposits found all over the headspace of two pilot reactors (R1 and R2) as a result of operating under such conditions for 7 and 15 months (respectively) were sampled and removed. After restarting micro-oxygenation, $H_2S$-free biogas was rapidly obtained, and the $O_2$ demand of R2 decreased. This highlighted the need for a cleaning interval of less than 14 months in order to minimise the micro-oxygenation cost. The $H_2S$ removed from R2 after approximately 1 month was recovered from its headspace as $S^0$, thus indicating that the biogas desulphurisation did not take place at the liquid interface. Denaturing gradient gel electrophoresis indicated that the composition, species richness and size of the sulphide-oxidising bacteria population depended on the location, and, more specifically, moisture availability, and indicated increasing species richness over time. Additionally, a possible succession was estimated.

Abbreviations: DGGE, denaturing gradient gel electrophoresis; HS, headspace; PCR, polymerase chain reaction; SOB, sulphide-oxidising bacteria; TS, total solids

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**Keywords**

Biogas desulfurization; headspace; microaerobic; sulfide-oxidizing bacteria; sulfur

## 1. Introduction

$H_2S$ is a common biogas compound arising from the anaerobic digestion of proteins and S-containing compounds. It can exceed concentrations of 0.05%v/v and up to 2.0%v/v, which inevitably causes corrosion problems in combustion engines, and the release of $SO_x$ in flue gases (Fortuny et al., 2008). Therefore, most manufacturers of combined heat and power installations recommend a biogas sulphide content of less than 0.01 or 0.03%v/v, depending on the equipment concerned (Peu et al., 2012).

$H_2S$ can be controlled either at the source, by controlling the feedstock, at the end, by desulphurising the biogas in a later stage, or at process level, directly inside the anaerobic digester (Peu et al., 2012). The first solution is not realistic, and it is in fact the latter $H_2S$ removal from biogas which is the most established method in practice, as it can be carried out during digestion or in an additional unit (Cirne et al., 2008). The most common end-of-pipe techniques for $H_2S$ removal are based on physical-chemical processes. However, their high costs of both operation and by-product disposal have encouraged research and the application of biological processes (Park et al, 2011). Specifically, biological desulphurisation has been reported to be approximately 62% cheaper than chemical absorption (Burgess et al., 2001). Furthermore, it can achieve more complete removal due to the extremely high affinity of sulphide-oxidising bacteria (SOB) for the substrate (Kobayashi et al., 2012).

The most widespread biotechnologies for $H_2S$ removal are biofilters, biotrickling filters and bioscrubbers, in which aerobic species of chemolitotrophic SOB oxidise the sulphide mainly

to $S^0$ or $SO_4^{2-}$, depending on the $O_2$ availability (Tang et al., 2009). Besides requiring fourfold less $O_2$, the conversion of $H_2S$ into $S^0$ is preferred over conversion to $SO_4^{2-}$ due to the fact that $S^0$ is harmless and can be recovered from liquid streams and reused in bioleaching and agriculture (Kleinjan, 2005). Inside these biological systems, sulphide can be also chemically oxidised to $S_2O_3^{2-}$ (Lohwacharin et al., 2010). Importantly, this oxidation mechanism can be catalysed by any metal ion present in the bioreactor (Kleinjan, 2005). As a result, SOB have to contend with chemical sulphide oxidation for $O_2$. According to Robertson and Kuenen (2006), they compete effectively at very low $O_2$ and sulphide concentrations. However, the contribution of the chemical mechanisms increases at high sulphide loads due to limitations in biological activity.

As an economically attractive alternative to employing additional units (that is, a process-level solution), $H_2S$ can be removed from biogas simply by imposing microaerobic conditions in the anaerobic reactor. In Europe, this technique has been applied by injecting air directly into the headspace (HS) of the digester in order to maintain 4-6% of air in the biogas, and as a result, $S^0$ deposits have been found at the liquid interface and on other surfaces of the gas space (Abatzoglou and Boivin, 2009). This is partly consistent with the results of Díaz et al. (2010), who demonstrated that the desulphurisation process basically occurs in the HS independently of both the $O_2$ (or air) dosing point and the mixing method, but in this case neither $S^0$ nor SOB were found at the liquid interface . Similarly, Rodríguez et al. (2012) only identified representatives of this microbial group in the $S^0$-rich biomass attached to the HS, although micro-oxygenation was introduced from the bottom of the reactor.

It is essential to know how the increasing accumulation of $S^0$ in the headspace over time

affects the $O_2$ transfer conditions and, therefore, the performance of the biogas desulphurisation, since this could lead to a reduction in the intervals of time at which the digester must be cleaned. Although the $S^0$ accumulation could also significantly reduce the volume of the gas space in microaerobic reactors in the long-term, Ramos et al. (2012) demonstrated that a biogas residence time of approximately 1h sufficed to achieve $H_2S$ removal efficiencies similar to those obtained at around 7h. Díaz and Fdz-Polanco (2012) reported that the desulphurisation performance in a microaerobic digester treating sewage sludge was very similar just before HS cleaning and 30h later, after almost 21 months intercalating anaerobic and microaerobic experiments. Moreover, they highlighted the rapidity with which the $H_2S$ was removed from the biogas just after cleaning the HS, which suggested extremely high activity levels of SOB at the liquid interface and/or a great contribution by the chemical oxidation mechanisms. With regard to this, it must be noted that Ramos et al. (2012) provided evidence that this process is predominantly biological.

Likewise, it is of utmost importance to know how SOB grow in the HS to optimise the efficiency of $H_2S$ removal from biogas in microaerobic reactors. However, only Kobayashi et al. (2012) have provided valuable information in this area. They showed that both cell density and bacterial activity in the HS were much higher in the areas nearest the liquid phase, which was attributable to an increased availability of water and nutrients.

Based on the points outlined above, the main objectives of this study were:

- to evaluate the impact of HS cleaning on the efficiency of biogas desulphurisation
- to investigate where exactly the biogas desulphurisation takes place in the HS
- to characterise and locate the SOB population that is removing $H_2S$ during sewage

sludge digestion

- to approach the temporal differences in the SOB population

## 2. Materials and Methods

### *2.1. Pilot plant scale reactors*

Research was carried out in two continuous stirred tank reactors (R1 and R2) with 200L (250L of total volume) treating sewage sludge with a variable organic and sulphur load at 19d of hydraulic retention time. A diagram of the digesters is shown in Fig. 1. Temperature (35ºC) was monitored by probes and was regulated by electric resistors surrounding their walls, which were in turn covered with insulation. Mixing was carried outat approximately 50L/h by peristaltic pumps. Microaerobic conditions were implemented by making a single-point injection of pure $O_2$ into the HS using mass flow controllers. Biogas composition was determined by gas chromatography (Díaz et al., 2010), and its production was measured volumetrically.

### *2.2. Digestion monitoring*

Digestion performance was assessed by measuring total and soluble chemical oxygen demand (COD), total solids (TS), volatile solids (VS), volatile fatty acids, total kjeldahl nitrogen and ammonia according to APHA (1998). Total dissolved sulphide and $SO_4^{2-}$ concentrations were measured by the potentiometric and the chromatographic method, respectively (APHA, 1998). $S_2O_3^{2-}$ was measured by high liquid performance chromatography according to the procedure described by van der Zee et al. (2007). A LECO CS-225 was utilised to determine elemental composition in terms of S and C.

*2.3. Experimental procedure*

The operational sequence is schematised in Fig. 2. The HS of R2 was cleaned at t=0 (Fig. 2); however this reactor was operated for several months before beginning this research under the aforementioned conditions. Seven months afterwards (at t=8), R1 was started up with sludge from R2. Thereafter, both digesters operated in parallel and under the above conditions. Until t=15, they basically operated under microaerobic conditions; micro-oxygenation was rarely interrupted.

At t=15, the ceiling of both reactors was removed. Six samples (A, B, C, D, E and F) were taken from different points of the HS for TS, elemental, and microbial analysis (Fig. 3a). As indicated in Fig. 2, A1, B1, C1, D1, E1 and F1 were retrieved from R1, and A2, B2, C2, D2, E2 and F2 belonged to R2. The A and B samples were taken from the walls (the lowest and the upper area, respectively), the C samples were taken from the ceiling, the D and E samples were taken from the dip tube (the upper and the lowest area, respectively), and the F samples were taken from the liquid interface (Fig. 3a). After sampling, all the surfaces were cleaned, and the liquid interface (approximately 250mm of sludge from the surface) was removed.

Once sealed, the digesters were operated under anaerobic conditions for 1 month (Fig. 2). Micro-oxygenation was restarted at t=16. At t=17, R2 was uncovered again, and the $S^0$-rich deposits accumulated in the HS were retrieved separately according to Fig. 3a. As shown in Fig. 2, those samples were called A3, B3, C3, D3 E3 and F3. After drying them, they were weighed and characterised in terms of S and C percentages in order to estimate the amount of $S^0$ deposited.

*2.4. Bacterial analysis*

The bacterial community established in the HS of the reactors at t=15 was characterized by denaturing gradient gel electrophoresis (DGGE) analysis. Samples were stored at -20ºC. Extraction of genomic DNA, polymerase chain reaction (PCR) amplification and DGGE analysis were performed according to Lebrero et al. (2013).

The DGGE profiles were processed by GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). The Shannon-Wiener diversity index (*H*) of every sample and the pair-wise similarity coefficient were both calculated according to Lebrero et al. (2013). The desired bands were excised from the gels according to the procedure described by Lebrero et al. (2011). The taxonomic position of the sequenced DGGE bands was obtained by the RDP classifier tool at a confidence level of 50% (Wang et al. 2007). Moreover, the closest matches to every band were obtained from the Blast search tool at the National Centre for Biotechnology Information (McGinnis and Madden, 2004). Sequences alignment (ClustalW) and phylogenetic analysis were performed using the MEGA software (version 6.0). The phylogenetic trees were constructed using the neighbor-joining method (1,000-fold bootstrap analysis). The sequences were deposited in the GenBank database under accession numbers KF148033-KF148052.

## 3. Results and Discussion

### *3.1. Deposition of $S^0$*

#### *3.1.1. Medium-long term operation*

Fig. 3b, c and d show the state of different surfaces of the HS of R1 at t=8 (Fig. 2). As in R2, $S^0$ was present all over the HS; however, it was not observed at the liquid interface of either of the two digesters. The S content of the samples F1 and F2 (Fig. 3a) were indeed negligible in

comparison with most of the rest of the samples (Table 1). In this regard, it must be noted that although the digesters were continuously recirculated, an inefficient mixing was maintained in the highest area of the liquid phase due to the fact that both the recirculation and the effluent streams left the reactor below the interface level (Fig. 1). This explains the great difference in TS content between F1 and F2 (which both contained approximately 60g/kg) and the respective effluents of R1 and R2 (containing around 19g/kg). Therefore, if $H_2S$ had been oxidised there, presumably some $S^0$ would have been observed at the liquid interface.

The $S^0$-rich deposits covering the walls of R2 were thicker than in R1, which was consistent with the operation time. By contrast, the $S^0$-rich deposits that accumulated on the ceiling and the dip tube of R2 were inexplicably thinner than in R1. The aspect of the walls and the dip tube of R1 was similar to that of the walls of R2 in terms of the proportion of $S^0$ (yellow part) to digested sludge (black part) againstheight. Obviously, the lowest parts of the HS were more frequently touched by sludge as a result of droplets and even momentary liquid level rises; hence the stratification pattern shown in Fig. 3b. This was indeed analytically proved; in general, the shorter the distance from the liquid surface, the higher the C content and the lower the S percentage (Table 1).

Regarding the dip tube in R2, it inexplicably did not present the aforementioned stratification pattern. In fact, although both samples D2 and B2 were taken at approximately the same height, D2 had a significantly lower S content, and its C concentration was approximately the double. Conversely, C1 and D1 had more similar S and C contents (90 and 8%w/w compared with 82 and 11%w/w, respectively). At this point, it should be highlighted that the digestate and the $S^0$ were intercalated in the different deposits (Fig. 3d); nevertheless, and in contrast to

Kobayashi et al. (2012), no specific stratification pattern was identified.

The different moisture levels maintained in both HS corresponded only partly to the above reasoning, that is, the larger the distance from the liquid phase, the lower the moisture content, or equivalently, the higher the level of dryness. The dryness of the samples is expressed as TS content in Table 1. The TS concentration in the samples taken from the walls and the ceiling of both reactors was fairly similar, and it was in turn considerably higher than in their respective dip tubes. Presumably, the moisture and sludge reaching these surfaces gradually dried. Regarding the relatively high moisture content in the samples taken from the ceiling (where the sludge made hardly, if any, contact), this was attributed to water condensation since this area was less well insulated than the walls.

*3.1.2. Short-term operation*

The total amount of $H_2S$ removed from t=16 to t=17 was estimated according to the daily biogas production and the $H_2S$ concentration recorded just before t=16 (under anaerobic conditions) (Fig. 2). Considering the weight and the elemental composition of the different $S^0$-rich deposits (A3, B3, C3, D3 and E3 in Table 1), all the $H_2S$ removed during that period (approximately 26g) was deposited equivalently in the form of $S^0$ on both the walls (excluding the highest area, as shown in Fig. 3e) and the ceiling. Namely, it was specifically accumulated where TS concentration was higher at t=15. A negligible amount of $S^0$ was recovered from the dip tube. Furthermore, $S^0$ was not observed at the liquid interface. In fact, as at t=15, comparison of the S concentration in F3 and in the effluent pointed to negligible (if any) $H_2S$ oxidation in this area. Consequently, nutrient accessibility did not seem to be a limiting factor for biogas desulphurisation; it presumably decreased with the increasing

distance from the liquid interface. Moisture level could indeed be the key factor for the process, which could be in turn related to the $O_2$ availability, since dryness may increase $O_2$ transfer.

*3.2. Recovery after cleaning*

As noted, microaerobic conditions were restored at t=16 (Fig. 2). The $O_2$ flow rate was frequently adjusted according to the evolution of the $O_2$ and the $H_2S$ concentrations in the biogas (Fig. 4a and b); the objective was to achieve the minimum amount of $O_2$ leaving the reactor and at least a 97% $H_2S$ removal efficiency. In R1, a biogas flow rate of 201NL/d containing approximately 0.27%v/v of $H_2S$ was entirely desulphurised within 6h (Fig. 4a). By then, the biogas $O_2$ content was 0.08%v/v, and the average molar ratio of $O_2$ supplied to $H_2S$ produced was approximately 2.0. Nonetheless, this relationship was further reduced during the following days; as shown in Table 2, an $O_2/H_2S$ molar ratio of about 0.9 was achieved. As a result, the $O_2$ content of the biogas decreased to 0.02%, which implied that only 3% of the $O_2$ supplied left the digester (Table 2). Assuming that all the $H_2S$ oxidised was converted into $S^0$ due to the limited $O_2$ availability, it was estimated that around 54% of the $O_2$ injected was consumed in $H_2S$ removal from biogas, and 43% was employed in other oxidative processes.

In R2, the biogas production at t=16 was similar to that inR1 (194NL/d). However, the $H_2S$ concentration recorded under anaerobic conditions was significantly higher (0.37%v/v); hence the higher $O_2$ flow rate reached (Fig. 4b). Nevertheless, the response of R2 to $O_2$ injection was slower than that of R1; the $O_2/H_2S$ molar ratio after 10h ofmicro-oxygenation was 7.6, while the $H_2S$ removal efficiency was only 65%. However, the next day, the biogas was entirely desulphurised and, therefore, the $O_2$ supply to R2 was reduced. Equivalent efficiencies were

eventually achieved in both digesters; an $O_2/H_2S$ molar ratio of approximately 1.0 was achieved, and approximately 47 and 45% of the $O_2$ supplied was consumed in the $H_2S$ removal from biogas and in unidentified processes, respectively (Table 2).

Although faster in R1, the rapidity with which the biogas was desulphurised in both reactors at t=16 suggested an important contribution of the chemical mechanisms of sulphide oxidation. Although the proportion of $H_2S$ removed by each of the mechanisms (chemical and biological) could not be estimated, at least at that point the biological oxidation rate was considered to be negligible. Due to the tasks carried out at t=15 (Fig. 2), the presence of a significant population of SOB in the HS or at the liquid interface at t=16 were ruled out. It should be taken into account that the absence of $O_2$ (electron acceptor) during the preceding anaerobic period (from t=15 to t=16) prevented SOB growth.

*3.3. Desulphurisation performance over time*

The impact of the operation time on the process of biogas desulphurisation was evaluated by comparing the $H_2S$ removal efficiency achieved just before and after cleaning the HS in R1 and R2 (Fig. 2). At t=14, 100 and 97% of the $H_2S$ produced in R1 and R2, respectively, was oxidised (Table 2). The performance of R2 was more unstable than in the same period in R1, however, due to the variability of the feed sludge, this could not be unequivocally attributed to the longer operation time. At t=16, the $H_2S$ removal efficiency in R1 and R2 remained stable at around 100 and 99%, respectively.

The efficiency of $O_2$ usage in R2 increased substantially after cleaning the HS, and a similar yield was observed in R1 (Table 2). In both reactors, the amount of $O_2$ consumed in

unidentified processes at t=16 was slightly lower than at t=14, probably as a result of the removal of the $O_2$-using microorganisms growing on the HS and at the liquid interface (Fig. 2). Considering the inefficient mixing conditions maintained in this area, it is possible that uniform mixing would have reduced the $O_2$ demand of the digesters. Besides, the amount of $O_2$ leaving the digester decreased, especially in R2, where it declined by 19%, while in R1, a decrease of only 6% was estimated. This suggested improved $O_2$ transfer in R2, and highlighted the need for a cleaning interval of less than 14 months in order to minimise the micro-oxygenation cost.

### *3.4. Composition and structure of the microbial communities*

#### *3.4.1. Bacterial diversity*

In general, the HS of both digesters showed a high species evenness and richness at t=15; *H* typically ranges from 1.5 to 3.5 (McDonald, 2003). In R1, *H* ranged from 3.2 to 3.6 (Fig. 5a), while it varied between 2.5 and 3.5 in R2 (Fig. 5b). The diversity indices of the samples A2, B2 and C2 were considerably higher than those of the samples D2, E2 and F2. Moreover, C1 and C2 presented the highest and the lowest *H*, respectively. With regard to this, it should be mentioned that the samples taken from the ceilings were expected to present significantly less *H*, even lower than in C2, due to the large distance from the liquid phase and the presumably deficiency in nutrients availability. Hence, taking into account that A2, B2 and C2 presented the highest TS concentrations in relation to the rest of the samples (including those taken from R1), it was hypothesised that the moisture levels maintained on the walls and the ceiling of R2 limited the bacterial community diversity (Table 1). Maybe the $O_2$ transfer was highest there, which certainly could prevent the growth of a wide group of microorganisms (those with no or low tolerance to $O_2$).

*3.4.2. Analysis of the DGGE profiles*

Overall, the pair-wise similarity indices indicated a low-moderate correspondence between the bacterial communities growing inside each HS at t=15 (Table 3). In R1, the highest similarity coefficients were found between samples A, B and C (68-72%), and between D, E and F (58-64%). C1 presented a similarity of 48-54% with D1, E1 and F1, while the rest of the samples presented low coefficients (13-28%). The samples with similar TS content were found to have the highest similarity. Hence, taking into account Table 1, it was the moisture level that determined the bacterial community. In fact, although the similarity indices calculated for R2 did not present so much variability, they roughly led to this conclusion. The similarity coefficients between A2 and C2 (59%), B2 and C2 (66%), and E2 and F2 (84%) were the highest.

As shown in Table 3, the pair-wise similarity indices of A1 and B1 (68%) and D1 and F1 (65%) were considerably higher than those of A2 and B2 (28%) and D2 and F2 (39%). Conversely, the similarity between A and D, B and F, and E and F was between 26 and 29% lower in R1. The rest of the sample pairs presented relatively low differences (1-13%).

*3.4.3. DGGE analysis*

According to the RDP classifier tool, from the 19 bands sequenced from the DGGE gel of R1 (Fig. 5a), 6 and 7 and were assigned to the Proteobacteria and Firmicutes phylum, respectively (Table 4). In addition, the phyla Actinobacteria (3 bands) and Verrucomicrobia (1 bands) were identified. It is worth noting that 2 bands could not be classified. From the bacterial DGGE profile of R2 (Fig. 5b), 8, 9 and 5 bands were placed within the

Proteobacteria, Firmicutes, and Actinobacteria phylum, respectively (Table 5). Only 1 band remained unclassified. Fig. 6a and b depict the phylogenetic relationships between the bands sequenced from the DGGE gel of R1 and R2 (respectively) and their closest relatives in GenBank (obtained by the Blast search tool).

The RDP classifier tool affiliated all the SOB genera found in R1 (DGGE bands 3, 4 and 6 in Fig. 5a) to the Proteobacteria phylum. They were members of two families: Epsilonproteobacteria (*Arcobacter* sp., *Sulfuricurvum* sp.) and Gammaproteobacteria (*Acidithiobacillus* sp.). Hence, at least three SOB species grew in the HS of R1 (Table 4). Both *Arcobacter* sp. and *Acidithiobacillus* sp. were found by Kobayashi et al. (2012) growing on the HS of a microaerobic digester. *Sulfuricurvum* was indicated by Kodama and Wanatabe (2004) as a chemolithoautotrophic and sulphur-oxidising genus capable of thriving under microaerobic and anaerobic conditions. Nonetheless, the Blast search tool also identified the genus *Alyciclobacillus* (band 13, within the Firmicutes phylum); it indicated a similarity of up to 100% with the specie reported in the study of Díaz et al. (2010). It must be highlighted that *Alyciclobacillus* was the only SOB genus found in F1.

According to the RDP classifier, five genera of SOB grew in the HS of R2. Nonetheless, the genus *Alyciclobacillus* was also identified by the Blast search tool. Along with the four SOB genera found in R1, *Acinetobacter* sp. (bands 10-12 in Fig. 5b) and *Rhodococcus* sp. (band 20), which are representatives of the Proteobacteria and Actinobacteria phyla, respectively, were found (Table 5). *Acinetobacter* sp. was reported by Omri et al. (2011) to be instrumental in desulphurising the air stream in a biofiltration system. Zhang et al. (2009) utilised a strain of *Rhodococcus* sp. to successfully remove $H_2S$ in a biotrickling filter. It should be

highlighted that *Rhodococcus* sp., *Acinetobacter* sp. and *Acidithiobacillus* sp. were found in F2.

In R1, the highest species richness of SOB was concentrated on the walls and the ceiling; only the genera *Arcobacter* and *Alicyclobacillus* were identified in the samples taken from the dip tube and the liquid interface, respectively (Table 4). Additionally, the intensity of the bands representing SOB in the lanes of the samples D1, E1 and F1 was substantially lower than in A1, B1 and C1 (Fig. 5a), which suggested that the size of the sulphide-oxisiding population was significantly larger in the walls and the ceiling.

According to Table 4 and 5, the species richness of SOB at the different locations in the HS was higher in R2, which could be at least partially related to the longer operation time. Sample A2 presented the highest SOB species richness; two or three SOB genera were found in the rest of the samples taken from R2 (Table 5). It must be noted that the genus *Rhodococcus* was found only in D2, E2 and F2, whereas *Alicyclobacillus* sp. was present only in A2, B2 and C2. Furthermore, although the difference between the samples was not as significant as in R1, the intensity of the bands representing SOB in A2 and C2 was higher than in the rest of the samples (Fig. 5b). Therefore, the moisture level seemed to determine the composition, species richness, and size of the SOB population, which is indeed consistent with the previous observations.

Obviously, the growing conditions in both HS changed over time as a result of $H_2S$ oxidation. Along with the availability of water and $O_2$, pH, trophic property, and the ability to utilise different S-compounds probably conditioned the order of appearance of the SOB species in

the HS. So, although both reactors operated normally under $O_2$-limiting conditions in order to completely convert $H_2S$ into $S^0$, some $S_2O_3^{2-}$ and $SO_4^{2-}$ could also be formed as a result of occasional increases in sulphide load or in $O_2$ availability, respectively, especially in R2, which operated for longer. Hence, presumably the genera of SOB found in R1 was more accurate in representing the population carrying out the $H_2S$ oxidation at the early stage of the microaerobic operation than those identified in R2. Namely, it was possible to estimate a succession of SOB.

## 4. Conclusions

Biogas desulphurisation took place in the HS of both reactors, excluding the liquid interface. A cleaning interval of less than 14 months was found to be necessary in order to minimise the micro-oxygenation cost. Once microaerobic conditions were restored after the HS cleaning, all the $H_2S$ was rapidly removed from the biogas, which suggested chemical oxidation. The moisture level determined the composition, species richness and size of the SOB population at the various locations within the HS.

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## References

1. Abatzoglou, N. and Boivin, S., 2009. A review of gas purification processes. Biofuels Bioprod. Biorefin. 3, 42-71.
2. Amann, R.J., Binder, B.J., Olson, R. J., Chisholm, S.W., Devereux, R., Stahl, D.A., 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microbiol. 56, 1919-1925.
3. APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th edn. American Public Health Association/American Water Works Association/Water Environmental Federation, Washington DC, USA.
4. Burgess, J. E., Parsons, S. A., Stuetz, R. M., 2001. Developments in odour control and waste gas treatment biotechnology: a review. Biotechnol. Adv. 19, 35-63.
5. Cirne, D. G., van der Zee, F. P., Fdz-Polanco, M., Fdz-Polanco, F., 2008. Control of sulphide during anaerobic treatment of S-containing wastewaters by adding limited amounts of oxygen or nitrate. Rev. Environ. Sci. Biotechnol. 7, 93-105.
6. Daims, H., Brühl, A., Amann, R., Schleifer, K.H., Wagner, M., 1999. The domain-specific probe EUB338 is insufficient for the detection of all bacteria: development and evaluation of a more comprehensive probe set. Syst. Appl. Microbiol. 22, 813-820.
7. Díaz, I., Pérez, S.I., Ferrero, E. M., Fdz-Polanco, M., 2010b. Effect of oxygen dosing point and mixing on the microaerobic removal of hydrogen sulphide in sludge digesters. Bioresour. Technol. 102, 3768-3775.
8. Díaz, I., Fdz-Polanco M., 2012. Robustness of the microaerobic removal of hydrogen sulfide from biogas. Water Sci. Technol. 65, 1368-1374.
9. Fortuny, M., Baeza, J.A., Gamisans, X., Casas, C., Lafuente, J., Deshusses, M.A., Gabriel, D., 2008. Biological sweetening of energy gases mimics in biotrickling filters. Chemosphere 71, 10-7.

10. Kleinjan, W., 2005. Biologically produced sulfur particles and polysulfide ions: effects on a biotechnological process for the removal of hydrogen sulfide from gas streams. PhD Thesis. Wageningen University. Wageningen, The Netherlands.

11. Kobayashi, T., Li, Y. Y., Kubota, K., Harada, H., Maeda, T., Yu, H.-Q., 2012. Characterization of sulfide-oxidizing microbial mats developed inside a full-scale anaerobic digester employing biological desulfurization. Appl. Microbiol. Biotechnol. 93, 847-857.

12. Kodama, Y., Watanabe, K., 2004. Sulfuricurvum kujiense gen. nov., sp. nov., a facultatively anaerobic, chemolithoautotrophic, sulfur-oxidizing bacterium isolated from an underground crude-oil storage cavity. Int. J. Syst. Evol. Microbiol. 54, 2297-2300.

13. Lebrero, R., Rodríguez, E., García-Encina, P.A., Muñoz, R., 2011. A comparative assessment of biofiltration and activated sludge diffusion for odour abatement. J. Hazard. Mater. 190,622-630.

14. Lebrero, R., Volckaert, D., Pérez, R., Muñoz, R., Van Langenhove, H., 2013. A membrane bioreactor for the simultaneous treatment of acetone, toluene, limonene and hexane at trace level concentrations. Water Res. 47, 2199-2212.

15. Lohwacharin, J., Annachhatre, A. P., 2010. Biological sulfide oxidation in an airlift bioreactor. Bioresour. Technol. 101, 2114-2120.

16. McDonald, G., 2003. Biogeography: Introduction to Space, Time and Life. Wiley, New York.

17. McGinnis, S., Madden, T.L., 2004. BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Res. 32, W20-25.

18. Omri, I., Bouallagui, H., Aouidi, F., Godon, J. J., Hamdi, M., 2011. $H_2S$ gas biological removal efficiency and bacterial community diversity in biofilter treating wastewater odor.

Bioresour. Technol. 102, 10202-10209.

19. Park, J., Evans, E. A., Ellis, T. G., 2011. Development of a biofilter with tire-derived rubber particle media for hydrogen sulfide odor removal. Water Air Soil Pollut. 215,145-153.

20. Peu, P., Picard, S., Diara, A., Girault, R., Béline, F., Bridoux, G., Dabert, P., 2012. Prediction of hydrogen sulphide production during anaerobic digestion of organic substrates. Bioresour. Technol. 121, 419-424.

21. Ramos, I., Díaz, I., Fdz-Polanco, M., 2012. The role of the headspace in hydrogen sulfide removal during microaerobic digestion of sludge. Water Sci. Technol. 66, 2258-2264.

22. Robertson, L. A., Kuenen, J. G., 2006. The Genus Thiobacillus. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer K. H., Stackebrandt, E. (eds). The Prokaryotes. Vol 5. 3rd ed. Springer, New York, 2006, 812-827.

23. Rodríguez, E., Lopes, A., Fdz-Polanco, M., Stams, A. J., García Encina, P. A., 2012. Molecular analysis of the biomass of a fluidized bed reactor treating synthetic vinasse at anaerobic and micro-aerobic conditions. Appl. Microbiol. Biotechnol. 93, 2181-2191.

24. Tang, K., Baskaran, V., Nemati, M., 2009. Bacteria of the sulphur cycle: An overview of microbiology, biokinetics and their role in petroleum and mining industries. Biochem. Eng. J. 44, 73-94.

25. van der Zee, F.P., Villaverde, S., García-Encina, P.A., Fdz-Polanco, F., 2007. Sulphide removal by moderate oxygenation of anaerobic sludge environments. Bioresour. Technol. 98, 518-524.

26. Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261-5267.

27. Zhang, L., Wang, Q., Tian, S., Wang, X., Xie, W., Sun, P., 2009. Biodegradation of hydrogen sulphide by inoculated *Rhodococcus* sp.zw11 in a pilot-scale biotrickling filter. International J. Environ. Pollut. 37, 450-465.

**Figure Captions**

**Fig. 1.** Digesters diagram.

**Fig. 2.** Operational sequence. ■ : microaerobic period; ■ : anaerobic period.

**Fig. 3.** HS diagram and samples codes (a). Walls of the HS (b), ceiling and dip tube (c and d) of R1 at t=15. Walls of the HS and liquid interface of R2 at t=17 (e).

**Fig. 4.** $O_2$ flow rate (discontinuous line), and concentrations of $H_2S$ (▲) and $O_2$ (○) in the biogas from R1 and R2 at t=16.

**Fig. 5.** Bacterial DGGE profiles of the 16S rRNA amplicons of the samples retrieved from the HS of R1 (a) and R2 (b) at t=15 with their respective diversity indices.

**Fig. 6.** Bacterial phylogenetic tree based on neighbour-joining analysis of 16S rRNA sequences from the HS of R1 (a) and R2 (b) (in boldface) and their closest relatives (similarity ≥ 97%) in GenBank obtained by the Blast search tool. Accession numbers are indicated. Numbers on the nodes indicate bootstrap values of 50% and higher (1,000 replicates). The scale bar indicates 10% sequence difference.

## Tables and Figures

**Table 1**. Elemental composition of the samples retrieved from the HS of the reactors.

| | R1 | | | | | | R2 | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | t=15 | | | | | | t=15 | | | | | | t=17 | | | | | | |
| Sample | A1 | B1 | C1 | D1 | E1 | F1 | A2 | B2 | C2 | D2 | E2 | F2 | A3 | B3 | C3 | D3 | E3 | F3 | Effluent |
| TS (g/kg) | 296 | 279 | 297 | 150 | 114 | 63 | 425 | 387 | 371 | 167 | 126 | 57 | - | - | - | - | - | - | 14 |
| S (%w/w) | 26 | 90 | 89 | 82 | 1 | <1 | 10 | 84 | 97 | 62 | 3 | <1 | 28 | 90 | 85 | 85 | 7 | <1 | <1 |
| C (%w/w) | 22 | 8 | 5 | 11 | 20 | 26 | 25 | 6 | 2 | 13 | 8 | 28 | 24 | 8 | 12 | 12 | 26 | 30 | 27 |

**Table 2.** Assessment of the desulphurisation performance before and after t=15.

| Reactor | Time | Biogas production (NL/d) | $H_2S^{AN}$ (%v/v) | $O_2/H_2S$ (mol/mol) | $H_2S^{MA}$ (%v/v) | $O_2^{MA}$ (%v/v) | $O_2$ to $S^0$ (%) | $O_2$ in biogas (%) | $O_2$ other processes (%) |
|---|---|---|---|---|---|---|---|---|---|
| R1 | t=14 | 176 | 0.55 | 1.3 | 0.00 | 0.05 | 36 | 9 | 55 |
| R1 | t=16 | 173 | 0.53 | 0.9 | 0.00 | 0.02 | 54 | 3 | 43 |
| R2 | t=14 | 139 | 0.48 | 2.5 | 0.02 | 0.20 | 20 | 27 | 53 |
| R2 | t=16 | 141 | 0.48 | 1.0 | 0.00 | 0.05 | 47 | 8 | 45 |

$^{AN}$ anaerobic conditions; $^{MA}$ microaerobic conditions

**Table 3.** Similarity indices (%R1/%R2) between the samples taken from the HS of the reactors at t=15.

| Sample | B | C | D | E | F |
|---|---|---|---|---|---|
| A | 68/28 | 68/59 | 13/39 | 16/25 | 16/17 |
| B | - | 72/66 | 28/25 | 21/30 | 18/47 |
| C | - | - | 54/51 | 43/37 | 48/35 |
| D | - | - | - | 64/54 | 65/39 |
| E | - | - | - | - | 58/84 |

**Table 4.** Taxonomic placement of the bacterial DGGE bands of the gel obtained from the samples taken from R1 at t=15 according to the RDP classifier at 50% of confidence level, and closest relatives in GenBank obtained by the Blast search tool showing sequence similarity and environments from which they were retrieved. "×" indicates presence of the band in the sample; high-intensity bands are depicted with a bold cross.

| Taxonomic placement | Band n° (accession nº) | A1 | B1 | C1 | D1 | E1 | F1 | Closest relatives in Blast (accession nº) | Similarity (%) | Source of origin |
|---|---|---|---|---|---|---|---|---|---|---|
| Phylum Proteobacteria | 1 (KC306914) | × | | | | | | Uncultured bacterium (AB286499) | 95 | Activated sludge |
| Class Epsilonproteobacteria | | | | | | | | | | |
| Order Campylobacterales | 2 (KF130769) | | | × | | | | Uncultured epsilon proteobacterium (DQ295695) | 99 | Floating microbial mat in sulfidic groundwater, Movile Cave |
| | | | | | | | | Uncultured bacterium (AB286499) | 98 | Activated sludge |
| Family Campylobacteraceae | | | | | | | | | | |
| Genus *Arcobacter*[a] | 3 (KC306915) | × | × | × | × | × | | Uncultured *Arcobacter* sp. (HQ392829) | 99 | Headspace of a digester of sewage sludge under microaerobic conditions |
| | | | | | | | | Uncultured *Arcobacter* sp. (HQ392823) | 99 | Headspace of a digester of sewage sludge under microaerobic conditions |
| Family Helicobacteraceae | | | | | | | | | | |
| Genus *Sulfuricurvum*[a] | 4 (KC306917) | × | × | × | | | | Uncultured bacterium (AB286499) | 99 | Activated sludge |
| | | | | | | | | Uncultured bacterium (EU662592) | 97 | Floating microbial mat from sulfidic water |
| Class Deltaproteobacteria | | | | | | | | | | |
| Order Syntrophobacterales | | | | | | | | | | |
| Family Syntrophaceae | | | | | | | | | | |
| Genus *Syntrophus* | 5 (KC130770) | × | × | × | × | × | × | Uncultured Deltaproteobacteria bacterium (CU926874) | 99 | Mesophilic anaerobic digester which treats municipal wastewater sludge |
| | | | | | | | | Uncultured bacterium (JQ085713) | 99 | Anaerobic digester |
| Class Gammaproteobacteria | | | | | | | | | | |
| Order Acidithiobacillales | | | | | | | | | | |
| Family Acidithiobacillaceae | | | | | | | | | | |
| Genus *Acidithiobacillus*[a] | 6 (KC130771) | × | × | × | | | | *Thiobacillus* sp. (AJ459802) | 99 | Culture collection |
| | | | | | | | | *Acidithiobacillus* sp. (FJ915156) | 99 | Culture collection |
| Phylum Firmicutes | | | | | | | | | | |
| Class Clostridia | | | | | | | | | | |
| Order Clostridiales | 7 (KC306921) | | × | × | × | × | | Uncultured bacterium (FJ978625) | 96 | Feces |
| Family Syntrophomonadaceae | | | | | | | | | | |
| Genus *Thermohydrogenium* | 8 (KC130772) | × | × | × | | | | Uncultured bacterium (GQ259594) | 95 | Bioreactor |
| | 9 (KC306923) | × | × | × | | × | | Uncultured bacterium (GQ259594) | 96 | Bioreactor |
| | 10 (KC306924) | × | × | × | × | | × | Uncultured bacterium (GQ259594) | 96 | Bioreactor |
| Family Peptostreptococcaceae | | | | | | | | | | |
| Genus *Clostridium XI* | 11 (KC306922) | × | × | × | × | × | × | Uncultured soil bacterium (JX489929) | 99 | Soil |
| | | | | | | | | Uncultured bacterium (FJ660495) | 99 | Activated sludge |
| Family Lachnospiraceae | 12 (KC306925) | × | × | × | | × | | *Clostridium* sp. (GU247219) | 93 | Waste water of a |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | pesticides firm |
| Class Bacilli<br>Order Bacillales[a] | 13 (KC306927) | × | × | × | × | × | × | Uncultured *Alicyclobacillus* sp. (HQ392831) | 100 | Headspace of a digester of sewage sludge under microaerobic conditions |
| | | | | | | | | *Bacillus solfatarensis* (AY518549) | 98 | Culture collection |
| Phylum Actinobacteria<br>Class Actinobacteria<br>Subclass Actinobacteridae<br>Order Actinomycetales<br>Suborder Corynebacterineae<br>Family Dietziaceae<br>Genus *Dietzia* | 14 (KC306928) | × | × | × | × | × | × | *Dietzia* sp. (FJ529029) | 95 | Excess sludge of municipal wastewater treatment plant |
| Family Mycobacteriaceae<br>Genus *Mycobacterium* | 15 (KF130773) | | | × | × | × | × | Uncultured bacterium (EU677397) | 97 | Soil |
| Family Nocardiaceae<br>Genus *Gordonia* | 16 (KF130774) | | | × | × | × | × | *Gordonia hirsuta* (NR_026297) | 99 | Biofilter of an animal rendering plant |
| | | | | | | | | Uncultured bacterium (CU925412) | 99 | Mesophilic anaerobic digester which treats municipal wastewater sludge |
| Phylum Verrucomicrobia<br>Class Optitutae<br>Order Optitutales<br>Family Optitaceae<br>Genus *Alterococcus* | 17 (KC306929) | | × | × | | × | × | Uncultured bacterium (FN985251) | 99 | Long-term biogas completely stirred tank reactor |
| | | | | | | | | Uncultured Verrucomicrobia bacterium (CU918353) | 99 | Mesophilic anaerobic digester which treats municipal wastewater sludge |
| Unclassified bacteria | 18 (KC306930) | | | | × | × | × | Uncultured bacterium (FN985598) | 99 | Long-term biogas completely stirred tank reactor |
| | | | | | | | | Uncultured bacterium (AB175392) | 99 | A mesophilic anaerobic BSA digester |
| | 19 (KC306931) | × | × | × | × | × | × | Uncultured Firmicutes bacterium (CU923016) | 97 | Mesophilic anaerobic digester which treats municipal wastewater sludge |

[a] Putative SOB

**Table 5**. Taxonomic placement of the bacterial DGGE bands of the gel obtained from the samples taken from R2 at t=15 according to the RDP classifier at 50% of confidence level, and closest relatives in GenBank obtained by the Blast search tool showing sequence similarity and environments from which they were retrieved. "×" indicates presence of the band in the sample; high-intensity bands are depicted with a bold cross.

| Taxonomic placement | Band n° (accession nº) | A2 | B2 | C2 | D2 | E2 | F2 | Closest relatives in Blast (accession nº) | Similarity (%) | Source of origin |
|---|---|---|---|---|---|---|---|---|---|---|
| Phylum Firmicutes | | | | | | | | | | |
| Class Clostridia | | | | | | | | | | |
| Order Clostridiales | 1 (KF148033) | | × | | | | | Uncultured bacterium (JF937217) | 99 | Anaerobic fluidized bed reactor treating vinasse |
| Family Syntrophomonadaceae | | | | | | | | | | |
| Genus *Thermohydrogenium* | 2 (KF148034) | × | × | × | × | × | × | Uncultured bacterium (GQ259594) | 95 | Bioreactor |
| | 3 (KF148035) | × | × | × | × | × | × | Uncultured bacterium (GQ259594) | 96 | Bioreactor |
| Family Lachnospiraceae | 4 (KF148036) | | | | × | × | | Uncultured bacterium (CR933122) | 99 | Every municipal wastewater treatment plant |
| | | | | | | | | Uncultured bacterium (JX627844) | 99 | Membrane bioreactor treating acetone, toluene, limonene and hexane |
| Genus *Hespellia* | 5 (KF148037) | | | | × | × | | Uncultured bacterium (CR933122) | 99 | Every municipal wastewater treatment plant |
| | | | | | | | | Uncultured bacterium (JX627844) | 99 | Membrane bioreactor treating acetone, toluene, limonene and hexane |
| Family Peptostreptococcaceae | | | | | | | | | | |
| Genus *Clostridium XI* | 6 (KF148038) | × | × | × | × | × | × | Uncultured soil bacterium (JX489929) | 99 | Soil |
| | | | | | | | | Uncultured bacterium (GQ480145) | 99 | Activated sludge from wastewater treatment plant |
| Family Clotridiales_incertae sedis III | 7 (KF148039) | | × | | | | | Uncultured bacterium (JF937217) | 100 | Anaerobic fluidized bed reactor treating vinasse |
| Class Bacilli | | | | | | | | | | |
| Order Bacillales[a] | 8 (KF148040) | | × | | | | | Uncultured *Alicyclobacillus* sp. (HQ392831) | 99 | Headspace of a digester of sewage sludge under microaerobic conditions |
| | | | | | | | | *Bacillus solfatarensis* (AY518549) | 98 | Culture collection |
| | 9 (KF148041) | | × | | | | | Uncultured *Alicyclobacillus* sp. (HQ392831) | 100 | Headspace of a digester of sewage sludge under microaerobic conditions |
| | | | | | | | | *Bacillus solfatarensis* (AY518549) | 98 | Culture collection |
| Phylum Proteobacteria | | | | | | | | | | |
| Class Gammaproteobacteria | | | | | | | | | | |
| Order Pseudomonadales | | | | | | | | | | |
| Family Moraxellaceae | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Genus *Acinetobacter*[a] | 10 (KC306918) | × | | | × | | | *Acinetobacter johnsonii* (NR_044975) | 95 | Culture collection |
| | 11 (KF148042) | × | | | × | × | × | *Acinetobacter johnsonii* (NR_044975) | 97 | Culture collection |
| | 12 (KC306919) | × | | × | × | × | × | *Acinetobacter johnsonii* (NR_044975) | 99 | Culture collection |
| | | | | | | | | Uncultured bacterium (JX040380) | 99 | Wastewater |
| | | | | | | | | Uncultured *Acinetobacter* sp. (JN679102) | 99 | Membrane bioreactor |
| Order Acidithiobacillales | | | | | | | | | | |
| Family Acidithiobacillaceae | | | | | | | | | | |
| Genus *Acidithiobacillus*[a] | 13 (KF148043) | × | × | × | × | × | × | *Thiobacillus* sp. (AJ459802) | 99 | Culture collection |
| | | | | | | | | Uncultured *Acidithiobacillus* sp. ( EF612419) | 98 | Mine tailings |
| | | | | | | | | Uncultured bacterium (JQ906816) | 97 | Hydrogen sulfide biofilter |
| | 14 (KF148044) | × | × | × | | | | *Thiobacillus* sp. (AJ459802) | 99 | Culture collection |
| | | | | | | | | Uncultured bacterium (JQ906816) | 97 | Hydrogen sulfide biofilter |
| Order Enterobacteriales | | | | | | | | | | |
| Family Enterobacteriaceae | | | | | | | | | | |
| Genus *Raoultella* | 15 (KF148045) | × | | × | × | × | × | Uncultured bacterium (JF689907) | 97 | MFC anode biofilm |
| | | | | | | | | Enterobacteriaceae bacterium (HQ259701) | 97 | Activated sludge |
| Class Epsilonproteobacteria | | | | | | | | | | |
| Order Campylobacterales | | | | | | | | | | |
| Family Helicobacteraceae | | | | | | | | | | |
| Genus *Sulfuricurvum*[a] | 16 (KF148046) | × | | | | | | Uncultured epsilon proteobacterium (DQ295695) | 99 | Floating microbial mat in sulfidic groundwater,Movile Cave |
| | | | | | | | | Uncultured bacterium (AB248647) | 99 | A mesophilic anaerobic butyrate degrading reactor |
| Family Campylobacteraceae | | | | | | | | | | |
| Genus *Arcobacter*[a] | 17 (KF148047) | × | × | × | | | | Uncultured *Arcobacter* sp. (HQ392829) | 100 | Headspace of a digester of sewage sludge under microaerobic conditions |
| | | | | | | | | Uncultured *Arcobacter* sp. (HQ392823) | 99 | Headspace of a digester of sewage sludge under microaerobic conditions |
| Phylum Actinobacteria | | | | | | | | | | |
| Class Actinobacteria | | | | | | | | | | |
| Subclass Actinobacteridae | | | | | | | | | | |
| Order Actinomycetales | | | | | | | | | | |
| Suborder Corynebacterineae | | | | | | | | | | |
| Family Nocardiaceae | | | | | | | | | | |
| Genus *Gordonia* | 18 (KF148048) | | | | × | × | × | Uncultured Actinobacteria bacterium (CU925412) | 99 | Mesophilic anaerobic digester which treats municipal wastewater sludge |
| | | | | | | | | *Gordonia hirsuta* (NR_026297) | 98 | Biofilter of an animal rendering plant |
| | 19 (KF148049) | | × | | × | × | × | Uncultured Actinobacteria bacterium (CU925412) | 98 | Mesophilic anaerobic digester which treats municipal wastewater sludge |
| | | | | | | | | *Gordonia hirsuta* (NR_026297) | 98 | Biofilter of an animal rendering plant |
| Family Nocardiaceae | | | | | | | | | | |
| Genus *Rhodococcus*[a] | 20 (KF148050) | | | | × | × | × | *Rhodococcus* sp. (AJ007001) | 99 | Compost biofilter |
| | | | | | | | | *Rhodococcus* sp. (FR690460) | 98 | Sludge of a |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | bioreactor |
| Family Mycobacteriaceae<br>Genus *Mycobacterium* | 21 (KF148051) | × | | × | × | × | Uncultured bacterium (EU677397) | 99 | Soil |
| | | | | | | | Uncultured bacterium (JX627819) | 98 | Membrane bioreactor treating acetone, toluene, limonene and hexane |
| Unclassified bacteria | 22 (KF148052) | | | × | × | × | Uncultured bacterium (FN985598) | 99 | Long-term biogas completely stirred tank reactor |
| | | | | | | | Uncultured bacterium (AB175392) | 99 | A mesophilic anaerobic BSA digester |

[a] Putative SOB

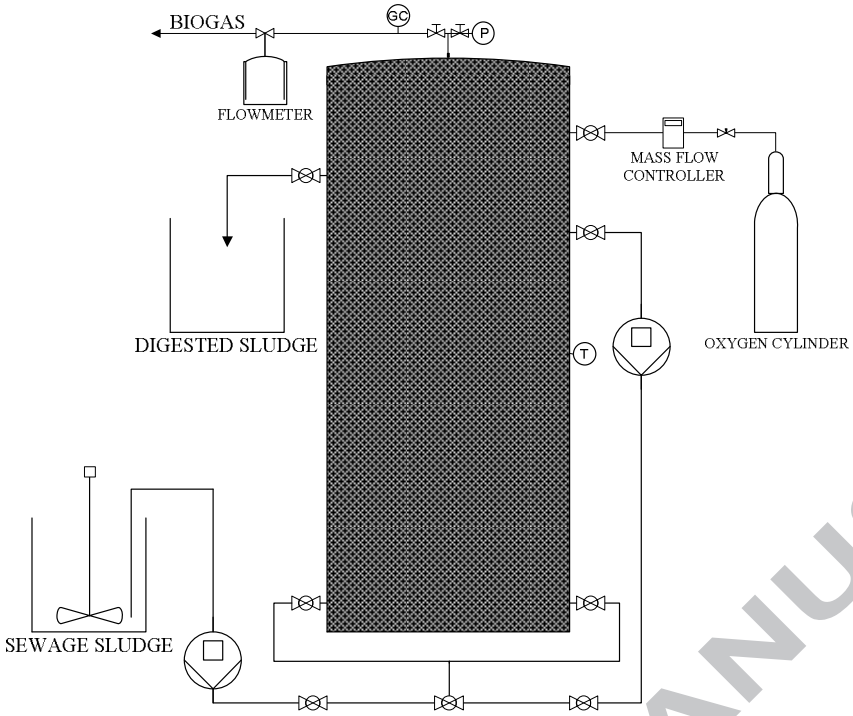



**Fig. 1.**

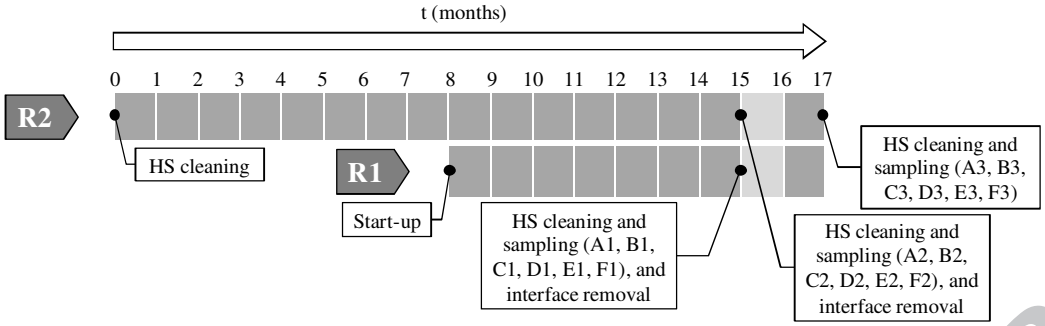



**Fig. 2.**

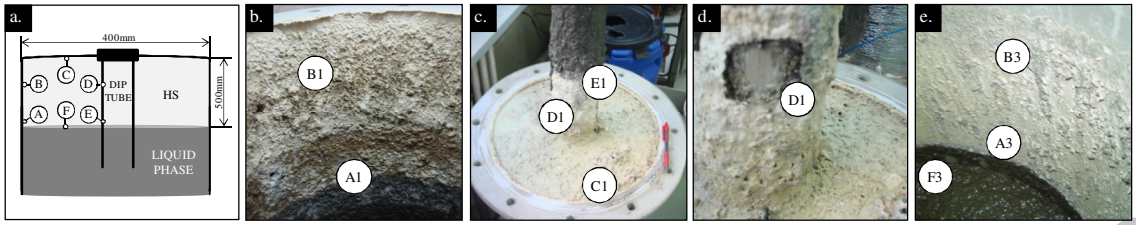
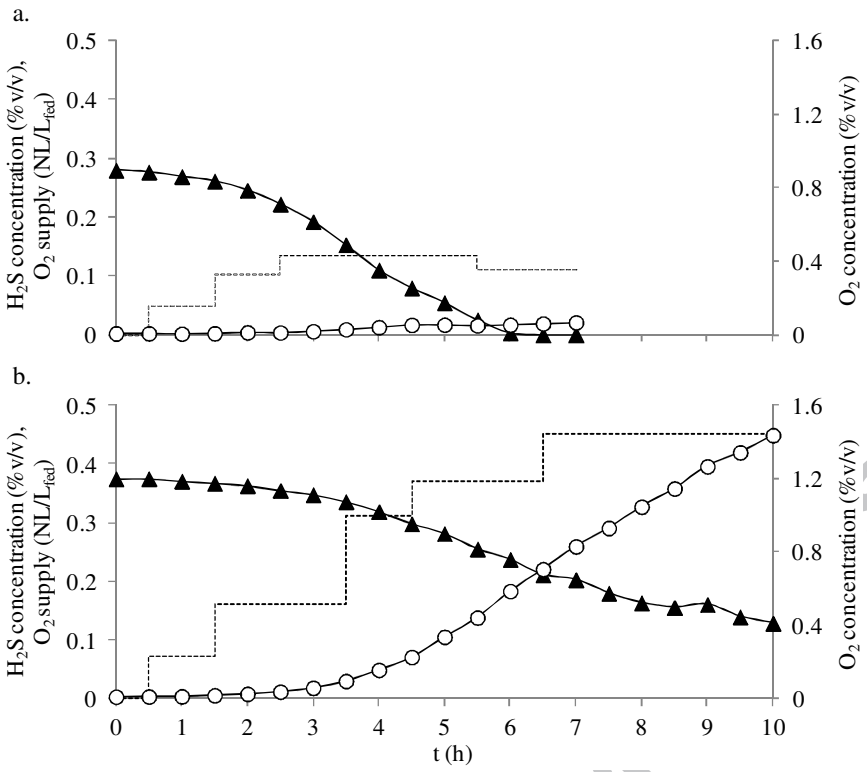



**Fig. 3.**

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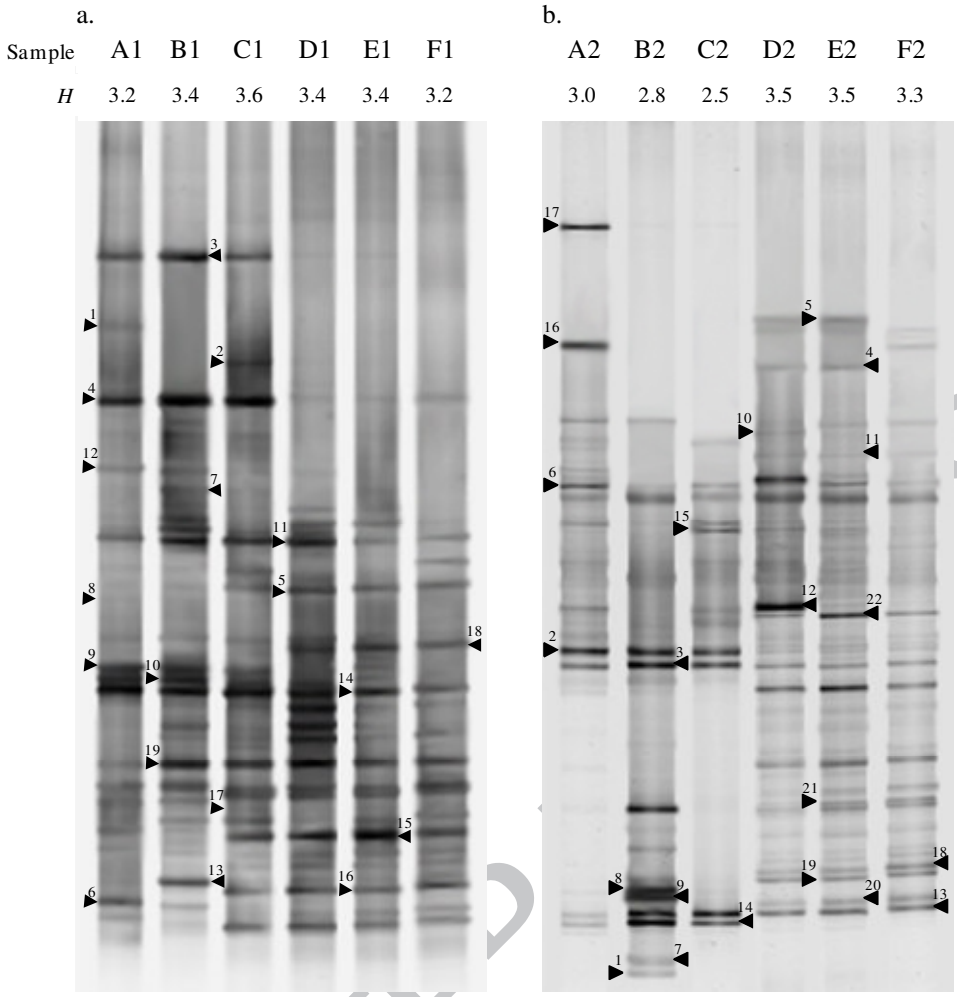


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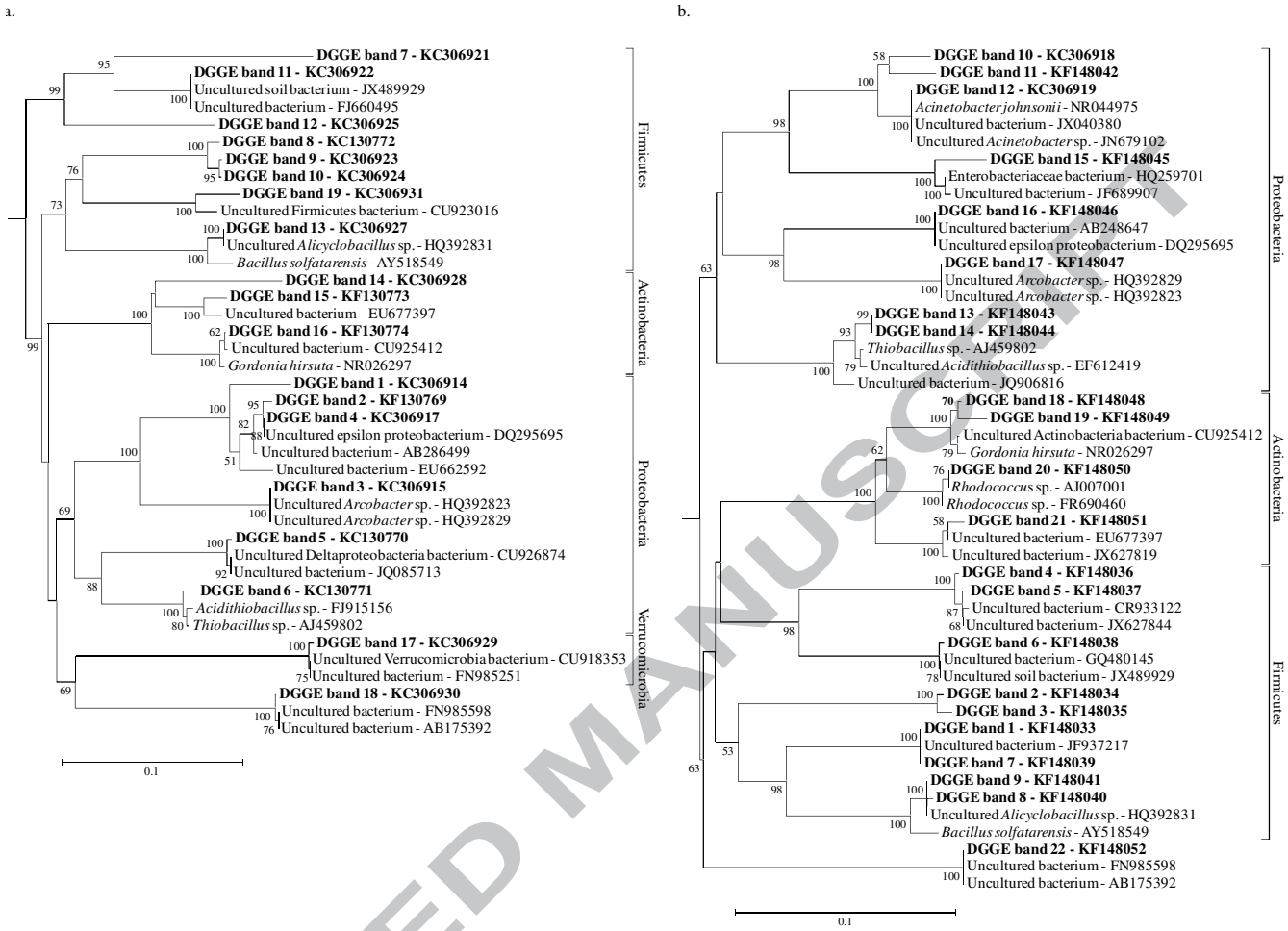


**Fig. 6.**

# 4

# The role of the headspace in hydrogen sulfide removal during microaerobic digestion of sludge

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# The role of the headspace in hydrogen sulfide removal during microaerobic digestion of sludge

I. Ramos, I. Díaz and M. Fdz-Polanco

## ABSTRACT

The role of the headspace (HS) in the microaerobic removal of hydrogen sulfide from biogas produced during sludge digestion was studied. Research was carried out in a pilot reactor with a total volume of 265 L, under mesophilic conditions. Biogas was successfully desulfurized (99%) by introducing pure oxygen (0.46 NL/$L_{fed}$) into the recirculation stream when the HS volume was both 50.0 and 9.5 L. The removal efficacy dropped sharply to ≈15% when the HS was reduced to 1.5 L. The system responded quickly to the operational changes imposed: micro-oxygenation stops and variations in supply, as well as HS volume reductions and increases. As the final result, the microaerobic process required a minimum surface into the gas space to occur, which along with the elemental sulfur deposition in this area indicated that the oxidation took place there. Additionally, the pattern of sulfur accumulation suggested that the removal occurred preferentially on certain materials, and pointed to a significant biological contribution.

**Key words** | anaerobic digestion, biogas desulfurization, headspace, hydrogen sulfide removal, microaerobic conditions

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## INTRODUCTION

The energy sector accounts for 64% of the world's greenhouse-gas emissions (IEA 2009). Biogas is a versatile and renewable energy source that can be used for the replacement of fossil fuels in several applications (Weiland 2010). Consequently, anaerobic digestion (AD) is gaining increasing attention worldwide as one of the most promising biotechnologies to produce it (Jagadabhi *et al.* 2010).

AD is considered an essential part in a wastewater treatment plant (WWTP), as it reduces the sludge volume, improves its character, and reduces the associated health problems (Appels *et al.* 2008). Many industrial wastewaters have high concentrations of sulfur compounds, such as sulfates (Zhou *et al.* 2007). This anion is not a direct threat to the treatment process; however, sulfate-reducing bacteria (SRB) use it for the oxidation of organic compounds and hydrogen under anaerobic conditions, thereby producing sulfide (Hulshoff Pol *et al.* 1998). As shown in equilibriums 1 and 2, dissolved sulfide exists in undissociated and dissociated form according to pH (Deublein & Steinhauser 2007). At pH characteristic of methanogenic systems, between 20 and 50% of the dissolved sulfide is in the undissociated form (Colleran *et al.* 1995), which is released to the gas phase according to a coefficient $\alpha$ (Hulshoff Pol *et al.* 1998). As the final result, this biogas component is in waste gases, wastewaters and sewage sludge:

$$H_2S \leftrightarrow H^+ + HS^- \left(K_1 = 1.0 \times 10^{-7}\right) \quad (1)$$

$$HS^- \leftrightarrow H^+ + S^{2-} \left(K_2 = 1.0 \times 10^{-14}\right) \quad (2)$$

$$[H_2S]_l = \alpha [H_2S]_g \quad (3)$$

Gaseous hydrogen sulfide causes malodour, contamination, energy performance deterioration, toxicity, and corrosion. Therefore, biogas desulfurization is required in order to prevent damage and standardize its quality according to the final biogas application (Appels *et al.* 2008). Physicochemical methods are widely employed for this purpose. Nonetheless, biological processes such as bioscrubbers, biotrickling filters, and biofilters are gaining attention as a result of their competitive performance and lower operational costs. These methods are based on the biological sulfur cycle; specifically, on the dissolved sulfide oxidation (Kleinjan 2005).

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Among the biodesulfurization techniques, the direct micro-oxygenation (or micro-aeration) of anaerobic reactors can be a more interesting alternative. Sulfide oxidation takes place both biologically and chemically, and the end product formation depends on the oxygen availability (van der Zee *et al.* 2007). Sulfate production involves the production of intermediate compounds such as polysulfide, thiosulfate, tetrathionate, and sulfur, which is the final product under oxygen limited conditions (Kleinjan 2005).

Under microaerobic conditions, sulfide removal competes effectively for oxygen versus other processes, and is faster than the re-reduction of oxidized sulfur compounds (van der Zee *et al.* 2007; Fdz-Polanco *et al.* 2009). So, biogas desulfurization efficacies higher than 97% have been achieved by both oxygen and air at equivalent rates with none impact on digestion performance (Díaz *et al.* 2010a). Jenicek *et al.* (2010), in contrast, reported that methane production could decrease because of aerobic substrate consumption. Nonetheless, that could be compensated by improved hydrolysis and increased biogas production (Johansen & Bakke 2006; Jenicek *et al.* 2008). Other oxygen benefits have been reported: sulfide toxicity suppression (Khanal & Huang 2003), improved degradation of recalcitrants organics (Jenicek *et al.* 2008), and reduction of volatile fatty acid (VFA) (Botheju *et al.* 2010).

Díaz *et al.* (2010b) demonstrated that oxygen transfer to the liquid phase was not required to achieve sulfide-free biogas, and observed elemental sulfur deposition in the walls and ceiling of the reactor headspace (HS), where different sulfide oxidizing bacteria (SOB) were found. Obviously, the contact between the oxygen and the liquid media is really restricted when it is injected into the HS, therefore almost all the oxygen is only accessible to microorganisms present in the gas space. However, Díaz *et al.* (2010b) achieved similar desulfurization efficacies by supplying oxygen into the HS, the recirculation stream and with the feed sludge. Thus, they deduced that the hydrogen sulfide removal took place in the HS. This is consistent with Fdz-Polanco *et al.* (2009), who suggested an oxidation mechanism in the liquid–gas interface. They reported similar biogas desulfurization performances when oxygen was injected into the recirculation stream and the feed stream, independently of the mixing method. Nevertheless, dissolved sulfide removal occurred only with biogas recirculation. It certainly resulted in larger contact area between the oxygen and the liquid phase, as the size of bubbles that entered into the digester rose. That led to increased oxygen flow across the gas–liquid interface and consequent higher intake by microorganisms. Accordingly, the oxygen mass transfer was indicated as the limiting step for hydrogen sulfide removal. In contrast, Jenicek *et al.* (2011) reported sulfur accumulation in the sludge as a result of hydrogen sulfide removal by introducing limited amount of air into the liquid media. Furthermore, that increase was consistent with the biogas desulfurization efficacy observed. Therefore, the oxidation seemed to occur in the liquid phase.

Considering the inconclusive results relative to the oxidation place, it is of interest to explore where the biogas desulfurization is carried out really. The aim of this study is to investigate the role of the HS in the microaerobic removal of hydrogen sulfide.

## MATERIALS AND METHODS

### Digester

Research was performed in a continuous stirred-tank reactor (CSTR) with a total volume of 265 L, whose top consisted of a conical cover with a detachable and transparent cylinder in the uppermost part (Figure 1). Mesophilic conditions were applied by an electric resistance. The bioreactor was operated under variable hydraulic retention time (HRT) because of the changes in the HS volume (20–26d). It was fed with sewage sludge from a WWTP. The liquid phase was recirculated at a constant rate of 50 L/h by a peristaltic pump. Oxygen from a cylinder was supplied by a mass flow controller into the recirculation. Due to the variability of the feeding in terms of organic matter content, the organic loading rate (OLR) fluctuated throughout the study.

### Monitoring and experimental analysis

Pressure and temperature were monitored on-line by probes. Tygon tubing led the biogas produced to an inverted cylinder, where it was measured by a fixed liquid volume displacement ($550 \pm 5$ mL), and then released by an electro-valve. A gas chromatograph (VARIAN CP-4900 MicroGC) was used for on-line analysis of biogas composition, as described by Díaz *et al.* (2010b).

Digestion performance was evaluated by conventional parameter analysis according to the standard methods (APHA 1998): total and soluble chemical oxygen demand (COD), total solids (TS) and volatile solids (VS).

Sulfate concentration was measured by ion chromatography. Thiosulfate was analyzed by high performance

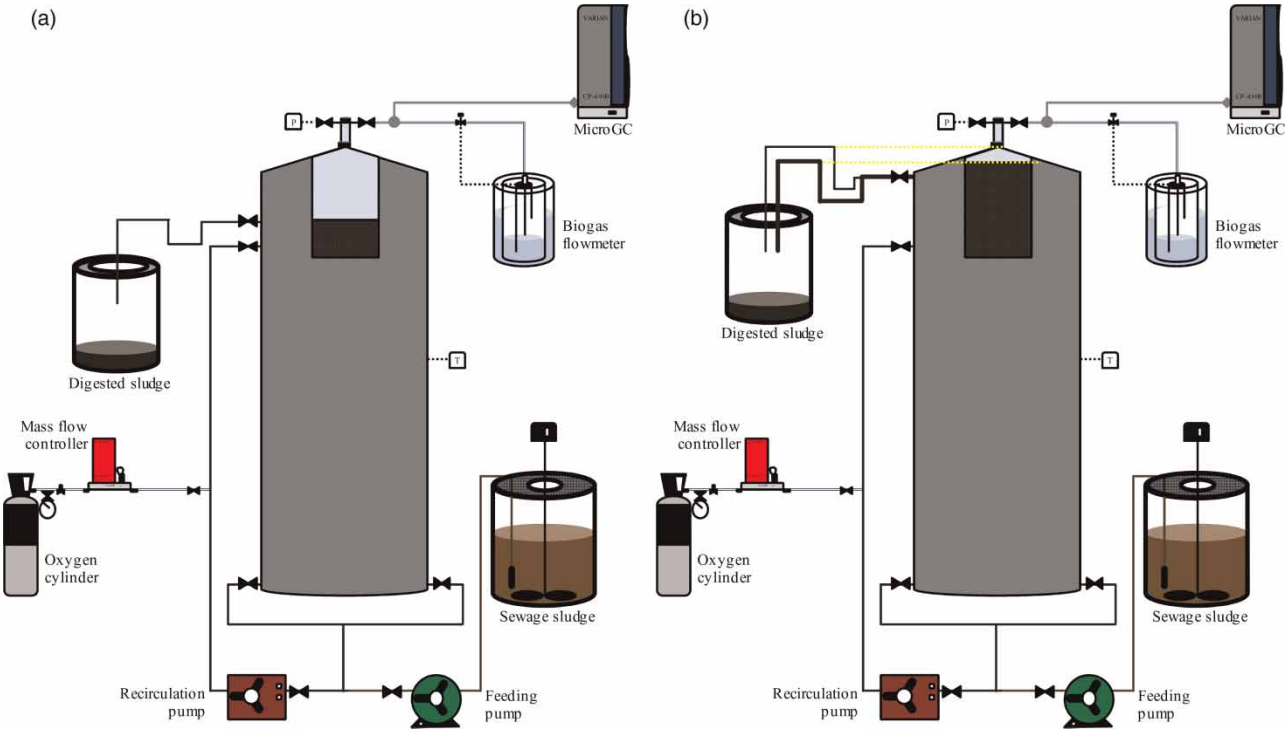


**Figure 1** | Digester diagram with HS of 50.0 (a) 9.5 and 1.5 L (b) effluent represented by a thick and thin line, respectively; liquid level indicated by a yellow dotted line. (The full colour version of this figure is available in the online version of this paper, at http://www.iwaponline.com/wst/toc.htm.)

liquid chromatography (HPLC), according to the procedure described by van der Zee *et al.* (2007). Sulfide was measured by potentiometry with selective electrode according to the standard method (APHA 1998).

## Experimental procedure

The study was divided into two phases (Table 1). At the beginning of the first phase, the digester operated under anaerobic conditions with a HS volume of 50.0 L (AN0). Once the hydrogen sulfide concentration remained stationary, the micro-oxygenation was started (MA0) according to Figure 1(a). Supply was raised gradually until the pollutant was removed.

At the beginning of phase 2, the reactor was operated under anaerobic conditions with a HS volume of 9.5 L (AN1). Microaerobic conditions were implemented at day 3 (MA1). As in the previous phase, oxygen was injected into the recirculation stream (Figure 1(b)). Once the residual hydrogen sulfide concentration was negligible (MA2), the liquid level of the reactor was increased until the HS was reduced to ≈1.5 L. After almost two days under such conditions (MA3), the HS volume was raised again to 9.5 L (MA4). Finally, the reactor worked under anaerobic conditions (AN2).

**Table 1** | Sequence of variations applied during the study, and digester response

| | Phase 1 | | Phase 2 | | | | | |
|---|---|---|---|---|---|---|---|---|
| | AN0 | MA0 | AN1 | MA1 | MA2 | MA3 | MA4 | AN2 |
| Time (d) | 0–2 | 2–14 | 0–3 | 3–4 | 4–5 | 5–6 | 6–7 | 7–8 |
| HS volume | 50.0 | 50.0 | 9.5 | 9.5 | 9.5 | 1.5 | 9.5 | 9.5 |
| Oxygen flow ($NL/L_{fed}$) | 0 | 0.16–0.46 | 0 | 0.43 | 0.46 | 0.46 | 0.46 | 0 |
| $VS_{fed}$ (g/L) | 29.6 | 32.0 | 39.0 | 29.7 | 29.7 | 31.6 | 31.6 | 31.6 |
| Biogas production (NL/d) | 158.2 | 169.0 | 207.5 | 166.4 | 157.0 | 173.8 | 179.0 | 174.2 |
| Methane yield ($NmL/g_{VS}$) | 337.1 | 325.5 | 329.4 | 353.9 | 335.4 | 352.0 | 353.9 | 341.6 |

## RESULTS AND DISCUSSION

### Phase 1

In AN0, the average hydrogen sulfide concentration was 0.35 ± 0.01%v/v (Figure 2). As a result of the first oxygen flow introduced to the reactor, the biogas sulfide content dropped to 0.15%v/v. Subsequently, the micro-oxygenation was increased by 50%, and the removal efficacy was 88%. Thereafter, the supply continued being raised to 0.27 NL/$L_{fed}$, 0.32 NL/$L_{fed}$, 0.36 NL/$L_{fed}$, 0.39 NL/$L_{fed}$, 0.43 NL/$L_{fed}$, and finally, 0.46 NL/$L_{fed}$. At 0.46 NL/$L_{fed}$, 99% of the pollutant produced was removed from the biogas. Therefore, as the stepwise supply adjustment assured the minimum biogas dilution, that value was considered the optimum micro-oxygenation level.

The desulfurization performance decreased slightly between the fifth and seventh day despite the increasing oxygen flow; it was raised at the beginning of 5th and 6th day (Figure 2). The efficacy loss recorded on the 5th day was attributed to a momentary increase in the OLR to the digester due to the sewage sludge variability (whose VS concentration varied widely throughout the study, as shown in Table 1). It also explained the biogas production peak recorded on the fifth day; it rose from 169.8 NL/d on fourth day to 201.2 NL/d, and decreased on the sixth day (161.8 NL/d). That is, the larger the amount of VS fed, the higher the release of sulfur compounds and biogas production. As a consequence, hydrogen sulfide generation and oxygen demand increased. Nevertheless, the biogas production fell on the next day and, remarkably, the biogas sulfide content continued rising. This suggested higher feeding sulfate content, which was detected shortly afterwards (data not shown). Note that the rise in the hydrogen sulfide production was verified in MA1.

### Phase 2

The first oxygen flow supplied removed 96% of the hydrogen sulfide produced (Figure 2). Then, it was raised to 0.46 NL/$L_{fed}$ (the optimum value in MA0), and the removal efficacy increased (99%). As in phase 1, the micro-oxygenation level reached by stepwise adjustment in phase 2 was considered the optimum. Furthermore, it was estimated that the hydrogen sulfide flow oxidized in MA0 and MA2 at 0.46 NL/$L_{fed}$ of oxygen supply was almost equal. Therefore, the microaerobic performance was not affected by the substantial reduction of the HS volume. The determination of the minimum HS needed to perform efficiently the biogas desulfurization could be interesting.

The higher working volume in phase 2 could result in poorer mixing (as recirculation rate was maintained constant) and longer contact time between the oxygen and the liquid phase. Furthermore, it is noteworthy that mixing was especially deficient in the upper part of the bioreactor because of the large distance between the liquid surface inside the reactor and both the exit point of digested sludge and the recirculation stream (Figure 1(b)). Whereas less efficient mixing could lower the oxygen transfer across the gas–liquid interface, higher contact time between both phases could improve it. However, as presented, the oxygen required for similar flows of hydrogen sulfide from biogas did not change from phase 1 to 2. This could mean that either the biogas desulfurization efficiency did not depend on the oxygen transfer to the liquid media, as Díaz *et al.* (2010b) suggested, or the reactor configuration set in phase

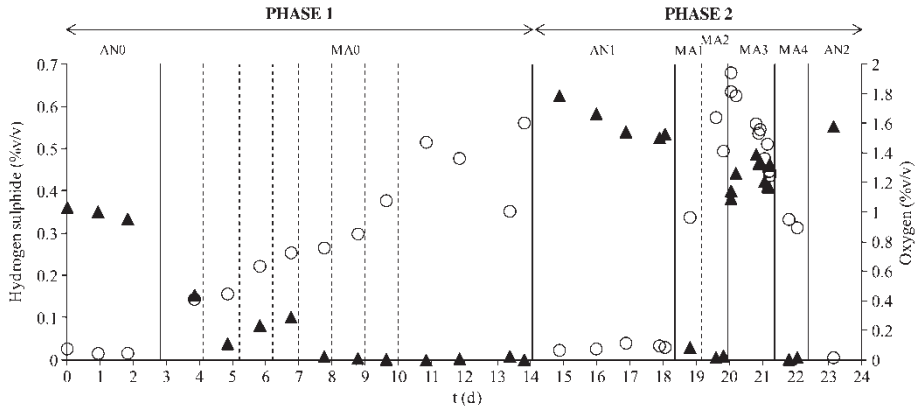


**Figure 2** | Hydrogen sulfide (▲) and oxygen (O) concentrations in biogas during the study. Vertical continuous lines indicate transition periods between anaerobic and microaerobic conditions and/or changes in HS volume; vertical discontinuous lines indicate changes in oxygen supply.

2 led to the same oxygen transfer rate as in phase 1. This will be clarified by estimating how oxygen was distributed into the digester in both phases (see below).

At day 5, the HS volume was reduced to 1.5 L, while the same oxygen flow was maintained. Shortly before, an increase of 0.37%v/v in the biogas sulfide content was recorded. The average pollutant concentration in MA3 was 0.44 ± 0.03%v/v, which resulted in a removal performance of ≈15%. This is consistent with the observations made during that experimental period relative to elemental sulfur deposition in HS (see below). Due to imminent clogging risks of the biogas outlet from the digester with sulfur, the liquid level was lowered. As a result, biogas was effectively desulfurized again (99%). The available surface area of the gas space seemed the limiting factor of the microaerobic process in MA3.

Although MA2 and MA4 were operated under the same conditions, oxygen contained in biogas was significantly different. Likewise, despite the low removal efficacy recorded in MA3, the oxygen concentration remained close to the values obtained in the previous period. Those oxygen responses resulted from the continuous changes in the biogas production due to the variable OLR to the digester (Table 1).

It must be emphasized that changes in the micro-oxygenation level and hydrogen sulfide flow are rapidly reflected in the biogas composition. Furthermore, the operating times to reach the stationary state at low biogas residence times (such as those fixed in this research) are very short. However, further research is being carried out in order to reduce safely the gas space (and as much as possible in an equivalent period to MA3) so that the response of the digester can be observed over a longer period of time.

## Sulfur deposition

During MA3, elemental sulfur accumulating on the sludge deposited inside the transparent cylinder of the cover could be seen (Figure 3(b)). One momentary liquid level rise above the level set just at the beginning of that stage was the cause of the sludge deposition. Meantime, though to a much lesser extent, sulfur accumulated in the valve of the biogas outflow (made of rigid polyvinyl chloride, PVC-U) and in the tygon tubing (Figure 3(a)). Whereas its deposition covering the sludge clearly pointed to biological biogas desulfurization, the possibility of chemical hydrogen sulfide oxidation in those areas free of sludge could not be ruled out. However, may be the affinity of SOB for transparent PVC (PVC-GLAS) was lower, and thus it was not

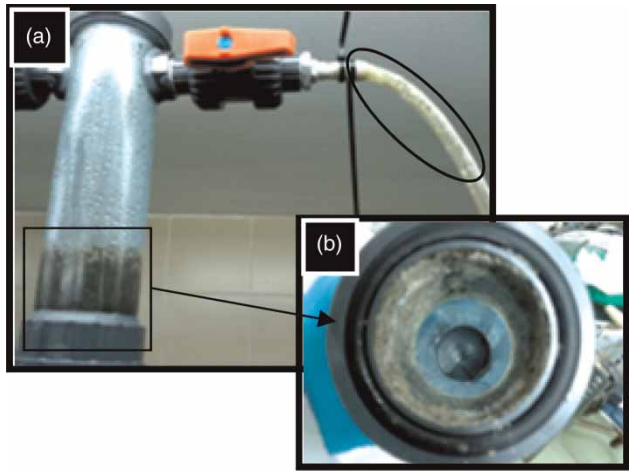


**Figure 3** | Elemental sulfur accumulated in the tygon tubing (delimited by an ellipse), sludge deposited in the transparent cylinder of the HS (delimited by a square) in MA3 (a), and sulfur accumulated on it from MA3 to MA4 (b).

deposited wherever sludge did not cover the cylinder, despite the shorter distance to the liquid surface and consequent higher accessibility to water and nutrients (and carbon sources to heterotrophic SOB). Overall, the preferential distribution of sulfur highlighted the predominance of the biological reactions of sulfide oxidation over the chemical ones.

When the transparent cylinder was removed for cleaning on 23rd day, sulfur was observed on those areas of the conical cover which were exposed to biogas after MA3 (Figure 1(b)). So, it was specifically deposited over the sludge that remained there after lowering the liquid level, which covered sulfur and sludge accumulated presumably in the preceding stages. Obviously, SOB could be more frequently provided by nutrients and water there when the HS volume was 9.5 L. Considering the short duration of MA4, that observation indicated that most of the sulfur shown in Figure 3(b) accumulated during MA3. Noteworthy is also that sulfur was not seen covering the surface of the liquid media on 23rd day; this was considered to be possibly due to the poor mixing conditions maintained around that area in phase 2. Moreover, it was not seen in the effluent either, which was examined daily. Along with the resulting removal performances, these observations confirmed that the hydrogen sulfide oxidation occurred in the HS.

Accordingly, the hypothesis is that SOB carried out the biogas desulfurization in the walls and/or the conical cover of the HS (both made of polypropylene homopolymer, PPH), during MA0, MA1 and MA2. SOB were carried along to those surfaces by biogas and sludge (due to splashes and even momentary liquid level rises), and once there, they

oxidized the gaseous sulfide that dissolved in the water deposited by condensation (droplets can be seen indeed in Figure 3(a)). Hence, certainly the oxidation process took place predominantly in the areas of the walls nearest the liquid media, where the growth conditions stimulated SOB. It is consistent with Kobayashi *et al.* (2012), who found that the shorter the distance from the liquid level of digested sludge, the higher the sulfide oxidizing activity. They also reported that SOB proliferated all over HS, including ceiling, walls, as well as components made of plastic, stainless and wooden placed around the middle of it.

### Oxygen utilization

The estimation of oxygen utilization into the reactor through the periods operated at 0.46 $NL/L_{fed}$ of oxygen supply is illustrated in Figure 4 (MA0 includes the results obtained from the 10th day). Note that neither sulfate nor thiosulfate were detected; so, elemental sulfur was the sole oxidation product. On the other hand, according to previous observations, it was assumed that the biogas sulfide content in MA0 was 0.5%. Likewise, it was considered constant from the 3th day of the second phase.

Figure 4 illustrates that the amount of oxygen employed to oxidize hydrogen sulfide in both MA0 and MA2 was almost equal (≈9%), which is in accordance with the optimum oxygen flows reached. However, it also shows a modest decrease in the oxygen consumed in unidentified processes (from 43 to 39%), which could be the final result of the poorer mixing and the longer contact time between phases (gas and liquid). This would imply that the mixing impact on the oxygen mass transfer was higher and, more importantly, that it was irrelevant to the desulfurization performance. The trend of the percentage of oxygen consumed in other processes over the study is indeed consistent with that. It must be noted, however, that it was the highest in MA4. Inexplicably, it rose by 14% over the equivalent period (MA2), and in the meantime, a 2% increase in the percentage of oxygen invested in partial hydrogen sulfide oxidation was estimated. Nevertheless, the higher surface providing favorable growth conditions for SOB as a result of the sludge deposition in most of the HS area could explain that slight rise.

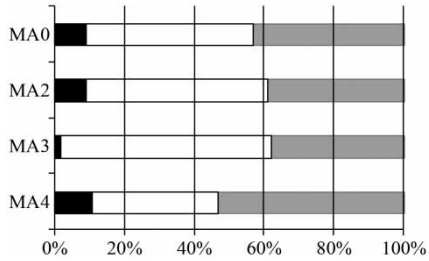


**Figure 4** | Oxygen utilization in the digester. Oxygen employed to produce elemental sulfur (■), oxygen consumed in other processes (▒), and oxygen in biogas (□).

Only a small amount of the oxygen supply was employed to remove hydrogen sulfide and, more importantly, it was the lowest in MA3 (2%). This is consistent with the low desulfurization efficacy recorded. The oxygen which ceased to be utilized for that end from MA2 left the digester with biogas instead of being utilized for other processes, which also pointed to hydrogen sulfide oxidation in HS. That is, there was no competition between other oxidizing microorganisms present in the liquid phase and SOB due to sulfide oxidizers only being able to consume the remaining oxygen that reached the HS, where they developed.

As noted, most of the oxygen left the reactor in biogas or was consumed in unidentified processes in all the periods evaluated. Among these processes, aerobic oxidation of readily available organic substrates hardly contributed to that result, as a correlation between microaerobic conditions and lower methane yield was not found (Table 1).

## CONCLUSIONS

Biogas was effectively desulfurized into the bioreactor at 0.46 $NL/L_{fed}$ of oxygen supply when the HS volume was 50.0 and 9.5 L. The removal performance dropped rapidly when the HS volume was lowered to 1.5 L, thereby showing that it strongly depended on the available surface area in the gas space. The deposition of elemental sulfur confirmed it. Moreover, the pattern of sulfur accumulation indicated that the oxidation occurred preferentially on certain materials, and suggested a significant biological contribution to the microaerobic process.

## ACKNOWLEDGEMENTS

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## REFERENCES

APHA 1998 *Standard Methods for the Examination of Water and Wastewater*, 20th edition. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.

Appels, L., Baeyens, J., Degrève, J. & Dewil, R. 2008 Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science* **34** (6), 755–781.

Botheju, D., Samarakoon, G., Chen, C. & Bakke, R. 2010 An experimental study on the effect of oxygen in bio-gasification – Part 2. In: *International Conference on Renewable Energies and Power Quality*, Granada.

Colleran, E., Finnegan, S. & Lens, P. 1995 Anaerobic treatment of sulfate-containing waste streams. *Antonie van Leeuwenhoek* **67** (1), 29–46.

Deublein, D. & Steinhauser, A. 2007 *Biogas from Waste and Renewable Resources: An Introduction*. Wiley-VCH, Weinheim.

Díaz, I., Lopes, A. C., Pérez, S. I. & Fdz-Polanco, M. 2010a Performance evaluation of oxygen, air and nitrate for the microaerobic removal of hydrogen sulfide in biogas from sludge digestion. *Bioresource Technology* **101** (20), 7724–7730.

Díaz, I., Pérez, S. I., Ferrero, E. M. & Fdz-Polanco, M. 2010b Effect of oxygen dosing point and mixing on the microaerobic removal of hydrogen sulfide in sludge digesters. *Bioresource Technology* **102** (4), 3768–3775.

Fdz-Polanco, M., Díaz, I., Pérez, S. I., Lopes, A. C. & Fdz-Polanco, F. 2009 Hydrogen sulfide removal in the anaerobic digestion of sludge by micro-aerobic processes: pilot plant experience. *Water Science and Technology* **60** (12), 3045–3050.

Hulshoff Pol, L. W., Lens, P. N. L., Stams, A. J. M. & Lettinga, G. 1998 Anaerobic treatment of sulfate-rich wastewaters. *Biodegradation* **9** (3–4), 213–224.

International Energy Agency 2009 *World Energy Outlook*. IEA Publications. Paris. Available from: http://www.iea.org/textbase/nppdf/free/2009/weo2009.pdf (accessed 11 April 2011).

Jagadabhi, P. S., Kaparaju, P. & Rintala, J. 2010 Effect of micro-aeration and leachate replacement on COD solubilisation and VFA production during mono-digestion of grass-silage in one-stage leach-bed reactors. *Bioresource Technology* **101** (8), 2818–2824.

Jenicek, P., Keclik, F., Maca, J. & Bindzar, J. 2008 Use of microaerobic conditions for the improvement of anaerobic digestion of solid wastes. *Water Science and Technology* **58** (7), 1491–1496.

Jenicek, P., Koubova, J., Bindzar, J. & Zabranska, J. 2010 Advantages of anaerobic digestion of sludge in microaerobic conditions. *Water Science and Technology* **62** (2), 427–434.

Jenicek, P., Celis, C. A., Koubova, J. & Pokorna, D. 2011 Comparison of microbial activity in anaerobic and microaerobic digesters. *Water Science and Technology* **63** (10), 2244–2249.

Johansen, J. E. & Bakke, R. 2006 Enhancing hydrolysis with microaeration. *Water Science and Technology* **53** (8), 43–50.

Khanal, S. K. & Huang, J. C. 2003 ORP-based oxygenation for sulfide control in anaerobic treatment of high sulfate wastewater. *Water Research* **37** (9), 2053–2062.

Kleinjan, W. 2005 Biologically produced sulfur particles and polysulfide ions: effects on a biotechnological process for the removal of hydrogen sulfide from gas streams. PhD Thesis. Wageningen Universiteit, Wageningen, The Netherlands.

Kobayashi, T., Li, Y. Y., Kubota, K., Harada, H., Maeda, T. & Yu, H. -Q. 2012 Characterization of sulfide-oxidizing microbial mats developed inside a full-scale anaerobic digester employing biological desulfurization. *Applied Microbiology and Biotechnology* **93** (2), 847–857.

van der Zee, F. P., Villaverde, S., García, P. A. & Fdz-Polanco, F. 2007 Sulphide removal by moderate oxygenation of anaerobic sludge environments. *Bioresource Technology* **98** (3), 518–524.

Weiland, P. 2010 Biogas production: current state and perspectives. *Applied Microbiology and Biotechnology* **85** (4), 849–860.

Zhou, W., Imai, T., Ukita, M., Li, F. & Yuasa, A. 2007 Effect of limited aeration on the anaerobic treatment of evaporator condensate from a sulfite pulp mill. *Chemosphere* **66** (5), 924–929.

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# 5 Where does the removal of $H_2S$ from biogas occur in microaerobic reactors?

# Where does the removal of $H_2S$ from biogas occur in microaerobic reactors?


## Abstract

In order to maximise the efficiency of biogas desulphurisation and minimise the oxygenation costs during microaerobic digestion, it is essential to know where the process predominantly occurs. For this purpose, a reactor with a total volume of 266L, treating around 10L/d of sewage sludge, was operated with 25.0L and virtually without headspace. Under anaerobic conditions, the $H_2S$ concentration in the biogas varied between 0.21 and 0.38%v/v. Next, $O_2$ was supplied from the bottom of the reactor. At 0.25-0.30N$LO_2/L_{fed}$, the biogas was entirely desulphurised, and its $O_2$ content remained below 1.03%v/v, when the digester had 25.0L of gas space. However, with almost no headspace, the $H_2S$ content in the biogas fluctuated from 0.08 to 0.21%v/v, while the average $O_2$ concentration was 1.66%v/v. The $H_2S$ removed accumulated in the outlet pipe of the biogas in the form of $S^0$ due to the insufficient headspace.

## 1. Introduction

Anaerobic digestion is a well-established technology that transforms a large part of the organic matter content of many wastes into a renewable energy source: biogas. It is utilised for heat, steam, electricity, cooling, chemical and protein production, as fuel for vehicles and fuel cells, and for injection into natural gas grids (Holm-Nielsen et al., 2009). Though substantially inferior to other common fuels such as compressed natural gas, which produces approximately 8,600 kcal per $m^3$, it has a good calorific value (around 5,000kcal/$m^3$) (Abbasi et al., 2012).

Biogas is a mixture of gases whose composition depends on the type of material to be digested, as well as on the operational conditions in the reactor (Noyola et al., 2006). It is generally composed of $CH_4$ and $CO_2$ in a ratio of 3:1, and other minor constituents; among them, $H_2S$ is of particular interest due to its corrosive, toxic and environmentally hazardous properties. In fact, along with $CH_4$, whose concentration in the biogas determines the particular calorific value therein, it has the greatest impact when the traditional applications of biogas are considered (Rasi et al., 2011). The biogas sulphide content can vary from 0.01 to 1.00%v/v (Tippayawong and Thanompongchart, 2010). However, as an example, for trouble free operation of combined heat and power stations, the $H_2S$ concentration in the biogas must be lower than 0.01 or 0.03%v/v, depending on the equipment concerned (Peu et al., 2012). Besides causing corrosion, $H_2S$ also causes the deterioration of the lubrication oil (Weiland, 2010). Consequently, $H_2S$ production must be prevented, or $H_2S$ must be removed from the biogas.

Due to the high technicality and costs of sulphide emission prevention by adding selective inhibitors of sulphidogenic bacteria or sulphide scavengers to precipitate sulphide directly to the digester, $H_2S$ removal from biogas is the most consolidated strategy in practice (Cirne et al., 2008; Peu et al., 2012). For this purpose, a wide range of physical, chemical and biological methods exist. The first two categories include techniques based on absorption and adsorption processes (reactive or non-reactive), while technologies using microorganisms capable of oxidising sulphide (such as bioscrubbers, biofilters and biotrickling filters) belong to the third category (Abatzoglou and Boivin, 2009). Though rapid and effective, the physical and chemical methods for $H_2S$ removal are costly and produce secondary wastes, which in turn gives rise to another pollution problem (Lin et al., 2013). The biological processes have the potential to overcome these disadvantages. Besides, they can achieve greater depth of desulphurisation (Kobayashi et al., 2012) and generate by-products ($S^0$) that can be used in other industrial processes (Kleinjan, 2005). In fact, chemical and biological processes are usually combined. In the system proposed by Ho et al. (2013), the $H_2S$ is first oxidised by ferric iron to generate $S^0$ in a chemical reactor, and the resulting ferrous iron is then oxidised in a biological reactor by iron-oxidising bacteria. Likewise, the only two patented technologies specifically developed for $H_2S$ removal from biogas consist of a chemical scrubber, in which the $H_2S$ is washed from the biogas, and a bioreactor, where the dissolved sulphide is utilised by sulphide-oxidising bacteria (SOB) (Fortuny et al., 2008). At this point, it should be mentioned that $H_2S$ can also be chemically oxidised in biological reactors, especially if the $H_2S$ load is high, and in this case $S_2O_3^{2-}$ is the main by-product (Lohwacharin and Annachhatre, 2010).

The direct injection of $O_2$ or air into anaerobic reactors was proposed in order to carry out both the production and desulphurisation of biogas in a single-unit; SOB are naturally present therein (Weiland, 2010). In fact, this process has been reported to proceed mainly through biological

reactions (Ramos et al., 2012). Under fully oxygenated conditions, SOB generate $SO_4^{2-}$, whereas under $O_2$-limiting conditions, they oxidise sulphide to $S^0$ (van der Zee et al., 2007). Evidently, both reactants, $O_2$ or air, are supplied in limited amounts in order to minimise both the surplus of $O_2$ and the presence of $N_2$ in the biogas leaving the digester, and the operating costs. It must be noted that $O_2$ transfer has been suggested to be the limiting step during $H_2S$ removal from biogas in these reactors, which are usually referred to as microaerobic reactors (Fdz-Polanco et al., 2009). Therefore, the use of $O_2$ is recommended instead of air (Díaz et al., 2010a); thus, additional dilution by $N_2$ is avoided (Jenicek et al., 2010; Díaz et al., 2010a). As a result, $S^0$ is the main by-product of $H_2S$ oxidation during microaerobic digestion.

Neither the digestion performance nor the productivity or the $CH_4$ content of biogas are significantly reduced under microaerobic conditions (Fdz-Polanco et al., 2009); they can even be increased (Jenicek et al., 2008). In fact, the introduction of limited amounts of $O_2$ is a general practice in agricultural reactors; an air flow rate of 2-6%v/v of the biogas production is introduced in the headspace (HS) or, occasionally, in the feed stream. As a result, $S^0$ has been reported to accumulate on the different surfaces in the gas space, or to leave the digester with the effluent, respectively (Cirne et al., 2008). Similarly, Kobayashi et al. (2012) found that the $S^0$ generated as a result of $O_2$ injection into the HS of a dairy cow manure digester and the consequent $H_2S$ oxidation, was deposited all over the HS. Likewise, Jenicek et al. (2011) indicated that the $H_2S$ conversion into $S^0$ when air was supplied to the recirculation stream of a reactor treating waste activated sludge occurred the liquid phase; the increase in the digestate S content was consistent with the efficiency of the biogas desulphurisation. However, Rodríguez et al. (2012) reported that the $S^0$ produced during the microaerobic digestion of synthetic vinasse was deposited in the HS despite the $O_2$ being introduced from the bottom of the system; this compound was indeed the main by-product of the $H_2S$ oxidation. Besides, they found SOB only in the gas space. These contradictory results certainly point to $O_2$ transfer limitations; Rodríguez et al. (2012) detected a considerable part of the $O_2$ injected into the reactor in the biogas, which still contained significant amounts of $H_2S$. Nevertheless, Díaz et al. (2010b) reported that increasing the $O_2$ transfer to the liquid phase of a sewage sludge digester did not lead to a higher efficiency of biogas desulphurisation, while the $O_2$ consumption in other oxidative processes rose. This was indeed consistent with the previous findings (Fdz-Polanco et al., 2009). Moreover, they also found SOB only in the HS. As a result, Díaz et al. (2010b) indicated that the process of biogas desulphurisation took place in the HS independently of both the $O_2$ dosing point and the mixing method. Accordingly, the optimum configuration of a microaerobic reactor aiming for biogas desulphurisation consists of $O_2$ or air injection into the HS and liquid recirculation.

Considering the inconsistent results concerning the predominant location for $H_2S$ removal from the biogas during microaerobic digestion, Ramos et al. (2012) designed an experiment which aimed to clarify this question. Although their results indicate that the process takes place predominantly in the gas space, they are not conclusive due to the short duration of the experimentation. The research presented here extends the results obtained in that preliminary study, and proposes the definitive principles of the process of biogas desulphurisation accordingly.

## 2. Materials and methods

### *2.1. Digester*

Digestion was carried out in a continuous stirred tank reactor with 266L of total volume. As shown in Fig. 1, it consisted of a conical ceiling with a transparent cylindrical piece on top. Before this study, the digester operated during several months under microaerobic conditions and HRT of 20d. The present research was conducted at 22 and 24d of hydraulic retention time (HRT) (Fig. 1a and c, and b, respectively), depending on the liquid level inside the digester, or equivalently, the presence (25.0L) or the absence (lower than 0.3L) of HS, respectively, while the feeding rate was maintained constant. The reactor volume was increased with digestate thereof. Mixed sludge from a municipal wastewater treatment plant was continuously fed to the bioreactor; its composition varied widely during the research (Table 1). The digestion temperature (35ºC) was maintained by an electric resistor surrounding the walls of the digester, which were in turn insulated. The ceiling was also covered by the insulator. Microaerobic conditions were implemented by supplying pure $O_2$ from the bottom of the system, just where the streams sludge recirculation and feeding converged. The recirculation flowed at a rate of approximately 50L/h. As shown in Fig. 1b, the level of the outflow valve of the recirculation stream was raised when the HS volume was reduced in order to ensure mixing in the upper part of the liquid phase.

**Table 1**. Sequence of variations applied, and response of the digester.

| Period[a] | HS volume (L) | HRT (d) | OLR ($kg_{VS}/m^3/d$) | $O_2$ supply ($NL/L_{fed}$) | Biogas production (NL/d) | $H_2S$ (%v/v) | $O_2$ (%v/v) |
|---|---|---|---|---|---|---|---|
| A1 | 25.0 | 22 | 0.8 | 0 | 77.3 | 0.31 | 0.03 |
| M1 | 25.0 | 22 | 0.9 | 0.21 | 95.3 | 0.03 | 0.86 |
| M2 | 0.3 | 24 | 1.0 | 0.25 | - | 0.14 | 1.83 |
| M3 | 25.0 | 22 | 1.4 | 0.28 | - | 0.00 | 0.59 |
| M4 | 0.3 | 24 | 1.0 | 0.30 | - | 0.13 | 1.41 |
| A2 | 25.0 | 22 | 0.8 | 0 | 89.4 | 0.24 | 0.03 |

[a] A=anaerobic, M=microaerobic

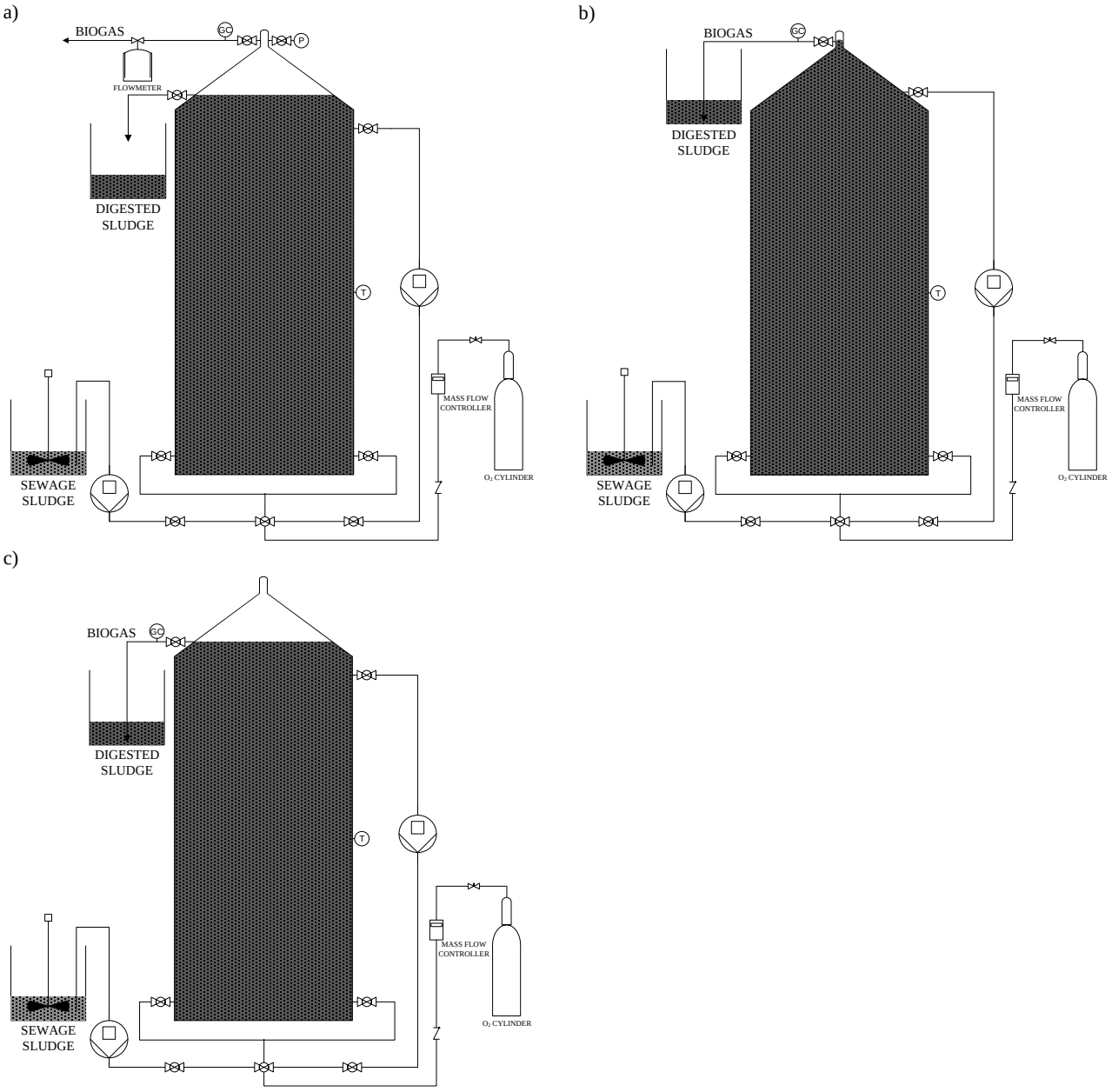


**Fig. 1**. Digester diagram in period A1, M1 and A2 (a), M2 and M4 (b), and MA3 (c).

### *2.2. Monitoring and experimental analysis*

Digestion pressure was monitored by a sensor (Fig. 1a). Temperature was measured by a PT100 probe (Fig. 1a, b and c). Biogas production was quantified volumetrically (Fig. 1a). The $CH_4$, $CO_2$, $N_2$, $O_2$ and $H_2S$ content of biogas was determined by gas chromatography (VARIAN CP-3800 GC) according to Díaz et al. (2010a); a 100µL-syringe was used to sample the biogas.

Total and soluble chemical oxygen demand, total solids, volatile solids (VS), volatile fatty acids, total kjeldahl nitrogen, ammonia, $S_2O_3^{2-}$ and $SO_4^{2-}$ were measured. Except for $S_2O_3^{2-}$, which was determined according to the procedure described by van der Zee et al. (2007), the rest of the parameters were analysed according to APHA (1998).

*2.3. Experimental procedure*

This research was divided into six periods, according to the HS volume of the digester (Table 1). In A1 and A2, the reactor operated under anaerobic conditions, and from M1 to M4, it operated under microaerobic conditions. The digester configuration in A1, M1 and A2 is shown in Fig. 1a; in these periods, the biogas production was quantified. Fig. 1b shows the reactor configuration maintained during M2 and M4, and Fig. 1c illustrates the reactor configuration in M3.

## 3. Results and discussion

*3.1. Experimental results*

*3.1.1. Period A1*

During the first 12 days of the research (period A1), the bioreactor operated under anaerobic conditions, and with 25.0L of HS (HRT=22d) (Fig. 1a). The $H_2S$ concentration in the biogas varied widely (Fig. 2), which was attributed to changes in the relative composition of the sewage sludge in terms of S-containing organic compounds, such as proteins, and/or variations in the feeding sulphide content. The concentration of S-containing anions ($SO_4^{2-}$ and $S_2O_3^{2-}$) in the raw sludge was negligible (data not shown), while the organic loading rate (OLR) remained almost constant ($0.8 kg_{VS}/m^3/d$), which in turn resulted in fairly stable biogas production (Table 1). Just before implementing microaerobic conditions, the biogas sulphide content was 0.36%v/v; this value was considered the baseline for the subsequent calculations.

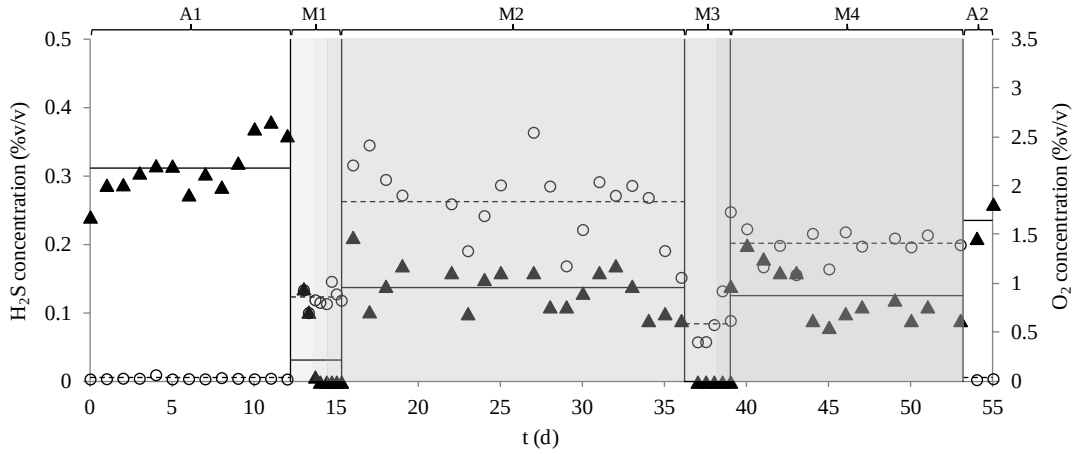


**Fig. 2**. $H_2S$ (▲) and $O_2$ (○) concentration in biogas. The intensity of the shaped area indicates the micro-oxygenation level: =0.18, = 0.23, = 0.25, = 0.30 (in $NL/L_{fed}$). The vertical lines indicate the average $H_2S$ (continuous lines) and $O_2$ concentration (discontinuous lines) in every period.

*3.1.2. Period M1*

At day 12 (period M1), $O_2$ was supplied to the reactor at a rate of $0.18 NL/L_{fed}$, equalling a ratio of $O_{2injected}/H_2S_{produced}$ of 5.4 (v/v) (Fig. 1a). This was set based on the previous study (Ramos et al., 2012), and considering the current gaseous sulphide flow rate. On the following day, the $H_2S$ removal efficiency was only 66%, and the $O_2$ concentration in the biogas was around 0.82%v/v. This

highlighted the inefficient $O_2$ transfer conditions in the digester, since approximately 48% of the $O_2$ supplied remained unused in the biogas.

On the 13$^{th}$ day, the $O_2$ flow rate was raised to 0.23NL/$L_{fed}$ in order to achieve $H_2S$-free biogas. Thus, the $O_{2injected}/H_2S_{produced}$ ratio rose to approximately 6.5 (v/v), which resulted in a higher gradient concentration across the gas-liquid interface, thereby increasing the $O_2$ transfer rate. The amount of $O_2$ leaving the digester as a percentage of the total $O_2$ supply specifically decreased to an average of 36%. As a result, although the biogas production rose slightly due to the somewhat higher OLR (0.9kg$_{VS}$/m$^3$/d), the biogas was entirely desulphurised, and its $O_2$ content hardly changed (Fig. 2).

Taking into account the significant fluctuations in the $H_2S$ production during A1, the $O_2$ supply was increased further on the 14$^{th}$ day (just before raising the liquid level) in order to ensure a removal efficiency of 100% from then on (Fig. 2). Moreover, although the reactor's content would be increased only by 9% (from 241 to almost 266L) when the liquid level was raised, it was considered that the amount of $O_2$ available per unit of volume of sludge would decrease slightly. On the other hand, increasing the reactor's content extended the contact time between the biogas and the liquid phase, which could slightly improve the $O_2$ transfer to the liquid media. At a micro-oxygenation rate of 0.25NL$O_2$/$L_{fed}$, the $O_2$ content in the biogas rose slightly due to the biogas production being somewhat lower; however, the additional $O_2$ supplied was consumed in the bioreactor (this being approximately 70% of the $O_2$ injected).

As indicated, the amount of $O_2$ consumed in the digester during M1 rose as the micro-oxygenation level was raised; it was specifically estimated to be about 62% of the $O_2$ injected, on average. Thus, the ratio of accessible $O_2$ to $H_2S$ produced was increased from approximately 2.2 to 5.9. At such high $O_2$ availabilities, $H_2S$ could be converted into $SO_4^{2-}$, $S_2O_3^{2-}$ and $S^0$. However, the $SO_4^{2-}$ and $S_2O_3^{2-}$ concentrations in the digestate were both negligible (data not shown). Moreover, although the absence of $S^0$ in the liquid phase was not analytically ruled out, it is worth noting that $S^0$ was not observed when the reactor's content was sampled This pointed to the removal of $H_2S$ from the biogas in the HS; in this location, these by-products probably accumulated on the areas nearest the liquid phase due to the greater availability of moisture and nutrients. Kobayashi et al. (2012) reported that these were the key factors controlling the activity levels of SOB. Although many researchers have reported the presence of $S^0$ all over the gas space (Díaz et al., 2010b; Kobayashi et al., 2012; Ramos et al., 2012; Rodríguez et al., 2012), it could be expected that the $H_2S$ removal from the biogas in early stages of the process might occur in the areas nearest the liquid phase, where the growing conditions for SOB are more favorable. In fact, although its characteristic yellowish-white colour enabled the $S^0$ to be visually recognisable, it was not deposited in the cylindrical piece on top of the digester (Fig. 1a).

### *3.1.3. Period M2*

On the 15$^{th}$ day, the liquid level in the reactor was raised in order to virtually eliminate the gas space (period M2), while the $O_2$ flow rate was maintained at 0.25NL/$L_{fed}$ (Table 1). Thus, the HRT was increased to 24d; and here it should be emphasised that the feeding rate was not changed. Under such conditions, both the effluent and the biogas left the reactor by the same pipe, and the digestate

overflowed from the digester 60mm below the uppermost point of the reactor (Fig. 1b). Consequently, sludge was deposited all over the cylindrical piece of the HS due to splashes, which made it impossible to see what happened in that area thereafter. For this reason, the HS was estimated to be 0.3L at most. In order to keep the reactor under pressure, a liquid column was always maintained in the effluent collection tank.

On day 16, the biogas composition was almost equal to that in M1, which was attributed to the existence of sulphide-oxidising activity in the outlet pipe of the biogas and the digestate due to the relatively large amount of $S^0$ accumulated therein over the last day (Fig. 1b). This compound was attached at both shores of the digestate stream, in addition to some $S^0$ in the digestate inside the effluent collection tank. However, it was not observed when the reactor's content was sampled, which suggested that it formed in the pipe and was dragged by the effluent stream; in fact, it is possible that some $H_2S$ could be converted into $S^0$ inside the effluent tank. Therefore, in order to obtain a sample which was as representative as possible of the biogas leaving the HS of the digester, the outlet pipe was cleaned at pressure in order to remove the biomass attached until then, and once the air was displaced from the pipe (around 20min after cleaning), the biogas was sampled again. As a result, the $H_2S$ and $O_2$ content of the biogas both increased significantly in relation to M1 (Fig. 2); they were 0.21 and 2.22%v/v, respectively. The large increase in the $O_2$ concentration suggested that the $H_2S$ was oxidised mainly to $SO_4^{2-}$ during the preceding period.

The sampling procedure described above was applied daily until the 53$^{rd}$ day. $H_2S$ concentrations of up to 0.10%v/v were recorded in M2 (Fig. 2). However, it was proven that the more time that elapsed since the outlet pipe was cleaned, the lower the concentration of $H_2S$. The highly favourable growing conditions for SOB in the outlet pipe were considered to be the key factor for the rapidity of the sulphide oxidation; it must be considered that fresh digested sludge flowed continuously therein (Fig. 1b). Although the habitat for SOB in the 0.3L-HS was also highly favourable, presumably negligible amounts of $H_2S$ were removed there; $S^0$ was not observed in the outlet valve of the biogas and digestate when the pipe was removed for cleaning. In fact, if this compound had formed in the 0.3L-HS, clogging problems would probably have arisen. Besides, the biogas residence time (BRT) in the HS was even lower than in the pipe, whose volume was approximately 0.7L.

Considering the OLR (Table 1), the BRT in the outlet pipe during M2 was estimated to be at most 12min, which could certainly limit the transfer of $O_2$, thereby preventing $H_2S$ conversion into $SO_4^{2-}$ and promoting $S^0$ formation. The large amounts of this compound deposited from day to day thus indicated that $S^0$ was indeed the only by-product of the sulphide-oxidising activity. By contrast, the BRT maintained in M1 (approximately 6h) could certainly suffice to provide the different surfaces of the HS with the $O_2$ required for further oxidation of $H_2S$.

The correlation between the profile of the $H_2S$ and the $O_2$ concentration in biogas in M2 was high; in general, the higher the $O_2$ concentration, the lower the biogas sulphide content, which suggested higher consumption of $O_2$ due to biogas desulphurisation (Fig. 2). Furthermore, both profiles varied widely, and this was attributed to fluctuations in biogas production and $H_2S$ concentration; these variables determined both transfer and demand of $O_2$, respectively. Specifically, the higher the biogas flow rate was, the shorter the BRT, and the higher the turbulence in the digester. Moreover, the lower the $H_2S$ flow rate, the lower the demand of $O_2$, and the higher the efficiency of biogas

desulphurisation. As in A1, although the content of S-containing anions ($SO_4^{2-}$ and $S_2O_3^{2-}$) of the feeding was negligible, the $H_2S$ concentration could oscillate during M2. Moreover, since the OLR increased up to 1.2$kg_{VS}/m^3/d$ from approximately the 25$^{th}$ day, presumably the biogas production increased significantly; the OLR remained stable around 0.8$kg_{VS}/m^3/d$ until that day (Table 1).

As noted, $S^0$ was not observed in the samples retrieved directly from the reactor. Additionally, neither $SO_4^{2-}$ nor $S_2O_3^{2-}$ were detected in significant amounts. Therefore, and considering the above observations, it was concluded that the $O_2$ utilised in $H_2S$ oxidation during M1 left the HS unused with the $H_2S$-laden biogas during M2 due to the gas space being insufficient for biogas desulphurisation. Next, the pipe functioned as an external HS, a concept that has indeed already been exploited (Ramos et al., 2013). $H_2S$ probably dissolved all over the outlet pipe; besides sludge, presumably water was deposited through the condensation of moisture contained in the biogas. However, as noted, it was oxidised on the areas surrounding the effluent stream, namely, on both the sludge remaining attached in the pipe, and the wet areas due to condensation which were occasionally reached by sludge droplets. This was related to the higher availability of both water and nutrients for SOB, and also the presence of catalysts (metal ions), which could in turn promote the abiotic $H_2S$ oxidation (Kleinjan, 2005).

### *3.1.4. Period M3*

The liquid level was lowered on the 36$^{th}$ day in order to increase the HS volume to 25.0L again (period M3). Importantly, the configuration of the reactor was not modified in order to sample biogas under identical conditions and by using the same procedure (Fig. 1c). Although the $O_2$ flow rate was maintained at 0.25$NL/L_{fed}$, the biogas was entirely desulphurised, and the biogas $O_2$ content decreased to around 0.56%v/v (Fig. 2), which was consistent with the previous results; it suggested that $SO_4^{2-}$ was the main by-product of the $H_2S$ oxidation during M3. In contrast to M2, $S^0$ was not deposited in the outlet pipe. Nevertheless, this compound was not observed in the samples taken from the reactor, which either had significant concentrations of $SO_4^{2-}$ or $S_2O_3^{2-}$.

Although presumably the biogas production in M3 was significantly higher than in the preceding periods due to the larger OLR, which could certainly result in a higher $H_2S$ flow rate to remove, the $O_2$ supply was still sufficient to achieve $H_2S$-free biogas (Table 1). An increased $H_2S$ production could indeed justify the lower $O_2$ concentration maintained in M3, in comparison with the last days of M1 (days 14 and 15), when the digester operated at the same micro-oxygenation level (Fig. 2). Nonetheless, the presumably larger biogas production could improve the $O_2$ transfer to the liquid phase, which could also explain the lower biogas $O_2$ content.

As in M1, the $O_2$ flow rate was raised further from the 38$^{th}$ day (just before raising the liquid level) in order to ensure $H_2S$-free biogas thenceforth; the micro-oxygenation level was set at 0.30$NL/L_{fed}$. The biogas sulphide content remained at 0, and a slight increase in the $O_2$ concentration was detected; however, considering that the OLR rose from approximately 1.3 to 1.5$kg_{VS}/m^3/d$ during M3, and the results obtained in M1 (see above), at least a part of the additional $O_2$ injected into the bioreactor could be consumed therein. The larger amount of $O_2$ available in the liquid phase was expected to encourage biogas desulphurisation there. As noted, although a considerable percentage of the $O_2$ supplied was consumed in the liquid phase until then, it did not seem to be utilised in this

process; it must be considered that many facultative microorganisms grow in the sludge along with SOB.

### *3.1.5. Period M4*

On the 39$^{th}$ day, the HS was virtually eliminated by raising the liquid level in the digester again (period M4). The biogas composition was determined just before and immediately after that; its content of both $H_2S$ and $O_2$ rose, from 0 and 0.63%v/v to 0.14 and 1.74%v/v, respectively (Fig. 2). Moreover, $S^0$ was seen once again to accumulate in the outlet pipe of the biogas and the digestate (Fig. 1b). In fact, the average $H_2S$ and $O_2$ concentration in biogas during M4 were 0.13 and 1.41%v/v, respectively. In the meantime, as in M2, increasing amounts of $S^0$ were deposited from day to day, and this compound was not observed either in the HS, when the pipe was removed for cleaning, or in the sludge retrieved from the reactor. Furthermore, the $SO_4^{2-}$ and $S_2O_3^{2-}$ concentrations in the digester were both negligible. Assuming that the amount of $H_2S$ produced in A2 was similar to that produced in M4, at least from the 44$^{th}$ day, when the OLR remained almost constant, it was estimated that around 121mg of $S^0$ accumulated daily in the pipe (Table 1). At this point, it is worth mentioning that the significant decrease in the $H_2S$ concentration in biogas occurring on the 44$^{th}$ day was related to a change in the OLR, which specifically dropped from 1.4 to 0.8$kg_{VS}/m^3/d$ (Table 1). Therefore, $O_2$ transfer to the liquid phase was proved unnecessary in order to desulphurise biogas due to the fact that the process did not take place there, but in the gas space.

### *3.1.6. Period A2*

In A2, the digester operated with 25.0L of HS, and under anaerobic conditions and approximately 0.8$kg_{VS}/m^3/d$ of OLR (Table 1). The biogas sulphide content was approximately 0.24%v/v (Fig. 2).

## *3.2. Principles of biogas desulphurisation*

The $O_2$ supplied from the bottom of the reactor dissolved only partially in the liquid phase due to $O_2$ transfer limitations. Although the resulting $O_2$ availability was sufficient for $H_2S$ oxidation, the habitat was more favourable for other microorganisms instead of SOB, such as perhaps facultative acidogens, for whom $O_2$ has been reported to increase the yields (Botheju and Bakke, 2011). Hence, the unidentified oxidising microorganisms consumed all the dissolved $O_2$ more rapidly than SOB due to their higher activity levels. Additionally, assuming that biogas was also desulphurised by chemical mechanisms, the reaction rates of the abiotic $H_2S$ oxidation should be also lower than the yields of those facultative microorganisms.

Next, the $H_2S$-laden biogas reached the HS along with the $O_2$ not dissolved in the liquid phase. The gas space (the lower part of the walls, mostly) was covered with sludge due to splashes and even occasional momentary rises in the liquid level. The upper area (that is, the conical ceiling) could also contain some moisture from the condensation of water contained in the biogas, due to it being not surrounded with electric resistor. As a result, $H_2S$ and $O_2$ both dissolved on different surfaces of the HS, thereby enabling SOB to develop. Since that environment was more stringent than that existing in the liquid phase due to much more limited availability of nutrients and organic substrates, the activity rates of other $O_2$-utilising microorganisms, such as acidogenic bacteria, was limited. Some SOB seem to have relatively low nutrients requirements indeed (Ramos et al., 2013). As a result,

$H_2S$ was oxidised, preferentially on the area nearest the sludge due to the higher accessibility of water and nutrients in this area, and maybe also the increased availability of the catalyst for chemical oxidation. Finally, the biogas laden with the $O_2$ unused in either the $H_2S$ oxidation or in other oxidative processes, left the reactor.

## 4. Conclusions

A 266L-reactor was operated with and virtually without HS in order to investigate where the process of biogas desulphurisation predominantly took place when $O_2$ was injected into the liquid phase. $H_2S$ was entirely removed from the biogas when the digester had 25.0L of HS. However, at equal $O_2$ supplies, and with almost no HS, the $H_2S$ concentration approached anaerobic values, and the biogas $O_2$ content doubled. Moreover, the $H_2S$ removed under such conditions was not oxidised inside the reactor due to insufficient HS, but was deposited in the form of $S^0$ in the outlet pipe of the biogas.

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## References

1. Abatzoglou, N., Boivin, S., 2009. A review of gas purification processes. Biofuels Bioprod. Biorefin. 3, 42-71.

2. APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association/American Water Works Association/Water Environmental Federation, Washington, DC, USA.

3. Abbasi, T., Tauseef, S.M., Abbasi, S.A., 2012. Anaerobic digestion for global warming control and energy generation-An overview. Renew. Sustain. Energy Rev. 16, 3228-3242.

4. Botheju, D., Bakke, R., 2011. Oxygen effects in anaerobic digestion - A review. Open Waste Manag. J. 4, 1-19.

5. Cirne, D. G., van der Zee, F. P., Fdz-Polanco, M., Fdz-Polanco, F., 2008. Control of sulphide during anaerobic treatment of S-containing wastewaters by adding limited amounts of $O_2$ or nitrate. Rev. Environ. Sci. Biotechnol. 7, 93-105.

6. Díaz, I., Pérez, S. I., Ferrero, E., Fdz-Polanco, M., 2010a. Performance evaluation of oxygen, air and nitrate for the microaerobic removal of hydrogen sulphide in biogas from sludge digestion. Bioresour. Technol. 101, 7724-7730.

7. Díaz, I., Pérez, S.I., Ferrero, E.M., Fdz-Polanco, M., 2010b. Effect of oxygen dosing point and mixing on the microaerobic removal of hydrogen sulphide in sludge digesters. Bioresour. Technol. 102, 3768-3775.

8. Fdz-Polanco, M., Díaz, I., Pérez, S. I., Lopes, A. C., Fdz-Polanco, F., 2009. Hydrogen sulfide removal in the anaerobic digestion of sludge by micro-aerobic processes: pilot plant experience. Water Sci. Technol. 60, 3045-3050.

9. Fortuny, M., Baeza, J.A., Gamisans, X., Casas, C., Lafuente, J., Deshusses, M.A., Gabriel, D., 2008. Biological sweetening of energy gases mimics in biotrickling filters. Chemosphere 71, 10-17.

10. Ho, K.-L., Ho, K.-L., Lin, W.-C., Chung, Y.-C., Chen, Y.-P., Tseng, C.-P., 2013. Elimination of high concentration hydrogen sulfide and biogas purification by chemical-biological process. Chemosphere 92, 1396-1401.

11. Holm-Nielsen, J. B., Al Seadi, T., Oleskowicz-Popiel, P., 2009. The future of anaerobic digestion and biogas utilization. Bioresour. Technol. 100, 5478-5484.

12. Jenicek, P., Keclik, F., Maca, J. & Bindzar, J., 2008. Use of microaerobic conditions for the improvement of anaerobic digestion of solid wastes. Water Sci. Technol. 58, 1491-1496.

13. Jenicek, P., Koubova, J., Bindzar, J., Zabranska, J., 2010. Advantages of anaerobic digestion of sludge in microaerobic conditions. Water Sci. Technol. 62, 427-434.

14. Jenicek, P., Celis, C.A., Koubova, J., Pokorna, D., 2011. Comparison of microbial activity in anaerobic and microaerobic digesters. Water Sci. Technol. 63, 2244-2249.

15. Kleinjan, W., 2005. Biologically Produced Sulfur Particles and Polysulfide Ions: Effects on a Biotechnological Process for the Removal of Hydrogen Sulfide from Gas Streams. PhD Thesis. Wageningen University, Wageningen, The Netherlands.

16. Kobayashi, T., Li, Y.Y., Kubot, A.K., Harada, H., Maeda, T., Yu, H.-Q., 2012. Characterization of sulfide-oxidizing microbial mats developed inside a full-scale anaerobic digester employing biological desulfurization. Appl. Microbiol. Biotechnol. 93, 847–857.

17. Lin, W.-C., Chen, Y.-P., Tseng, C.-P., 2013. Pilot-scale chemical-biological system for efficient $H_2S$ removal from biogas. Bioresour. Technol. 135, 283-291.

18. Lohwacharin, J., Annachhatre, A. P., 2010. Biological sulfide oxidation in an airlift bioreactor. Bioresour. Technol. 101, 2114-2120.

19. Noyola, A., Morgan-Sagastume, J. M., López-Hernández, J. E., 2006. Treatment of biogas produced in anaerobic reactors for domestic wastewater: odor control and energy/resource recovery. Rev. Environ. Sci. Biotechnol. 5, 93-114.

20. Peu, P., Picard, S., Diara, A., Girault, R., Béline, F., Bridoux, G., Dabert, P., 2012. Prediction of hydrogen sulphide production during anaerobic digestion of organic substrates. Bioresour. Techonol. 121, 419-424.

21. Ramos, I., Díaz, I., Fdz-Polanco, M., 2012. The role of the headspace in hydrogen sulfide removal during microaerobic digestion of sludge. Water Sci. Technol. 66, 2258-2264.

22. Ramos, I., Pérez, R., Fdz-Polanco, M., 2013. Microaerobic desulphurisation unit: A new biological system for the removal of H2S from biogas. Bioresour. Techonol. 142, 633-640.

23. Rasi, S., Läntelä, J., Rintala, J., 2011. Trace compounds affecting biogas energy utilisation – A review. Energy Convers. Manag. 52, 3369-3375.

24. Rodríguez, E., Lopes, A., Fdz-Polanco, M., Stams, A. J., García Encina, P. A., 2012. Molecular analysis of the biomass of a fluidized bed reactor treating synthetic vinasse at anaerobic and micro-aerobic conditions. Appl. Microbiol. Biotechnol. 93, 2181-2191.

25. Tippayawong, N., Thanompongchart, P., 2010. Biogas quality upgrade by simultaneous removal of $CO_2$ and $H_2S$ in a packed column reactor. Energy 35, 4531-4535.

26. van der Zee, F.P., Villaverde, S., García-Encina, P.A., Fdz-Polanco, F., 2007. Sulphide removal by moderate oxygenation of anaerobic sludge environments. Bioresour. Technol. 98, 518-524.

27. Weiland, P., 2010. Biogas production: current state and perspectives. Appl. Microbiol. Biotechnol. 85, 849-860.

# Microaerobic digestion of sewage sludge on an industrial-pilot scale: the efficiency of biogas desulphurisation under different configurations and the impact of $O_2$ on the microbial communities

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# Microaerobic digestion of sewage sludge on an industrial-pilot scale: the efficiency of biogas desulphurisation under different configurations and the impact of $O_2$ on the microbial communities


## Abstract

Biogas produced in an industrial-pilot scale sewage sludge reactor ($5m^3$) was desulphurised by imposing microaerobic conditions. The $H_2S$ removal efficiency was evaluated under various configurations: different mixing methods and $O_2$ injection points. Biogas was entirely desulphurised under all the configurations set, while the $O_2$ demand of the digester decreased over time. Although the $H_2S$ removal seemed to occur in the headspace, $S^0$ (which was found to be the main oxidation product) was scarcely deposited there. $O_2$ did not have a significant impact on the digestion performance; the VS removal remained around 47%. Conversely, DGGE revealed that the higher $O_2$ transfer rate to the sludge maintained by biogas recirculation increased the microbial richness and evenness, and caused an important shift in the structure of the bacterial and the archaeal communities in the long term. All the archaeal genera identified (*Methanosaeta*, *Methanospirillum* and *Methanoculleus*) were present under both anaerobic and microaerobic conditions.

## 1. Introduction

Anaerobic digestion (AD) is one of the oldest and most widely used processes for wastewater sludge stabilisation. It successfully reduces the quantity of solids, destroys pathogenic organisms, minimises odour problems, and produces an agricultural fertiliser, the digestate, and a renewable and versatile energy source, the biogas (Ward et al., 2008). Thus, AD optimises the costs of wastewater treatment plants (WWTPs) (Appels et al., 2008).

The biogas produced during AD is commonly used for heat and electricity production (Rasi et al., 2007) in relatively small and easy-to-manage industrial units (Antoni et al., 2007). A well-operated anaerobic reactor produces more energy (in the form of biogas) than that required on site, and the excess is usually sold to a local utility for domestic use (Turovskiy and Mathai, 2006). Alternatively, it can be fed to the gas grid, or used as a fuel in combustion engines, cars, or fuel cells (Appels et al., 2008)

Biogas from sewage digesters usually contains around 55-65% of $CH_4$, 35-45% of $CO_2$, and 1% of $N_2$. Nevertheless, it typically also presents traces of $H_2S$ and other S-containing compounds (Rasi et al., 2007). Besides its bad smell, $H_2S$ is highly non-desirable in energy-recovery processes because it converts into $SO_2$ and $H_2SO_4$, which are highly corrosive, unhealthy and environmentally hazardous compounds (Abatzoglou and Boivin, 2009). Besides, $H_2S$ itself is reactive with most metals (Appels et al. 2008); it attacks iron, copper, cement, etc (Noyola et al., 2006). As a result, the biogas quality in the different technologies for energy recovery is assessed as $H_2S$ content.

The biogas generated during AD of sewage sludge can present $H_2S$ concentrations above the technical limits recommended by the facility manufacturers; it can contain up to 10,000ppmv. Nonetheless, the usual $H_2S$ concentration in the biogas produced in WWTPs is about 1,000ppmv. Therefore, with traditional boilers and internal combustion engines, in most cases the biogas can be used without any treatment, since the recommendations are that hydrogen sulphide content should not be more than 1,000 ppmv. Conversely, when the biogas is not used on site, the exhaustiveness required in the desulphurisation is much higher. The strictest limits of $H_2S$ are set for fuel cells, where its concentration in the biogas must not exceed 0.1ppmv (Rasi et al., 2011).

The removal of $H_2S$ from biogas can be carried out by biological and physico-chemical treatments. Besides being ecologically cleaner, the biological desulphurisation techniques (the most notable of which are biofilters, biotrickling filters and bioscrubbers) are inexpensive in terms of capital outlay and operating costs (Burgess et al., 2001). Nevertheless, physico-chemical processes are the most common methods for biogas desulphurisation. Among them, the dosing of iron chloride to the digester is one of the most widespread solutions (Persson and Wellinger, 2006), especially for sewage sludge digesters, where $H_2S$ concentrations of below 150ppmv can be achieved (Deublein and Steinhauser, 2008). However, it implies high costs due to large chemical consumption and iron sludge production (Abatzoglou and Boivin, 2009). The rest of the popular physico-chemical methods are applied in the gas stream and in the upgrading process, such as adsorption on activated carbon (Krischan et al., 2012) and water scrubbing (Appels et al., 2008), respectively.

As an alternative to internally and inexpensively desulphurise biogas, air/$O_2$ can be injected into the anaerobic reactor; thus, there is no hazardous reagent usage and chemical sludge production and $H_2S$ levels of less than 50ppmv can be achieved (Díaz et al., 2010b). The basis of this method lies in the presence of sulphide-oxidising bacteria (SOB) in the feedstock (Weiland, 2010), which convert dissolved sulphide into $S^0$ and $SO_4^{2-}$ (van der Zee et al., 2007). The ratio of $SO_4^{2-}/S^0_{produced}$ depends on the dissolved $O_2$ concentration; limited $O_2$ availability and high sulphide loads favour $S^0$ production (Fortuny et al., 2008). Moreover, under such conditions, SOB can effectively compete with the chemical sulphide oxidation (Robertson and Kuenen, 2006). In comparison to the other biological processes, microaeration/microoxygenation of "anaerobic" digesters has a smaller footprint, and entails lower capital outlay and only a minor modification of the process flowchart.

The feasibility of sulphide oxidation during digestion under microaerobic conditions has been widely demonstrated; the process successfully competes for $O_2$ with other oxidative processes, and is faster than re-reduction of the oxidised sulphur species (van der Zee et al., 2007). Moreover, the presence of $O_2$ has been proved not to have a negative effect on either the organic matter removal, the biogas or the methane productivity (Fdz-Polanco et al., 2009; Díaz et al., 2010a; Díaz et al., 2010b). In fact, the full-scale experiments carried out by Jenicek et al. (2008, 2010) revealed that, besides efficiently desulphurising biogas, an intimate contact between the $O_2$ and the liquid phase can improve organic matter degradation, digestate quality and productivity of both biogas and $CH_4$ during municipal sludge digestion. This can arise from higher hydrolysis rates (Botheju et al., 2010; Lim and Wang, 2013), alleviation of sulphide toxicity (Jenicek et al., 2010; Zhou et al., 2007), and shifts in microbial activity and populations (Jenicek et al., 2011; Rodríguez et al., 2012; Tang et al., 2004; Zhou et al., 2007). Specifically, Zhou et al. (2007) observed that the rod-shaped methanogens almost disappeared and were replaced by cocci-shaped ones after imposing microaerobic conditions. Tang et al. (2004) reported a dramatic increase in the population size of *Methanoculleus* due to microaeration. In the meantime, they found a significant decrease in the population of *Methanosarcina*, which suggested that *Methanoculleus* sp. out-competed *Methanosarcina* sp. due to a higher tolerance for $O_2$.

The most profitable oxidative reactant for imposing microaerobic conditions during digestion is $O_2$ since air implies further biogas dilution (Díaz et al., 2010a; Jenicek et al. 2010). On the other hand, Díaz et al. (2010b) demonstrated that the most economical configuration for desulphurising biogas during the pilot-scale digestion of sewage sludge consisted of $O_2$ supply to the headspace and liquid recirculation, which is consistent with the fact that the $H_2S$ removal takes place in the gas space of the reactor (Ramos et al., 2012). However, further research is required in order to corroborate these findings on a larger scale. Hence, the main aims of this research were:

- to investigate the efficiency of the process of biogas desulphurisation in an industrial-pilot scale digester under different configurations (mixing method and concentrated $O_2$ dosing point)
- to study the impact of micro-oxygenation on the microbial communities

## 2. Materials and methods

### *2.1. Industrial-pilot scale reactor*

The research was carried out in a continuous stirred tank reactor with 5$m^3$ (7$m^3$ of total volume) located at the WWTP of Villalonquéjar (Burgos, Spain). Temperature (35ºC) was maintained by a water heat exchanger and monitored by probes (Fig. 1). Mixed sludge produced in the aforementioned municipal WWTP was continuously pumped to the digester by a screw pump. It presented a highly variable organic load, with a VS concentration ranging between 24.7 and 44.9g/L. As a result, its rheology was highly changeable, which in turn led to fluctuations in the feeding flow rate. Hydraulic retention time (HRT) was approximately 20d. The reactor's content was recirculated by a peristaltic pump at a rate of approximately 25$m^3$/d. Depending on the operational period, a compressor provided 21$m^3$/d of biogas recirculation (Fig. 1). Microaerobic conditions were implemented from the period P2 to P8 using an $O_2$ concentrator (Table 1), which achieved a purity of 92-98%. $O_2$ was introduced intermittently (approximately every 10min). Fig. 1 shows the $O_2$ dosing point in each operational period.

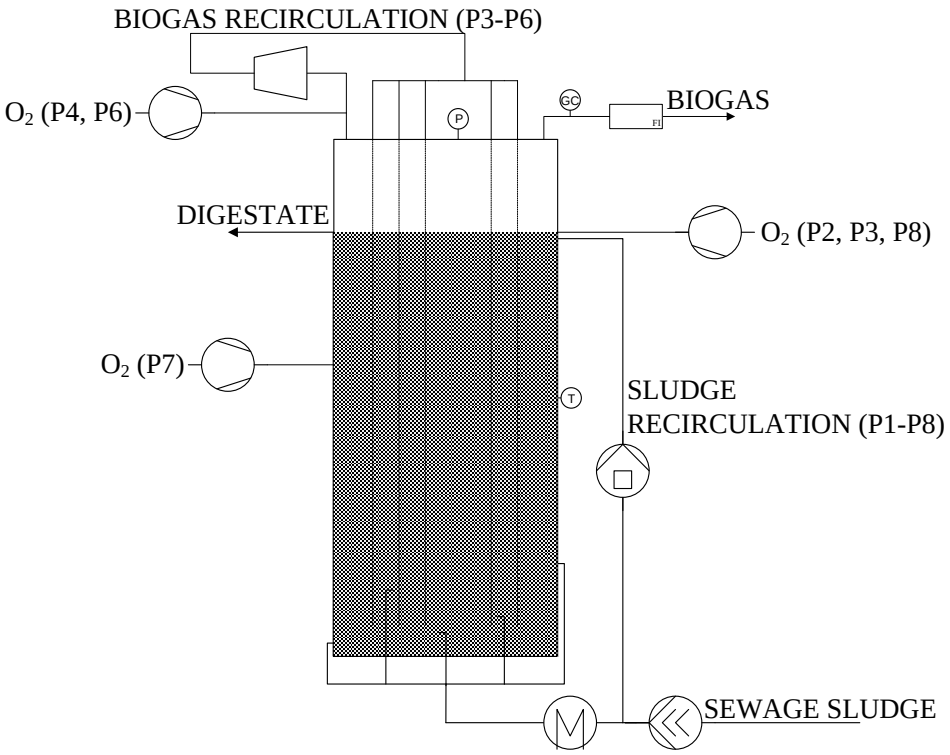


**Fig. 1**. Digester diagram. Operational periods (P1-P8) are indicated in brackets.

**Table 1**. Sequence of variations applied to the digester.

| Period | Conditions | Recirculation | $O_2$ dosing point | $O_2$ flow rate ($L/m^3/d$) |
|---|---|---|---|---|
| P1 | Anaerobic | Sludge | - | - |
| P2 | Microaerobic | Sludge | Headspace | 12-34 (increasing) |
| P3 | Microaerobic | Sludge and biogas | Headspace | 34-14 (decreasing) |
| P4 | Microaerobic | Sludge and biogas | Biogas recirculation | 14 |
| P5 | Anaerobic | Sludge and biogas | - | - |
| P6 | Microaerobic | Sludge and biogas | Biogas recirculation | 14 |
| P7 | Microaerobic | Sludge | Liquid phase | 14-5 (decreasing) |
| P8 | Microaerobic | Sludge | Headspace | 5 |

Pressure was monitored by a sensor. Biogas production was quantified by a thermal mass flow meter, and its composition was determined by gas chromatography (GC) (CP-4900 Micro-GC) according to Díaz et al. (2010b) from the beginning of the study to the 87$^{th}$ day. From the 117$^{th}$ day, it was measured by a gas analyser (GA-3000) whose precision in determining $CH_4$, $O_2$ and $H_2S$ was ±3%v/v, ±1%v/v, and ±5% of the result or 500ppm (the largest value), respectively. SCADA software was used for monitoring; data (pressure, temperature, biogas production and recirculation rate) were displayed and stored in real-time in a computer.

*2.2. Experimental procedure*

The reactor was inoculated with sludge from a full-scale anaerobic reactor located at the aforementioned WWTP. It was started-up under anaerobic conditions, using sludge recirculation as the mixing method. HRT was set at about 24d until the 50$^{th}$ day; thereafter, it was around 20d. At day 135 (day 0 in Fig. 2), it was considered that a stationary state had been achieved.

The study was divided into eight operational periods (Table 1): two anaerobic (P1 and P5) and six microaerobic (P2, P3, P4, P6, P7 and P8). In P1, P2, P7 and P8, the reactor's content was mixed by sludge recirculation (Fig. 1). In the rest of the experimental periods, the digester operated with both biogas and sludge recirculation.

In P2, $O_2$ was supplied to the gas space, just above the liquid interface, where the $H_2S$ removal from biogas reportedly occurs (Abatzoglou and Boivin, 2009; Weiland, 2010), and the flow rate was gradually raised from approximately 12 to 34$L/m^3/d$. During P3, the micro-oxygenation level was gradually lowered to 14$L/m^3/d$. In P4, the $O_2$ dosing point was changed; it was injected into the biogas recirculation at a constant flow rate (14$L/m^3/d$). Micro-oxygenation was interrupted in order to evaluate the $H_2S$ production during P5. In P6, the configuration and the $O_2$ flow rate set in P4 were restored. During P7, the biogas recirculation was stopped, and $O_2$ was injected into the liquid phase, just above the midpoint of the digester. As shown in Table 1, the $O_2$ flow rate was gradually lowered from 14 to 5$L/m^3/d$. In the last experimental period (P8), the configuration applied during P2 was restored, while the micro-oxygenation level remained constant (5$L/m^3/d$).

### *2.3. Chemical analysis*

The feed and the digested sludge were analysed in terms of total and soluble chemical oxygen demand (TCOD and SCOD), total and volatile solids (TS and VS), carbon (C) and sulphur (S), total kjeldahl nitrogen (TKN), N-$NH_4^+$, $NO_2^-$ , $NO_3^-$, $PO_4^{3-}$, sulphide, $SO_4^{2-}$, and $S_2O_3^{2-}$. Specifically, $NO_2^-$ , $NO_3^-$, $PO_4^{3-}$ were analysed by UV-visible spectroscopy (APHA, 1998), $SO_4^{2-}$ and $S_2O_3^{2-}$ concentrations were determined by ion chromatography (APHA, 1998) and high-performance liquid chromatography (van der Zee et al., 2007), respectively. Dissolved sulphide was analysed by potentiometric titration (APHA, 1998), and elemental analysis (C and S) were carried out by IR spectroscopy (LECO CS-225). The pH of the reactor was monitored by a pH-meter with a temperature probe.

### *2.4. Microbial analysis*

In order to evaluate how the presence of $O_2$ affected the microbial community, four biomass samples (S1, S2, S3 and S4) were collected and stored immediately at -20ºC. Samples S1, S2, S3 and S4 were taken in P1 ($18^{rd}$ day), P2 ($67^{rd}$ day), P3 ($109^{th}$ day) and P4 ($165^{th}$ day), respectively.

The V6-V8 and the V2-V3 regions of the bacterial and the archaeal 16S rRNA genes were amplified by polymerase chain reaction (PCR) using the universal bacterial primers 968-F-GC and 1401-R, and A 109(T)-F and 515-GC-R (Sigma- Aldrich, St. Louis, MO, USA), respectively (Rodríguez et al., 2012). The PCR mixture contained 1µL of each primer (10 ng µL-1 each primer), 25µL of BIOMIX ready-to-use 2× reaction mix (Bioline, Ecogen), PCR reaction buffer and deoxynucleotide triphosphates, 2µL of the extracted DNA, and Milli-Q water up to a final volume of 50 µL. PCR was performed in a iCycler Thermal Cycler (Bio Rad Laboratories, Inc.) applying the thermo-cycling program described by Rodríguez et al. (2012). DGGE analysis of the bacterial and archaeal amplicons were performed according to Rodríguez et al. (2012). DGGE profiles were compared using the GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). The Shannon-Wiener diversity index (*H*) of every sample and the pair-wise similarity coefficient were both calculated according to Lebrero et al. (2013). Similarity dendrograms were constructed by using UPGMA clustering with error resampling (500 resampling experiments).

Individual bands were excised from the archaeal DGGE gel. Both DNA extraction and purification of PCR products were carried out according to Rodríguez et al. (2012). The taxonomic position of the sequences was obtained using the RDP classifier tool (80% confidence level) (Wang et al., 2007). Moreover, the sequences were compared with those included in GenBank by the BLAST search tool at the National Centre for Biotechnology Information (NCBI) website (McGinnis and Madden, 2004). Along with BLAST, DECIPHER was used as the chimera checking tool (Wright et al., 2012). Finally, the sequences were deposited in GenBank under accession numbers KJ402278-KJ402293.

## 3. Results and discussion

### *3.1. Biogas quality*

#### *3.1.1. $H_2S$ and $O_2$*

The average sulphide content of the biogas produced in P1 (under anaerobic conditions) was 0.49%v/v (Table 2). Microaerobic conditions were applied on the 29$^{th}$ day (period P2) by injecting around 12L$O_2$/m$^3$/d into the gas space, just above the liquid interface (by a single point) (Table 1). Under such a configuration, presumably the $O_2$ transfer rate to the sludge was significantly limited. Moreover, it is possible that $O_2$ could only be transferred to a limited area in the headspace (around the injection point). Within 24h, the $H_2S$ concentration decreased to 0.35%v/v (Fig. 2a). It indeed remained at around that value until the 54$^{th}$ day, despite the gradual increase in the $O_2$ supply. Thereafter, at micro-oxygenation levels above 29L/m$^3$/d, the biogas sulphide content remained below 0.10%v/v. Specifically, from the 70$^{th}$ to the 74$^{th}$ day (at approximately 34L$O_2$/m$^3$/d), the average $H_2S$ and $O_2$ concentration in biogas was 0.02 and 4.3%v/v, respectively. It is worth noting that at even the highest micro-oxygenation level, the biogas $N_2$ content did not exceed 0.1%v/v.

**Table 2**. Biogas quality.

| Period | $O_2$ (%v/v) | $H_2S$ (%v/v) | $CH_4$ (%v/v) | $CH_4/CO_2$ |
|---|---|---|---|---|
| P1 | 0.0 | 0.49 | 63.5 | 1.8 |
| P2 | 1.7 | 0.21 | 62.4 | 1.8 |
| P3 | 0.9 | 0.01 | 62.0 | 1.7 |
| P4 | 0.1 | 0.02 | 59.1 | 1.5 |
| P5 | 0.0 | 0.25 | 58.8 | 1.5 |
| P6 | 0.1 | 0.01 | 59.4 | 1.5 |
| P7 | 0.0 | 0.00 | 60.1 | 1.5 |
| P8 | 0.0 | 0.00 | 59.2 | 1.4 |

On the 74$^{th}$ day (period P3), the biogas recirculation was started, while the $O_2$ supply was maintained at approximately 34L/m$^3$/d (Table 1). As expected, the biogas $O_2$ content dropped significantly (to approximately 1.2%v/v), which was attributed to improved $O_2$ transfer due to higher contact between the gas and the liquid phase. The $H_2S$ concentration in biogas also decreased, and stabilised at around zero. Hence, from the 77$^{th}$ to the 87$^{th}$ day, the $O_2$ flow rate was gradually reduced from approximately 31 to 19L$O_2$/m$^3$/d. This hardly affected the $H_2S$ and the $O_2$ concentration in the biogas. Regarding the $O_2$ content in the biogas, it presumably remained constant due to lesser $O_2$ consumption in unidentified processes, which could in turn result from the lower gradient of $O_2$ in the liquid phase.

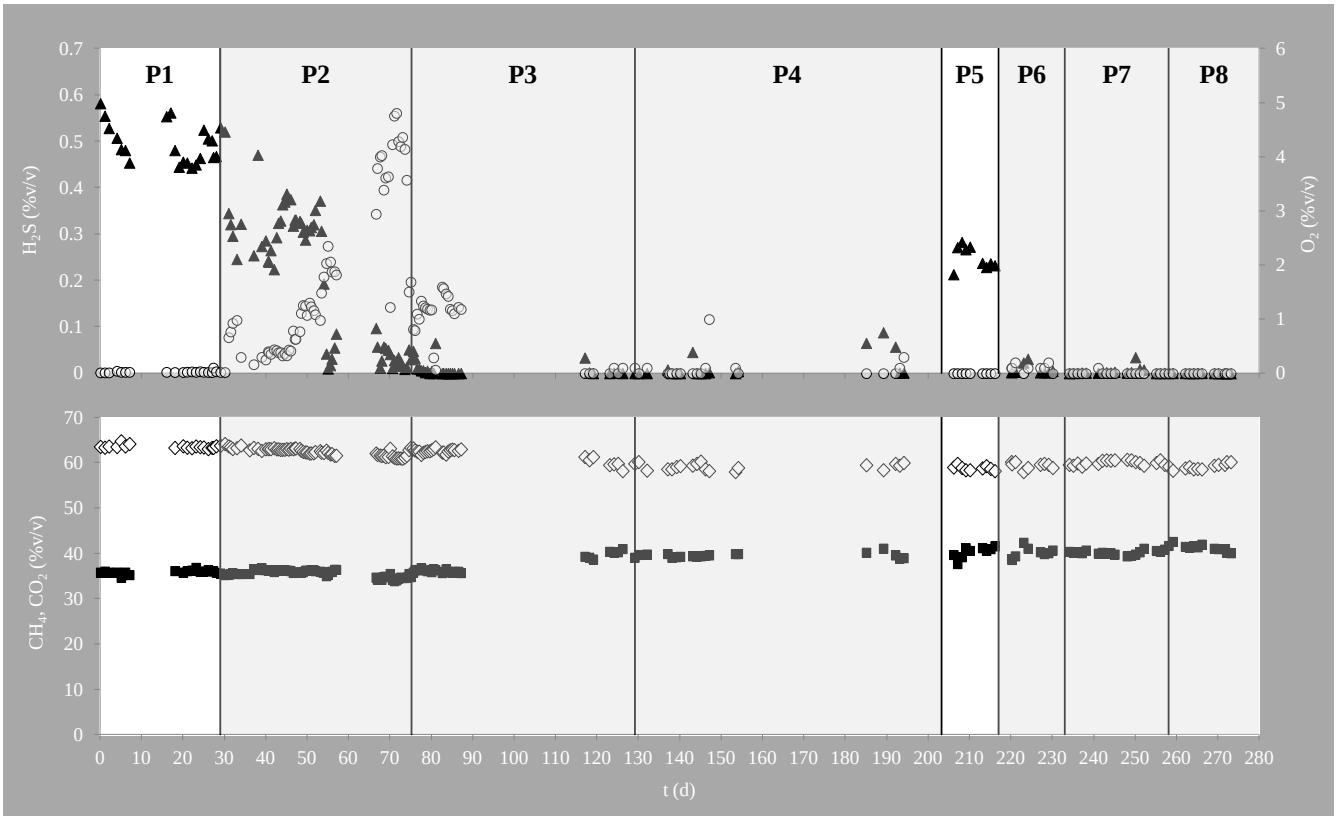


**Fig. 2**. Concentration of $H_2S$ (▲) and $O_2$ (○) (a), and $CH_4$ (◊) and $CO_2$ (■) (b). Anaerobic periods are represented in white.

In contrast to Díaz et al. (2010b), who reported a decrease in dissolved sulphide in the digester from approximately 130mg/L to less than 40mg/L due to the implementation of biogas recirculation, the digestate sulphide content in P3 did not decrease significantly in relation to the previous operational periods. The feeding sulphide content was around 26mg/L during the whole study, while the sulphide concentration in the effluent in P1, P2 and P3 was 58, 63 and 50mg/L, respectively.

During P4, the $O_2$ was introduced in the biogas recirculation instead of in the gas space (Table 1). As expected, the $H_2S$ and the $O_2$ concentration in the biogas remained almost constant (around zero). Regarding the $O_2$ concentration, it must be noted that the difference between the first and the last 12 days of P3 was due to the change in the measuring device (Fig. 2a).

In P5, the micro-oxygenation was stopped in order to evaluate the $H_2S$ concentration under anaerobic conditions (Table 1). The biogas sulphide content remained around 0.25%v/v (Table 2). The considerable decrease in the $H_2S$ concentration in relation to P1 was related to the sludge variability. At this point, it must be noted that, besides a variable organic load, the feeding presented a highly variable concentration of S. Specifically, the C and S content of the sewage sludge was in the range of 29-37 and 0.5-2.0%w/w, respectively.

When microaerobic conditions were restored with the same configuration as in P4 (period P6), the sulphide content of the biogas dropped to an average of 0.01%v/v, while the surplus of $O_2$ hardly rose (Fig. 2a). It must be mentioned that the peaks of $H_2S$ concentration detected on the 223$^{rd}$ and 224$^{th}$ day could result from the increase in the biogas production recorded during those days. Once

the biogas recirculation was stopped (period P7), the $H_2S$ concentration decreased negligibly. Regarding the biogas $O_2$ content, it hardly changed, which could be related to the precision of the gas analyser; it was expected to rise due to lower $O_2$ transfer to the sludge. In fact, from the 235$^{th}$ day, although the $O_2$ supply was gradually lowered, the biogas composition remained almost constant in terms of both $O_2$ surplus and $H_2S$ concentration. From the 250$^{th}$ day until the end of the study, the $O_2$ flow rate was maintained at around 5L/m$^3$/d.

In P8, the $H_2S$ and $O_2$ concentrations in the biogas were almost equal to those in P7 (Table 2), which highlighted that the success of the biogas desulphurisation was independent of the $O_2$ transfer rate to the liquid phase. At this point, it should be mentioned that the objective of the configuration applied in P7 was to maintain a successful $O_2$ transfer to the all the surfaces of the headspace. As a result, it was concluded that the biogas desulphurisation took place predominantly in the gas space.

Despite applying the same configuration, the molar ratio of $O_2$ supplied to $H_2S$ produced which was required to achieve similar $H_2S$ concentrations in the biogas in P2 was almost four times higher than in P8 (8 against 2v/v, respectively) (Table 1). Maybe the population size of SOB in the area of the headspace with sufficient $O_2$ availability during P2 (that around the $O_2$ injection point) did not suffice to achieve efficient biogas desulphurisation. Presumably the SOB population in the gas space increased over time due to the accessibility of both $H_2S$ and $O_2$.

### *3.1.2. $CH_4$*

The average $CH_4$ content of the biogas produced in P1 was 63.5%v/v (Table 2). During P2, while the surplus of $O_2$ in the biogas increased, the $CH_4$ concentration declined gradually; it was specifically 62.4%v/v on average (Fig. 2b). It should be noted that this oscillated between 61.7 and 60.9%v/v from the 87$^{th}$ day. Subsequently, in P3, due to the implementation of biogas recirculation and the consequently higher $O_2$ consumption in the reactor, the biogas $CH_4$ content rose, remaining around 62.7%v/v until the 67$^{th}$ day. Consequently, the decrease in the $CH_4$ concentration observed in P2 was attributed to dilution rather than aerobic oxidation of methanogenic substrate. In fact, the ratio of $CH_4$ to $CO_2$ was 1.8 in both P1 and P2 (Table 2). In P3, before the change in the measuring device of biogas composition, this variable was negligibly lower (1.7), which indicated that the additional $O_2$ was scarcely employed to oxidise soluble organic matter by aerobic respiration; this finding was confirmed in the following operational periods. Nonetheless, it must be considered that slight changes in the biogas composition could arise from the variability of the feeding composition.

In P3, after the 117$^{th}$ day, due to the replacement of the measuring device, the $CH_4$ and $O_2$ concentrations were significantly lower, and the $CH_4/CO_2$ ratio remained around 1.5 (Fig. 2b). The average biogas $CH_4$ content in P3 from the aforementioned day was specifically 60.0%v/v. In the rest of the operational periods, including P4, P5 (anaerobic), P6, P7 and P8, it was 59.1, 58.8, 59.4, 60.1 and 59.2%v/v (respectively) (Table 2). Regarding the $CH_4/CO_2$ ratio, it varied negligibly, which along with the biogas production (Table 3) suggested that $O_2$ did not cause a significant impact on the reactor productivity, independently of its availability.

*3.2. Digestion performance*

Due to the continuous changes in the organic loading rate to the reactor arising from the variability of the feeding composition, the digestion performance (organic matter removal and biogas productivity) could not be estimated with the necessary precision in order to identify significant differences between the various operational periods, specifically, between P1, P2, P3 and P4, that is, the periods with a sufficiently long duration. With regard to this, it must be considered that the reactor was started-up and operated under the configuration set in P1 for 135 days.

**Table 3**. Biogas production, digestate quality and VS removal.

| | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 |
|---|---|---|---|---|---|---|---|---|
| Biogas production ($m^3/m^3/d$) | 0.86 | 0.92 | 0.94 | 0.97 | 1.01 | 1.05 | 1.08 | 1.05 |
| TS (g/L) | 31.6 | 31.9 | 32.8 | 32.8 | 31.1 | 29.5 | 29.8 | 30.0 |
| VS (g/L) | 17.0 | 17.4 | 18.1 | 17.8 | 17.0 | 17.3 | 17.4 | 17.6 |
| VS removal (%) | 45 | 46 | 44 | 46 | 48 | 47 | 49 | 47 |

The VS removal in P1, which was considered the baseline period, was 45% on average and, during P2, P3 and P4, this variable fluctuated around that value (Table 3). Likewise, neither the digestate quality deteriorated significantly nor the biogas production or its $CH_4$ content decreased substantially during the different stages in relation to P1. Similarity to the $CH_4$ concentration in the biogas, the TS and VS content of the digestate and the biogas production both fluctuated within a narrow range all through the research (31.6-32.8g/L, 17.0-18.1g/L, and 0.86-1.08$m^3/m^3/d$, respectively). Moreover, these variables rose concurrently, which was consistent with the increasing VS content of the feeding (data not shown). These results are in turn consistent with those reported in literature, since even on an industrial scale, it has been broadly demonstrated that the presence of limited amounts of $O_2$ in digesters does not negatively affect digestion performance (Jenicek et al., 2008, 2010).

*3.3. Sulphide oxidation products*

According to the $SO_4^{2-}$ and $S_2O_3^{2-}$ analysis, $H_2S$ was mostly oxidised to $S^0$. $S_2O_3^{2-}$ was detected in negligible amounts in both the sewage and the digested sludge. The average $SO_4^{2-}$ concentration in the feeding from P1 to P4 was between 141 (P4) to 238mg/L (P1), while the effluent $SO_4^{2-}$ content remained between 24 (P1) and 29mg/L (P2). According to the $H_2S$ production and the operational conditions, the expected increase in the digestate S content during the microaerobic periods in relation to P1 was also negligible. Moreover, even if it had been significant, it would hardly have been detectable due to the aforementioned variable S concentration in the feeding. Nevertheless, it is worth noting that the effluent S and C content of the digested sludge oscillated continuously between 0.7 and 2.0%w/w, and between 22 and 28%w/w, respectively.

After approximately 415 days of operation (240 days under microaerobic conditions), the ceiling of the reactor was removed. A relatively thick deposit with 19%w/w of C and 58%w/w of S was observed at the area of the walls surrounding the liquid phase. Presumably it formed due to the

slight, unavoidable fluctuations in the liquid level and even sludge splashes. The rest of the headspace was covered with a really thin, dry layer with 6 and 86%w/w of C and S, respectively. The high degree of dryness observed in most of the surfaces of the gas space after the operation shutdown suggested that moisture availability could limit the growth of SOB in the headspace. This could be the cause of the low desulphurisation performance and relatively high $O_2$ demand maintained at the early stage of the microaerobic operation (Fig. 2a).

Considering that the biogas desulphurisation appeared to occur predominantly in the headspace (see 3.1.1), and, as noted above, $H_2S$ was mostly converted into $S^0$, larger amounts of this compound were expect to be found all over the headspace. Presumably the $S^0$ formed there fell into the sludge, thereby leaving the reactor with the effluent. Since the main factor affecting the profitability of the implementation of microaerobic conditions in full-scale reactors is the required periodicity for cleaning the headspace (Díaz, 2011), this observation could be of utmost importance.

### *3.4. Microbial communities*

#### *3.4.1. Biodiversity*

In order to evaluate the richness and evenness of species (diversity) in the reactor in P1 (sample S1), P2 (sample S2), P3 (sample S3), and P4 (sample S4) the Shannon-Wiener index (*H*) was calculated from both DGGE gels (Fig. 3a and b). This index typically ranges from 1.5 to 3.5 (McDonald, 2003). All the samples showed a moderate archaeal diversity, with *H* ranging from 2.4 to 2.8 (Fig. 3a). The diversity indices calculated from the bacterial DGGE gel were in the range of 2.6 to 3.1, which highlighted a moderately high bacterial richness and evenness (Fig. 3b).

The archaeal diversity of the sample S3 (2.4) was negligibly lower than that of S2 and S1 (2.5), while S4 presented the highest *H* (2.8) (Fig. 3a). On the other hand, S1 and S4 exhibited the lowest and the highest bacterial diversity (2.6 and 3.1, respectively), while S2 and S3 presented the same *H* (2.9) (Fig. 3b). Therefore, the $O_2$ availability in the sludge maintained during P2 sufficed to cause a significant increase in the bacterial diversity. Likewise, the archaeal and the bacterial richness and evenness both rose in P4. This indicated that the increase in the $O_2$ transfer rate to the liquid phase arising from the implementation of biogas recirculation in P3 positively affected the microbial diversity in the long term (in period P4).

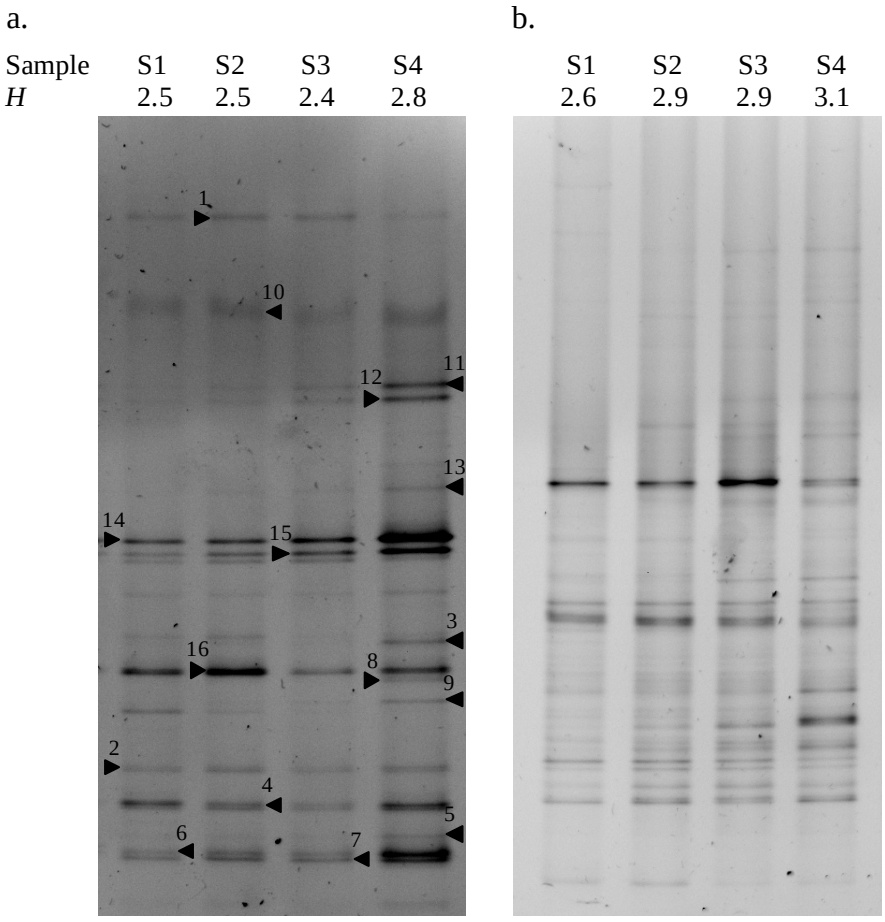


**Fig. 3**. Archaeal (a) and bacterial (b) DGGE profiles of the 16S rRNA amplicons of the samples S1-S4 with their respective diversity indices.

*3.4.2. Similarity*

The pair-wise similarity indices indicated a moderate-high correspondence between the microbial communities growing in the sludge at the different periods of the study (Fig. 4). The similarity coefficients between the archaeal communities fluctuated between 60 and 91% (Fig. 4a). Sample S1 and S2 presented the highest similarity, which suggested that the archaeal communities were only slightly affected by the injection of $O_2$ into the headspace. This was consistent with the presumably low $O_2$ transfer to the sludge maintained during P2. Likewise, a high similarity was also found between sample S3 and S4 (87%). By contrast, the similarity indices between the rest of the sample pairs were lower than 70%. Therefore, the implementation of biogas recirculation in P3 and the resulting increase in the $O_2$ transfer rate to the sludge caused a significant impact on the structure of the archaeal communities.

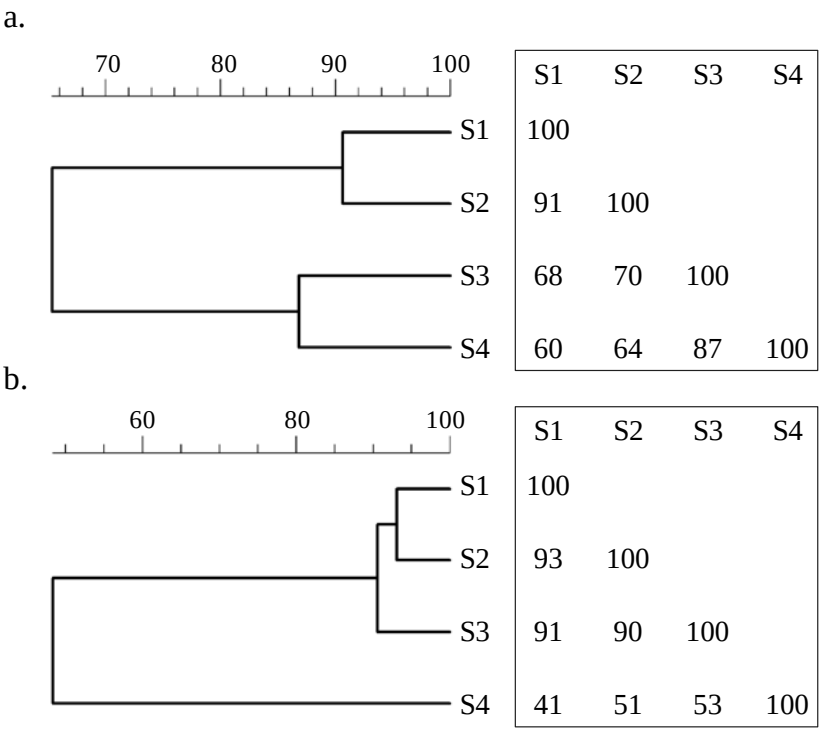


**Fig 4.** Archaeal (a) and bacterial (b) similarity dendrogram (UPGMA clustering) and matrix with error resampling (500 resampling experiments).

The similarity coefficients between the bacterial communities fluctuated from 41 to 93% (Fig. 4b). The highest similarities were found between S1 and S2 (93%), S1 and S3 (91%), and S2 and S3 (90%). Conversely, S4 presented a similarity of between 41 and 53% with the rest of the samples. Hence, as in the archaeal communities, the bacterial communities were only slightly affected when $O_2$ was injected into the gas space. The increase in the $O_2$ transfer rate to the liquid phase during P3 also had a limited impact on them; however, in the long term (P4), it caused a significant change in the structure of the bacterial communities.

### *3.4.2. Archaeal DGGE dynamics*

From the archaeal DGGE gel (Fig. 3a), sixteen bands were sequenced (Table 4). According to the RDP classifier (confidence threshold of at least 80%), all of them belonged to the Euryarchaeota phyla. Band 1 and 2 remained unclassified, and the rest were all ascribed to one class, Methanomicrobia, and two orders, Methanosarcinales (band 3-9) and Methanomicrobiales (band 10-16). According to the BLAST search tool, band 1 and 2 could belong to the Methanosarcinales order. The rest of the results obtained from this database were consistent with those given by the RDP classifier.

**Table 4**: RDP classification of the archaeal DGGE bands sequenced with at least 80% of confidence level, and corresponding closest relatives in Genbank obtained by the BLAST search tool with their similarity percentages, and environments from which they were retrieved. Intensity < 35 = ×, 35 ≤ intensity ≥ 80 = ××, intensity > 80 = ×××.

| Taxonomic placement (confidence threshold ≥ 80%) | Band | S1 | S2 | S3 | S4 | Closest relatives (accession nº in Genbank) | Similarity (%) | Source of origin |
|---|---|---|---|---|---|---|---|---|
| Phylum Euryarchaeota | 1 (KJ402278) | × | × | × | × | Uncultured archaeon (CU917025) | 98 | Mesophilic anaerobic digester which treats municipal wastewater sludge |
| | | | | | | Uncultured *Methanosaeta* sp. (KC769082) | 97 | Active sludge |
| | 2 (KJ402279) | × | × | × | × | Uncultured archaeon (HM639839) | 98 | Activated sludge wastewater treatment plant |
| | | | | | | Uncultured Methanosarcinaceae (AJ879026) | 97 | Rice rhizosphere |
| Class Methanomicrobia<br>Order Methanosarcinales<br>Family Methanosaetaceae<br>Genus *Methanosaeta* | 3 (KJ402280) | | | | × | Uncultured archaeon (KC412610) | 99 | Lab-scale anaerobic digester of agricultural waste material |
| | | | | | | Uncultured *Methanosaeta* sp. (HQ290282) | 98 | Anaerobic digester fed with distillers grains |
| | 4 (KJ402281) | ×× | ×× | × | ×× | Uncultured archaeon (FJ222220) | 99 | Biogas plant supplied with cattle liquid manure, cattle dung, maize silage, gras silage, grains |
| | | | | | | *Methanosaeta concilii* (AB679168) | 97 | Culture collection |
| | 5 (KJ402282) | × | × | × | × | Uncultured archaeon (EU636895) | 99 | Biogas plant (mesophilic CSTR) supplied with cattle liquid manure and maize silage |
| | | | | | | *Methanosaeta concilii* (AB679168) | 97 | Culture collection |
| | 6 (KJ402283) | × | ×× | × | ××× | Uncultured archaeon (EU926764) | 99 | Anaerobic biogas reactor fed with core silage |
| | | | | | | *Methanosaeta concilii* (AB679168) | 97 | Culture collection |
| | 7 (KJ402284) | × | ×× | × | ×× | Uncultured archaeon (KC412572) | 99 | Lab-scale anaerobic digester |
| | | | | | | *Methanosaeta concilii* (AB679168) | 97 | Culture collection |
| | 8 (KJ402285) | | | | × | Uncultured archaeon (HM639803) | 97 | Activated sludge wastewater treatment plant |
| | | | | | | Uncultured archaeon (AJ576227) | 97 | Landfill leachate |
| | 9 (KJ402286) | | × | × | × | Uncultured archaeon (KC412593) | 97 | Lab-scale anaerobic digester |
| | | | | | | Uncultured archaeon (EU926764) | 97 | Anaerobic biogas reactor fed with core silage |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Order Methanomicrobiales | | | | | | | | |
| Family Methanospirillaceae | | | | | | | | |
| Genus *Methanospirillum* | 10 (KJ402287) | × | × | × | × | Uncultured euryarchaeote (AB175351) | 99 | Mesophilic anaerobic BSA digester |
| | | | | | | Uncultured Methanomicrobiales (CU917420) | 99 | Mesophilic anaerobic digester which treats municipal wastewater sludge |
| | 11 (KJ402288) | × | × | × | ×× | Uncultured Methanomicrobiales (CU916501) | 99 | Mesophilic anaerobic digester which treats municipal wastewater sludge |
| | | | | | | Uncultured *Methanospirillum* sp. (HQ290290) | 98 | Anaerobic digester fed with distillers grains |
| | 12 (KJ402289) | × | × | × | ×× | Uncultured euryarchaeote (AB248620) | 99 | Mesophilic anaerobic butyrate degrading reactor |
| | | | | | | Uncultured Methanomicrobiales (CU916636) | 99 | Mesophilic anaerobic digester which treats municipal wastewater sludge |
| | 13 (KJ402290) | | | × | × | Uncultured euryarchaeote (AB175351) | 99 | Mesophilic anaerobic BSA digester |
| | | | | | | Uncultured Methanomicrobiales (JX023174) | 99 | Sludge samples of anaerobic digesters treating sewage sludge or mixture of sewage sludge and food waste |
| | 14 (KJ402291) | ×× | ×× | ××× | ××× | Uncultured euryarchaeote (AB248620) | 99 | Mesophilic anaerobic butyrate degrading reactor |
| | | | | | | Uncultured Methanomicrobiales (CU915986) | 99 | Mesophilic anaerobic digester which treats municipal wastewater sludge |
| | 15 (KJ402292) | × | ×× | ×× | ××× | Uncultured euryarchaeote (AB175351) | 99 | Mesophilic anaerobic BSA digester |
| | | | | | | Uncultured Methanomicrobiales (JX023211) | 99 | Sludge samples of anaerobic digesters treating sewage sludge or mixture of sewage sludge and food waste |
| Family Methanomicrobiaceae | | | | | | | | |
| Genus *Methanoculleus* | 16 (KJ402293) | ×× | ××× | ×× | ××× | Uncultured *Methanoculleus* sp. (JN052756) | 99 | Anaerobic digestion of animal waste |
| | | | | | | *Methanoculleus marisnigri* (NR_074174) | 98 | Culture collection |

Most of the sequences were close to sequences of already-known methanogenic genera retrieved from reactors (Leclerc et al., 2004). The sequences affiliated to the Methanosarcinales order were assigned to the *Methanosaeta* genus (Table 4), which contains acetotrophic methanogens capable of utilising acetate. On the other hand, from all the DGGE bands ascribed to the Methanomicrobiales order, six (10-15) were assigned to *Methanospirillum* sp., and band 16 was assigned to *Methanoculleus* sp.. These two genera have been reported to consist of hydrogenotrophic methanogens (Robertson and Kuenen, 2006).

All the genera identified were present in the four samples. Moreover, according to the bands intensity, $O_2$ increased the evenness of all these genera (Table 4). Therefore, in contrast to Tang et al. (2004), micro-oxygenation did not seem to cause a particular impact on any of the archaeal populations.

## 4. Conclusions

Biogas produced in an industrial-pilot reactor was efficiently desulphurised independently of the mixing method and the $O_2$ dosing point. The $O_2$ demand of the digester decreased with time. The $H_2S$ removal seemed to occur in the headspace; however, $S^0$, which was found to be the main oxidation product, scarcely accumulated there. $O_2$ did not have a significant impact on digestion. In the long term, the relatively high transfer rate of $O_2$ to the sludge maintained by the biogas recirculation caused a significant change in the structure and a considerable increase in the richness and evenness of the microbial communities.

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## References

1. Abatzoglou, N., Boivin, S., 2009. A review of gas purification processes, Biofuels Bioprod. Biorefin. 3, 42-71.
2. Antoni, D., Zverlov V. V., Schwarz, W. H., 2007. Biofuels from microbes. Appl. Microbiol. Biotechnol. 77, 23-35.
3. APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th edn. American Public Health Association/American Water Works Association/Water Environmental Federation, Washington DC, USA.
4. Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. 34, 755-781.
5. Botheju, D., Samarakoon, G., Chen, C. and Bakke, R., 2010. An experimental study on the effects of oxygen in bio-gasification; Part 1. In: International Conference on Renewable Energies and Power Quality, Granada.
6. Burgess, J.E., Parsons, S.A., Stuetz, R.M., 2001. Developments in odour control and waste gas treatment biotechnology: a review. Biotechnol. Adv. 19, 35-63.
7. Deublein, D., Steinhauser, A., 2008. Biogas from Waste and Renewable Resources: An Introduction. Wiley-VCH, Weinheim.

8. Díaz, I., Lopes, A.C., Pérez, S.I., Fdz-Polanco, M., 2010a. Performance evaluation of oxygen, air and nitrate for the microaerobic removal of hydrogen sulphide in biogas from sludge digestion. Bioresour. Technol. 101, 7724-7730.
9. Díaz, I., Pérez, S.I., Ferrero, E.M., Fdz-Polanco, M., 2010b. Effect of oxygen dosing point and mixing on the microaerobic removal of hydrogen sulphide in sludge digesters. Bioresour. Technol. 102, 3768-3775.
10. Díaz, I., 2011. $H_2S$ removal from biogas. From anaerobic to microaerobic. PhD Thesis. University of Valladolid, Valladolid, España.
11. Fdz-Polanco, M., Díaz, I., Pérez, S. I., Lopes, A. C. & Fdz-Polanco, F., 2009. Hydrogen sulfide removal in the anaerobic digestion of sludge by micro-aerobic processes: pilot plant experience. Water Sci. Technol. 60, 3045-3050.
12. Fortuny, M., Baeza, J.A., Gamisans, X., Casas, C., Lafuente, J., Deshusses, M.A., Gabriel, D., 2008. Biological sweetening of energy gases mimics in biotrickling filters. Chemosphere 71, 10-17.
13. Jenicek, P., Keclik, F., Maca, J., Bindzar, J., 2008. Use of microaerobic conditions for the improvement of anaerobic digestion of solid wastes. Water Sci. Technol. 58, 1491-1496.
14. Jenicek, P., Koubova, J., Bindzar, J. & Zabranska, J., 2010. Advantages of anaerobic digestion of sludge in microaerobic conditions. Water Sci. Technol. 62, 427-434.
15. Jenicek, P., C. A., Celis, J., Koubova, D., Pokorna, 2011. Comparison of microbial activity in anaerobic and microaerobic digesters. Water Sci. Technol. 63, 2244-2249.
16. Krischan, J., Makaruk, A., Harasek, M., 2012. Design and scale-up of an oxidative scrubbing process for the selective removal of hydrogen sulfide from biogas. J. Hazard. Mater. 215-216, 49-56.
17. Lebrero, R., Volckaert, D., Pérez, R., Muñoz, R., Van Langenhove, H., 2013. A membrane bioreactor for the simultaneous treatment of acetone, toluene, limonene and hexane at trace level concentrations. Water Res. 47, 2199-2212.
18. Leclerc, M., Delgènes, J. P., Godon, J. J., 2004. Diversity of the archaeal community in 44 anaerobic digesters as determined by single strand conformation polymorphism analysis and 16S rDNA sequencing. Environ. Microbiol. 6, 809-819.
19. Lim, J. W., Wang J.-Y., 2013. Enhanced hydrolysis and methane yield by applying microaeration pretreatment to the anaerobic co-digestion of brown water and food waste. Waste Manag. 33, 813-819.
20. McGinnis, S., Madden, T. L., 2004. BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Res. 32, W20-W25.
21. Noyola, A., Morgan-Sagastume, J.M., López-Hernández, J.E., 2006. Treatment of biogas produced in anaerobic reactors for domestic wastewater: odor control and energy/resource recovery. Rev. Environ. Sci. Biotechnol. 5, 93-114.
22. Persson, M., Wellinger, A., 2006. Biogas upgrading and utilization, Task 37, IEA Bioenergy.
23. Ramos, I., Díaz, I., Fdz-Polanco, M., 2012. The role of the headspace in hydrogen sulfide removal during microaerobic digestion of sludge. Water Sci. Technol. 66, 2258-2264.
24. Rasi, S., Veijanen, A., Rintala, J., 2007. Trace compounds of biogas from different biogas production plants. Energy 32, 1375-1380.
25. Rasi, S., Läntelä, J., Rintala, J., 2011. Trace compounds affecting biogas energy utilisation - a review. Energy Convers. Manag. 52, 3369-3375.
26. Robertson, L. A., Kuenen, J. G., 2006. The Genus Thiobacillus, in: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer K. H., Stackebrandt, E. (Eds), The Prokaryotes, Vol 5, third ed. Springer, New York, pp. 812-827.
27. Rodríguez, E., Lopes, A., Fdz-Polanco, M., Stams, A. J., García Encina, P. A., 2012. Molecular analysis of the biomass of a fluidized bed reactor treating synthetic vinasse at anaerobic and micro-aerobic conditions. Appl. Microbiol. Biotechnol. 93, 2181-2191.

28. Tang, Y., Shigematsu, T., Ikbal, Morimura, S., 2004. The effects of micro-aeration on the phylogenetic diversity of microorganisms in a thermophilic anaerobic municipal solid-waste digester. Water Res. 38, 2537–2550.
29. van der Zee, F.P., Villaverde, S., García-Encina, P.A., Fdz-Polanco, F., 2007. Sulphide removal by moderate oxygenation of anaerobic sludge environments. Bioresour. Technol. 98, 518-524.
30. Wang, Q., Garrity, G. M., Tiedje, J. M., Cole, J. R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261-5267.
31. Ward, A. J., Hobbs, P. J., Holliman, P. J., Jones, D. L., 2008. Optimisation of the anaerobic digestion of agricultural resources. Bioresour. Technol. 99, 7928-7940.
32. Weiland, P., 2010. Biogas production: current state and perspectives. Appl. Microbiol. Biotechnol. 85, 849-860.
33. Wright, E.S., Yilmaz, L.S., Noguera, D.R., 2012. DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences. Appl. Environ. Microbiol. 78, 717-725.
34. Turovskiy, I. S., Mathai, P. K., 2006. Wastewater Sludge Processing, John Wiley & Sons, Inc., Hoboken, New Jersey.
**35.** Zhou, W., Imai, T., Ukita, M., Li, F., Yuasa, A., 2006. Effect of limited aeration on the anaerobic treatment of evaporator condensate from a sulfite pulp mill. Chemosphere 66, 924-929.

# END-OF-PIPE CONTROL

# Microaerobic desulphurisation unit: A new biological system for the removal of $H_2S$ from biogas

7

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# Microaerobic desulphurisation unit: A new biological system for the removal of $H_2S$ from biogas

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## HIGHLIGHTS

- A new biotechnology for biogas desulphurisation is presented.
- Microaerobic conditions are implemented in an additional unit.
- Digested sludge is an efficient and durable reaction media.
- The MDU demonstrates a high robustness against fluctuations in BRT, $O_2/H_2S$ ratio.
- $S^0$ is the main by-product, which settles to the bottom of the system.

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Oxygen

## ABSTRACT

A new biotechnology for the removal of $H_2S$ from biogas was devised. The desulphurisation conditions present in microaerobic digesters were reproduced inside an external chamber called a microaerobic desulphurisation unit (MDU). A 10 L-unit was inoculated with 1 L of digested sludge in order to treat the biogas produced in a pilot digester. During the 128 d of research under such conditions, the average removal efficiency was 94%. The MDU proved to be robust against fluctuations in biogas residence time (57–107 min), inlet $H_2S$ concentration (0.17–0.39% v/v), $O_2/H_2S$ supplied ratio (17.3–1.4 v/v), and temperature (20–35 °C). Microbiological analysis confirmed the presence of at least three genera of sulphide-oxidising bacteria. Approximately 60% of all the $H_2S$ oxidised was recovered from the bottom of the system in the form of large solid $S^0$ sheets with 98% w/w of purity. Therefore, this system could become a cost-effective alternative to the conventional biotechniques for biogas desulphurisation.



## 1. Introduction

In the current energy context, the possibility of generating heat or electricity generation from biogas are attractive reasons for the application of anaerobic digestion to treat organic wastes (Pöschl et al., 2010). Biogas can also be used as vehicle fuel and for chemicals production (Appels et al., 2008). Whatever the application, its quality is crucial in terms of both $CH_4$ content (which determines its calorific value) and purity.

$H_2S$ is typically the main pollutant in biogas derived from sludge digesters in municipal wastewater treatment plants, with concentrations ranging from 1.0% v/v to 0.1% v/v (10,000–1000 ppmv) (Rasi et al., 2011). It is produced by the anaerobic degradation of S-containing compounds (mainly proteins) and the reduction of anionic species (particularly $SO_4^{2-}$) contained in the feedstock of the digester (Stams et al., 2003). $H_2S$ has a great influence when the different uses of biogas are considered, due to the corrosion problems that it causes in the installations for energy recovery. Therefore, manufacturers of biogas facilities establish limit concentrations; e.g., the biogas sulphide content should not be more than 1000 and 0.1 pp mv, respectively, in internal combustion engines and molten carbonate fuel cells (Rasi et al., 2011). Likewise, in combined heat and power plants, which are mainly implemented for the utilisation of biogas, levels below 250 ppmv are required (Weiland, 2010). Nonetheless, $H_2S$ removal is also required for health and safety reasons. State laws and regulations have been issued in Europe to minimise its presence in all parts of the biogas plants, including in bioreactors, gasholders, ignition and storage tanks, etc. (Deublein and Steinhauser, 2008).

Most of the commercial technologies for $H_2S$ removal are based on physicochemical processes; namely, absorption and adsorption. In order to overcome the chemical, energy and disposal costs of these desulphurisation methods, biologically-based removal processes were developed. Biological methods are the cost-effective and environmentally friendly solution to these techniques, since

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they can proceed at lower temperatures and pressures, and with limited or no reagent consumption (Syed et al., 2006).

Biogas can be biologically desulphurised in additional units, represented mainly by biofilters, biotrickling filters, and bioscrubbers, or directly into the anaerobic reactor, that is, by applying microaerobic conditions during digestion. All these processes are based on the S cycle, and more specifically, in $H_2S$ oxidation. In the aforementioned extra units, $H_2S$ is solubilised in a humid packed bed where aerobic species of sulphide-oxidising bacteria (SOB) are immobilised and grown as a biofilm in the presence of $O_2$ (Noyola et al., 2006). By contrast, these microorganisms are naturally present inside reactors; many organic wastes treated by AD have SOB (Weiland, 2010).

Elemental sulphur ($S^0$) (Eq. (1)) and $SO_4^{2-}$ (Eq. (3)) are the thermodynamically stable by-products from biological $H_2S$ oxidation, which, it has been proposed, proceeds through several intermediates. Duan et al. (2005) suggested the following pathway of chemoautotrophs: $H_2S \rightarrow S^0 \rightarrow S_2O_3^{2-} \rightarrow S_4O_6^{2-} \rightarrow S_3O_6^{2-} \rightarrow SO_3^{2-} \rightarrow SO_4^{2-}$. The main reactions carried out by SOB are shown below (Tang et al., 2009). At this point, it should be noted that $H_2S$ oxidation in biological systems occurs concurrently with chemical reactions (van der Zee et al., 2007), where $S_2O_3^{2-}$ is the main by-product (Janssen et al., 1995).

$$H_29 + 0.5O_2 \rightarrow S^0 + H_2O; \Delta G^0 = -209.4\ kJ/reaction \quad (1)$$

$$S^0 + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+; \Delta G^0 = -587.41\ kJ/\text{reaction} \quad (2)$$

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+; \Delta G^0 = -798.2\ kJ/\text{reaction} \quad (3)$$

$$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2SO_4^{2-} + 2H^+; \Delta G^0 = -818.3\ kJ/reaction \quad (4)$$

Considerable efforts are still required concerning the packed-media based biotechnologies; though effective, they have strict requirements in terms of both monitoring and maintenance due to the bacteria's high sensitivity to fluctuations in operational conditions (Burgess et al., 2001), which translates into costs. Thiopaq® (Paques) and Biopuric® (Biothane Corporation) are indeed the only two industrial biotechnologies that have been specifically developed for the $H_2S$ removal from biogas. Both of them combine a chemical scrubber and a bioreactor (Fortuny et al., 2008). Additionally, the Thiopaq® process includes a settler to separate the formed solid $S^0$ from the liquid phase (http://www.paques.nl/). Besides as fertilizer, $S^0$ can be applied in bioleaching and processes, and is suitable for producing $H_2SO_4$ (Kleinjan, 2005).

In microaerobic reactors, the sulphide-oxidising population specifically develop in the headspace, and as a result, the biogas desulphurisation and the consequent $S^0$ deposition both take place in this area (Díaz et al., 2010; Kobayashi et al., 2012). Therefore, periodic cleaning is required in order to prevent clogging problems and ensure stable $H_2S$ removal efficiency, which in turn implies extra costs (Díaz and Fdz-Polanco, 2011).

According to the above observations, a potentially ideal external process for the removal of $H_2S$ from biogas would integrate the simplicity of the desulphurisation process in microaerobic digesters. On this basis, a new biological technology was devised. The objective of this research was to evaluate the feasibility of this system, and to carry out a preliminary assessment of some of the variables that could affect its performance.

## 2. Methods

### 2.1. Experimental set-up and performance monitoring

In this research, a variable flow rate of biogas with a changeable $H_2S$ content from a pilot-scale anaerobic digester of sewage sludge was treated (Table 1). As illustrated in Fig. 1, it was mixed with pure $O_2$, the dose being regulated by a mass flow controller (Bronkhorst EL-FLOW Select). Prior to entering a thermostated chamber, the untreated biogas was analysed by a VARIAN MicroGC in terms of $CH_4$, $CO_2$, $N_2$, $O_2$, $H_2$ and $H_2S$ according to Díaz et al. (2010). Subsequently, it passed through a 10 L-system called a microaerobic desulphurisation unit (MDU), where it was desulphurised in the presence of 1 L of inoculum taken from the anaerobic digester used as the biogas source. Thus, considering the amount of biogas yet to be treated, a biogas residence time (BRT) of approximately 60 min would be maintained. This period was sufficient to achieve an efficient hydrogen sulphide removal in a microaerobic reactor (Ramos et al., 2012). Then, the biogas composition was determined again. Finally, the biogas flow rate leaving the system was measured volumetrically by water displacement. Treatment performance was assessed by calculating mass loading rate (MLR), elimination capacity (EC) and removal efficiency (RE).

**Table 1**
Sequence of variations applied and desulphurisation performance.

| Period | Reaction media | Biogas injection point | Temperature (°C) | BRT (min) | Inlet $H_2S$ (% v/v) | Outlet $H_2S$ (% v/v) |
|---|---|---|---|---|---|---|
| A | Water | Liquid phase | 34 | 68 | 0.30 | 0.26 |
| B1 | Digestate | Liquid phase | 34 | 68 | 0.33 | 0.01 |
| B2 | Digestate | Gas space (middle) | 34 | 61 | 0.29 | 0.01 |
| B3 | Digestate | Gas space (top) | 34 | 74 | 0.28 | 0.01 |
| B4 | Digestate | Gas space (top) | 29 | 88 | 0.37 | 0.05 |
| B5 | Digestate | Gas space (top) | 34 | 72 | 0.32 | 0.02 |
| B6 | Digestate | Gas space (top) | 20–35 | 80 | 0.29 | 0.02 |
| B7 | Digestate | Gas space (top) | 34 | 84 | 0.30 | 0.02 |
| B8 | Digestate | Gas space (top) | 34 | 100 | 0.28 | 0.27 |

In order to increase the support area for SOB, wires, plastic tubing and paper strips (i.e., objects with low specific surface area) were tied to a small metal grid hanging from the top of the MDU (Fig. 1). It should be noted that they were partially submerged in the liquid phase.

### 2.2. Chemical analysis

The inoculum was characterised in terms of total and volatile solids (TS and VS, respectively) according to APHA (1998).

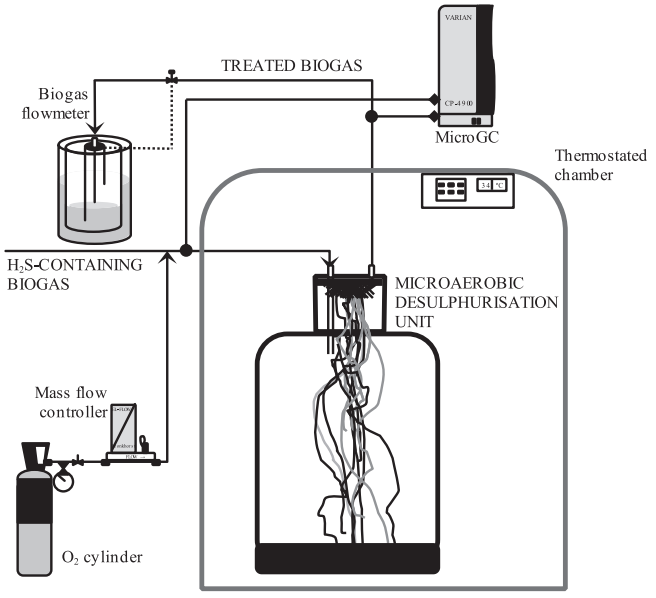


**Fig. 1.** MDU diagram.

Additionally, C and S concentrations in the inoculum and $S^0$-rich biomass accumulated inside the MDU were determined by a LECO CS-225.

### 2.3. Microbiological analysis

In order to identify the bacteria present in the MDU, an inoculum sample was drawn and frozen immediately after the system shutdown. DNA extraction, polymerase chain reaction (PCR) amplification and denaturing gradient gel electrophoresis (DGGE) analysis were carried out according to Lebrero et al. (2012). The universal bacterial primers 968-F-GC and 1401-R were used for the PCR amplification procedure (Sigma–Aldrich, St. Louis, MO, USA).

The DGGE profile was processed using the GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). After image normalisation, bands were defined by the programme search algorithm. Subsequently, the Shannon-Wiener diversity index ($H$) was determined according to the expression: $H = -\Sigma\ [P_i \ln(P_i)]$, where $P_i$ is the importance probability of the bands in a lane, and is calculated as $n_i/n$, where $n_i$ is the height of an individual peak, and $n$ the sum of all peak heights in the densitometric curves of the DGGE profile. Therefore, this index reflects both the sample richness (relative number of DGGE bands) and evenness (relative intensity of every band). According to McDonald (2003), it ranges from 1.5 to 3.5 (low and high species evenness and richness, respectively).

Based on a visual analysis of the gel, some DGGE bands were excised according to Lebrero et al. (2011). The closest matches to each sequenced band within a confidence level of 50%, and its respective taxonomic position were obtained by the Blast search tool at the National Centre for Biotechnology Information (McGinnis and Madden, 2004), and the Ribosomal Database Project (RDP) classifier tool (Wang et al., 2007), respectively. All the nucleotide sequences were deposited in the GenBank database under accession numbers KC306914 to KC306931.

## 3. Results and discussion

### 3.1. Feasibility of the system

An abiotic experiment was performed initially (period A in Table 1). It was contemplated that some SOB could reach the MDU carried along by the biogas. Therefore, the objective of that stage was to evaluate $H_2S$ removal by bacteria reaching the system in this way. In order to provide sufficient moisture conditions, 1 L of water was introduced into the MDU. It must be noted that a significant $H_2S$ removal by adsorption and/or absorption during the stage A was ruled out by passing biogas through the system during the preceding 3 days.

RE ranged between 23% and 31% during the first 2 days of the period A (Fig. 2b). However, $O_2$ consumption was negligible (or even zero), which pointed to metal sulphides formation on the grid surface (Fig. 2a). Furthermore, RE was seen to decline thereafter, and although a higher $O_2$ flow was supplied to increase the $O_2$ availability, RE continued decreasing, suggesting saturation of the metal surface.

Subsequently, (period B1 in Table 1), the MDU was inoculated with digestate from the well-functioning bioreactor used as the biogas source, which operated under anaerobic and microaerobic conditions intermittently over the months preceding this study. The TS and VS content of the inoculum is shown in Table 2.

In B1, a significant linear correlation was observed between MLR and EC (Fig. 3a). As a result, REs higher than 94% were

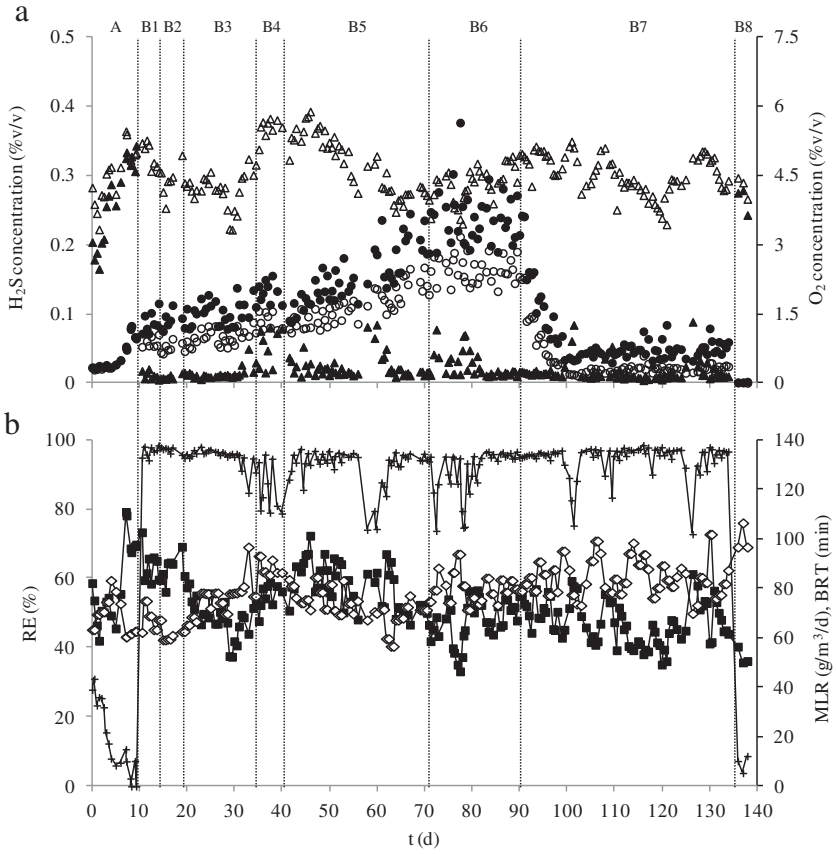


**Fig. 2.** (a) Input $H_2S$ (Δ) and $O_2$ (●) concentrations, and output $H_2S$ (▲) and $O_2$ (o) concentrations, and (b) RE (+), MLR (■) and BRT (◇) during the research.

**Table 2**
Elemental composition of the inoculum and $S^0$-rich biomass deposited in the MDU.

| | Inoculum | | Walls | Sheets |
|---|---|---|---|---|
| | After | Before | | |
| C (% w/w) | 30 | 13 | 6 | 1 |
| S (% w/w) | 1 | 50 | 90 | 98 |
| TS (g/L) | 24 | 41 | – | – |
| VS (g/L) | 15 | 27 | – | – |

achieved. Moreover, the inlet and outlet $O_2$ concentrations in the biogas suggested $H_2S$ oxidation (Fig. 2a and b), which was confirmed in the last period of the research (period B8 in Table 1). Once the micro-oxygenation had stopped, the biogas sulphide content leaving the MDU increased rapidly. In B8, REs of 4–8% were recorded, and the $O_2$ consumption was negligible. Since the inlet $H_2S$ concentration could frequently fluctuate due to the biogas source, 8% was not considered to be a significant removal.

The desulphurisation process in the MDU was considered to have occurred due to both chemical and biological reactions; some substances present in the inoculum could certainly have catalysed the chemical mechanisms (Kleinjan, 2005).

### 3.2. Effect of operational conditions

#### 3.2.1. Biogas injection point

Once the feasibility of the MDU was confirmed, the effect of the biogas inlet point on its performance was studied. In B2 and B3, the biogas ceased to be bubbled into the sludge, and was injected at the middle and at the top of the gas space, respectively (Table 1). A substantial reduction in RE was expected in relation to B1; besides a lower transfer of $O_2$, it was anticipated that a substantial flow rate of untreated biogas left the MDU directly (especially in B3). However, RE remained fairly stable until the 31st day (Fig. 2b). Moreover, similar ECs were reached at almost equal MLRs (Fig. 3a). In this regard, it must be noted that as in B1, both parameters increased and decreased simultaneously. Therefore, the overall MDU performance was concluded to be independent of the biogas injection point.

#### 3.2.2. Residence time and $H_2S$ content of biogas

The average RE from B1 to B3 was 96%. Such successful performance was achieved under variable BRT, which specifically ranged from 59 to 97 min (Fig. 2b). Furthermore, the biogas $H_2S$ content during B1, B2 and B3 oscillated between 0.22% v/v and 0.35% v/v (Fig. 2a). Hence, the MDU proved its robustness towards changes in both the flow rate of biogas yet to be treated and the inlet concentration of $H_2S$. Additionally, it was deduced that $O_2$ availability was not a factor limiting the process.

#### 3.2.3. Inlet $O_2/H_2S$ ratio

From B1 to B3, it was observed that the $O_2/H_2S_{consumed}$ ratio (v/v) increased and decreased concurrently with the $O_2/H_2S_{supplied}$ ratio (v/v) (Fig. 3b). It was at least partially attributed to variations in the $SO_4^{2-}/S^0_{produced}$ ratio. Fortuny et al. (2008) showed that even at an $O_2/H_2S_{supplied}$ ratio of 1.6, 3–4% of the $H_2S$ removed in a laboratory-scale biotrickling filter was oxidised to $SO_4^{2-}$, and its production increased up to 70% when that ratio was raised to 5.3; obviously, the rest of the $H_2S$ was converted into $S^0$. This agrees with the relatively high $O_2/H_2S_{consumed}$ ratios maintained in this study even at the lowest $O_2/H_2S_{supplied}$ ratios. Nonetheless, it must be considered that reactions such as those showed in Eqs. (2) and (4) could also take place. It is worth noting the ruling out of the possibility that some species of SOB might become dominant according to the $O_2/H_2S_{supplied}$ ratio; SOB have been proved to be capable of switching from $SO_4^{2-}$ to $S^0$ formation really fast, and vice versa (Janssen et al., 1995). As a result, the system performance was found to withstand variations in $O_2/H_2S_{supplied}$ ratio.

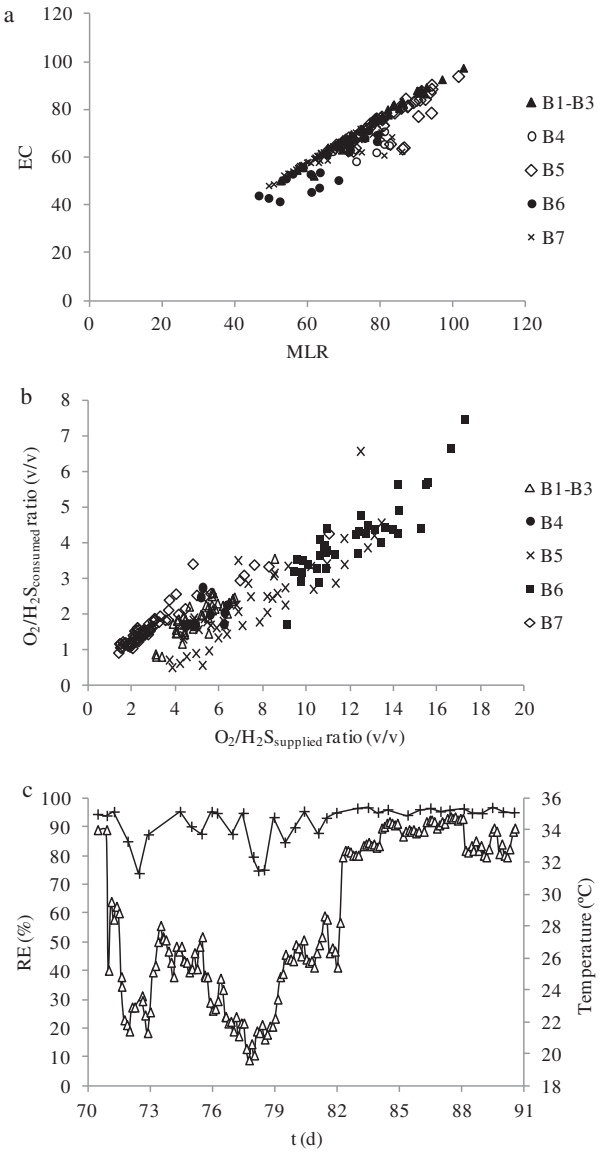


**Fig. 3.** (a) MLR against EC, and (b) $O_2/H_2S_{supplied}$ ratio against $O_2/H_2S_{consumed}$ ratio in the different experimental periods. (c) RE (+) and temperature (Δ) in B6.

In order to determine the minimum $O_2/H_2S_{supplied}$ ratio needed in order to achieve high REs, the $O_2$ supply was gradually lowered during the period B7 (Table 1). The decline in this variable is reflected in Fig. 2a. As the $O_2/H_2S_{supplied}$ ratio decreased, the efficiency of $O_2$ utilisation inside the MDU increased; hence the relatively high relationship between the $O_2/H_2S_{supplied}$ ratio and the $O_2/H_2S_{consumed}$ ratio in relation to the preceding experimental periods. Specifically, the $O_2$ percentage leaving the system decreased by approximately 23%; in the preceding periods, only 30–40% of the $O_2$ supplied was consumed inside the MDU. As a result, 95% of the $H_2S$ reaching the system in B7 was removed (Fig. 2b). The decreases in RE were attributed to insufficient $O_2$ availability; specifically, at $O_2/H_2S_{supplied}$ ratios below 2.1, RE ranged from 73% to 97%.

A significant change in the relationship between the $O_2/H_2S_{supplied}$ ratio and the $O_2/H_2S_{consumed}$ ratio was observed when the $O_2/H_2S_{supplied}$ ratio decreased below approximately 4.0 (Fig. 3b).

According to Janssen et al. (1995), it could be related, at least in part, to variations in the relative contribution of the mechanisms of $H_2S$ oxidation. They pointed out that limitations in biological activity can take place at low $H_2S/O_{2supplied}$ ratios, and as a result, rates of chemical $H_2S$ oxidation can rise.

### 3.2.4. Temperature

Temperature was maintained at 29 °C from the 35th to 40th day (period B4 in Table 1). RE decreased in relation to the last days of the period B3 (86 against 91%), when the system operated at 34 °C (Fig. 2b). In fact, it started to increase immediately after restoring the temperature to 34 °C (period B5 in Table 4), and continued rising during the following 2 days, when a RE of 96% was achieved. Nevertheless, values of up to 86% were reached shortly afterwards (44th day). In fact, taking into account the profile of this parameter during B5, the deterioration in the system performance during B4 could not be unequivocally linked with the reduction in temperature.

It is worth noting that a lower correlation between the $O_2/H_2S_{supplied}$ ratio and the $O_2/H_2S_{consumed}$ ratio was observed during B4 and a part of B5 (until the 51st day). Additionally, the relationship between these variables decreased significantly once the temperature was restored to 34 °C. The average $O_2$ percentage consumed inside the system up until the 45th day was only 17%; it re-normalised thereafter. Therefore, temperature did seem to influence the SOB population.

In order to further assess the influence of temperature on the process performance, the MDU was subject to continuous temperature fluctuations from the 70th day onwards (period B6 in Table 1). As shown in Fig. 2b, although the average temperature was significantly lower during the first 11 days of the B6 period (25 °C) than in the B4 period (29 °C), even reaching 20 °C (it specifically oscillated between 20 and 29 °C), the average RE was slightly higher in B6 (88 against 86%). Hence, further research about the temperature effect on the MDU performance is needed.

The average RE in B6, with temperatures ranging from 20 to 35 °C, was 91% (Fig. 3c). It must be noted that the lowest REs (≈75%) coincided with the largest temperature drops, and above 32 °C, this parameter remained over 95%. Therefore, the MDU demonstrated considerable robustness towards fluctuations. Moreover, it is worth mentioning that the correlation between the $O_2/H_2S_{consumed}$ ratio and the $O_2/H_2S_{supplied}$ ratio was fairly changeable in B6 (Fig. 3b), which corroborated the evidence that temperature did affect the process.

### 3.3. $S^0$ deposition, recovery and characterisation

Since the walls of the MDU were translucent, $S^0$ accumulation in the walls could be observed. Immediately after the start-up, $S^0$ started to accumulate in the area nearest the liquid media; it specifically covered the sludge deposited on the MDU walls during the inoculation process and due to sludge splashes resulting from the biogas bubbling (Fig. 4a). Obviously, SOB grew there due to humidity and the availability of nutrients. In fact, when the system was opened on the 14th day, $S^0$ was also observed on both the sludge surface (Fig. 4b) and the additional supports (namely, in the area nearest the liquid media).

$S^0$ continued accumulating on the walls during B2 (Fig. 4c). Nevertheless, the deposition rate slowed thereafter; as reflected in Fig. 4f, minor differences were found between the system state on the 19th day and the 138th day. This was attributed to the depletion of both humidity and nutrients. Sludge reached those areas only occasionally, due to the slight movements performed for the visual examination of the MDU, which also caused sporadic contacts between the walls and the additional supports; hence the presence of $S^0$ in the areas furthest from the liquid phase (Fig. 4f). Along with the previous observations, it suggested that most of this by-product was accumulating on the gas–liquid interface, where growth conditions were more favourable. That was indeed confirmed later (Fig. 4d and e). Note that the degree of $S^0$ accumulation on the extra supports hardly changed in relation to the 14th day (Fig. 4g).

$S^0$ particles started to consolidate into thin sheets before the 60th day (Fig. 4e); many of them were found immersed in the sludge on the last day of the research (Fig. 4h). They appeared to form on the liquid surface and settle at a later time. Hence the morphological differences observed between its two sides; one side was smooth and the other was rough (Fig. 4i). With a magnifying

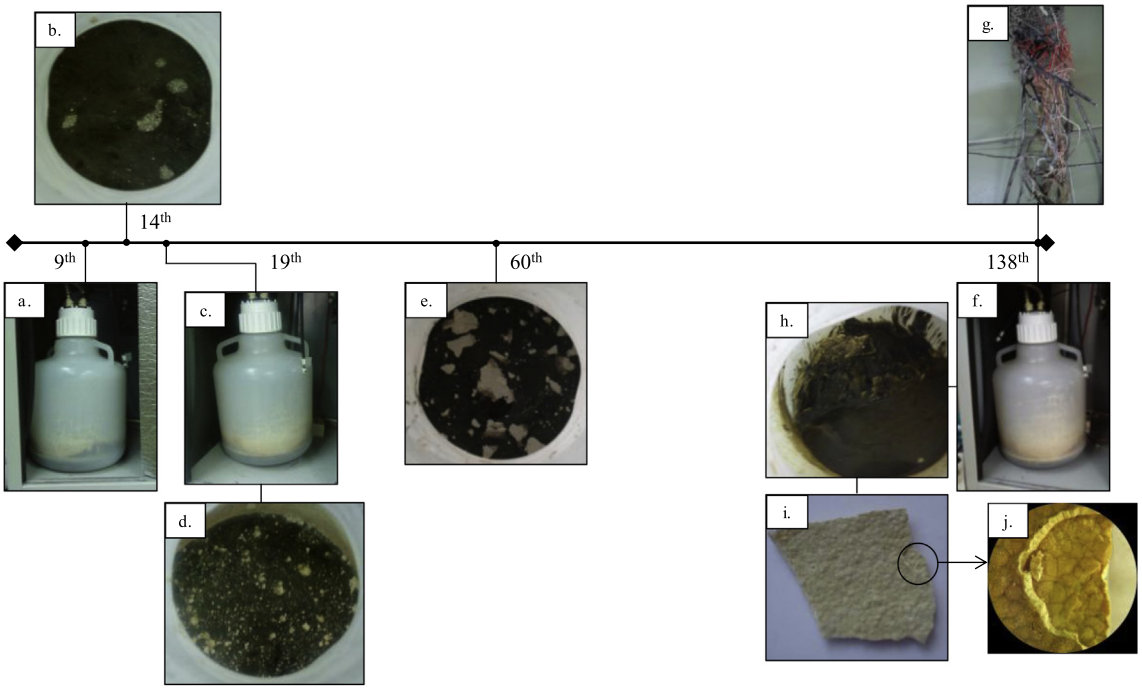


**Fig. 4.** MDU state with time.

glass, it could be shown that $S^0$ sheets were formed from multiple layers (Fig. 4j).

The largest possible amount of $S^0$ sheets was collected, cleaned and dried immediately after the system shutdown. Elemental analysis revealed a content of S and C of 98% w/w and 1% w/w, respectively (Table 2). Therefore, assuming that all the $H_2S$ removed during the study was partially oxidised to $S^0$, the recovered sheets accounted for 60%. The amount of $S^0$-rich biomass collected from both the walls and the additional supports was negligible. Nonetheless, it must be highlighted that due to their brittleness, many fragments of different sizes were resealed from them into the inoculum. As a result, the inoculum had a heterogeneous content of these $S^0$ structures, which explained the extremely high S content of the sample analysed. It must be noted that 0.8 L of inoculum was measured at the end of the research. This reduction in the sludge volume was consistent with the substantial increase in the concentration of both TS and VS. Moreover, it should be indicated that the C concentration in the inoculum decreased considerably, which suggested that the inoculum was further degraded inside the MDU. Therefore, the MDU exhibited a high capability to oxidise $H_2S$ to easily recoverable $S^0$; large aggregates with good settling properties were formed.

### 3.4. Characterisation and diversity of the bacterial community

From the DGGE gel, 17 bands belonging to four different phyla were sequenced (Table 2): *Proteobacteria* (6 bands), *Firmicutes* (7 bands), *Actinobacteria* (1 band) and *Verrucomicrobia* (1 band), while two bands remained unclassified. In general, the results given by the RDP classifier tool were consistent with the results obtained from the Blast search tool (Table 3).

**Table 3**
RDP classification of the DGGE bands sequenced with a 50% of confidence level, and corresponding matches according to the Blast search tool, with their similarity percentages, and environments from which they were retrieved.

| Taxonomic placement | Band No. | Closest relatives (accession No.) | Similarity (%) | Source of origin |
|---|---|---|---|---|
| Phylum *Proteobacteria* | 1 | Uncultured bacterium (AB286499) | 95 | Activated sludge |
| Class *Epsilonproteobacteria* | | | | |
| Order *Campylobacteraceae* | | | | |
| Family *Campylobacteraceae* | | | | |
| Genus *Arcobacter* | 2 | Uncultured *Arcobacter* sp. (HQ392829) | 99 | Headspace of a digester of sewage sludge under microaerobic conditions |
| | 3 | Uncultured *Arcobacter* sp. (HQ392829) | 100 | Headspace of a digester of sewage sludge under microaerobic conditions |
| Family *Helicobacteraceae* | | | | |
| Genus *Sulfuricurvum* | 4 | Uncultured *Epsilonproteobacterium* (DQ295695) | 99 | Floating microbial mat in sulfidic groundwater (Movile Cave) |
| Class *Gammaproteobacteria* | | | | |
| Order *Pseudomonadales* | | | | |
| Family *Moracellaceae* | | | | |
| Genus *Acinetobacter* | 5 | Uncultured *Acinetobacter* sp. (EU567041) | 95 | Oil field soil |
| | 6 | Uncultured *Acinetobacter* sp. (JN679106) | 99 | Membrane bioreactor |
| Phylum *Firmicutes* | | | | |
| Class *Clostridia* | | | | |
| Order *Clostridiales* | 7 | Uncultured bacterium (FJ978625) | 96 | Faeces |
| Family *Peptostreptococcaceae* | | | | |
| Genus *Clostridium XI* | 8 | Uncultured bacterium (JQ085717) | 99 | Anaerobic digester |
| Family *Syntrophomonadaceae* | | | | |
| Genus *Thermohydrogenium* | 9 | Uncultured bacterium (HE681331) | 96 | Bioreactor |
| | 10 | Uncultured bacterium (GQ259594) | 96 | Bioreactor |
| Family *Clostridiales incertae sedis* | | | | |
| Genus *Soehngenia* | 11 | Uncultured bacterium (AB114320) | 90 | Thermophilic anaerobic municipal solid waste digester |
| Family *Lachnospiraceae* | 12 | *Clostridium* sp. (GU247219) | 93 | Waste water of a pesticides firm |
| Class *Bacilli* | | | | |
| Order *Bacillales* | 13 | Uncultured *Alicyclobacillus* sp. (HQ392831) | 100 | Headspace of a digester of sewage sludge under microaerobic conditions |
| Phylum *Actinobacteria* | | | | |
| Class *Actinobacteria* | | | | |
| Subclass *Actinobacteridae* | | | | |
| Order *Actinomycetales* | | | | |
| Suborder *Corynebacterineae* | | | | |
| Family *Dietziaceae* | | | | |
| Genus *Dietzia* | 14 | Uncultured *Dietzia* sp. (JN882177) | 95 | Crude oil samples |
| Phylum *Verrucomicrobia* | | | | |
| Class *Optitutae* | | | | |
| Order *Optitutales* | | | | |
| Family *Optitaceae* | | | | |
| Genus *Alterococcus* | 15 | Uncultured *Verrucomicrobia* (CU918353) | 99 | Mesophilic anaerobic digester which treats municipal wastewater sludge |
| Unclassified Bacteria | 16 | Uncultured bacterium (AB175392) | 99 | Mesophilic anaerobic BSA digester |
| | 17 | Uncultured *Firmicutes* (CU923016) | 97 | Mesophilic anaerobic digester which treats municipal wastewater sludge |

*Proteobacteria* was the predominant phylum of SOB inside the MDU, with the genera *Acinetobacter*, *Arcobacter* and *Sulfuricurvum* as representatives. A strain of *Acinetobacter* sp. (DGGE bands 4 and 5) was partially responsible for $H_2S$ removal in the bioscrubber system developed by Potivichayanon et al. (2006) in the 28–33 °C temperature range. Members of the genus *Arcobacter* (DGGE bands 2 and 3) were found in the headspace of microaerobic digesters treating dairy cow manure (Kobayashi et al., 2012) and sewage sludge (Díaz et al., 2010) at 35 °C. Finally, a bacterium belonging to the genus affiliated to the DGGE band 4, *Sulfuricurvum kujiense*, was described by Kodama and Watanabe (2004) as a chemolithoautotrophic SOB capable of oxidising $H_2S$, $S^0$ and $S_2O_3^{2-}$ under microaerobic conditions in the 10–35 °C and 6–8 temperature and pH range, respectively.

From the *Firmicutes* phylum, the Blast search tool assigned the DGGE band 13 to the genus *Alicyclobacillus* with an identity of 100%. Species belonging to this genus were reported in the study of Díaz et al. (2010).

Despite the stringent growing conditions inside the MDU, the Shannon–Wiener diversity index was 3.5, which revealed the high bacterial community diversity of the inoculum.

### 3.5. Competitive advantages of the MDU

Although further research is being carried out in order to optimise operational conditions and thus evaluate the maximum removal capacity of the MDU, a preliminary comparative assessment in relation to the current biotechnologies for biogas desulphurisation can be performed based on the results of this study.

In contrast to other bioprocesses involving a chemically-based previous step (such as a scrubber), the MDU is a single-stage process, which could translate into lower space requirements and capital investment. It must be taken into account that additional equipment such as pumps, storage tanks or settlers are not needed. With regard to this, it should be emphasised that the $S^0$ formed during this research settled by itself inside the MDU.

Due to the extremely simple configuration of the MDU, its construction would be easier than that of other types of bioreactors. Furthermore, it is worth noting that, whereas the biomass growth could increase the treatment capacity of the MDU over time, this is a common problem in packed media-based bioreactors (clogging) (Kennes et al., 2009). Therefore, the MDU would largely reduce the frequency of supervision tasks and shutdowns.

The MDU proved to be at least in the medium-term self-sustaining and self-regulating. Conversely, other bioprocess require a strict control of the operational conditions, such as pH, moisture content or nutrient balance, which in turn entails the need for specific instrumentation and frequent monitoring tasks. Hence, besides the chemicals required in the previous chemical process, they require nutrients solutions. Therefore, liquid effluents are constantly generated. Overall, the MDU could largely reduce the capital cost, as well as the outlays for operation and maintenance.

## 4. Conclusions

A novel alternative to the conventional biotechniques for biogas desulphurisation was developed. 1 L of digested sludge was proved to be an efficient reaction media for $H_2S$ removal in a 10 L-system during 128 d. Although the MDU was subjected to several fluctuations in BRT, concentration of $H_2S$, $O_2/H_2S_{supplied}$ ratio, and temperature, and neither nutrients nor water were added, the average RE was 94%. The system presented a really high bacterial diversity, including at least three genera of SOB. $S^0$ was the major by-product; it accumulated in the form of extremely pure multilayered sheets, which settled to the bottom of the system.

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## References

APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association/American Water Works Association/Water Environmental Federation, Washington, DC, USA.

Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. 34, 755–781.

Burgess, J.E., Parsons, S.A., Stuetz, R.M., 2001. Developments in odour control and waste gas treatment biotechnology: a review. Biotechnol. Adv. 19, 35–63.

Deublein, D., Steinhauser, A., 2008. Biogas from Waste and Renewable Resources: An Introduction. Wiley-VCH, Weinheim.

Díaz, I., Pérez, S.I., Ferrero, E.M., Fdz-Polanco, M., 2010. Effect of oxygen dosing point and mixing on the microaerobic removal of hydrogen sulphide in sludge digesters. Bioresour. Technol. 102, 3768–3775.

Díaz, I., Fdz-Polanco, M., 2011. Robustness of the microaerobic removal of $H_2S$ from biogas. Water Sci. Technol. 65, 1368–1374.

Duan, H., Yan, R., Koe, L.C., 2005. Investigation on the mechanism of $H_2S$ removal by biological activated carbon in a horizontal biotrickling filter. Appl. Microbiol. Biotechnol. 69, 350–357.

Fortuny, M., Baeza, J.A., Gamisans, X., Casas, C., Lafuente, J., Deshusses, M.A., Gabriel, D., 2008. Biological sweetening of energy gases mimics in biotrickling filters. Chemosphere 71, 10–17.

Janssen, A.J.H., Sleyster, R., van der Kaa, C., Jochemsen, A., Bontsema, J., Lettinga, G., 1995. Biological sulphide oxidation in a fed-batch reactor. Biotechnol. Bioeng. 47, 327–333.

Kennes, C., Rene, E.R., Veiga, M.C., 2009. Bioprocesses for air pollution control. J. Chem. Technol. Biotechnol. 84, 1419–1436.

Kleinjan, W., 2005. Biologically Produced Sulfur Particles and Polysulfide Ions: Effects on a Biotechnological Process for the Removal of Hydrogen Sulfide from Gas Streams. PhD Thesis. Wageningen University, Wageningen, The Netherlands.

Kobayashi, T., Li, Y.Y., Kubot, A.K., Harada, H., Maeda, T., Yu, H.-Q., 2012. Characterization of sulfide-oxidizing microbial mats developed inside a full-scale anaerobic digester employing biological desulfurization. Appl. Microbiol. Biotechnol. 93, 847–857.

Kodama, Y., Watanabe, K., 2004. *Sulfuricurvum kujiense* gen. nov., sp. nov., a facultatively anaerobic, chemolithoautotrophic, sulfur-oxidizing bacterium isolated from an underground crude-oil storage cavity. Int. J. Syst. Evol. Microbiol. 54, 2297–2300.

Lebrero, R., Rodríguez, E., García-Encina, P.A., Muñoz, R., 2011. A comparative assessment of biofiltration and activated sludge diffusion for odour abatement. J. Hazard. Mater. 190, 622–630.

Lebrero, R., Rodríguez, E., Estrada, J.M., García-Encina, P.A., Muñoz, R., 2012. Odor abatement in biotrickling filters: effect of the EBRT on methyl mercaptan and hydrophobic VOCs removal. Bioresour. Technol. 109, 38–45.

McDonald, G., 2003. Biogeography: Introduction to Space. Time and Life, Wiley, New York.

McGinnis, S., Madden, T.L., 2004. BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Res. 32, W20–W25.

Noyola, A., Morgan-Sagastume, J.M., López-Hernández, J.E., 2006. Treatment of biogas produced in anaerobic reactors for domestic wastewater: odor control and energy/resource recovery. Rev. Environ. Sci. Biotechnol. 5, 93–114.

Potivichayanon, S., Pokethitiyook, M., Kruatrachue, M., 2006. Hydrogen sulfide removal by a novel fixed-film bioscrubber system. Process Biochem. 41, 708–715.

Pöschl, M., Warda, S., Owende, P., 2010. Evaluation of energy efficiency of various biogas production and utilization pathways. Appl. Energy 87, 3305–3321.

Ramos, I., Díaz, I., Fdz-Polanco, M., 2012. The role of the headspace in hydrogen sulfide removal during microaerobic digestion of sludge. Water Sci. Technol. 66, 2258–2264.

Rasi, S., Läntelä, J., Rintala, J., 2011. Trace compounds affecting biogas energy utilisation – a review. Energy Convers. Manage. 52, 3369–3375.

Stams, A.J.M., Oude Elferink, S.J.W.H., Westermann, P., 2003. Metabolic interactions between methanogenic consortia and anaerobic respiring bacteria. Adv. Biochem. Eng. Biotechnol. 81, 31–56.

Syed, M., Soreanu, G., Faletta, P., Béland, M., 2006. Removal of hydrogen sulfide from gas streams using biological processes – a review. Canadian. Biosyst. Eng. 48, 2.1–2.14.

Tang, K., Baskaran, V., Nemati, M., 2009. Bacteria of the sulphur cycle: an overview of microbiology, biokinetics and their role in petroleum and mining industries. Biochem. Eng. J. 44, 73–94.

van der Zee, F.P., Villaverde, S., García-Encina, P.A., Fdz-Polanco, F., 2007. Sulphide removal by moderate oxygenation of anaerobic sludge environments. Bioresour. Technol. 98, 518–524.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.

Weiland, P., 2010. Biogas production: current state and perspectives. Appl. Microbiol. Biotechnol. 85, 849–860.

## Web reference

http://www.paques.nl/ (accessed 20.04.13).

# 8 Performance evaluation of the microaerobic desulphurisation unit under different operational conditions

# Performance evaluation of the microaerobic desulphurisation unit under different operational conditions

## Abstract

The microaerobic desulphurisation unit (MDU) is a promising alternative to the conventional biotechnologies for $H_2S$ removal from biogas. A 1.0L-MDU with 0.6L of microaerobic biomass was tested for its ability to desulphurise biogas under different inlet concentrations and mass loading rates (MLR) of $H_2S$, biogas residence times (BRT), $O_2/H_2S$ supplied ratios, and temperatures. High removal efficiencies (97-99%) were achieved under all the operational conditions set. At inlet $H_2S$ concentrations of approximately 0.48%v/v, MLR of 0.7kg/$m^3$/d, BRT of 12min, $O_2/H_2S$ supplied ratio (v/v) of 1.8 and 35ºC, almost equal removal efficiencies were achieved in a 0.5L-MDU with 0.1L of microaerobic inoculum. Although temperature (20-35ºC) did not have a significant effect on the system performance during steady state operation, relatively high temperatures at the start-up could be key to achieving successful operation. $S^0$ accumulated on the liquid interface and the walls of the gas space, which highlighted the importance of promote SOB growth on these areas. The design of this system has to be optimised accordingly.

## 1. Introduction

Biogas produced from anaerobic digestion is one of the most favourable bioenergy forms mainly because of the high net energy yields and the substrate flexibility. Currently, biogas production is mainly based on sewage sludge digestion in municipal wastewater treatment plants (WWTPs) (Börjesson and Mattiasson, 2007), and the most popular biogas utilisation pathway is the production of combined heat and power (CHP) by means of internal combustion engines (Makaruk et al., 2010). Some of the produced heat and power is used by the biogas plant as process heating and to meet the electricity demand on site, and the rest can be sold to the grid and distributed through the district heating system to the consumers. After an enrichment and upgrading process (by which most of the $CO_2$ and the impurities are removed), the biogas (95-98% of $CH_4$) can be used for transport fuel production (Murphy et al., 2004) or injected to the natural gas grid (Holm-Nielsen et al., 2009).

Biogas consists of $CH_4$ (50-80%), $CO_2$ (20-50%) and traces of, for example, $H_2S$ (0-0.4%) (Lantz et al., 2007). This is one of the most common reduced S-compounds present in biogas (Maestre et al., 2010), whose concentration depends mainly on the raw material (Fernández et al., 2013). $H_2S$ is a colourless, toxic and flammable gas with a characteristic odour of rotten eggs (Ramírez et al., 2009) that shortens she combustion engines life-span by corrosion and, upon combustion, forms harmful $SO_2$ (Maestre et al., 2010). Therefore, in typical boilers and internal combustion engines, the specifications are that $H_2S$ content should be no more than 0.10%v/v (Rasi et al., 2011).

Biogas desulphurisation methods are classified into two categories: those entailing physicochemical phenomena (reactive or non-reactive absorption, and reactive or non-reactive adsorption) and those involving biological processes ($H_2S$ consumption by bacteria resulting in less harmful and odorless compounds) (Abatzoglou and Boivin, 2009). Physicochemical processes are in common use today, but biological desulphurisation is an increasingly adopted alternative due to economical and environmental reasons (Potivichayanon et al., 2006). Biotechnologies offer removal efficiencies (REs) higher than 90% under relatively low temperatures (15-35ºC), optimum pH ranges between 6.8 and 7.0, and atmospheric pressure (Vergara-Fernández et al., 2007).

As the physiochemical techniques, biological methods for $H_2S$ removal can be applied at different levels: during the anaerobic process itself (a), or at a later process unit (b). Approach (a) implies injecting limited amounts of air or $O_2$, or nitrate or nitrite directly into the anaerobic digester, namely, implementing microaerobic or microanoxic conditions, respectively (Cirne et al., 2008). Approach (b) implies the utilisation of an additional treatment unit, being biofilters, biotrickling filters, and bioscrubbers the most popular types of bioreactors. As approach (a), $H_2S$ removal in these systems is generally based on aerobic methods (Fernández et al., 2013).

The basic mechanisms of aerobic $H_2S$ removal in all the biological processes are the same. After absorption (Eq. 1), species of aerobic sulphide-oxidising bacteria (SOB) such as *Thiobacillus*, *Acidithiobacillus, Halothiobacillus*, *Thiomonas* and *Thiofaba* (Díaz et al., 2010; Lin et al., 2013; Rodríguez et al., 2012) oxidise sulphide according to Eq. 2 and 3 (Rodríguez et al., 2013). The ratio of $O_2$ to $H_2S$ is the key parameter determining the $SO_4^{2-}/S^0$ produced ratio (Fortuny et al., 2008).

$H_2S \leftrightarrow HS^- + H^+$ (1)

$HS^- + 0.5O_2 \rightarrow S^0 + OH^-$ (2)

$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+$ (3)

Abiotic oxidation of sulphide to $S_2O_3^{2-}$ (Eq. 4) can take place in parallel with the above reactions, and this compound can be in turn biologically oxidised to $SO_4^{2-}$ according to Eq. 5 (Fortuny et al., 2011). Depending on the redox conditions, further oxidation of $S^0$ to $SO_4^{2-}$ (Eq. 6) can occur if sulphide is limited (Fortuny et al., 2011).

$2H_2S + 2O_2 \rightarrow S_2O_3^{2-} + H_2O + 2H^+$ (4)

$S_2O_3^{2-} + 2O_2 + H_2O \rightarrow 2SO_4^{2-} + 2H^+$ (5)

$S^0 + 3/2O_2 \rightarrow H_2O + SO_4^{2-} + 2H^+$ (6)

Despite its several advantages, there are many fewer full-scale bioscrubbers in operation than biofilters and bioscrubbers. This is probably related to the excessive biomass growth therein and the consequent high amounts of sludge needing for disposal, and the fact that two process units (absorption column and bioreactor) are required instead of one (Burgess et al., 2001; Kennes et al., 2009; Mudliar et al., 2010). Moreover, the solubility of hydrogen sulphide limits the applicability of bioscrubbing for its removal (Ramírez et al., 2009). This technique has been reported to be useful for pollutants with a non-dimensional Henry's coefficient (*H*) lower than 0.01, while for $H_2S$, *H*=0.92 (at 25ºC) (Kennes et al., 2009; Mudliar et al., 2010). On the other hand, clogging is one of the most important drawbacks (if not the main one) in operation of biofilters and biotrickling filters (Estrada et al., 2013; Montebello et al., 2012; Rodríguez et al., 2013; Yang et al., 2010). Insufficient $O_2$ availability can intensify this problem due to $S^0$ formation (Fernández et al., 2013; Fortuny et al., 2008). Therefore, and also due to it can lead to the re-reduction of the oxidised S species, the existence of microaerobic conditions is avoided during biofiltration (Mudliar et al., 2010). As a result, the biogas is often severely diluted. During the experiments of Montebello et al. (2013), the dilution factor due to aeration in a biotrickling filter ranged from 6 to 32%. Similarly, the air concentration in the biogas treated by a DMT Bio-Sulfurex® biological desulphurisation reactor (full-scale biotrickling filter) was around 9%v/v (http://www.dirkse-milieutechniek.com). Stringent requirements of monitoring and control and complicated start-up in biofilters and biotrickling filters constitute other important limitations in the utilisation of these systems (Mudliar et al., 2010).

In contrast to the external biological techniques, the process of $H_2S$ removal from biogas during microaerobic digestion has been reported to be simple and economical (Abatzoglou and Boivin, 2009). It has been widely proved that microaerobic and anaerobic reactors yield equivalently (Díaz et al., 2010), or even further (Jenicek et al., 2010). However, the findings of Díaz (2010) indicate that the profitability of this method in comparison with other alternatives (specifically, ferric chloride addition to the digester and usage of an iron-sponge filter inoculated with thiobacteria) is contingent upon the required periodicity of headspace cleaning. This is due to the $H_2S$ oxidation takes place predominantly in the gas space and, as a result, $S^0$ accumulates there (Díaz et al., 2010; Ramos et al., 2012).

Based on the above observations, a new process for the removal of $H_2S$ from biogas was recently developed: the microaerobic desulphurisation unit (MDU, patent pending) (Ramos et al., 2013). This

novel external system integrates the simplicity of the desulphurisation process in microaerobic digesters. The objective of this research was to evaluate the characteristics of the biogas desulphurisation process in MDUs, and deduce the criteria for successful operation and optimum design of these systems.

## 2. Methods

### *2.1. Experimental set-up and process monitoring*

The experimental set-up was described in the previous study (Ramos et al., 2013). The biogas produced in a pilot-scale anaerobic reactor was mixed with $H_2S$ (to increase and control its sulphide content) and $O_2$ from two bottles (100% pure both). The $H_2S$ and the $O_2$ flow rate were regulated by the means of mass flow controllers. After being characterised by gas chromatography (GC) in terms of $CH_4$, $CO_2$, $N_2$, $O_2$, $H_2S$ and $H_2$ content, biogas was desulphurised in a MDU (0.5 or 1.0L) with 0.4L of gas space (Table 1) at temperatures between 20 and 35ºC, depending on the operational stage. As shown in Table 1, the system was inoculated with microaerobic or anaerobic biomass, and biogas entered therein from the top or the bottom. The anaerobic and the microaerobic sludge came from two pilot reactors treating municipal sewage sludge under mesophilic conditions, and was characterised in terms of total and volatile solids (TS and VS, respectively) by standard methods (APHA, 1998). The biogas leaving the system was characterised by GC and quantified by water displacement (±120mL).

It should be noted that the significant variations in the BRT reflected on Table 1 were due the variable organic load supplied to the reactor used as the biogas source. This in turn explains the fairly different inlet $H_2S$ concentrations recorded under the same MLR.

### *2.2. Experimental procedure*

The research was divided into three experiments. The specific operational conditions are shown in Table 1. Experiment 1 was carried out in a 0.5L-MDU with 0.1L of microaerobic sludge containing 29.5 and 17.4g/L of TS and VS, respectively, at 35ºC. Biogas entered and left the system by the top thereof. The objective was to evaluate the feasibility of the MDU itself, since the $H_2S$ mass loading rate (MLR) and the biogas residence time (BRT) were both significantly higher than in the previous study (Ramos et al., 2013).

**Table 1**. Operational conditions in the different experiments of the study.

| | **Experiment 1** | **Experiment 2** | | | | **Experiment 3** | |
|---|---|---|---|---|---|---|---|
| | | Period 1 | | | Period 2 | Period 1 | Period 2 |
| | | Phase 1 | Phase 2 | Phase 3 | | | |
| $V_{total}$ (L) | 0.5 | 1 | 1 | 1 | 1 | 1 | 1 |
| $V_{gas}$ (L) | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Biogas injection point | Top | Top | Top | Top-bottom | Bottom | Bottom | Bottom |
| Inoculum* | MA | MA | MA | MA | MA | AN | AN |
| Temperature (ºC) | 35 | 35 | 35 | 35 | 35-20 | 20-35 | 35-20 |
| BRT (min) | 12 | 12 | 19 | 23 | 37 | 40 | 18 |
| MLR ($kg/m^3/d$) | 0.7 | 0.7 | 1.0 | 1.3 | 0.7 | 0.7 | 0.7 |
| $H_2S$ (%v/v) | 0.47 | 0.49 | 1.31 | 1.77 | 1.28 | 1.22 | 0.64 |
| $O_2/H_2S_{supplied}$ (v/v) | 1.8-3.8 | 1.8 | 1.2-1.4 | 1.0-1.4 | 1.8 | 1.8 | 1.8 |

*MA=microaerobic; AN=anaerobic

Experiment 2 was carried out in a MDU with a total volume of 1.0L and 0.6L of microaerobic inoculum (31.0 and 17.3 of TS and VS, respectively). It was divided into two periods (Table 1). In Period 1, the response of the system to fluctuations in the inlet concentration and the MLR of $H_2S$ was studied. For this purpose, the MDU operated at 35ºC and three different MLRs and inlet $H_2S$ concentrations. Therefore, this period was in turn divided into three phases, according to the values of the aforementioned variables. As shown in Table 1, the biogas injection point was changed in Phase 3 and, thereafter, biogas bubbled into the inoculum. In Period 2, the MDU operated at different temperatures (20-35ºC) in order to evaluate the influence of this parameter in its steady-state performance.

In Experiment 3, a 1.0L-MDU with 0.6L of gas space was inoculated with anaerobic sludge (16.1 and 10.0g/L of TS and VS, respectively) in order to investigate the impact of the start-up temperature on the process efficiency (Table 1). For this purpose, the system was started-up at 20 (Period 1) and 35ºC (Period 2).

## 3. Results

### *3.1. Experiment 1: 0.5L-MDU with 0.1L of microaerobic sludge*

The results obtained in Experiment 1 are represented in Fig. 1. During the first 20h, the MDU operated without $O_2$ supply. Once $H_2S$ removal by non-oxidative mechanisms was ruled out, micro-oxygenation was started at an $O_2/H_2S_{supplied}$ ratio (v/v) of approximately 1.8. The $O_2$ flow rate was set according to the previous research (Ramos et al., 2013), in which $O_2/H_2S_{supplied}$ ratios (v/v) lower than 2.1 provided REs of up to 97%. Within 1h, the $H_2S$ concentration in the biogas leaving the system dropped to 0.22%v/v. Although the $O_2/H_2S_{supplied}$ ratio was raised, the outlet $H_2S$ concentration remained around that value until the $7^{th}$ day, thus maintaining approximately 56% of RE. As a result of the successive raises in the $O_2/H_2S_{supplied}$ ratio, the $O_2$ content of the outlet biogas

stream (from 0.9 to approximately 2.5%v/v) and the ratio of $O_2/H_2S_{consumed}$ both rose, which pointed to increased ratio of $SO_4^{2-}/S^0_{produced}$. This indicated that the $O_2$ availability was not limiting the performance of the process, which was also observed in the previous study (Ramos et al., 2013). It was indeed confirmed from the 14 day, when the $O_2/H_2S_{supplied}$ was maintained at 1.8 again. Regarding the BRT, it declined gradually from 14 to 12min until the 7$^{th}$ day, which did not seem to have any effect on the system performance either.

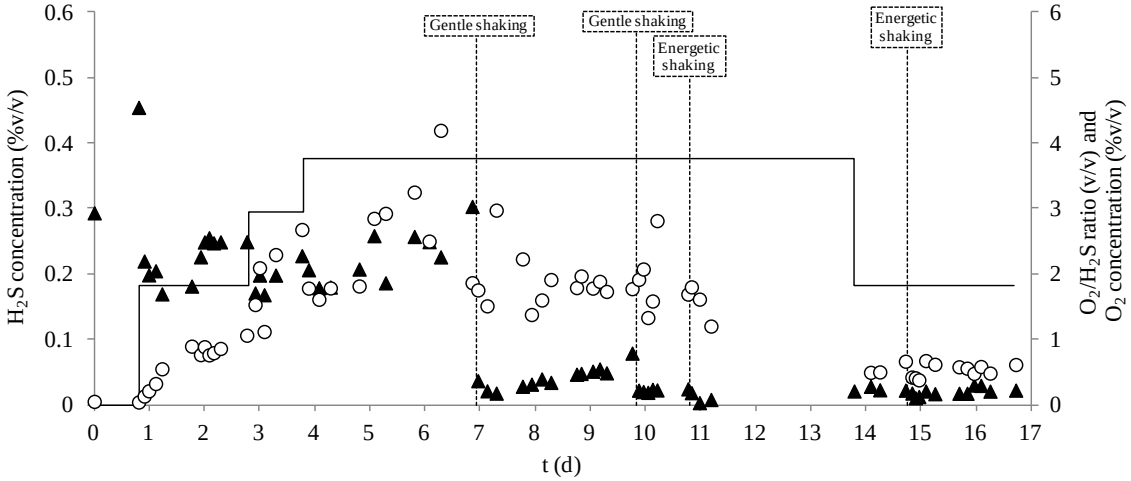


**Fig. 1**. Outlet $H_2S$ (▲) and $O_2$ (○) concentrations, and micro-oxygenation level (continuous line) in Experiment 1.

Fig. 2a shows the state of the MDU on the 2$^{nd}$ day. It demonstrates the rapidity with which $H_2S$ was removed from the biogas and oxidised to $S^0$. Specifically, this compound accumulated at the liquid interface and on the lowest area of the walls, just above the interface. Conversely, $S^0$ did not form in the liquid media. This was consistent with the previous research (Ramos et al., 2013).

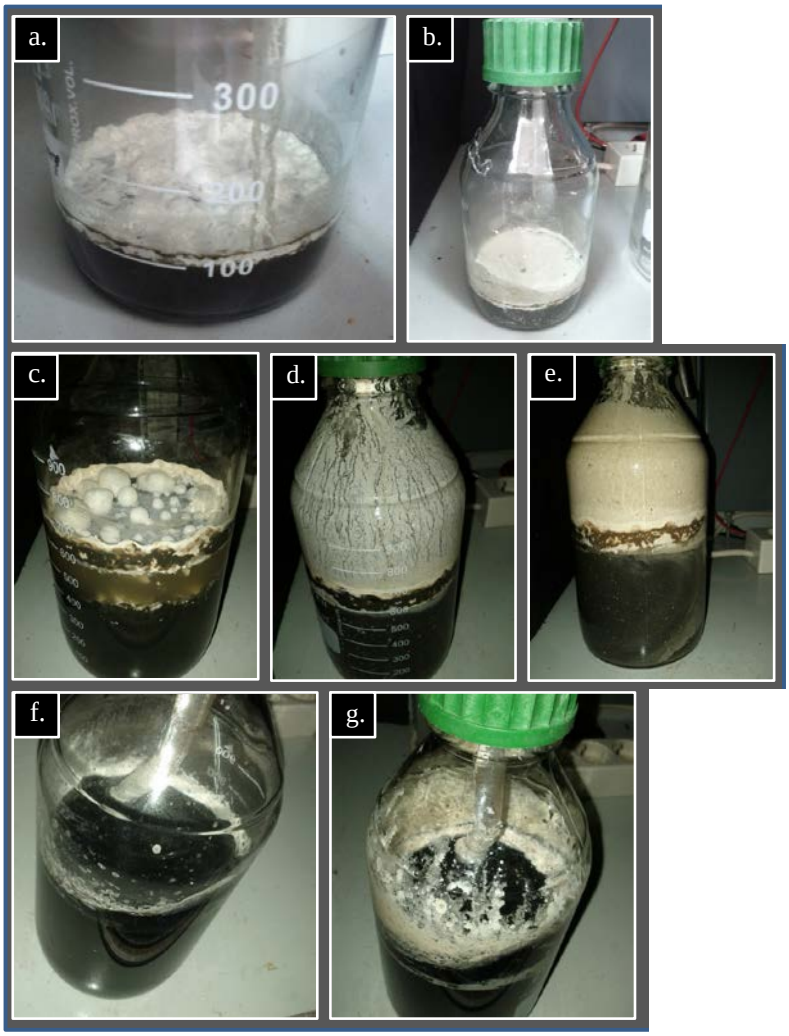


**Fig. 2**. State of the MDU on the 2nd (a) and the 8th day (b) in Experiment 1, the 3rd (c), 13th (d) and 61st day in Experiment 2, and the 2nd (e), and 13th (f) day in Period 2 of Experiment 3.

On the 7th day, the system was gently shaken and, as a result, the $S^0$ deposited at the liquid interface shank (which justifies its presence in the sludge in Fig. 2b). In addition, the lowest area of the walls was moistened; however, it dried rapidly. The outlet $H_2S$ concentration dropped rapidly (Fig. 1). It remained below 0.04%v/v until the 8th day, when it started to increase slightly and gradually. Fig. 2b shows the state of the MDU on that day. It demonstrates that $S^0$ accumulated rapidly at the liquid interface again. Hence, this improvement in the RE could be related to a raise of the SOB settled at the bottom of the system to the liquid interface and the lowest area of the walls, where the $H_2S$ oxidation seemed to predominantly occur. Presumably the size of the SOB population attached to the $S^0$ deposited on these areas rose, and was sufficient to maintain high RE thenceforth (Fig. 1). Specifically, from the 7th to the 10th day, the average RE was 90%. It should be noted that the BRT remained almost constant (11-12min).

On the 10th day, the system was gently shaken again (Fig. 1). The outlet $H_2S$ concentration decreased rapidly from approximately 0.08%v/v to 0.02%v/v. The next day, the MDU was energetically shaken in order to moisten the walls. As previously, they rapidly dried, and the outlet $H_2S$ concentration decreased; specifically, it reached zero. Nonetheless, this was only a momentary effect, as was also

proved at day 14, when the MDU was vigorously shaken again. These brief and limited rises in the RE were attributed to momentary increases in the nutrients availability on the lowest area of the walls and the liquid interface, since $S^0$ was only deposited there. From the 15$^{th}$ day, the RE was 95% on average, and the $O_2$ content of the outlet biogas stream was around 0.55%v/v. Regarding the BRT, it remained almost constant from the 10$^{th}$ day (11-10min).

### *3.2. Experiment 2: 1.0L-MDU with 0.6L of microaerobic sludge*

As noted, this experiment was divided into two periods (Table 1). In the first period, the MDU operated at constant temperature (35ºC), and during the second one, it run between 20 and 35ºC. Period 1 was in turn divided into three phases. In Phase 1, the MLR was 0.7kg/m$^3$/d, and the inlet $H_2S$ concentration was 0.49%v/v on average. In Phase 2 and 3, the MLR was raised to 1.0 and 1.3kg/m$^3$/d, and the $H_2S$ content of the outlet biogas stream was 1.31 and 1.77%v/v, respectively.

#### *3.2.1. Period 1: impact of the inlet H2S concentration and MLR*

The results obtained in this period are represented in Fig. 3a. Until the 19$^{th}$ day (Phase 3), the biogas was injected at the top of the gas space, as in Experiment 1. Thereafter, it was introduced from the bottom of the system, thus bubbling into the inoculum.

Since the absence of non-oxidative removal mechanisms in the MDU was confirmed in the previous experiment, in Experiment 2 (Phase 1) micro-oxygenation was started at day 0 (Fig. 3a). The $O_2/H_2S_{supplied}$ ratio (v/v) was set at 1.8, since it sufficed to maintain high REs in Experiment 1 (Fig. 1). Only 12h after, the $H_2S$ content of the biogas leaving the system was 0.02%v/v. Although the BRT rose gradually from 14 to 10min, the outlet $H_2S$ concentration remained around this value during the rest of Phase 1, thus maintaining an average RE of 97%. Therefore, at such BRTs (of at least 10min), this variable did not seem to limit the system performance.

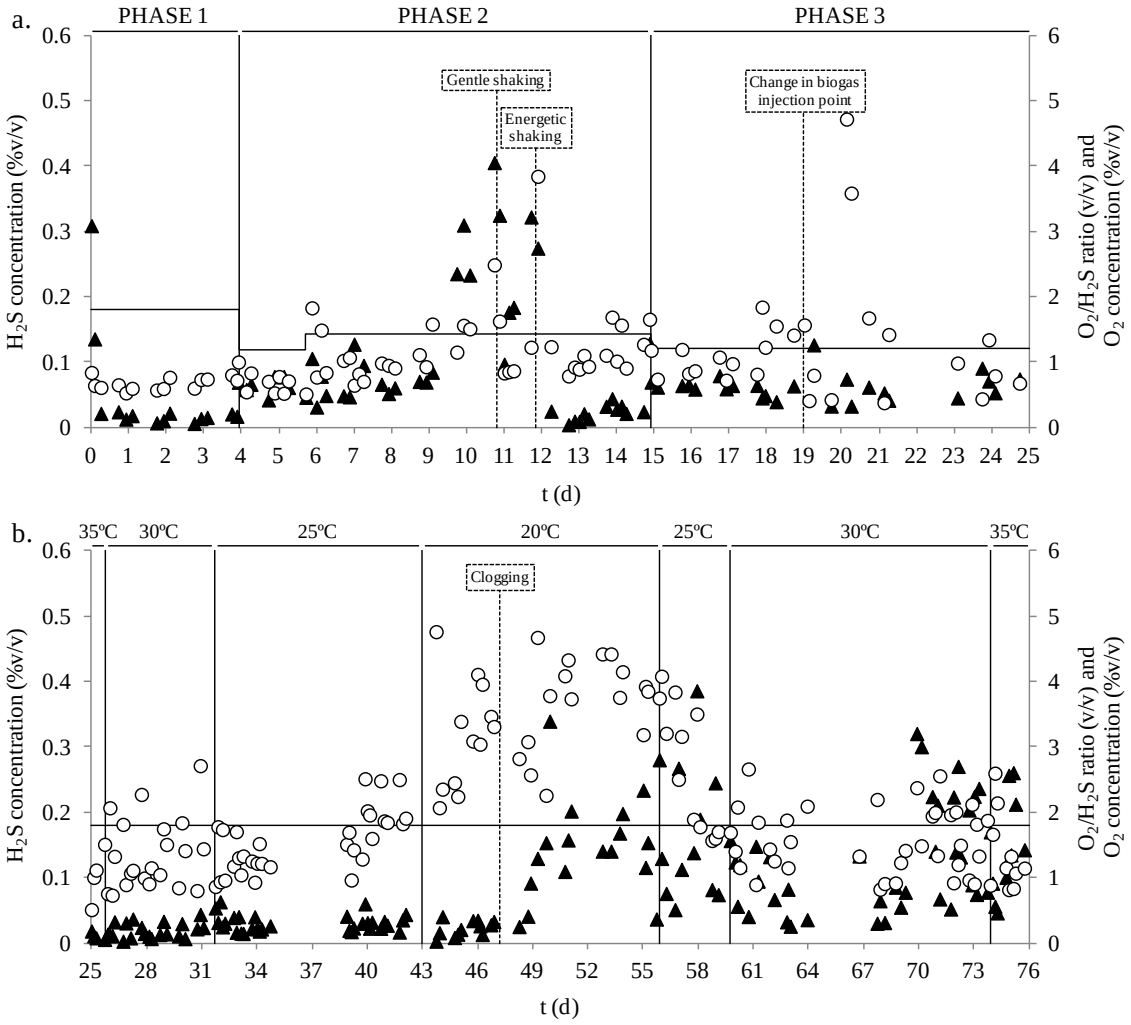


**Fig. 3**. Outlet $H_2S$ (▲) and $O_2$ (○) concentrations, and micro-oxygenation level (continuous line) in Period 1 (a) and 2 (b) of Experiment 2.

As in the previous experiment, $S^0$ accumulated rapidly at the liquid interface and on the lowest area of the walls, just above the interface. Nevertheless, this compound was also observed floating on the bottom of the supernatant. As shown in Fig. 2c, which illustrates the state of the MDU on the 3rd day, the sludge stratified due to sedimentation. Therefore, $S^0$ could form at the interface and then settled, or maybe it formed directly there due to sufficient $O_2$ transfer rate to that surface, where, as in the lowest area of the walls and the liquid interface, besides water, nutrients were presumably present. Accordingly, and taking into account that this phase and Experiment 1 were both carried out under the same conditions (Table 1), besides to the larger liquid interface and surface of the walls in contact to it, the faster onset of effective $H_2S$ removal and slightly higher REs achieved in this phase in relation to the previous experiment could also be partially due to the larger area with favourable growing conditions (namely, sufficient nutrients, water and $O_2$ availability).

In Phase 2, the $H_2S$ content of the biogas leaving the MDU rose from approximately 0.02 to 0.06%v/v (Fig. 3a). Until the 5th day, the $O_2/H_2S_{supplied}$ was significantly lower than in Phase 1 (1.2 against 1.8); therefore, it was raised to 1.4 (v/v). As in Experiment 1, this led to an increase in the $O_2/H_2S_{consumed}$ ratio, while the RE remained almost constant. Therefore, the increase in the outlet

$H_2S$ concentration was not attributable to insufficient $O_2$ availability, but the higher MLR and $H_2S$ content of the inlet biogas stream. Regarding the BRT, it rose significantly from the 6$^{th}$ day (from approximately 16 to 21min), which did not have any effect of the system performance. This was consistent with the previous observations. Hence, since the BRT during the rest of the research was longer than 10min, it was concluded that this variable did not limit the system performance during this study.

Until the 8$^{th}$ day, the RE remained around 94%. Thereafter, the performance of the system decreased and, hence, the system was gently shaken on the 10$^{th}$ day (Fig. 3a). As in Experiment 1, that resulted in a momentary increase in the RE. The next day, the system was vigorously shaken in order to moisten the walls, which explained the state of the MDU at day 13 (Fig. 2d). Although they dried, significant amounts of $S^0$ were deposited there, and the outlet $H_2S$ concentration dropped to 0.01%v/v From the 13$^{th}$ day, it stabilised around 0.02%v/v, thus maintaining 98% of RE (on average) during the rest of Phase 2. Therefore, in contrast to Experiment 1, the system performance was at least partially recovered by increasing the reaction area in the gas space.

In Phase 3, the $H_2S$ content of the outlet biogas stream rose to approximately 0.06%v/v, and the RE efficiency decreased to 97% (Fig. 3a). The BRT was significantly longer than in Phase 1 and 2 (Table 1). Regarding the $O_2/H_2S_{supplied}$ ratio, it was slightly lower than in the two previous phases, and therefore, it was raised to 1.4 (v/v) (the same as in Phase 2) on the 17$^{th}$ day. This led to a negligibly increase in the RE, which indicated that an $O_2/H_2S_{supplied}$ ratio (v/v) of 1.0 was the minimum required for successful biogas desulphurisation. At this point, worthy of noting is that the average outlet $O_2$ concentration during Phase 1, 2 and 3 was 0.96%v/v.

From the 19$^{th}$ day, the $O_2/H_2S_{supplied}$ ratio (v/v) was set at 1.0 again, and the biogas was injected at the bottom of the system. Therefore, the inoculum was continuously stirred, thus maintaining a homogeneous reaction media. Considering the previous responses of the system to shaking, it was expected to slightly increase the RE. Nevertheless, the RE rose negligibly, which was consistent with the presumably scarce amount of nutrients present in the system at that point of the experiment. Moreover, it must be considered that along with nutrients, sulphide and $O_2$ were also homogeneously and continuously distributed as a result of the change in the biogas injection point. At the end of this phase, after the $O_2/H_2S_{supplied}$ ratio (v/v) was lowered to 0.9, an insignificant decrease in the RE was recorded, which confirmed that the optimum value of this variable was around 1.0.

### *3.2.2. Period 2: impact of temperature*

The results obtained in this period are represented in Fig. 3b. As shown in Table 1, the biogas was injected at the bottom of the system, which operated at constant MLR (0.7kg/m$^3$/d) and $O_2/H_2S_{supplied}$ ratio (Table 1).

Until the 26$^{th}$ day (at 35ºC), the $H_2S$ content of the outlet stream remained around 0.01%v/v (Fig. 3b), and the average RE was 99%. Therefore, although the MDU operated at the same MLR, $O_2/H_2S_{supplied}$ ratio and temperature, and higher inlet $H_2S$ concentration (Table 1), the performance of the MDU rose in relation to Phase 1 of the previous period (Fig. 3a). Since the BRT was found not to be a limiting factor at the operating values of this study, and the change in the biogas injection point

did not increase the RE in Phase 3 of Period 1, this was related to the larger reaction area in the gas space. Thus, the importance of promoting SOB growth all over the gas space was revealed.

From the 26$^{th}$ day, temperature was lowered stepwise to 30, 25 and 20ºC (Fig. 3b). The RE and the $H_2S$ concentration in the biogas leaving the system were 0.03%v/v and 98% on average (respectively) until the 47$^{th}$ day. Regarding the increase in the $O_2$ content of the outlet biogas stream recorded on the 45$^{th}$ day, it was due to a substantial rise in the BRT (to approximately 58min). Hence, the MDU performed similarly under all the temperatures tested. This was consistent with the studies carried out by Chung et al. (1996), who reported negligible variations in the performance of a biofiltration system between 20 and 35ºC.

On the 47$^{th}$ day, the inlet pipe of biogas clogged and, as a result, SOB were subjected to a 20h-period of starvation. Once the operation was restarted, a gradual deterioration of the MDU performance was recorded (Fig. 3b). As in biofiltration systems, a rise in temperature was expected to increase SOB activity (Vergara-Fernández et al., 2007). However, the system did not recover neither at 20ºC nor at higher temperatures. Only momentary increases in the RE took place just after raising temperature. The presumable scarce amount of nutrients present inside the system at that point of the research could prevent SOB growth. This highlighted the importance of the biological mechanisms of sulphide oxidation for achieving efficient $H_2S$ removal.

The increase in the amount of $S^0$ present into the inoculum since the biogas was injected at the bottom of the system was negligible, as demonstrated by Fig. 4d and e. Likewise, when the system was opened on the 76$^{th}$ day, this compound did not cover the liquid interface. By contrast, Fig. 4e demonstrates that significant amounts of sulphide were oxidised on the walls of the system (highest and lowest area). Therefore, the constant agitation of the system seemed to prevent $S^0$ accumulation at the liquid interface (this was indeed confirmed in Experiment 3) and promote sulphide-oxidising activity on the walls. It should be highlighted that the biogas bubbling did not cause splashes, only gentle waves on the inoculum surface. In fact, sludge drops were not observed on the walls.

### *3.3. Experiment 3: 1.0L-MDU with 0.6L of anaerobic sludge*

As noted, Experiment 3 was divided into two periods, according to the start-up temperature. Fig. 4a and b show the results obtained when the system was started-up at 20ºC (Period 1) and 35ºC (Period 2), respectively. Once the RE stabilised, temperature was raised to 35ºC and lowered to 20ºC stepwise, respectively (Table 1).

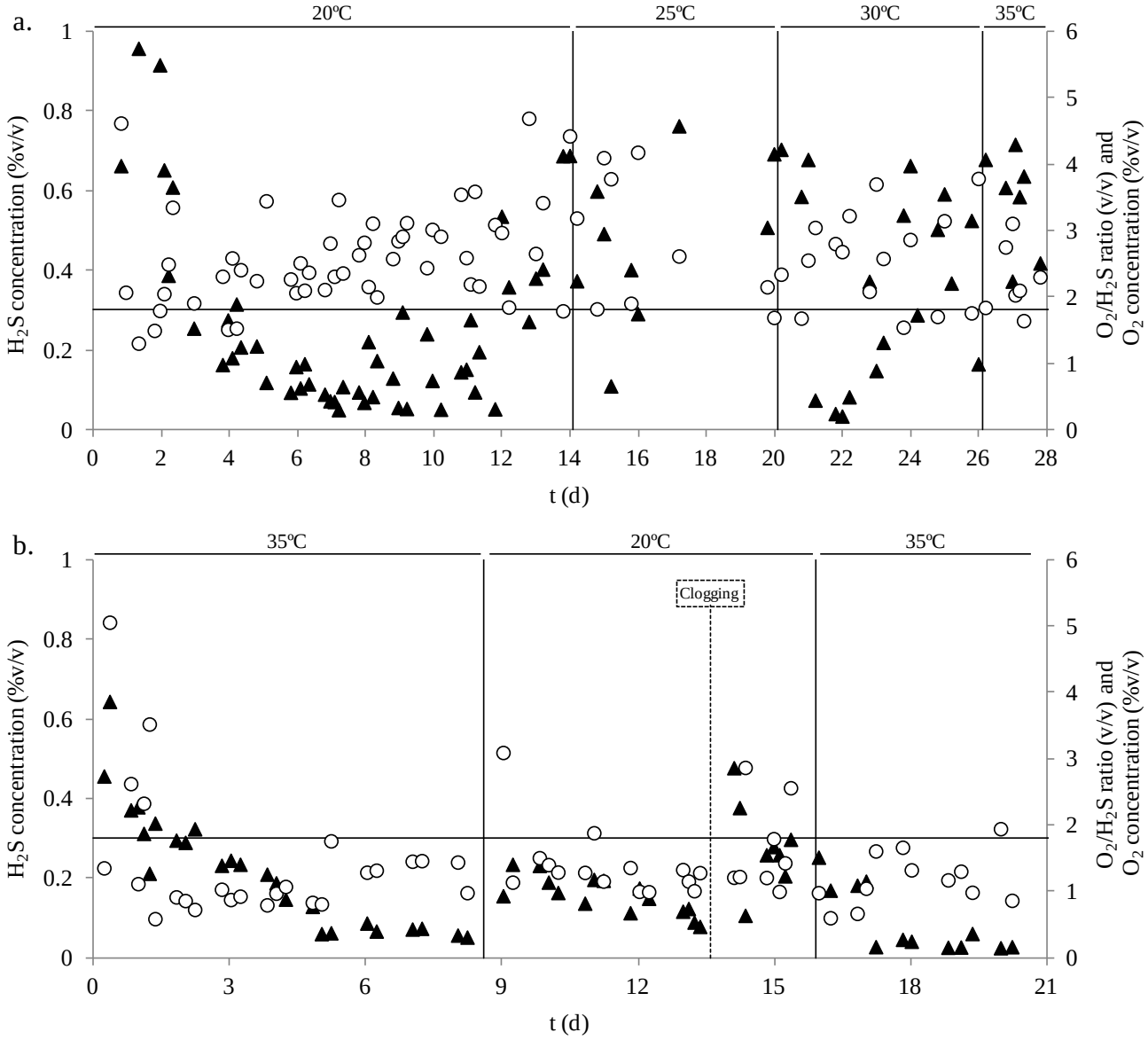


**Fig. 4**. Outlet $H_2S$ (▲) and $O_2$ (○) concentrations, and micro-oxygenation level (continuous line) in Period 1 and 2 of Experiment 3.

*3.3.1. Period 1: start-up at 20ºC*

The results obtained in this period are represented in Fig. 4a. As shown in Table 1, the inlet biogas stream presented approximately 1.25%v/v of $H_2S$. As Experiment 2, this experiment was started-up at day 0 at an $O_2/H_2S_{supplied}$ ratio (v/v) of 1.8. The $H_2S$ concentration in the biogas leaving the MDU decreased gradually until approximately the 8$^{th}$ day. Thereafter, it fluctuated widely; specifically, it ranged between 0.28 and 0.05%v/v until the 12$^{th}$ day. The average outlet $H_2S$ concentration and the RE from the 8$^{th}$ to the 12$^{th}$ day were 0.14%v/v and 88%, respectively. The system performance deteriorated substantially from the 12$^{th}$ day and, as in Experiment 2, it was not recovered either at 20ºC or at higher temperatures.

In this period, $S^0$ deposits were not found either at the liquid interface or in the sludge. This compound accumulated on the lowest area of the walls, which was consistent with the observations made in the preceding experiment.

*3.3.2. Period 2: start-up at 35ºC*

The results obtained in this period are represented in Fig. 4b. As shown in Table 1, the $H_2S$ content of the inlet biogas stream was significantly lower than in the previous period, which arose from the shorter BRT. The $H_2S$ concentration in the biogas leaving the MDU decreased gradually, and stabilised around 0.07%v/v from the $5^{th}$, thus maintaining 91% of RE until the $9^{th}$ day. Hence, as expected, the time required for achieving similar REs was shorter at 35 than at 20 ºC.

On the $8^{th}$ day, temperature was lowered to 20ºC (Fig. 4b). At such temperature, the outlet $H_2S$ concentration showed a decreasing profile, which suggested an adaptation period. Unfortunately, the inlet pipe of biogas clogged on that day. As in Experiment 2, after approximately a 20h-starvation period, the system performance decreased. Although the results obtained at day 15 pointed to recovery, temperature was raised to 35ºC in order to possibly accelerate the process. Around 1 day after, the outlet $H_2S$ concentration and the RE re-stabilised at approximately 0.04%v/v and 94%, respectively. Presumably the nutrients availability was higher than after the starvation period occurring in Experiment 2, which allowed recovery. This is consistent with the increase in the RE observed in relation to the days before the pipe clogging, which suggested increasing SOB population.

Although temperature did not have a significant effect on the RE during steady state operation (Fig. 3b), taking into account the results obtained in Period 1 and 2 (Fig. 4a and b), relatively high temperatures at the start-up could be key to achieving successful operation.

Although the system was not shaken, $S^0$ accumulated on the walls, as demonstrated by Fig. 2f and g. As in Experiment 2, $S^0$ did not accumulated at the liquid interface (probably due to the biogas bubbling), but on the walls, just above the interface. Moreover, this compound did not seem to form in the sludge.

## Conclusions

The MDU was proved to be robust towards fluctuations in the inlet $H_2S$ concentration, MLR, BRT, $O_2/H_2S$ ratio, and temperature. During steady state operation, the outlet $H_2S$ concentration fluctuated between 0.01 and 0.07%v/v, thus maintaining REs between 96 and 99%. Moreover, temperature (20 to 35ºC) did not influence the system performance. Conversely, higher temperatures at the star-up period were found to be necessary for successful operation, and to accelerate the start-up. The importance of maintain favourable growing conditions to SOB in the gas phase and the MDU design was highlighted.

## References


1. Abatzoglou, N., Boivin, S., 2009. A review of gas purification processes. Biofuels Bioprod. Biorefin. 3, 42-71.
2. APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th edition. American Public Health Association/American Water Works Association/Water Environmental Federation, Washington DC, USA.
3. Börjesson, P., Mattiasson, B., 2007. Biogas as a resource-efficient vehicle fuel. Trends in Biotechnol. 26, 7-13.
4. Burgess, J. E., Parsons, S. A., Stuetz, R. M., 2001. Developments in odour control and waste gas treatment biotechnology: a review. Biotechnol. Adv. 19, 35-63.
5. Chung, Y.-C., Huang, C., Tseng, C.-P., 1996. Operation optimization of *Thiobacillus thioparus* CHl 1 biofilter for hydrogen sulfide removal. J. Biotechnol. 52, 31-38.
6. Cirne, D. G., van der Zee, F. P., Fdez-Polanco, M., Fdez-Polanco, F., 2008. Control of sulphide during anaerobic treatment of S-containing wastewaters by adding limited amounts of oxygen or nitrate. Rev. Environ. Sci. Biotechnol., 7, 93-105.
7. Díaz, I., Pérez, S.I., Ferrero, E. M., Fdz-Polanco, M., 2010. Effect of oxygen dosing point and mixing on the microaerobic removal of hydrogen sulphide in sludge digesters. Bioresour. Technol. 102, 3768-3775.
8. Díaz, I., 2011. $H_2S$ removal from biogas. From anaerobic to microaerobic. PhD Thesis. University of Valladolid, Valladolid, España.
9. Estrada, J. M., Quijano, G., Lebrero, R., Muñoz, R., 2013. Step-feed biofiltration: A low cost alternative configuration for off-gas treatment. Water Res. 47, 4312-4321.
10. Fernández, M., Ramírez, M., Pérez, R. M., Gómez, J. M., Cantero, D., 2013. Hydrogen sulphide removal from biogas by an anoxic biotrickling filter packed with Pall rings. Chem. Eng. J. 225, 456-463.
11. Fortuny, M., Baeza, J.A., Gamisans, X., Casas, C., Lafuente, J., Deshusses, M.A., Gabriel, D., 2008. Biological sweetening of energy gases mimics in biotrickling filters. Chemosphere 71, 10-7.
12. Fortuny, M., Gamisans, X., Deshusses, M. A., Lafuente, J., Casas, C., Gabriel, D., 2011. Operational aspects of the desulfurization process of energy gases mimics in biotrickling filters. Water Res. 45, 5665-5674.
13. Holm-Nielsen, J. B., Al Seadi, T., Oleskowicz-Popiel, P., 2009. The future of anaerobic digestion and biogas utilization. Bioresour. Technol. 100, 5478-5484.
14. Jenicek, P., Koubova, J., Bindzar, J., Zabranska, J., 2010. Advantages of anaerobic digestion of sludge in microaerobic conditions, Water Sci. Technol. 62, 427-434.
15. Kennes, C., Rene, E. R., Veiga, M. C., 2009. Bioprocesses for air pollution control. J. Chem. Technol. Biotechnol. 84, 1419-1436.
16. Lantz, M., Svensson, M., Björnsson, L., Börjesson, P., 2007. The prospects for an expansion of biogas systems in Sweden - Incentives, barriers and potentials. Energy Policy 35, 1830-1843.
17. Lin, W.-C., Chen, Y.-P., Tseng, C.-P., 2013. Pilot-scale chemical-biological system for efficient H2S removal from biogas. Bioresour. Technol. 135, 283-291.
18. Maestre, J. P., Rovira, R., Álvarez-Hornos, F. J., Fortuny, M., Lafuente, J., Gamisans, X., Gabriel, D., 2010. Bacterial community analysis of a gas-phase biotrickling filter for biogas mimics desulfurization through the rRNA approach. Chemosphere 80, 872-880.
19. Makaruk, A., Miltner, M., Harasek, M., 2010. Membrane biogas upgrading processes for the production of natural gas substitute. Sep. Purif. Technol. 74, 83-92.
20. Montebello, A., M., Fernández, M., Almenglo, F., Ramírez, M., Cantero, D., Baeza, M., Gabriel, D., 2012. Simultaneous methylmercaptan and hydrogen sulfide removal in the desulfurization of biogas in aerobic and anoxic biotrickling filters. Chem. Eng. J. 200-202, 237-246.

21. Mudliar, S., Giri, B., Padoley, K., Satpute, D., Dixit, R., Bhatt, P., Pandey, R., Juwarkar, A., Vaidya, A., 2010. Bioreactors for treatment of VOCs and odours - A review. J. Environ. Manag. 91, 1039-1054.

22. Murphy, J. D., McKeogh, E., Kiely, G., 2004. Technical/economic/environmental analysis of biogas utilization. Appl.Energy 77, 407-427

23. Potivichayanon, S., Pokethitiyook, M., Kruatrachue, M., 2006. Hydrogen sulfide removal by a novel fixed-film bioscrubber system. Process Biochem. 41, 708-715.

24. Ramírez, M., Gómez, J. M., Aroca, G., Cantero, D., 2009. Removal of hydrogen sulfide by immobilized Thiobacillus thioparus in a biotrickling filter packed with polyurethane foam. Bioresour. Technol. 100, 4989-4995.

25. Ramos, I., Díaz, I., Fdz-Polanco, M., 2012. The role of the headspace in hydrogen sulfide removal during microaerobic digestion of sludge. Water Sci. Technol. 66, 2258-2264.

26. Ramos, I., Pérez, R., Fdz-Polanco, M., 2013. Microaerobic desulphurisation unit: A new biological system for the removal of $H_2S$ from biogas. Bioresour. Technol. 142, 633-640.

27. Rasi, S., Läntelä, J., Rintala, J., 2011. Trace compounds affecting biogas energy utilisation – A review. Energy Convers. Manag. 52, 3369-3375.

28. Rodríguez, E., Lopes, A., Fdz-Polanco, M., Stams, A. J. M., García-Encina, P. A., 2012. Molecular analysis of the biomass of a fluidized bed reactor treating synthetic vinasse at anaerobic and micro-aerobic conditions, Environ. Biotechnol. 93, 2181-2191.

29. Rodriguez, G., Dorado, A. D., Fortuny, M., Gabriel, D., Gamisans, X., 2013. Biotrickling filters for biogas sweetening: Oxygen transfer improvement for a reliable operation. Process Saf. Environ. Prot., DOI: http://dx.doi.org/10.1016/j.psep.2013.02.002.

30. Vergara-Fernández, A., Molina, L. L., Pulido, N. A., b , Aroca, G., 2007. Effects of gas flow rate, inlet concentration and temperature on the biofiltration of toluene vapors. J. Environ. Manag. 84, 115-122.

31. Yang, C., Chen, H., Zeng, G., Yu, G., Luo, S., 2010. Biomass accumulation and control strategies in gas biofiltration. Biotechnol. Adv. 28, 531-540.

# CONCLUDING REMARKS

The introduction of precise amounts of oxygen to reactors can remove efficiently the sulphide content in the biogas, while reducing and increasing negligibly the methane and the oxygen concentration (respectively), thereby improving the overall biogas quality. In the meantime, it can lead to the formation of more stable digestion systems, which can be related to the fact that micro-oxygenation can increase the richness and evenness of the microbial communities. As a result, microaerobic reactors can present increased ability to deal successfully with process imbalances. However, during steady state operation, oxygen does not have a significant impact on the digestion process.

The parameter to regulate the oxygen supply during microaerobic digestion in order to accurately control the sulphide content in biogas must be selected according to the operational conditions of the bioreactor. Under variable organic load and steady sulphur load, the oxygen supply to microaerobic reactors can be precisely regulated as a function of the biogas production by deducing the optimum doses under different hydrogen sulphide concentrations. However, under variable sulphur load or more stringent control requirements, biogas sulphide content can be the basis for the development of precise and consistent control strategies.

The optimum oxygen dose to a microaerobic reactor can increase or decrease over time, depending on the moisture conditions at the different surfaces of the gas space. This is due to the hydrogen sulphide removal from biogas takes place predominantly in this area, although SOB also develop at the liquid interface. Therefore, high efficiencies of biogas desulphurisation are achieved under all the possible configurations of the process.

Under sufficient moisture availability, SOB grow all over the headspace and, as a result, high amounts of elemental sulphur accumulate at the different locations in this area. The moisture level determines the composition, richness and size of the SOB population growing at the different surfaces of the gas space. Nevertheless, biogas is rapidly and efficiently desulphurised after headspace cleaning. By contrast, at low moisture availability in the headspace, elemental sulphur hardly accumulates there. The performance of biogas desulphurisation and the oxygen demand of the reactor are low and relatively high at the start-up of the microaerobic operation, and increases and decreases substantially over time (respectively).

The implementation of microaerobic conditions on full-scale is the next, final step for the culmination of the research presented in this thesis. The findings obtained on lab-pilot and industrial-pilot scale must be tested on a larger scale.

The MDU is a promising end-of-pipe technology for hydrogen sulphide control. A preliminary comparative assessment highlights that this novel system can fulfil all the desirable characteristics of bioreactors for hydrogen sulphide removal from biogas: simple configuration, no hydraulic problems, low oxygen demand, high capability to transform sulphide into elemental sulphur, easy recovery of the generated elemental sulphur, no nutrient requirements, and robustness to fluctuations in operational conditions. Nonetheless, its design and operating conditions must be still optimised in order to evaluate its maximum removal capacity and the optimum criteria for scale-up. Obviously, the volume of the system has to be minimised. For this purpose, strategic installation of specific supports for SOB in the gas space of the system could be key.