UNIVERSIDAD DE VALLADOLID

ESCUELA DE INGENIERIAS INDUSTRIALES

Grado en Ingeniería Química
Semestre Internacional

El reciclaje de nitratos a partir del centrado como estrategia para mitigar la liberación de olores

Autor:
Kweinor Tetteh, Emmanuel

Tutor:
Muñoz Torre, Raúl,

Cotutor:
Aizpuru, Aitor

Dpto. Ingeniería Química y Tecnología del Medio Ambiente
Valladolid, Junio de 2017

UNIVERSITY OF VALLADOLID

SCHOOL OF INDUSTRIAL ENGINEERING

Chemical Engineering Degree
International Semester

Nitrate recycling from centrate as a strategy to mitigate odour release

Author:

Kweinor Tetteh, Emmanuel

Tutor:
Muñoz Torre, Raúl,

Cotutor:
Aizpuru, Aitor

Dept. of Chemical Engineering and Environmental Technology
Valladolid, Junio de 2017

Resumen

En este trabajo fin de grado se utilizó un biorreactor integrado a escala piloto (anaeróbico-anóxico-aeróbico) con un volumen de trabajo de 12,9 L acoplado sedimentador de separación de biomasa para tratar aguas residuales mediante un proceso A_2O. Se evaluó a escala laboratorio la recirculación de nitratos (provenientes de la oxidación de centrados) como estrategia de prevención de olores, estudiando tanto el proceso de tratamiento de agua residual como las cinéticas de oxidación de H2S con nitrato. Los parámetros de operación utilizados fueron velocidad de carga orgánica (OLR) de 0.079-0.132gTOC/Ld y tiempo de retención hidráulica (HRT) de 36-24h y tiempo de retención de los lodos (SRT) de 10 días. La operación del proceso a un HRT de 24h, El rendimiento de depuración de aguas residuales fue superior al 75% de eliminación para los contaminantes, con nitrificación completa del amoníaco afluente. De la cinética se establece que el nitrato que sirve como aceptor de electrones para la eliminación del sulfuro de hidrógeno y el proceso sería técnicamente viable.

Palabras clave; Cinética, Control de olores, Lodos activos Biomasa, Nitrato, Sulfuroato de hidrógeno

Abstract

A pilot-scale A_2O integrated bioreactor (anaerobic-anoxic-aerobic) with a working volume of 12.9 L coupled with a settler to harvest the biomass was used to treat wastewater. Nitrate recycling from centrates as a strategy to prevent odour emission was evaluated on a bench scale, via study of the wastewater treatment performance and kinetics of sulphide oxidation with nitrate. The operational parameters here used were: organic loading rate (OLR) of 0.079 to 0.132 gTOC/Ld and the hydraulic retention time (HRT) of 36—24h and a sludge retention time (SRT) of 10 days. Process operation at a HRT of 24h resulted in wastewater treatment efficiencies of 75% for most contaminants with a complete nitrification of the influent ammonia. Kinetics studies revealed that nitrate served as the electron acceptor for the removal of hydrogen sulphide and the process would be technical viable.

Keywords; Activated sludge, Hydrogen sulphide, Kinetics, Nitrate, Odour control

Table of content

1. INTRODUCTION

1.0 INTRODUCTION

The demand for water quality and quantity is on the increase globally. The use of water for various activities by man has led to increased pollution in water sources thus affecting the quality of water. Huge amounts of polluted water are generated from domestic uses and from industrial processes such as food, petrochemical, pulp and paper industries .etc. Wastewater treatment plant (WWTPs) plays important role in treating these polluted waters before they are discharged into receiving bodies.

From the WWTPs, in particular, from the sludge handling processes, odours emitted in the form of gaseous pollution have become a significant source of environmental irritation. When treating high-sulphate wastewater, high concentrations of sulphur compounds hinder wastewater treatment and effluent quality. This phenomenon results from the microbiological reduction of sulphates into sulphides, with increase in cost of production (Gostelow et al., 2001, Chan et al., 2009, Munz et al., 2015) Therefore, a considerable and economical approach using recycling of nitrate to enhance WWTP, while preventing odorant formation before discharge is very important.

In the recent urbanisation development and wastewater treatment, odour related abatement and control has raised a major environmental compliance with implementation of stricter regulatory obligations by governmental bodies to minimise its effect on the neighbouring communities (Chan et al., 2009, Greben et al., 2009, Lateef et al., 2013). To combat this, for instance, the European regulation on encroachment of housing on land near sewage treatment works and environmental legislation is increasingly becoming more stringent (Zub et al., 2008, Munz et al., 2015, Ahmad and O-Aljasser, 2013). On other hand, emerging biotechnologies to treat odorous emissions are limited with regulatory obligations, investment costs, land acquisition and undesirable economic returns (Birima et al., 2005, Deng et al., 2009, Estrada et al., 2015). Therefore, odour management has become a priority in the design and operation of WWTPs due to its consequence in poor public image of operating companies, avoiding toxicological effects of H_2S in

human health and to conform to environment regulation (Falahti-Marvast and Karimi-Jashni, 2015, Fang et al., 2015, Gostelow et al., 2001, Munoz et al., 2010).

Odours are a nuisance and have potential impacts on nearby quality of life associated with psychological stress and widespread of health-related indicators such as loss of appetite, nausea, headaches, insomnia and respiratory problems (Munoz et al., 2010, Munz et al., 2015). H2S compounds are malodorous and due to their toxic nature can also cause corrosion and deteriorating of digesters, pipe lines and process equipment (Zhang et al., 2008, Yuan and Bandosz, 2007, Kim et al., 2014). In addition, the emission of H2S gas into the atmosphere causes acid rain due to its interaction with ozone to form sulphuric acid (Birima et al., 2005, Hernández et al., 2012, Janssen et al., 1999).

Odours, unlike other wastewater parameters like turbidity, have both a sensorial and a chemical constituent, which makes their characterisation a challenging task. H_2S, as our focus and the main source of odour in WWTPs is a colourless and hazardous gas which has a rotten eggs smell, with an extremely low odour threshold (0.5 ppm) (Munoz et al., 2010), which accounts for 80-90% of the malodourous compounds in WWTPs (Munz et al., 2015). However, depending on the type of wastewater, odour emissions are generated by biochemical reactions occurring under anaerobic conditions by sulphate reducing bacteria or desulphurisation of organic compounds containing sulphur. In this context, the dissolved sulphide is biologically oxidised to other sulphur species (sulphur, thiosulphate and sulphate) using either oxygen (aerobic conditions) or nitrate (anoxic conditions) as a final electron acceptor (Fang et al., 2015, Estrada et al., 2015, Munz et al., 2015). Thus, the hydrogen sulphide and sulphur dioxide can be converted into elemental sulphur via the biological sulphur cycle as depicted in Figure 1. However, the recovery of sulphur as a valuable compound can be used in the production of sulphuric acid or applied for bioleaching processes (Chan et al., 2009, Munz et al., 2015). Therefore, a high effective odour prevention and removal technology is essential for the WWTPs as it will serve as means of income generation.

1.1 Odour management in WWTPs

The degree of pollution of the wastewater determines the type or combination technology to use. Most WWTPs consist of physico-chemical and biological processes. For impact minimization of odour, in the form of removing sulphide from wastewater streams, a number of physico-chemical processes are commonly used today, which involve direct air stripping, chemical precipitation and oxidation (Munz et al., 2015, Chan et al., 2009). Others involve increasing the redox potential to reduce the rate of H_2S production by adding oxygen, peroxide, nitrate or iron salts. Other techniques also includes pH adjustment, chlorination, and ozonation, addition of potassium permanganate or hydrogen peroxide treatment (Ahmad and O-Aljasser, 2013, Estrada et al., 2015, Janssen et al., 1999) .The high energy requirements or high operating costs and also the production of chemical by products which must be disposed of prior to discharge create important drawbacks of these methods (Munz et al., 2015)
According to Munz et al. (2015), the use of passive barriers such as trees or buffer zones to promote dilution of the odour serve as a simple strategy that has been applied in the WWTPs. However, their efficiency is limited and further more depends on the direction of the wind. In addition, the end –of –pipe technologies which are usually classified into physical–chemical and biological techniques have high abatement efficiency and robustness when operated and maintained properly. But they are limited by high operating costs especially at medium and high odour concentration, due to adsorption material and chemicals consumption, which also causes high environmental impacts (Munz et al., 2015, Zhang et al., 2008). And also, the physical–chemical mechanism via passive barriers installation or chemical agents spraying and end-of-pipe treatment address odour nuisance management, once odours have been produced and discharged from the wastewater. Thus with limited odour prevention option before been released into the environment. Hence, in this context, biological techniques create a more cost-effective and environmentally friendly alternative than the physical–chemical process in achieving high odour prevention as some techniques shown in Table 1.

With suitable technology design and environmental control, nearly all wastewaters containing biodegradable constituents can be treated without difficulty by biological means. In recent years, much attention has been paid to bioreactors for wastewater treatment to meet the strict constraints with respect to odour production (Gostelow et al., 2001, Kim et al., 2014). Thus, the integrated bioreactors coupled with anoxic, aerobic and anaerobic processes in a single reactor are seen as a viable alternative. Unfortunately, upgrading or re-designing of an already existing WWTP is very expensive. In this case, some operational practices are implemented such as maintaining aerobic or anoxic conditions in the wastewater where possible, frequent cleaning of process units, minimization of the sludge retention time in thickeners and dewatering systems or the use of buildings and covers to confine the odour emission in the key operation units (Van der Werf et al., 2010, Kim et al., 2000).

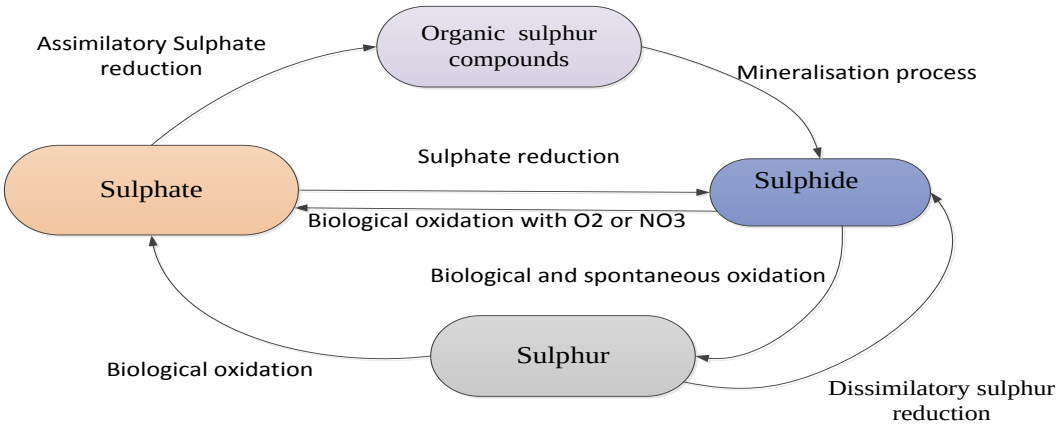


Figure 1. Biological oxidation cycle of hydrogen sulphide (odour emission) control (Hernández et al., 2012, Kim et al., 2000)

Table 1- Emerging technologies for odour control management (Estrada et al., 2015, Munz et al., 2015)

Application	Methods	Removal action	Main sulphur compound	Further treatment	Comments
Acidic pH control	Acid dose for stripping at pH (5)	Removal by adjusting pH to equilibrim of H_2S solution	H_2S dissolved	Gas stream H_2S removal	pH adjustment is difficult to maintain at equilibrim, H_2S is highly soluble
pH control alkali	Base dosing for maintaining pH for the solution at 9	Control by adjusting pH to equilibrim of H_2S solution	H_2S	Neutralisation for discharge	pH too high for biodegradation activity
Redox or Biological oxidation recycling	Oxygen Nitrate	Prevention , removal and control	SO_4	Maintenace of high redox or oxidation recycling	Maintain flux to enhance S and SO_4 reduction
Oxidation	H_2O_2 , Chlorine, ozone, hypochloride	Removal and control	S SO_4	Removal of solid or reconversion of H_2S	Expensive, toxic chemical handling consideration
Stripping	Air CO_2	Gas stripping for downstream gas phase treatment	H2S in carrier gas	Gas collection and scrubbing	Low pH required, removal of carrier gas
Precipitation	Ferrous sulphate	Reaction to acid prepitate	Fe_2S_3 solid	Solid removal and disposal	
Spraying/ Bactericidal	Acid/alkali chlorine	Removal of sulphur reduction species biologically by killing them	n/a	Ensure no biological activity	Application for corrosion and odour control in pipe lines

1.2 Biological Odour control technologies

Biological treatment processes are a promising technology to the attainment of revenue from Certified Emission Reduction (CER) credits. They are capable of converting the organic matter in the wastewater by microorganisms organisms such as bacteria (aerobically or anaerobically), thus, resulting in the formation H_2S and methane gas, which can be utilized as renewable energy (Estrada et al., 2015). Compounds such as ammonia and phosphorous which can be used as fertilizers are also produced (Fang et al., 2015). In view of this, to overcome the disadvantages of conventional methods, many biotechnologies have recently been developed using high rate bioreactors (such as up flow anaerobic sludge blanket (UASB), filter bioreactor, fluidized bed reactor, membrane

bioreactor) and are adopted in order to provide a treatment process which is both technologically and economically viable with the twofold goals of resource recovery and submission to current legislation for effluent discharge (Chan et al., 2009, Munz et al., 2015).

According to Chan et al. (2009) a more intensive form of biodegradation can be achieved by integrating anaerobic and aerobic zones within a single bioreactor. Essentially, some of these include (a) integrated bioreactors with physical separation of anaerobic–aerobic zone, (b) integrated bioreactors without physical separation of anaerobic–aerobic zone, (c) Sequencing Batch Reactors (SBR) based on temporal separation of the anaerobic and the aerobic phase, and (d) combined anaerobic–aerobic culture system based on the principle of limited oxygen diffusion in microbial biofilms. In addition, also sulphide oxidation under aerobic conditions can be carried out by using bacterial species such as Thiobacillus denitrificans, using nitrate or nitrite as an electron acceptor (Munz et al., 2015, Munoz et al., 2010) .

However, the integrated bioreactors, like any other biological system are centred on microbial physiology and are subject to failure whenever there is a shift in operational conditions or in the presence of inhibitory substances. Thus the sulphide oxidation bacteria are very sensitive towards unfavourable conditions. Some of these conditions includes, seeding, temperature, pH, carbon-nitrogen (C/N) ratio, volatile fatty acids (VFAs), organic loading rate (OLR), alkalinity, total volatile solids (VS), hydraulic retention time (HRT) and nutrients concentration(Chan et al., 2009, Falahti-Marvast and Karimi-Jashni, 2015). This effect can be either due to a direct inhibitory effect on the metabolism of sensitive microbial species or to an eventual modification of the conditions of the bioreactor environment such as pH changes. Therefore, process control and monitoring are engineered to boost the biochemical degradation, enhancing the pollutants and odour removal and stability of the sludge or centrates formation.

The integrated bioreactor coupled with anaerobic, anoxic and aerobic functioning as a single reactor can be widely applied to treat a wide-ranging of

industrial wastewaters. Thus it stands to have operationally and economically advantages in the treatment of high strength industrial wastewaters since it couples the benefit of anaerobic digestion (i.e. biogas production) with the benefits of aerobic digestion (i.e. better COD and VSS removal) (Munz et al., 2015). It also has the capability to biodegrade organic matter, such that, the sequential nitrogen removal including nitrification-denitrification, and reduction of Fe (III) and oxidation of Fe (II) with production of fine particles of iron hydroxide for adsorption of organic acids, phenols ammonium, and heavy metals (Ahmad and O-Aljasser, 2013). Therefore, this application for research and odour prevention in WWTPs sounds a promising technology.

1.2.1 Activated sludge recycling

The activated sludge recycling (ASR) as a biological process, is regularly employed for the treatment of a large number of industrial wastewaters. However, the knowledge or determination of the microbial kinetics of the wastewater is essential for designing the treatment facilities. In a typical WWTP using ASR, the influent wastewater is aerated with dissolved oxygen and total suspended solids concentration of 2–3 mg/L and 1000–10,000 mg VSS/L respectively via the bioreactor(Munz et al., 2015). The organic matter and other pollutants are then oxidised by the microorganisms under aerobic condition. Afterwards, a secondary clarifier separates the biomass from the treated water, where the settled sludge of about 4000–12,000 mg VSS/ L is recycled back to the anoxic or anaerobic tanks (METCALF and Eddy, 2003, Munz et al., 2015).Thus, this strategy involves the recycling of the waste or aerobic activated sludge from aerated biological reactors to the inlet of the WWTP headwork. This stimulates the consumption of odorous compounds before they are volatilised from the liquid phase. The two preventing mechanism that occur before the malodorous compounds are released from the subsequent wastewater treatment units are said to be adsorption followed by oxidation (Munz et al., 2015).

In view of that , Hernández et al. (2012) reported that the recycled activated sludge from the aeration basin or the settler contains substantial concentration

of oxygen (2–3 mg/L), and , or nitrate (6–10 mg/L) that act as electron acceptors for the oxidation of the odorants or the malodorous compound originators. In as much as odour oxidation can be done by aerobic oxidation, or anoxic oxidation coupled to denitrification biologically, limitation of oxygen or nitrate can result in production and precipitation of elemental sulphur. In addition, under anoxic conditions, it is also possible to find incomplete denitrification with nitrate being reduced to nitrite instead of elemental nitrogen (Munz et al., 2015, Zub et al., 2008).

Munz et al. (2015) reported trials carried out at the Lee County WWTP (Lee County, Florida) which revealed H_2S gas concentration reductions ranging from 87% to 98% and odour reductions of 69% (measured as odour detection threshold reduction) in recycling return activated sludge to-wastewater ratio. Kim et al. (2014) also reported ASR implementation at the Englewood Water Reclamation Facility (Englewood, Florida) as a strategy for odour control. The pilot tests resulted in H_2S and other gas concentration reductions of 93% and 96%, respectively. The continuous field scale implementation of the ASR strategy has revealed odour reductions of 89% (measured as odour detection threshold reduction) in the surge tanks at minimum capital and investment costs (Munz et al., 2015, Zub et al., 2008).

All the same, a major concern by WWTP operators is that recycling strategies might increase the abundance in sulphur consuming and filamentous bacteria, which might have adverse effects on the activated sludge floc sedimentation properties (Lateef et al., 2013). Prior to that, Zhang et al. (2008) activated the sludge and mixed mechanically for one or two days, improving sulphide reduction. The effect of iron salts, often added during wastewater treatment for phosphorus precipitation, present in the recycled sludge liquor can be also beneficial for odour prevention by promoting the precipitation of dissolved sulphide as ferrous sulphide (Zub et al., 2008, Zhang et al., 2008, Munz et al., 2015).

1.2.2 Oxidized Ammonium Recycling

The de-watering of the anaerobic digested sludge generates ammonia rich effluents (500–1000 mg/L) commonly referred to as centrates. This ammonia-rich effluent, representing up to 20% of the total ammonia load of the WWTP is recycled to the biological treatment to be removed by conventional nitrification–denitrification process. In order to conform to the nitrogen discharge limits, recycling of the centrates serves as an innovation technology for the oxidation of ammonia to nitrate and its further recycling to the WWTP inlet works to control odour emission (Zub et al., 2008, Munoz et al., 2015). It has also been reported that factors such as high ammonia concentration and low dissolved oxygen (DO) level can result in the disruption of the equilibrium between the nitrification - denitrification steps, resulting in significant reduction in the activities of nitrite oxidisers which can lead to toxic nitrite build-up and a subsequent failure of nitrification (Zhang et al., 2008).

1.2.3 Recycling of nitrate from digestate oxidation

Recently, attention has been drawn on autotrophic denitrification, during removal of nitrate from wastewaters containing high concentrations of sulphur or other sulphur compounds. In this process, nitrate-reducing and sulphide oxidizing bacteria (NR-SOB) use nitrate and reduced sulphur as electron acceptors and electron donors, respectively (Zub et al., 2008, Zhang et al., 2008, Munz et al., 2015). A study on reducing nitrate to nitrogen gas and oxidizing sulphur compounds to sulphate in wastewater-treatment plants from oil fields, and the petrochemical industry was conducted. It was reported that addition of nitrate as terminal electron acceptor resulted in autotrophic denitrification and led to desulfurization, thus controlling H_2S emissions from WWTPs (Zhang et al., 2008) . When sludge is not recycled together with the nitrate-rich effluent, the process will rely on the indigenous biodiversity present in the sewage to perform the anoxic oxidation of the malodorous compounds. Therefore, recycling of nitrate is therefore considered as an alternative technology in prevention of odours from WWTP.

A discharge of effluent with nitrogenous compounds is seen to be very unpleasant. This is because nitrate can stimulate eutrophication where pollution is caused in waterways by heavy algal growth. Thus nitrate polluted water supplies can to lead to outbreaks of infant disease as well as other illness. There is therefore, the need to protect public health from nitrate intake effects. Hence, the World Health Organisation has set a standard of 50 mg/L to regulate the nitrate concentration in drinking water (WHO, 2011).Therefore, complete nitrogen removal is necessary where the receiving water is a water supply source for downstream users, since eutrophication and nitrate enrichment should be avoided. As a result, development of economical and sustainable techniques for reducing the nitrogen content from wastewater has attracted a great deal of attention.

Biological denitrification is a convenient way to remove nitrogen from wastewater. In denitrification process nitrate is converted into nitrite, then into environmentally non-threatening nitrogen gas (N_2) with energy conservation The denitrifying sulphide removal process is an efficient way to bio-transform sulphide, nitrate, and organic carbon from industrial wastewater(Zub et al., 2008, Chan et al., 2009, Zhang et al., 2008, Munz et al., 2015).

According to Zhang et al. (2008) stoichiometrically, denitrification of 1 g N as nitrate (N- NO_3) needs 2.85 g chemical oxygen demand (COD); that is, almost 100 g COD for 1 L of a 150 g NO2,3/L of wastewater. But the addition of nitrate to the wastewater influent promotes anoxic conditions, where nitrate is used as an electron acceptor by microorganisms in order to oxidize dissolved sulphides and any readily biodegradable odorants, preventing their further release as malodorous emission (Kim et al., 2014). Munz et al. (2015), reported, assuming a conservative concentration of nitrate in the stream after the nitrification of an ammonia-rich centrates (500 mg NO_3 / L), that only a 0.2% recycling ratio would be needed for sulphide oxidation (e.g. 60 m^3/ day for a raw wastewater influent of 30,000 m^3/ day) (Munz et al., 2015, Falahti-Marvast and Karimi-Jashni, 2015). The stoichiometry reported in most literature ranged from 0.6 to 4.5 mg NO_3 -N / (mg/ S), with an average sulphide elimination of 90–100% (Zhang et al., 2008). However, the dosages varied with the nature and concentration of organic matter, biomass activity and ionic strength in the different wastewaters.

The biochemical reaction for sulphide oxidation in wastewater and hypothesized that Sulphate can be an important end-product under anoxic conditions when oxygen or nitrate is the electron acceptor are represented by following equations (1) and (2).

$$HS^- + NO_3^- + H^+ \rightarrow S^0 + NO_2^- + H_2O \quad (1)$$

$$S^0 + 3NO_3^- + H_2O + 3H^+ \rightarrow SO_4^{2-} + 3NO_2^- + 5H^+ \quad (2)$$

The nitrate concentration in the centrates or nitrified stream recycled will be the key parameter to provide enough sulphide oxidation potential. Unfortunately, due to the knowledge gap on combination of the high electron acceptor concentration in nitrified centrates, together with the biological activity of activated sludge, further research is needed. In addition, the lower kinetic rates of anoxic sulphide oxidation has to be taken into account before implementing nitrate-sulphide oxidation strategy on a bioreactors .Thus in order to allow sufficient residence time for the oxidation to occur before odorants are emitted.

1.3 Scope and objectives of study

To ensure the successful handling of industrial and municipal wastewater, integrated bioreactor (A20) was designed with the combination of (anaerobic, anoxic and aerobic) zones, to evaluate its efficiency to generate biomass and wastewater purification as a 12.9 L laboratory scale WWTP operated under anaerobic-aerobic conditions. Significantly, recycling of the nitrate from the digestate and monitoring its physical performance based on the total and volatile suspended solids (TSS/VSS) production. The wastewater treatment performance by the reactor was also evaluated based on the following parameters, total nitrogen (TN), total organic carbon (TOC), total carbon (TC), inorganic carbon (IC), Sulphate (SO_4^{2-}), phosphate (PO_4^{3-}),chlorides (Cl^-), ammonium (NH_4^+) , nitrate (NO_3-N) and nitrite (NO_2-N).

The specific objectives were

- To carry out, at batch scale, kinetics and stoichiometry oxidation of the sulphide - nitrate (S/N) as a technical route in which the nitrate serves

as the electron acceptor for the removal of the hydrogen sulphide or odorant compounds.

- To investigate and evaluate the nitrification–denitrification process with nitrate or sulphide in different S/N ratio with sludge from the bioreactor for S/N ratio (1:10 and 1:30) and sulphide to activated sludge(S/C) ratio (1:2 and 1:8) respectively.
- To investigate and evaluate the nitrification–denitrification process with nitrate or sulphide in different S/N ratio in the presence of activated sludge.

2. MATERIALS AND METHOD

2.0 MATERIALS AND METHODS

2.1 Wastewater and seed sludge

To ensure a constant nutrient and organic matter concentration in the raw wastewater along the entire experiment, a synthetic wastewater was used in this study instead of actual wastewater. The mineral salts of the wastewater as shown in Table 2 were supplied by PANREAC (Barcelona, Spain). A 25 folds stock solution was prepared, diluted with 25 L of distilled water and stored at 4°C for the first start up. However, for the second start up, tap water was used for the dilution of the stock solution and was not refrigerated. Peptone and glucose were used as carbon source, sodium bicarbonate ($NaHCO_3$) provided alkalinity to keep the pH of the wastewater neutral, urea and meat extract as a nitrogen source, while K_2HPO_4 served as orthophosphate source. The $CuCl_2.2H_2O$, NaCl and $CaCl_2.2H_2O$ were also used to balance the salinity of the wastewater and to provide trace elements.

Aerobic-anoxic activated sludge was obtained from a local WWTP located in Valladolid operated under a denitrification-nitrification configuration (Spain). The aerobic-anoxic sludge was used to inoculate the A2O bioreactor and to carry out the kinetics on the nitrification–denitrification process.

Table 2-Characteristics of the synthetic wastewater

Mineral salts	**Amount (g/L)**	**Purpose**
Peptone	4	carbon source
Glucose	2.75	
$NaHCO_3$	27.5	alkalinity to keep pH
Urea	0.75	nitrogen source
Meat extract	6.25	Nutrient
$MgSO_4.7H_2O$	0.05	Sulphate source
K2HPO4	0.7	orthophosphate source
$CuCl_2.2H_2O$	0.00125	Trace element /balance alkalinity
NaCl	0.175	
$CaCl_2.2H_2O$	0.1	

2.2 Experimental setup

2.2.1 Bioreactor description

The integrated bioreactor was fabricated with a Perspex plastic, commissioned and installed in the Gas treatment and Microalgae Technology Research Group laboratory under the Chemical Engineering and Environmental Technology, at Escuela de Ingenierias Industriales, Sede Dr. Mergelina, University of Valladolid (Spain). The bioreactor consisted of three compartments with a total working volume of 12.9L: A_1- anaerobic chamber (1.4L), A_2-anoxic chamber (2.7L), A_3-aerobic chamber (8.8L). A settler (4.5L) was also installed at the end the system to support biomass harvesting and recycling (**Error! Reference source not found.**). In the anaerobic and anoxic chambers, a complete-mix regime was achieved with a lower speed mixer (50rpm), while complete mixing in the aerobic chamber was achieve via an immersion water pump and process aeration. The influent ($F\text{-}A_1$) was introduced to the anaerobic chamber continuously, where the pilot plant also was equipped with one internal recycle line that connected the aerobic-anoxic chambers ($IR_1\text{-}A_3A_2$) and one external line that connected the settler-anaerobic chamber ($SR_2\text{-}SA_1$). This was done to enhance the nitrification and treatment efficiency. The top of the anaerobic and the anoxic chambers were also covered to maintain the dissolved oxygen below 1, to promote anaerobic and anoxic conditions, The sealing lids can be taken off and replaced after cleaning the headspace. The aerobic region is also equipped with diffusers with air controller, aerated from beneath to provide the required dissolved oxygen (2-3mg/L) for the biological process as well as complete mixing condition of the liquor in the reactor.

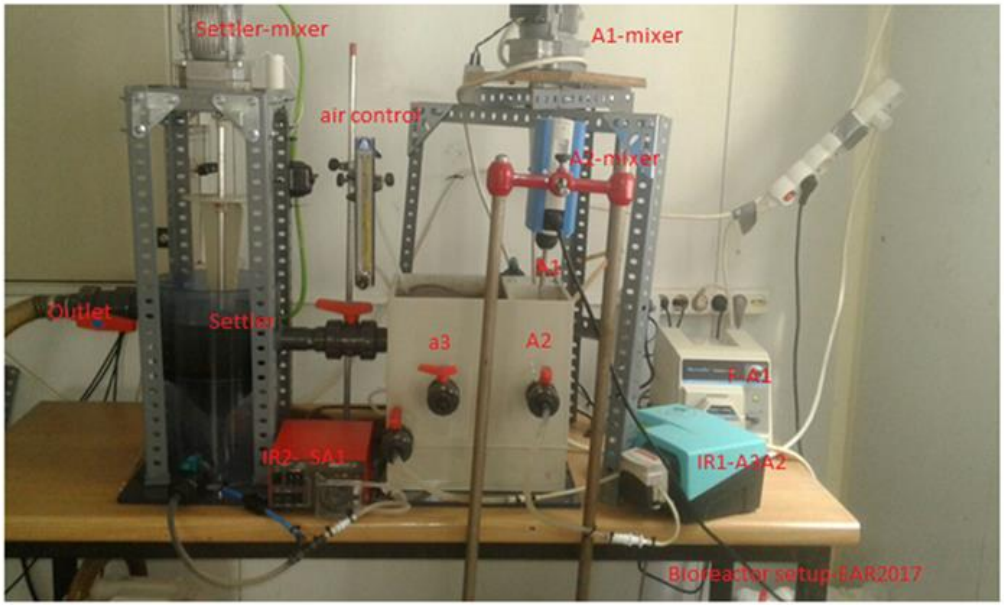


Figure 2. Schematic photo of the integrated bioreactor (A1-anaerobic, A2-anoxic, A3-aerobic and settler)

2.2.2 Bioreactor operation

The bioreactor, which was operated for a total period 90 days in two operational phases, was first commissioned with tap water to calibrate the pumps and mixers, check for leaks and dissolved oxygen injection. A detailed schematic flow diagram of the experimental setup is presented in Figure 3. Prior to the start-up, the reactor was inoculated with 10L of activated sludge. This was done when all the setup valves closed except X2. All the compartments were thus filled with the sludge to about 80% working volume of the reactor, resulting in initial TSS concentration of 3.5 g/L and 4.4g/L in the reactor for the first and second start up days, respectively. At this point it should be notice that a second start-up was needed due to an operational error during synthetic wastewater preparation.

The hydraulic retention time (HRT) was reduced from 36h to 24h, with the corresponding flowrates and operational conditions as shown in Table 3. The influent wastewater and the external biomass recycling were fed to the head of the anaerobic tank. While the influent pump (P1) operated continuously, the external biomass-recycling pump (P2) worked under intermittent operation in a 15-min cycle and 1h off at 50% of the influent flowrate to the head. The settler mixer also worked under intermittent operation of 5-min cycle and 1-h off to promote the periodic mixing of the settled sludge blanket. The solids retention time (SRT) was

kept constants for 10 days in both cases by drawing a varying wastage flowrate as a function of the biomass concentration in the settler and the bioreactor (~ 200-300mgTSS/L via valve X5). To promote nitrogen removal via denitrification, the aerated mixed liquour containing nitrified ammonium from the aerobic tank was also recycled to the head of anoxic tank at thrice the flowrate of the influent. This was done with a peristaltic pump (P3) where valves X7 and X8 were constantly kept opened. The diffuser coupled with air controller flowrate, the knob was adjusted where necessary to supply air to the aerobic tank. This was placed at the bottom of the aerobic tank along its wall to maintain the dissolved oxygen (2-3mg/L).

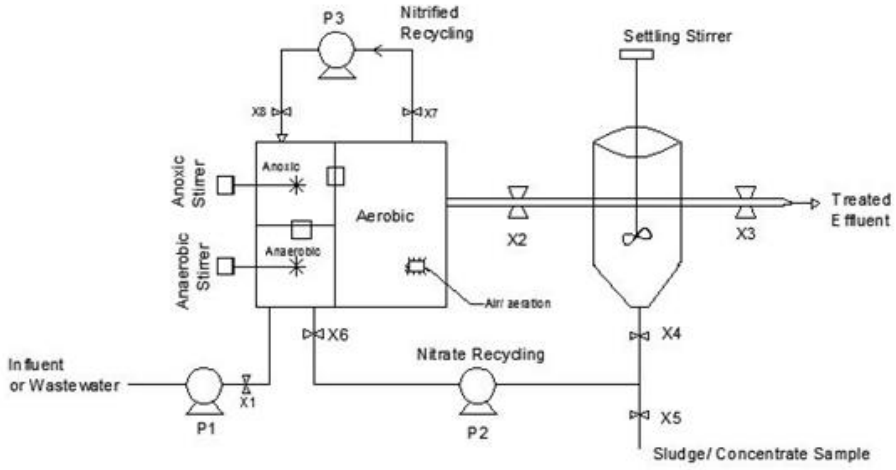


Figure 3. Schematic flow diagram of the integrated A2O bioreactor (A1-anaerobic, A2-anoxic, A3-aerobic and settler

Table 2- Experimental operational conditions

Operational parameter		Start up 1	Start up 2
Days operated		44	40
Organic loading rate(ml/min)			
	Feed(Q)	6.0	9.0
	Settler -anaerobic(0.5Q)	3.0	4.5
	aerobic-anoxic (3Q)	17.9	26.9
Pump speed (rpm)			
	Feed(P1)	90	80
	Settler -anaerobic(P2)	15	20
	aerobic-anoxic (P3)	38	38
Average aeration rate (ml/s)		50	60
HRT (h)		36	24
SRT(days)		10	10
Dilutant water type		Distilled	Tap
Initial sludge inoculant (mgTSS/L)		3.5	4.4
Expected effluent flux (L/d)		8	9

2.3 Nitrate oxidation kinetics

Batch tests were designed to evaluate the desulfurization and denitrification mechanism with four different mixtures such that the S/N ratio (1:1 and 1:3) , sulphide to activated sludge(S/C) ratio (1:2 and 1:8) and finally nitrate combination (Table 4). This was done via addition of potassium nitrate (KNO3 (101.1g/mol) and fresh activated sludge to synthetic wastewater (prepared according to Table 2 in different proportions while keeping sodium sulfide (Na2S.9H20 (240.18 g/mol)) concentration at about 15mg/L as depicted in Table 4. Run1 was inoculated at a biomass concentration of 3.5 mgTSS/L and supplied with nitrate and sulphide at 150 and 15 mg/L, respectively, to assess the influence on the aqueous matrix (tap water or synthetic wastewater) on the kinetics of sulphide oxidation with NO3-. Afterwards, synthetic wastewater was used to mimick the conditions prevailing in a WWTP. Before each run, the sludge was aerated and the TSS concentration was measured prior to inoculation (4.4 gTSS/L, 4.3 gTSS/L and 5.7 gTSS/L for the run2, run3 and run 4, respectively).

Each tests was prepared in duplicate. All the experiments were then performed in a 2L serum bottle with a 20% volume as a headspace. The serum bottles

were closed with butyl septa and sealed with aluminium lid to guarantee anaerobic conditions (Figure 4). Each bottle was agitated at 50 rpm. An infusion needle was inserted through the rubber stopper, which was connected on a Tedlar bag filled with Helium. This allowed to replace the liquid volume with helium from the airbag, which prevented the entrance of oxygen. The setup were kept at room temperature (25 ºC) and covered with aluminium foil to prevent H_2S photolysis and algal photosynthesis. In addition, to determine the desulfurization and denitrification kinetics, liquid samples were taken from 0 to 5 times (initial sampling), within a stipulated time interval of 2 hours. Samples were taken by inserting a needle through the rubber stopper and withdrawing the sample into a 10-mL syringe as necessary for the sulphide and nitrate concentration measurement.

Table 4 -Experimental Planning to assess the anoxic S oxidation.

Runs	Biomass (mgTSS/L)	Nitrate (mgN/L)	Sulphide (mgS/L)	Aqueous matrix type	S/N ratio	S/B ratio
R1	3.5	150	15	Tap and SWW	1	(1:0)
R2	4.4	150	15	SWW	1	(1:2) and(1:8)
R3	4.3	150	15	SWW	1	(1:2) and(1:8)
R4	5.7	(0, 150,450)	15	SWW	(1:1and1:3)	(1:2)

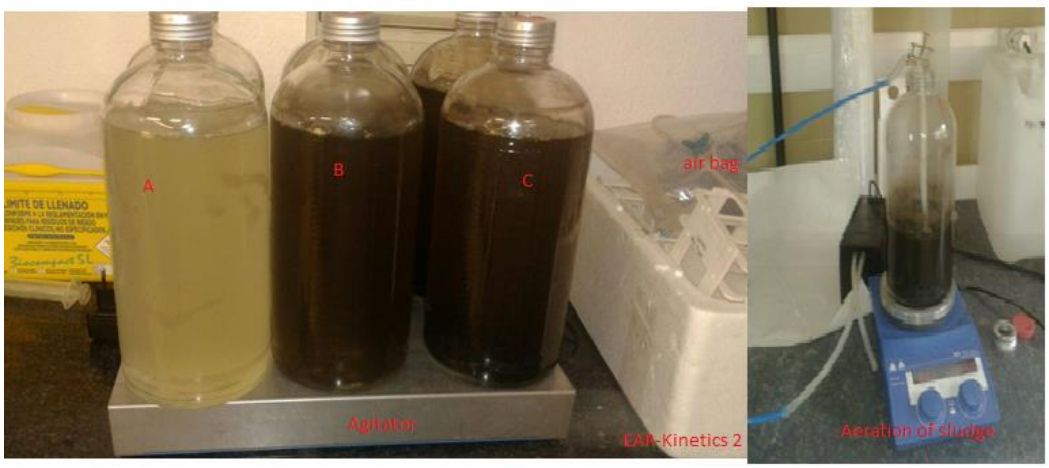


Figure 4. Schematic photo of the experimental setup used for S kinectic oxidation experiments (left picture) and aeration sludge system (right picture)

2.3.1Sulphide adaptation kinetics

A 2L serum bottle acting as an anoxic bioeactor was also set-up to promote the enrichment of a microbial community capable of oxidizing H_2S with NO_3^-.The sludge to the synthetic wastewater ratio was 50/50 by volume in the bottle, which was not connected to any helium bag. The sludge-wastewater mixture was then supplied with potassium nitrate (1.44g) and sodium sulphide (0.45g) in 4 sequential days. This resulted in initial NO_3^-/S ratio of X and an initial biomass concentration of X g/L. Nitrate and sulphide amendments were maintained for 12days, where samples were taken before and after injection, filtered before the nitrate and sulphide analysis were done according the to the protocols.

2.4 Analytical methods -nitrate oxidation and enrichment tests

The setup was constructed with 6 sampling points in the feed (BE1), anaerobic (BE2), anoxic (BE3) aerobic (BE4), settler (BE5) and the effluent (BE6). The TSS/VSS samples were collected from the BE2, BE3, BE4,BE5 and BE6 tanks twice a week, and the concentration were determined conferring to standard methods (APHA, 2005). The daily monitoring of the dissolved oxygen (DO) concentration and temperature was done with the DO meter (WTW Oxi 3310 Set 1 2BA301).The concentration of sulphate, phosphate, chlorides, ammonium, and nitrite and nitrate samples were taken from all sampling points and analysed once in week by HPLC-IC using a Waters 515 HPLC pump coupled with an ion conductivity detector (Waters 432), and equipped with an IC-PAK Anion HC column (4.6×150 mm) and an IC-Pak Anion Guard-Pak (Waters). The TOC and TN were also collected from all the sampling points and analysed once a week. This was done in accordance with the protocols of TOC-VCSH analyser (Shimadzu, Tokyo, Japan) coupled with a total nitrogen chemiluminescence detection module (TNM-1, Shimadzu, Tokyo, Japan).

2.4.1Bench top Sulphide analysis protocols

The concentration of the sulphur was measured with a Thermo Scientific Orion Dual Star pH/ISE Dual Channel Benchtop Meter in accordance the manufacturer's standards method. 1L buffer of antioxidant solution was prepared with EDTA (33.5g), ascorbic acid (17.5g) and NaOH (40g). In other to prepare the solution, 1L of deionised water was first degassed with helium. The NaOH was quickly mixed with the water, and the resulting solution heated by this exothermic reaction was then allowed to cool down for about 2 min before addition of the EDTA and ascorbic acid.

A standard solution of 50ml was then prepared using 0.112g of the sodium sulphide in 50ml of the buffer solution. The standard solution was then added to a flat bottom flask in sequence order 0.5ml, 1ml, 2.5ml, 5ml and 10ml, which resulted in calibration solutions of X, Y, Z, W and Q mg S/L (namely P1, P2, P3, P4 and P5, respectively). The determination of the exact sulphide concentration in each standards was estimated by measuring 30ml of the standard solution by addition of 0.2ml of 0.1M Pb^{2+} till a millivolt drop of 100mv is attained. This value was considered as the actual concentration of sulphur, which was then multiplied by the standard dilution factor of 1/200,1/100,1/40,1/20 and 1/10 to know the actual concentrations of P1, P2, P3, P4 and P5 respectively. Fresh calibrations were conducted prior to each sampling.

3. RESULTS AND DISCUSSION

3.0 RESULTS AND DISCUSSION

3.1 Bioreactor monitoring

The integrated bioreactor (anaerobic-anoxic-aerobic) system used in this study was monitored on daily basis for two sensitive parameters: dissolved oxygen (DO) and temperature, whilst the biomass formation was twice in a week. Other physiochemical analysis were carried out once in a week. The pH of the reactor was self-regulated due to biologically activities without neutralisation and was found to be within 6 and 8. This was in agreement with Hermendez et al (2012), they reported that, concurrent removal of NH_3, H_2S and organic compounds from odour treatment plants are usually reached with or without pH control due to their high solubility.

The DO and temperature had significant effects on the biomass growth, nitrification process and as well as the biological treatment efficiency of the wastewater. This is due to the fact that the rates of the microbial or biomass growth are reliant on chemical reactions which are influenced by temperature. During the experimental runs, both the temperature and the DO in the reactors were maintained within the range of 20–24 °C and 2-3 mg/L for the aerobic conditions, whilst the anaerobic and anoxic DO was less than 1 mg/L(Muñoz et al 2015, Zub et al 2008). Both the effluent and the two internal recirculation flux were kept constant where necessary.

The biomass carried over into the aerobic reactor was also crucial in the optimization of the anaerobic–aerobic system. As the aerobic reactor accepted an effluent from the anaerobic and anoxic chamber, a significant amount of microorganisms entered the aerobic reactor and are not quickly adapted to the aerobic conditions. These active microbes had an effect on the cell population in the aerobic reactor and could lead to a mixed microbial population of low oxygen utilization and biological activity. The anaerobic cells becomes inactive then increase the TSS and VSS, which puts an extra burden on the downstream, thus settle down as sludge or centrates (Chan et al 2009, Kim et al 2014). The

results presented in the following sections are with regards to the performance of the biomass.

3.1.1 Oxygen, temperature and effluent monitoring

With a low organic loading rate (OLR-6 ml/min), high HRT (36 h) and SRT (10 d), the daily monitory of start-up 1 and 2 are represented in figures (5-6) and (7-8) respectively. .It was found that the aerobic oxygen concentration was within 2-3 mg/L (Muñoz, et al 2015).

- Start up 1 evaluation

Figure 5 shows that the trend of the dissolved oxygen (DO) and the temperature at an average of 2.2 mg/L and 21°C respectively. The DO in the he aerobic reactor, DO was found to be stable on the 16th day. Similarly, both the anaerobic and anoxic DO concentrations were monitored, and were found to be high (1.6mg/L and 2.7mg/L) and as time progresses was reduced to (0.6 mg/L and 0.7mg/L) respectively.

From

Figure 6, it is seen that for the first 16 days, due to the high DO generated from the anaerobic and anoxic zones, the aeration was alternated to cushion the DO in the aerobic zone from the 20th to 30th , was stable at aeration rate of 50 ml/s when it was set. The average effluent flux was found to be 8 L/d.

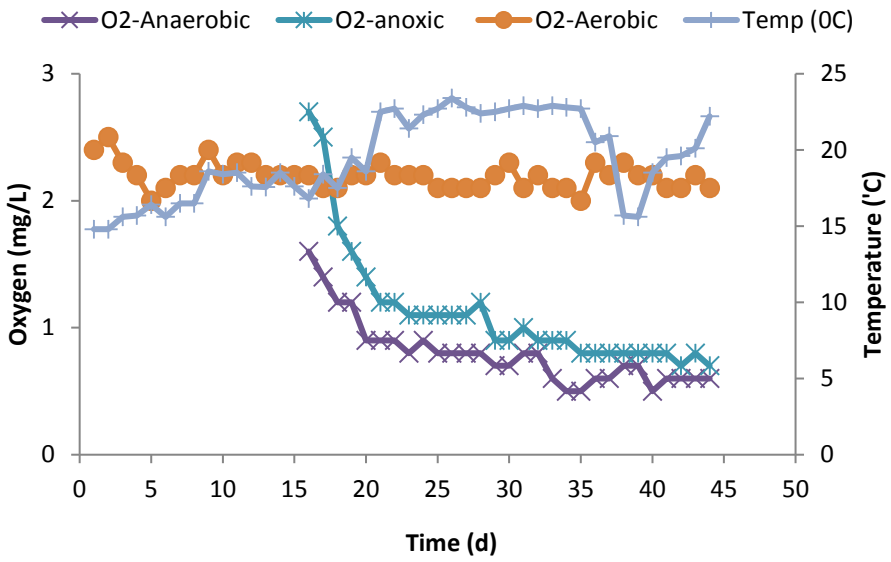


Figure 5. Start up1 oxygen (mg/L) and temperature (°C) daily monitoring; OLR (6 ml/min); HRT (36 h); OR (50 ml/s); SRT (10 d).

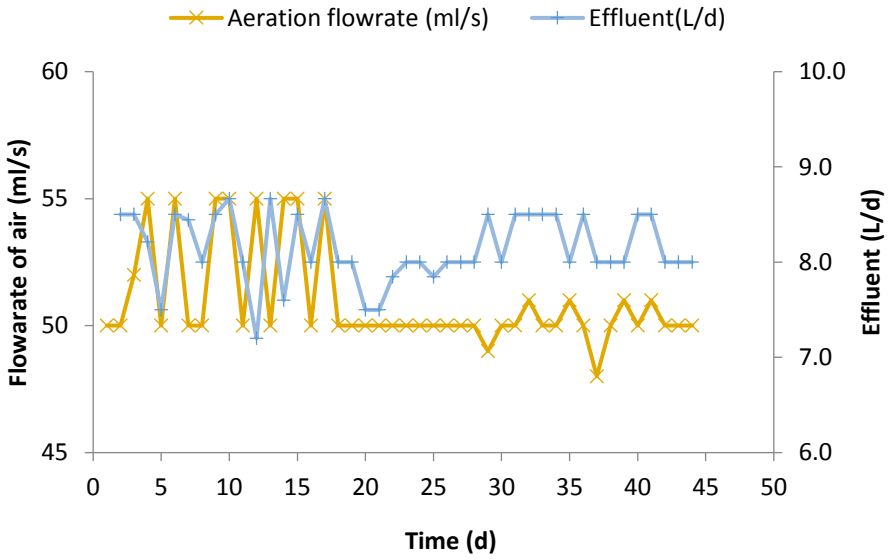


Figure 6 setup1 aeration (m/s) and effluent (L/d) daily monitoring; OLR (6 ml/min); HRT (36 h) ;OR(50 ml/s); SRT (10 d).

- **Startup 2 evaluation**

During the second stage of start-up, OLR was changed to 9 ml/min, HRT to 24 h and OR to 60 ml/s. This resulted in a tremendous change in the growth of the biomass in both the anaerobic and anoxic chambers (discussed next in Table 5).

Figure 7 shows that, for the first 20 days, there was a gradual decrease of the DO concentration in the reactor until a stable concentration was attained (values for anoxic and anaerobic). Both the DO and the temperature in the aerobic chamber were found to be stable at 2.2 mg/L and 22.5 °C respectively.

In

Figure 8, shows the aeration and effluent flux for the second start up. The aeration rate was found to be in steady state at 60ml/s.Then from the 20th day onwards, the aeration was kept constant at 60 ml/s which resulted in 2.1 mg/L of oxygen, this was found to be the optimal hence was maintained to enhance the operation. The average effluent flux was found to be 9L/d.

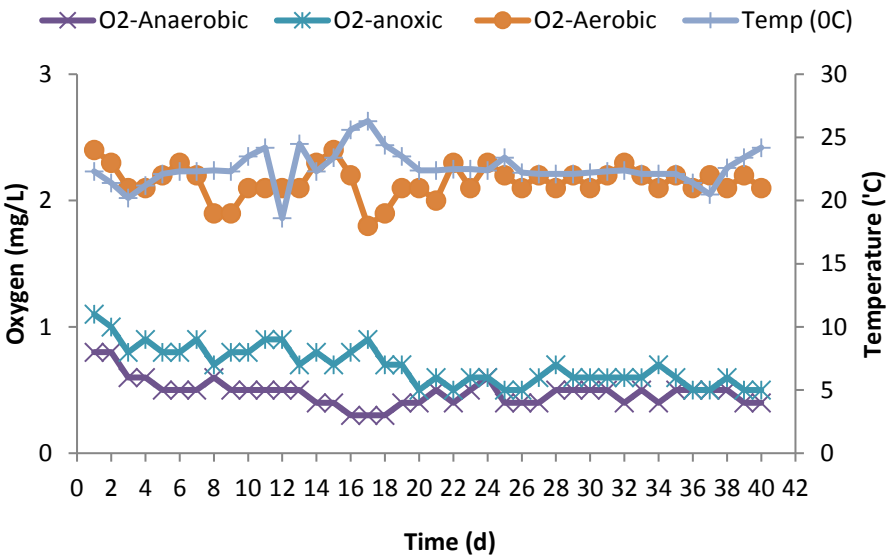
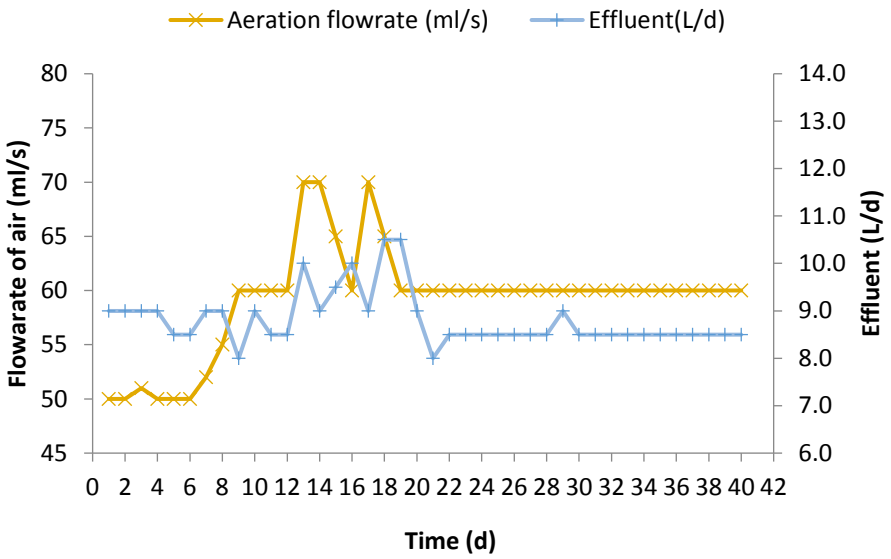


Figure 7. Start up 2 oxygen (mg/L) and temperature (oC) daily monitoring; OLR (9ml/min); HRT (24h) ;OR(60ml/s); SRT (10d)



.

Figure 8 setup 2 aeration (m/s) and effluent (L/d) daily monitoring; OLR (9ml/min); HRT (24h) ;OR(60ml/s); SRT (10d)

Comparative study on startup 1 and 2

The start up1 and 2 average operating condition performance is depicted in Table 3. It was established that the higher OLR (9 ml/min) with a low HRT (24h) decreased the DO present in the anaerobic (0.79-0.48 mg/L) and anoxic (1.11 mg/L-0.7mg/L) zones. With high a OLR, the food nutrient present for the microbial population activity is said to be in excess which generated a high amount of carbon, and thus a decrease in the DO. In order to balance the oxygen in the aerobic zone, a high aeration rate of (60ml/s) was required. In addition, the DO which were found to be 0.4 to 0.7 mg/L in the anaerobic and anoxic zones respectively had a beneficial effect on the denitrification process, and resulted in the biomass growth (8.44mgTSS/L; 6.8mgVSS/L) in the reactor for the second setup (Table 5). And the temperature and DO obtained in the aerobic zone were ideal conditions for working under biological treatment system. Thus operating DO for combined carbon oxidation-nitrification systems is 2 mg/l (Munoz et al 2015). Therefore using the second operation conditions to start up the reactor will enhance the performance rate with a short transient phase to achieve stability.

Table 3 -Comparing startups for biomass performance

Average parameter		Anaerobic (BE2)	Anoxic (BE3)	Aerobic (BE4)	Settler (BE5)	Effluent
Start up 1	Dissolved oxygen(mg/L)	0.79	1.11	2.17		
(44days)	Temperature(0C)		20.8			
	TSS(mg/L)	0.94	0.80	0.84	5.39	0.06
	VSS(mg/L)	0.85	0.73	0.71	4.83	0.06
	Biomass (gTOC/Ld)			0.079		0.132
Start up 2	Dissolved oxygen(mg/L)	0.48	0.7	2.16		
(40days)	Temperature(0C)		22.50			
	TSS(mg/L)	1.94	1.19	0.88	8.44	0.08
	VSS(mg/L)	1.62	0.98	0.72	6.81	0.07
	Biomass (gTOC/Ld)			0.132		0.132

3.1.2 Variation of biomass concentration over the time

Prior to the start-up 1 and 2, the reactor was inoculated with 10 L of activated sludge of initial TSS concentration of 3.5 g/L and 4.4g/L respectively. However, there were some significant changes resulting from the recirculation and the sedimentation tank to the anaerobic tank, and also dilution effect caused by the aerobic-anoxic circulations. The variation in the biomass concentration for the startup 1 and 2 is represented in Figures (9-11) and (12-14) respectively, and Figure 15 as a comparative study.

- Start up 1 evaluation

Figure 9 shows a gradually increase of the initial biomass concentration in terms of TSS as mentioned before , from the settler harvest, whilst the aerobic zone was found to be 1.5 gTSS/L, this led to the desludging of about 300 mlTSS/day in order to accommodate the biomass. However, there was sharp drop on the 3rd week (1.85gTSS/L), while on the 4th week there was significant increase to 8.73 gTSS/L,then a drop to 5.5TSS/L on the 5th week , and finally stabilizing around 5 gTSS/L, with some fluctuations on weeks 9 and 12. The effluents in the anaerobic and anoxic had an average value of TSS concentration of 0.05 gTSS/L, 1.43gTSS/L and 1.18 gTSS/L, respectively which eventually kept decreasing as the days progresses.

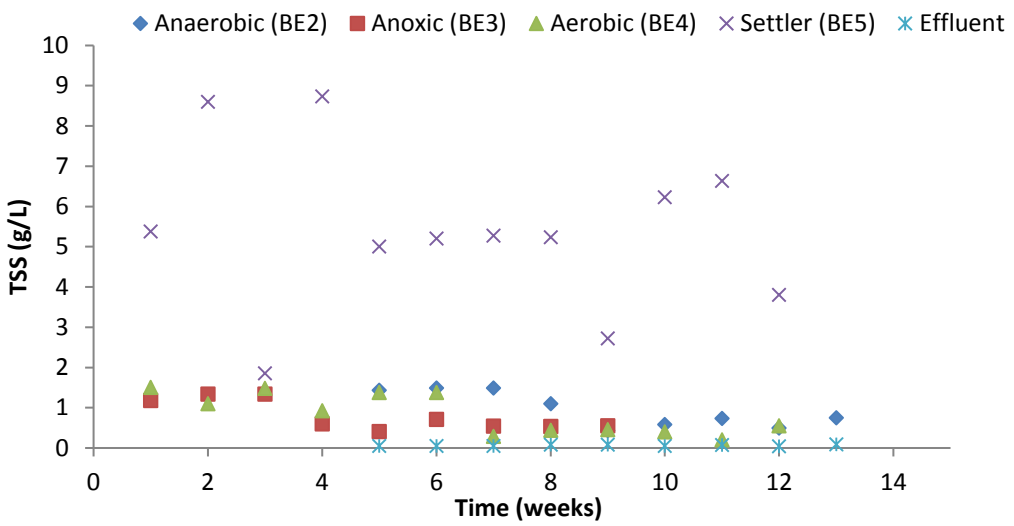


Figure 9. Variation of biomass in terms of TSS(g/L) weekly monitoring; OLR (6ml/min); HRT (36h) ;OR(50ml/s); SRT (10d).

Figure *10* shows the biomass performance in terms of the VSS. This also shows the same pattern as the TSS, where the initial harvest of the biomass concentration was increased from 4.46 gVSS/L to 7.1gVSS/L on the 2nd week, then a significant drop to 1.6 gVSS/L on the 3rd week due some operational changes. However, there was a shot up in growth from the 4th week and a drop on the 5th week from 7.31gVSS/L to 4.431gVSS/L. This was then maintained until a final biomass of 4.71gVSS/L was attained.

Figure *11* shows the daily production of the biomass as defined as the sum of the TSS by each reactor volume per the SRT ($TSS*V/SRT_1$). By maintaining the SRT at 10days, the average production was found to be 1.14 gTSS/day and desludging rate of 268 mlTSS/day. Hence, the higher the amount of biomass produced, the more amounts required to desludge.

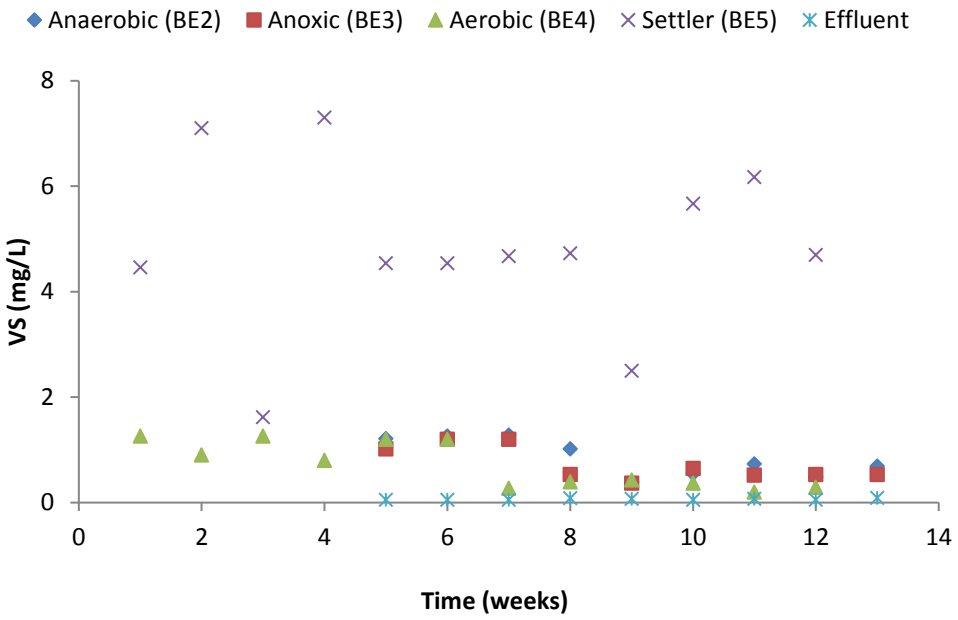


Figure 10. Variation of biomass in terms of VSS (g/L) weekly monitoring; OLR (6ml/min); HRT (36h) ;OR(50ml/s); SRT (10d).

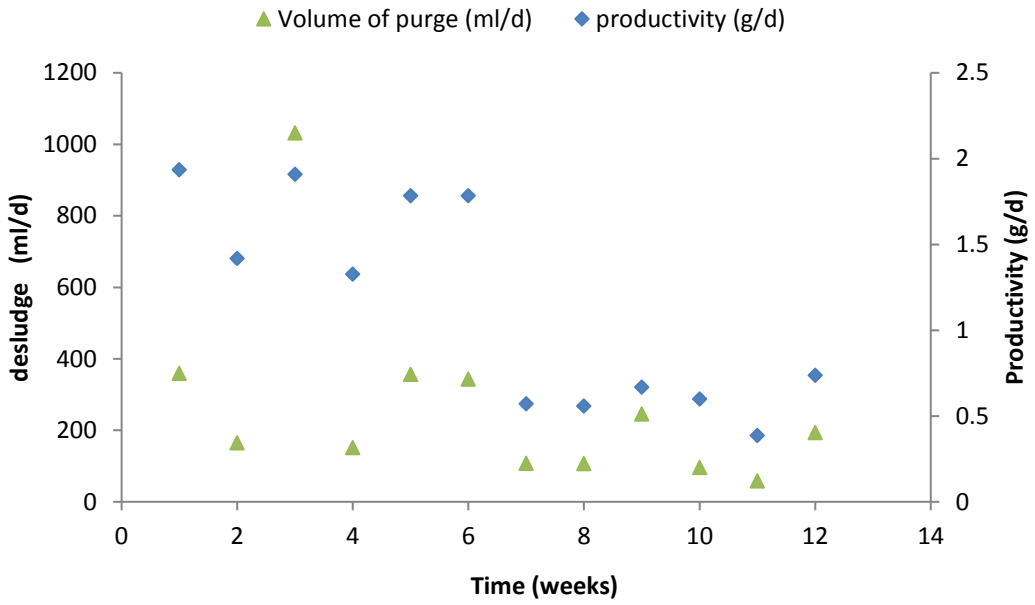


Figure 11. Biomass production and amount desludge per day; OLR (6ml/min); HRT (36h) ;OR(50ml/s); SRT (10d).

- **Start up 2 evaluation**

The change in OLR, HRT and OR to 9 ml/min, 24h and 60 ml/s respectively brought about a significant change in the biomass harvested by the settler. Figure *12* shows an increase from 4.4 gTSS/L to 12.4 gTSS/L for the two weeks, until the 3rd week were a significant drop to 6.83 gTSS/L was noticed. This was due to the transfer of a large amount of biomass from the aerobic zone to the anaerobic and anoxic zone. However, after the 4th week with stability of the system the biomass concentration was increased to 15 gTSS/L, afterwards, it was maintained until 3.9 gTSS/L have been attained finally. While the anaerobic, anoxic and aerobic zones biomass were within 0.9-2.7 gTSS/L, and that of the effluent was less than 1 gTSS

Error! Reference source not found. shows the same pattern as the TSS biomass performance of the startup 2, however there was significant drop from 9.67gVSS/L to 5.67gVSS/L for the 4th week, then an increase to 12.13gVSS/L for the 4th week. A biomass concentration of 3.9 gVSS/L was obtained finally.

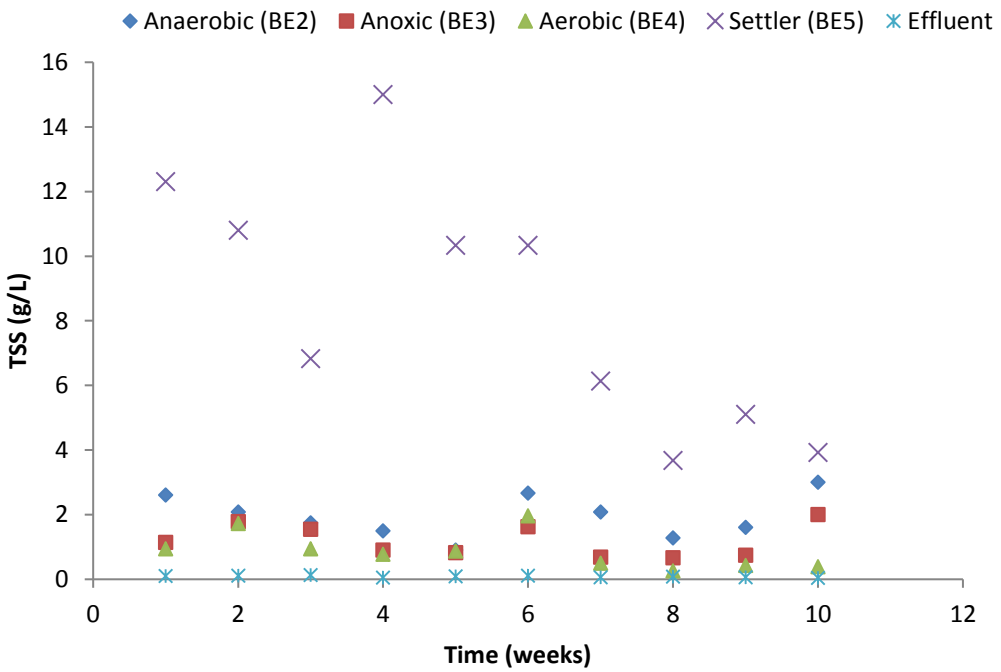


Figure 12. Variation of biomass in terms of TSS (g/L) weekly monitoring; OLR (9ml/min); HRT (24h) ;OR(60ml/s); SRT (10d).

Figure 14 shows the daily production of the biomass and amount of sludge required to be desludge in the second start up. By maintaining the SRT at 10 days, the average production was found to be 1.36 gTSS/day and desludging rate of 177 mlTSS/day. The higher the amount of biomass produced, the more amount required to desludge.

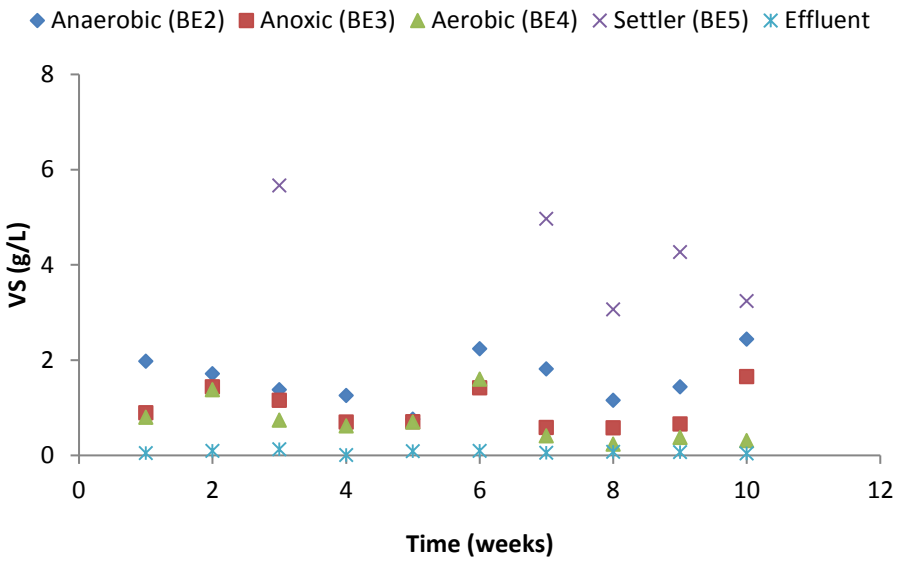


Figure 13. Variation of biomass in terms of VSS (g/L) weekly monitoring; OLR (9ml/min); HRT (24h) ;OR(60ml/s); SRT (10d).

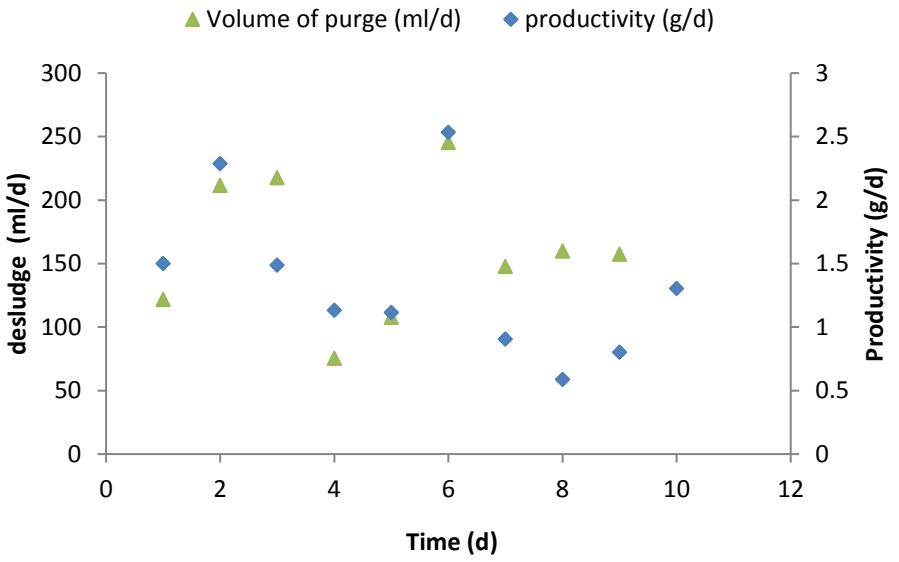


Figure 14. Biomass production and amount desludge per day; OLR (9ml/min); HRT (24h) ;OR(60ml/s); SRT (10d).

Comparative study on start-up 1 and 2

Figure 15 shows the average production of the biomass in each segment. It was found that an increase in OLR with reduction in HRT at a constant SRT led to an increase in biomass in all the zones. The harvest of the biomass from the settler was found to increase as days progress , in the likes of the biomass in the anaerobic zone was increased by 65% due to the increase in the feed to centrates ratio (1:3) (Chan et al 2009, Zub et al 2008). This therefore, increased the microorganism activity at that stable F/C ratio, with an increase in biomass with the reduction in the HRT, finally the biomass concentration reached 8.4 gTSS/L and 6.8 gVSS/L for TSS and VSS respectively. It seemed that at a constant SRT, the F/C ratio was independent of HRT, which also depends on the OLR and influent concentration. Thus, these results are due to increasing the biomass concentration at the lower HRTs and a constant F/C ratio during the entire study period. Thus a steady state anaerobic-aerobic operation will be able to generate more biomass and was found to be in agreement with the report by Chan et al (2009 and Kim et al (2014).

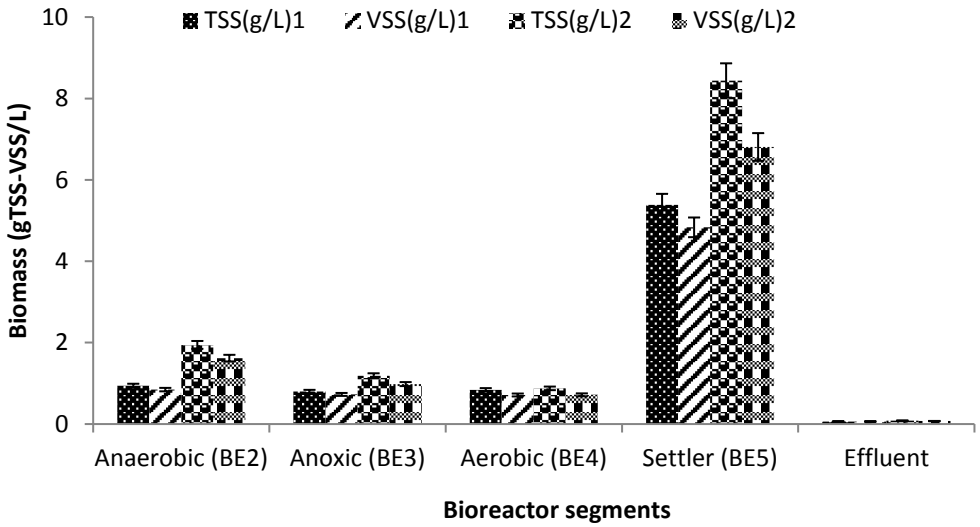


Figure 15-Average biomass performance in bioreactor segments

3.2 Bioreactor wastewater treatability performance

The integrated bioreactor as mentioned before, treated the wastewater via nitrification-denitrification process, hence the removal of the organics, nitrogen, phosphorus and sulphates were determined to evaluate its performance. In view of that the water quality parameters were focused on included the total nitrogen (TN), total organic carbon (TOC), total carbon (TC), inorganic carbon (IC), Sulphate (SO_4^{2-}), chlorides (Cl^-), ammonium (NH_4^+), nitrates and nitrite. However, the concentrations of NO_3 and NO_2 in the influent were practically zero. The average performance values during the study period is depicted in *Table 4* and discussed in the following sections.

Table 4-Summary of bioreactor treatability

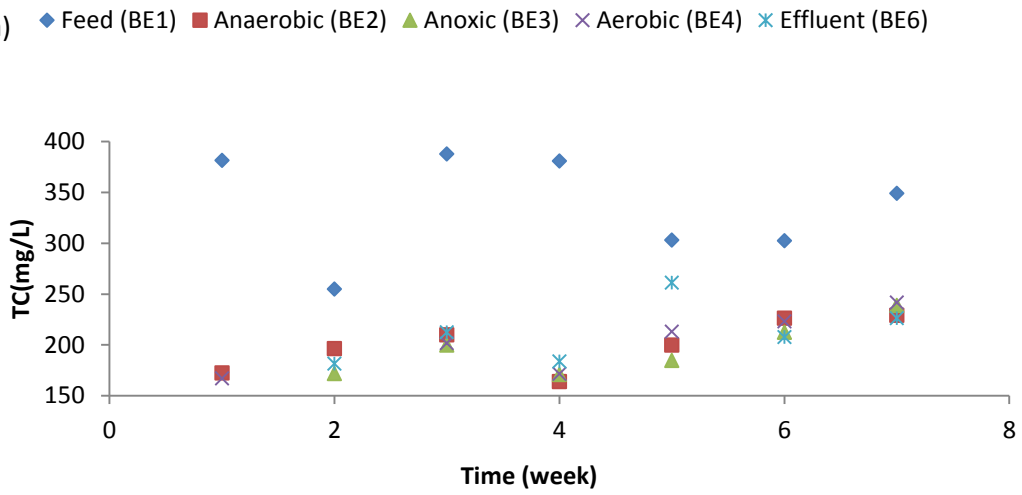
Runs	Parameter	Influent	Effleunt	Performance (%)
Startup1	TN(mg/L)	39.1	17.4	55%
	TOC (mg/L)	155.8	29.3	81%
	TC(mg/L)	337.1	212.1	37%
	IC(mg/L)	184.2	173.4	6%
	NH4(mg/L)	8.8	7.5	15%
	SO4 (mg/L)	14.5	12.3	15%
	Cl (mg/L)	26.3	25.7	2%
Startup2	TN(mg/L)	36.9	32.1	13%
	TOC (mg/L)	125.9	30.2	76%
	TC(mg/L)	321.3	247.3	23%
	IC(mg/L)	210.9	177.8	16%
	NH4(mg/L)	17.2	17.0	2%
	SO4 (mg/L)	25.3	20.4	19%
	Cl (mg/L)	26.3	25.7	2%

Removal of organic carbons

Organic carbons in the wastewater were in the form of the food nutrients, dissolved carbon and the decaying microorganisms. This was evaluated under the two different startups operating conditions as mentioned earlier.

Figure 16Figure 17Figure 18 shows the variations in TC, TOC, IC and effluent quality in the different reactors during the running period of the pilot plant. For the first startup, influent was characterised to have high amount of TC (337.1 mg/L) of which 37% was removed, although, the TC was made up of IC and TOC, of which 6% and 81% were removed respectively. However on the 8th week the first phase of the experiment ended and the reactor was stopped and prepared for the second phase. In the second phase of operation, the initial, TC in influent was 321.3 mgTC/L and a removal of 23%, 76% and 16% was observed for the TC, TOC and IC respectively. It is clear from the Figure 16Figure 17 Figure 18, and *Table 4* that the change in operating conditions and the water chemistry did affect the reactor performance. So, also an increase in the OLR from 6 ml/min to 9 ml/min, did increased organic nutrients in the reactor with less active microorganisms to enhance the degradation. However, about 76% removal of the TOC in both scenarios observed in the entire operation during stability has been reported by Falahti-Marvast and Karimi-Jashni, (2015) as one of the advantages of the integrated bioreactor.

(a)



(b)

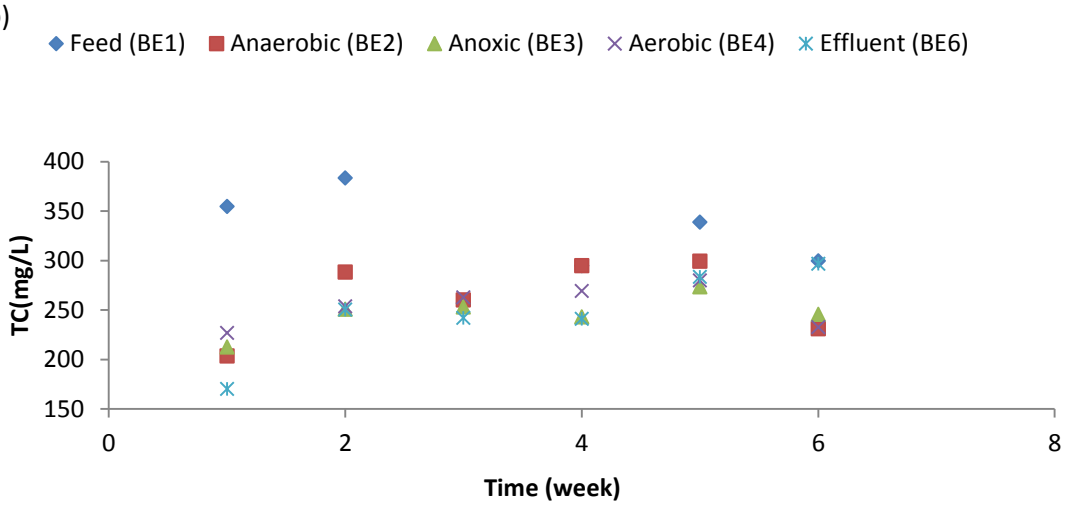


Figure 16.Total carbon (TC) concentrations at (a) startup1 and (b) startup 2.

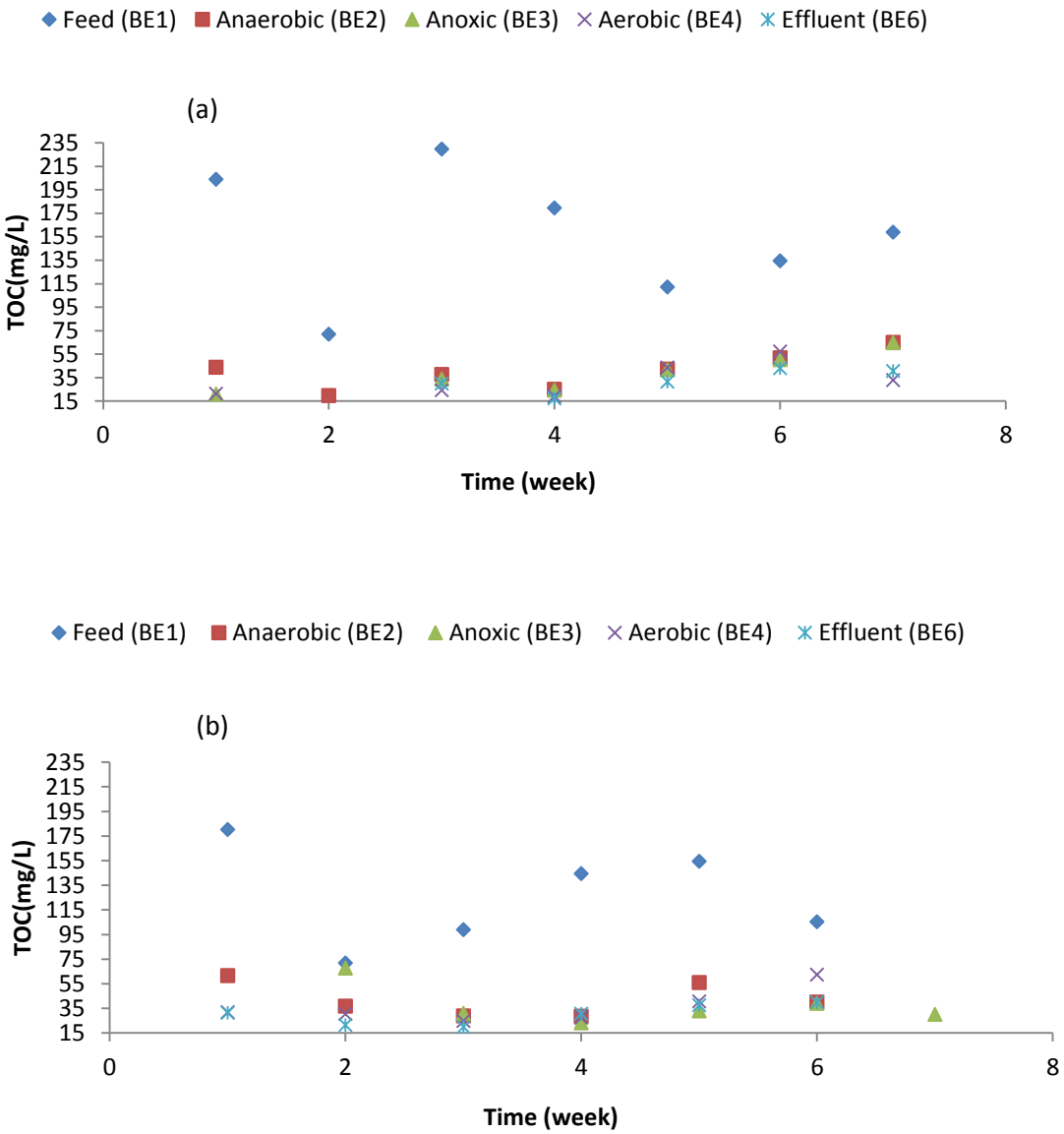


Figure 17.Total organic carbon (TOC) concentration at (a) startup1 and (b) startup 2.

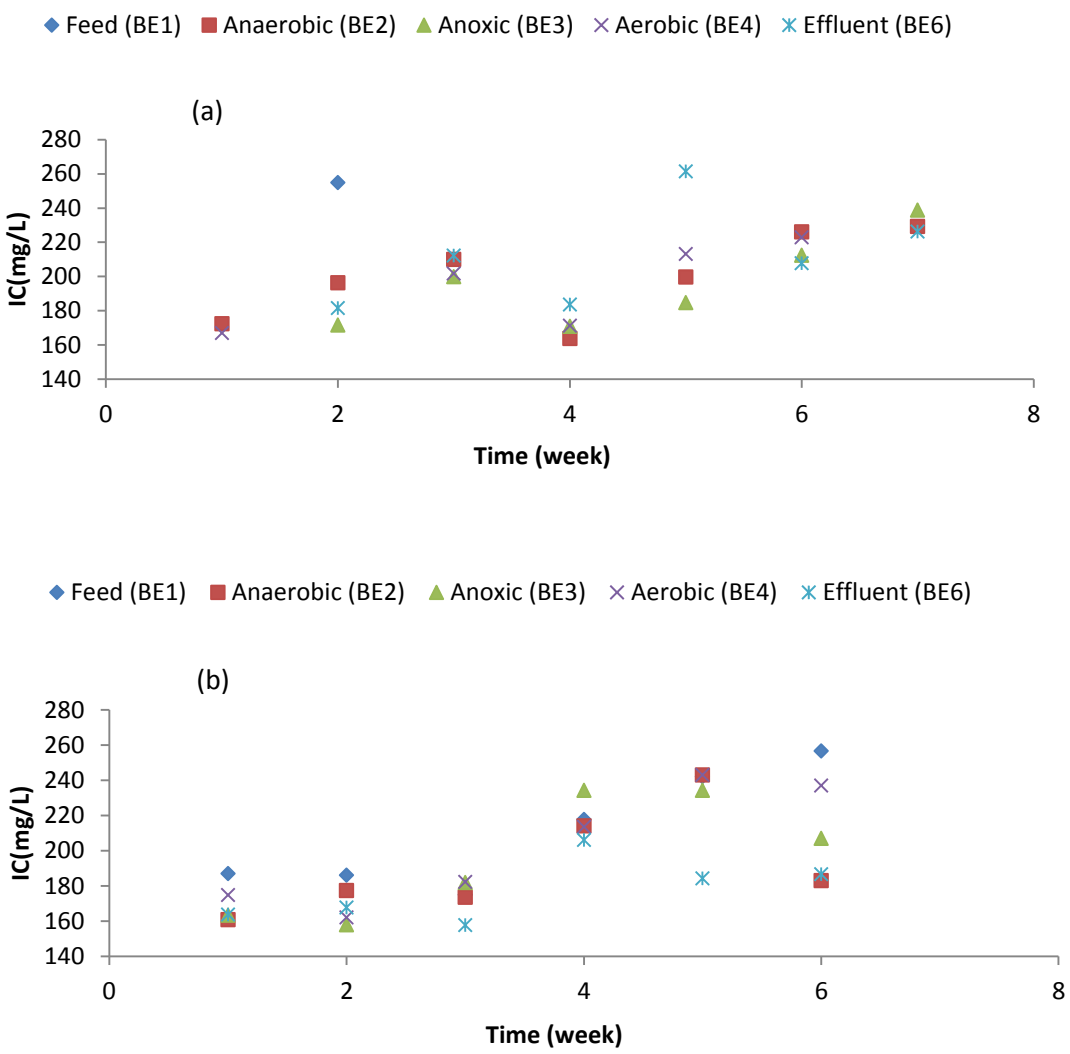


Figure 18.Inorganic carbon (IC) concentration at (a) startup1 and (b) startup 2.

Sulphate and Chloride removal

The biological purification enhances the sulphate and chloride elimination from the wastewater. This phenomenon occurrs in the anaerobic and the aerobic zone interchangeably via the denitrification mechanism (Muñoz et al 2015, Zub et al 2008, Falahti-Marvast and Karimi-Jashni, 2015). It has also been established that, the elimination mechanisms are bound to stop when the microorganisms becomes inactive.

Although, there was no sulphide addition to the influent, Figure 19 shows that the sulphate in both influent and effluent increased, and ranged from 12.34 to14.5 mg/L with 15% removal during the nitrification-denitrification process. However, a change in the OLR and HRT led to a significant increase in this range to 20.4 to 25.3 mg/L for the influent and effluent respectively and a 19% removal. Therefore, recycling of the nitrate back to the reactor is a guarantee for the sulphate removal. Figure 20 shows the chloride performance, it was found to be between 26 and 25 mg/L for the influent and effluent, with no significant removal. The low removal of the sulphate and chloride, might then be attributed to the low nitrate available during the denitrification process (Falahti-Marvast and Karimi-Jashni, 2015).

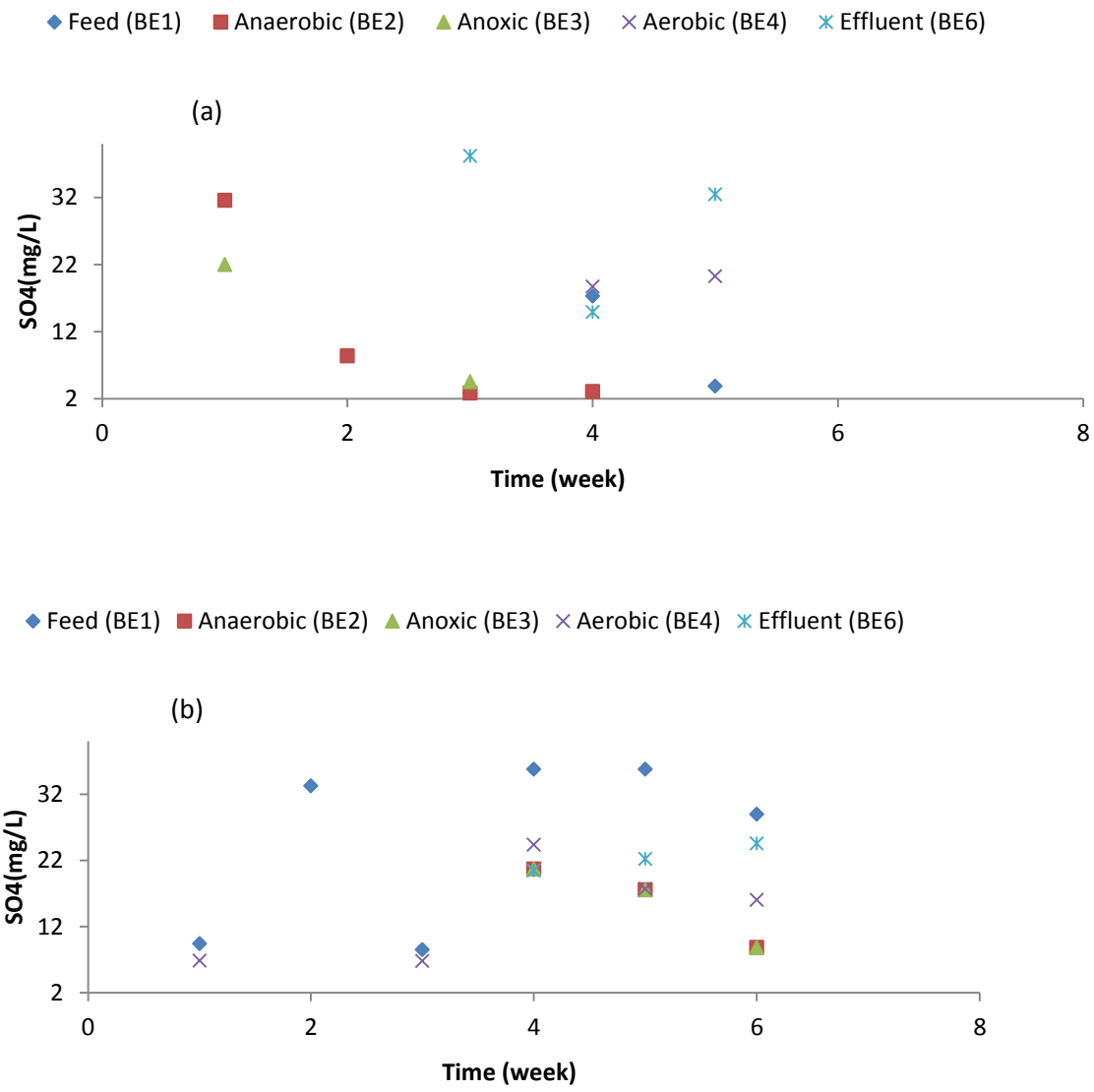


Figure 19. Sulphate concentration at (a) startup1 and (b) startup 2.

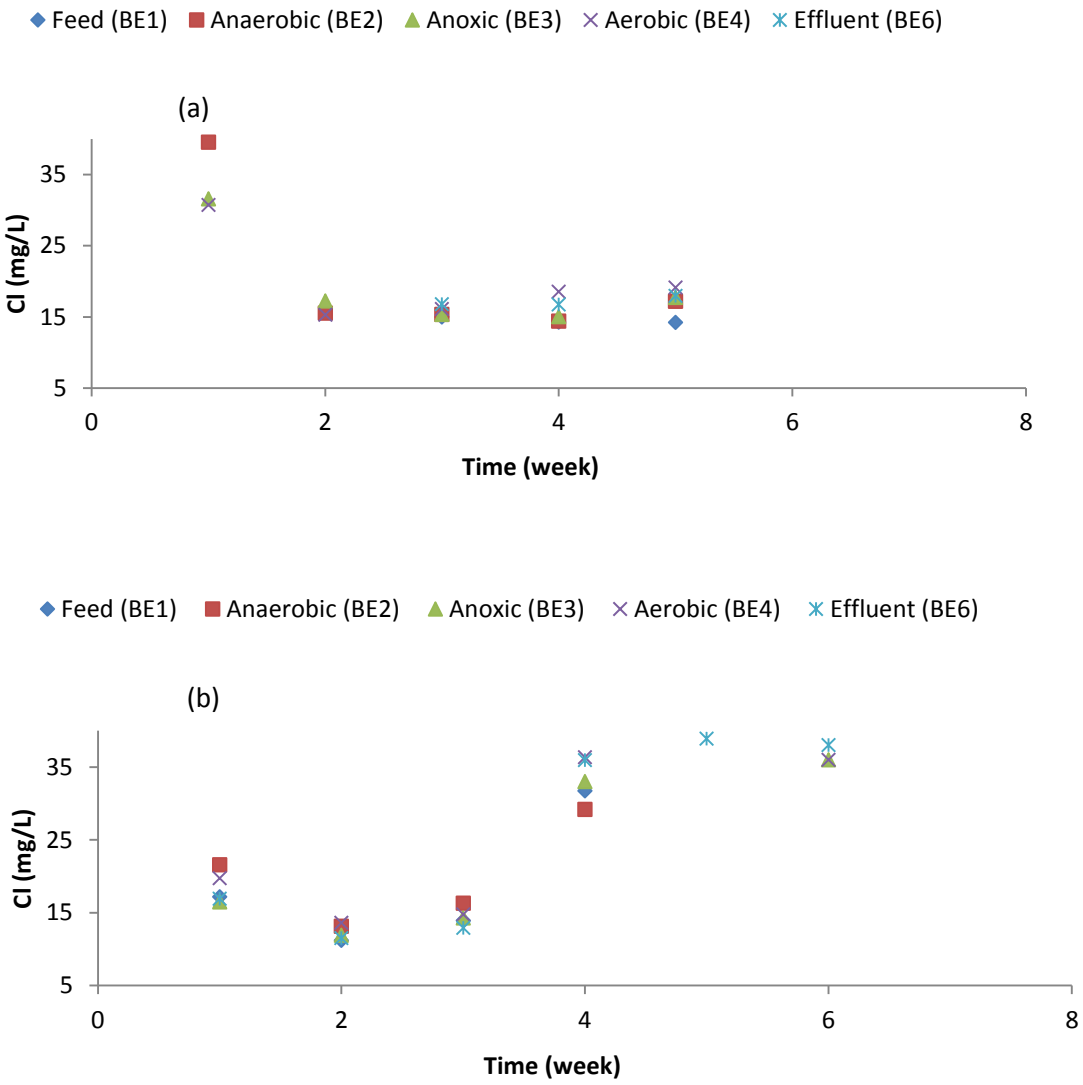


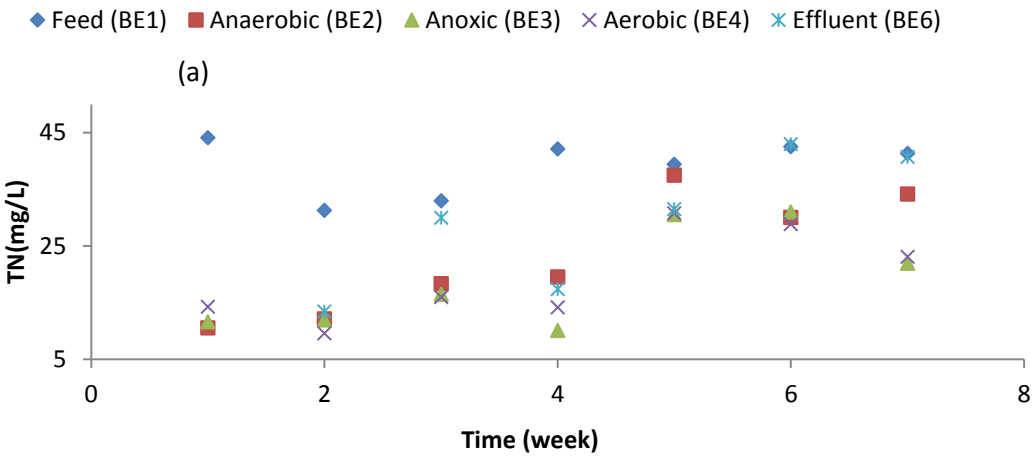
Figure 20. Chloride concentration at (a) startup1 and (b) startup 2.

Removal of total nitrogen (TN), ammonium (NH_4^+), nitrates (NO_3) and nitrite (NO_2)

The total nitrogen present in the wastewater was a result of the amino groups (in proteins and amino acids) as well as NH_4^+. The concentration of TN in the influent was 30–45 mg/L and 12–30 mg/L in the effluent. And the NH4 concentration in the influent was 6–25 mg/L and 7–17mg/L in the effluent.

Figure 21 shows the variation of the TN in the influent and effluent for the different operating conditions during the study periods. For the startup1, the concentration of the TN was reduced from 39.1 mg/L to 17.4 mg/L at a performance rate of 55%.While in the second phase, the concentration of the TN was 36.9 mg/L and was reduced to 32.1 mg/L giving a 13% removal (Table *4*-Summary of bioreactor treatability*Table 4*). It was found that, if the influent contains organic matter such such as the NO_3 and NO_2, they can be removed via the denitrification process (Munoz et al 2015, Zub et al 2008, Falahti-Marvast and Karimi-Jashni, 2015).

Figure shows the performance of the NH4 removal from wastewater. It was noticed that the concentration of the NH4 was low within the range of 7.5-17.2 mg/L, with performance rate of 15% and 2% for the startup 1 and 2 respectively (*Table 4*). However, the NH4 is nitrified and recycled to the inlet works where they undergo denitrification. Thus denitrification converts nitrogen to a harmless form, which has no significant effect on the environment. It was also observed that the nitrate circulation to the anoxic compartment was also efficient during the first start up, while the second start up there is a possibility of delay by denitrifying bacteria growth during the acclimatization period. And many researchers have reported that the nitrate concentration in the centrates or nitrified stream when recycled will be the key parameter to provide enough sulphide oxidation potential (Munoz et al 2015, Zub et al 2008). As reported by Munoz et al (2015) that a conservative concentration of nitrate in the stream after the nitrification of an ammonia-rich centrates (500 mg N03/L), only a 0.2% recycling ratio would be needed for sulphide oxidation.



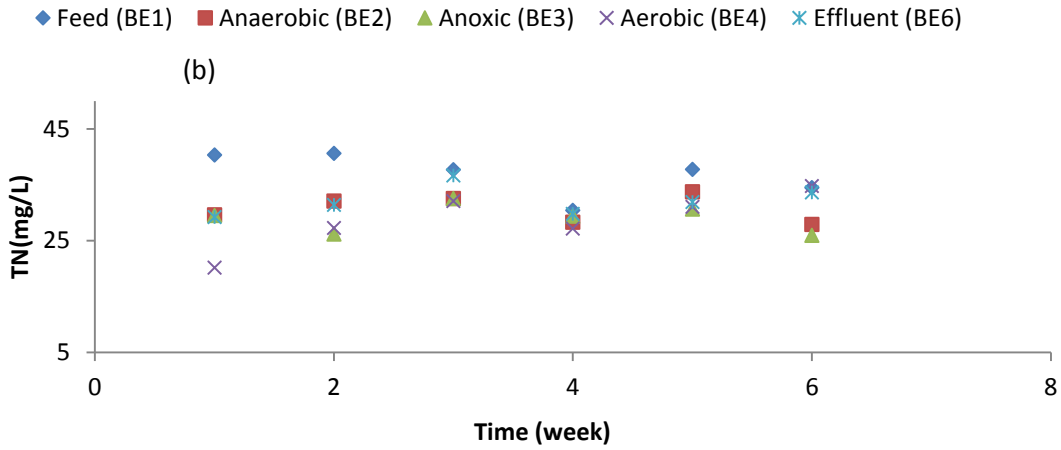


Figure 21. Total nitrogen (TN) concentration at (a) startup1 and (b) startup 2.

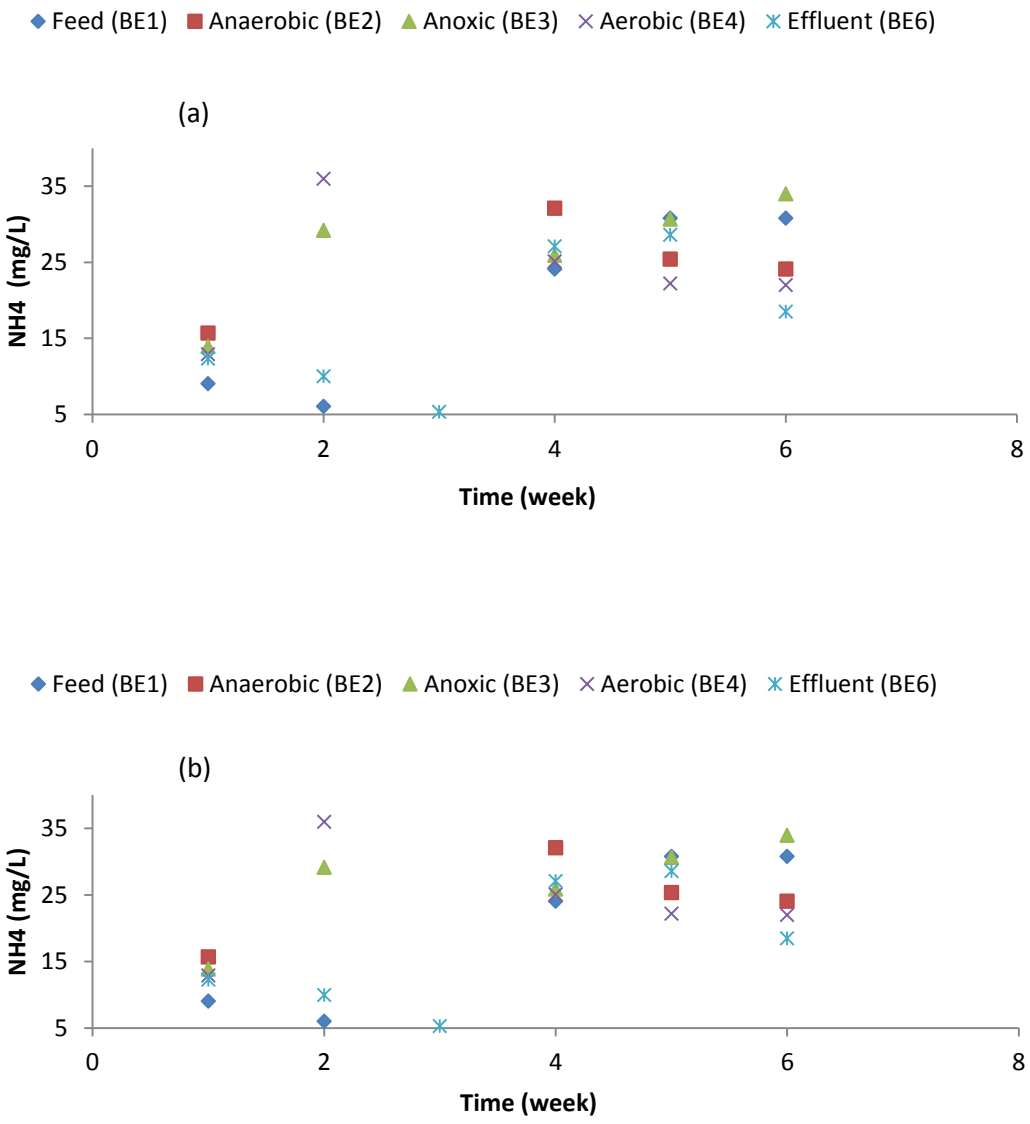


Figure 22. Ammonia concentration at (a) startup1 and (b) startup 2.

3.3 Nitrate-sulphide oxidation kinetics

The aim of the recycling the nitrate from the activated sludge was to enhance sulphide-oxidation, thus to prevent sulphur release via biological control. However, the sulphide was not applied on the bioreactor but rather a batch scale. Therefore, the kinetics and activated sludge adaptation for the oxidation of the sulphide using nitrate as the electron acceptor was evaluated and the result discussed in the following section.

Evaluating nitrate-sulphide oxidation in tap water and synthetic wastewater

The abiotic test, performed by addition of nitrate and sulphate in the absence of activated sludge to tap water and the synthetic wastewater (SWW) as per the experimental design, resulted in a reduction of the sulphate concentration in both cases. The initial sulphide concentration for throughout the experiment was 15mgS/L. Figure 23 shows how sulphur concentration in tap water was reduced from 15.77mgS/L to 14.67mgS/L, thus 71% over 10 hours period. Surprisingly, the use of the synthetic wastewater resulted in a reduction from 14.41 mgS/L to 6.38 mgS/L, thus the sulphur was reduced to 44% over the 10hrs period. Therefore, nitrate served as the electron acceptor for the chemical oxidation of hydrogen sulphide.

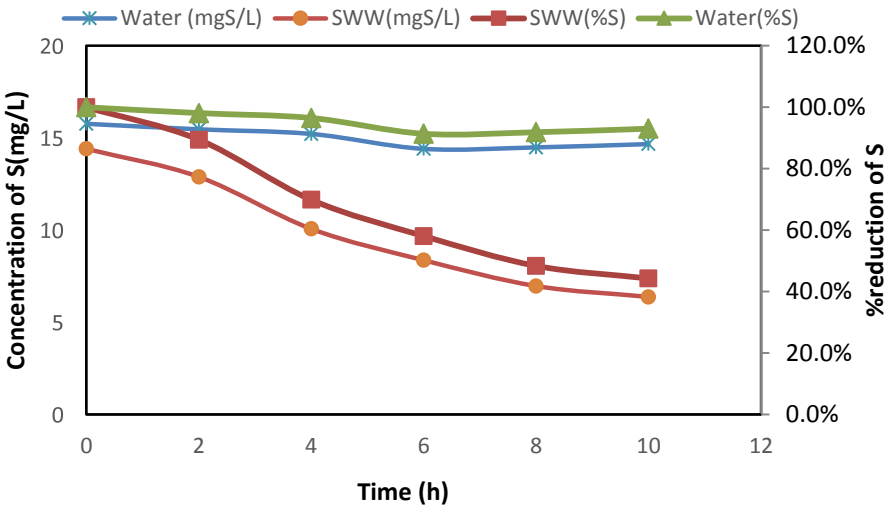


Figure23. Time course of the sulphide concentration in the abiotic test performed on tap water and synthetic wastewater (SWW)

Evaluating nitrate-sulphide oxidation at different nitrate/sulphide and S/biomass ratios

In this study, activated sludge addition was evaluated to emphasis the view on the water chemistry. Thus, from previous studies (gif) as it was found that the SWW acclimatization was the best option for the nitrate-sulphide oxidation. The variations in sulphide concentration over the time course of the experiment at different N/S ratios (1:1 and 3:1) are presented in Table 5.

Table 5. Results of nitrate-sulphide concentrations at different nitrate concentrations

Time (h)	SWW+N				SWW+0.15gN +0.5gS/TSS				sww+0.45gN+0.5gS/TSS			
	S(mg/L)	PO4 (mg/L)	SO4 (mg/L)	NO3 (mg/L)	S(mg/L)	PO4 (mg/L)	SO4 (mg/L)	NO3 (mg/L)	S(mg/L)	PO4 (mg/L)	SO4 (mg/L)	NO3 (mg/L)
0	11.5	10.8	16.6	2.5	9.9	10.1	22.3	2.8	10.0	9.1	27.8	2.9
6	11.2	13.2	28.7		10.0	25.1	25.9		10.5	16.0	20.8	
8	11.0	12.5	19.3		10.4	8.5	22.5		10.6	14.3	18.0	
10	10.5	9.9	21.3		10.2	13.4	22.3		10.1	10.8	15.7	

Figure 24 and 25 show the oxidation of sulphide to form sulphur at high and low activated sludge concentration ranges of sludge control, 2 and 8gS/gTSS and 0.1 and 0.5gS/gTSS, respectively. In figure24, 86%, 96% and 77% sulphur concentration removals were obtained using SWW only, 2gS/TSS and 8gS/TSS respectively. While in the lower sulphur/biomass range 87%, 98% and 98%

reduction of the initial sulphur were obtained during the study period as shown figure 25.

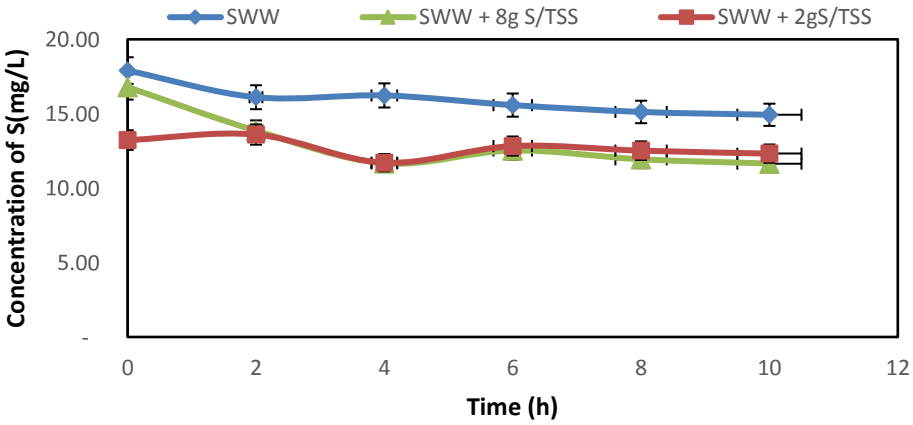


Figure 24 . Time course of the sulphur concentration in the range of high S to biomass ratios

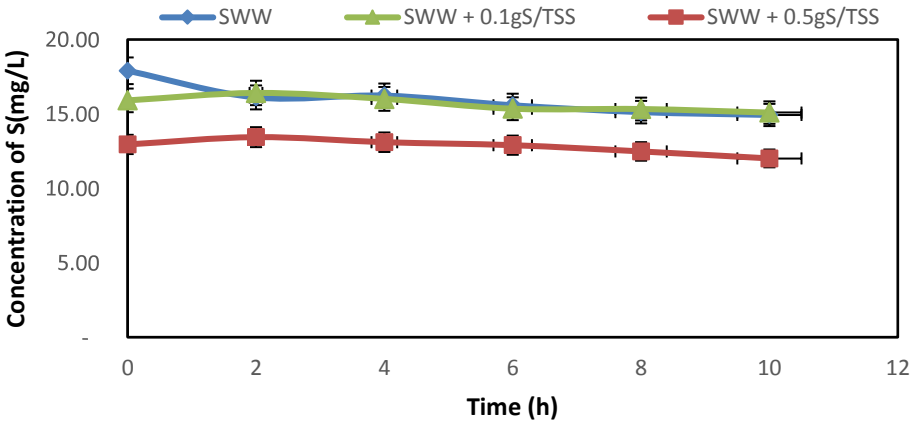


Figure 25. Time course of the sulphur concentration in the range of low S to biomass ratios

A further investigation was carried out to elucidate the influence of the nitrate to sulphide ratio. The results are presented in figure 26.Therefore, to enhance the total sulphur removal via the nitrate-sulphide oxidation mechanism, there is the need for more nitrate for the nitrification-denitrification cycle to go to completion.

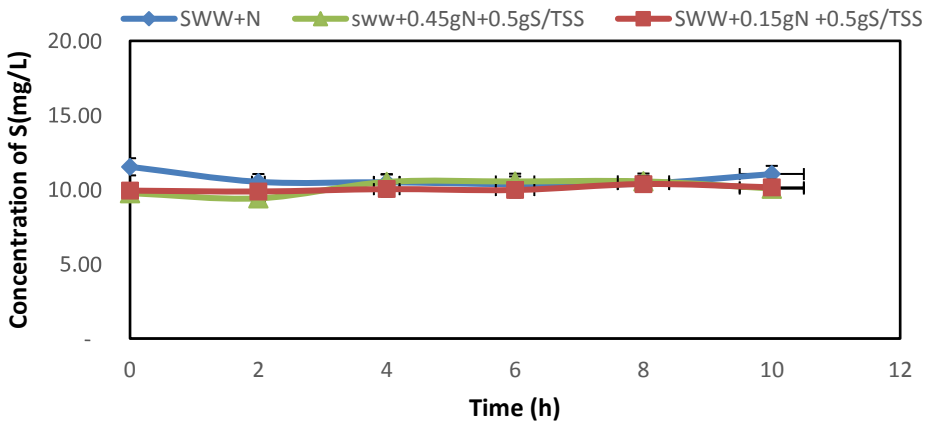


Figure 26. Time course of sulphide concentration at different nitrate concentrations

Adaptation of activated sludge

An activated sludge capable of oxidizing sulphide using nitrate as electron acceptor was acclimated with nitrate and sulphide and a sludge to wastewater ratio of 50%/50% per volume. Figure 27 and 28 show the accumulation of the nitrate-sulphate and its performance. In Figure 27. , sulphur concentration was reduced in the presence of nitrate from its initial concentration of 15mgS/L Thus, at time zero, the concentrations of the sulphide and nitrate were (1.44gNto 5.76gN) and (0.45gSto 1.8gS) respectively. The chemical analysis shows that 122.23mg/L, 205.6mg/L and 469 mg/L were the initial concentrations of sulphate, phosphate and nitrate, respectively. On the 4th run (12th day) it was found that there was a reduction in the concentration of sulphate and the nitrate to 57.3 mg/L and 106.9 mg/L respectively, and a complete removal of phosphate. The acclimation of the microbial population to a nitrate-driven sulphide oxidation will allow an efficient prevention of odour formation.

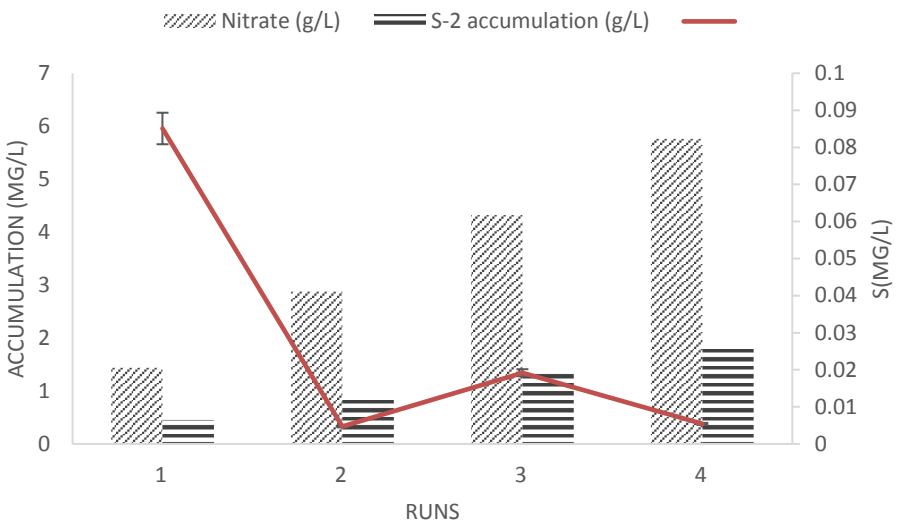


Figure 27. Adaptation of nitrate-sulphate over time period

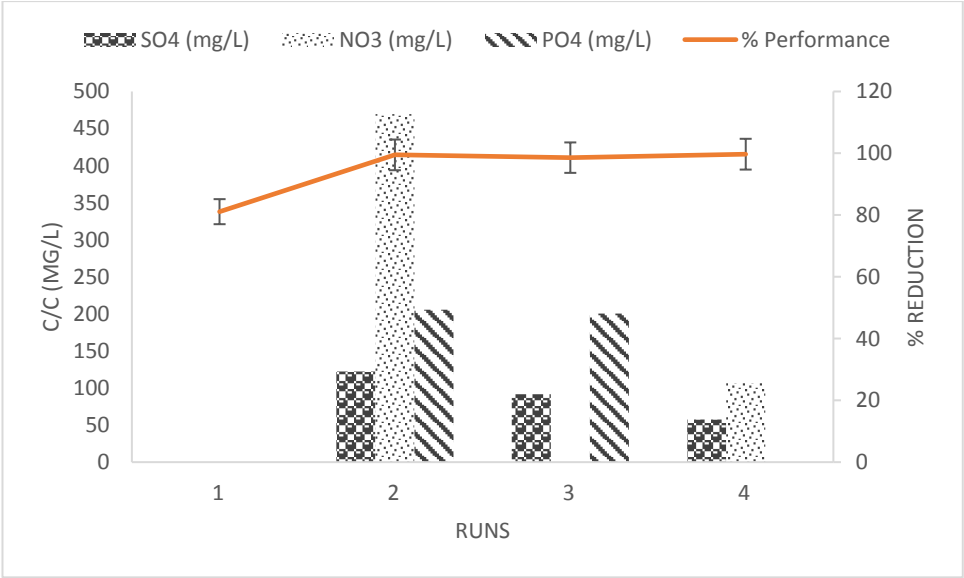


Figure 28 .Nitrate-sulphate concentrations after analysis and sulphur performance (%)

4.0 CONCLUSION

The A2O process integrating anoxic, aerobic and anaerobic conditions in a single bioreactor represented a suitable platform for the implementation of a low cost biotechnology for the abatement of odorous compounds such as sulphur in wastewater treatment plant. Thus, recycling of the nitrate generated from the oxidation of centrates can prevent odour emission in the headworks of wastewater treatment plants without any chemical consumption cost. The wastewater treatability performance, in this case using synthetic wastewater, was found to be more than 75% removal.

The activated sludge harvested from the bioreactor was used to evaluate the kinetics and stoichiometry oxidation of the sulphide with nitrate as the electron acceptor. The following conclusions were drawn from this study

- The oxygen concentration in the anaerobic and anoxic must be kept below 0.5mg/L and that of the aerobic within (2-3mg/L). Temperature must be maintain around (20-25^{0}C) to enhance pollutant biodegradation.

- The operating conditions in the bioreactors are highly sensitive, and when not controlled and monitored regularly can lead to undesirable performance and longer start up. To maintain microbial activity, desludging is encouraged as a practise when high amount of biomass is noted.

- The quantification of the nitrate-sulphide oxidation would allow an improved design and the operational control of the bioreactor during odour prevention. Therefore utilising nitrate to serve as an electron acceptor for the removal of the hydrogen sulphide or odorant compounds is a great investment for social economic development.

Future works

The limitations to this project was due to time factor, in view of this the following future works are proposed:

- Addition of quantified sulphide to the influent of the bioreactor and evaluating the nitrate-sulphide oxidation performance
- Optimising and simulating the operational hydrodynamics of the pilot scale integrated bioreactor as a function of nitrate-sulphide oxidation. This can help in the scaling up of the pilot scale plant unto a commercial design.

Acknowledgement

This project was conducted under a joint Erasmus+ KA107 Exchange Programme between Durban University of Technology (sending), South Africa and Valladolid University (host), Spain. Under the supervision of Prof. Raúl Muñoz Torre and Prof. Aitor Aizpuru, and conducted at Dpt Chemical Engineering and Environmental Technology. Escuela de Ingenierias Industriales, University of Valladolid, Spain.

I wish to acknowledge the following personals for their skills and expertise, my supervisors, Prof. Raul Muñoz Torre and Prof. Aitor Aizpuru for their guidance and encourangement through out the experiment and writeup.Ms Martha Chollom for proof reading and Ms Roxana Angeles Torre for her assistant during the experiment. Members and Technicians of the Gas treatment and Microalgae Research Group under Dpt Chemical Engineering and Environmental Technology. Escuela de Ingenierias Industriales, University of Valladolid, Spain, for their kind assistants during the experimental runs. And also Erasmus +KA107 (Valladolid) for the sponsorship for such a experience.

Reference

Ahmad, A. & O-Aljasser, A. 2013. Anaerobic Nitrogen, Sulfide, And Carbon Removal In Anaerobic Granular Bed Reactor. *Environmental Progress & Sustainable Energy***,** N/A-N/A.

Birima, A. H., Noor, M. M. M., Mohammed, T. A., Muyibi, S. A. & Idris, A. 2005. Simultaneous Organic And Nitrogen Removal Using Anoxic-Aerobic Membrane Bioreactor. *International Journal Of Engineering And Technology,* 2**,** 36-42.

Chan, Y. J., Chong, M. F., Law, C. L. & Hassell, D. G. 2009. A Review On Anaerobic–Aerobic Treatment Of Industrial And Municipal Wastewater. *Chemical Engineering Journal,* 155**,** 1-18.

Deng, L., Chen, H., Chen, Z., Liu, Y., Pu, X. & Song, L. 2009. Process Of Simultaneous Hydrogen Sulfide Removal From Biogas And Nitrogen Removal From Swine Wastewater. *Bioresour Technol,* 100**,** 5600-8.

Estrada, J. M., Kraakman, N. J., Lebrero, R. & Munoz, R. 2015. Integral Approaches To Wastewater Treatment Plant Upgrading For Odor Prevention: Activated Sludge And Oxidized Ammonium Recycling. *Bioresour Technol,* 196**,** 685-93.

Falahti-Marvast, H. & Karimi-Jashni, A. 2015. Performance Of Simultaneous Organic And Nutrient Removal In A Pilot Scale Anaerobic–Anoxic–Oxic Membrane Bioreactor System Treating Municipal Wastewater With A High Nutrient Mass Ratio. *International Biodeterioration & Biodegradation,* 104**,** 363-370.

Fang, Y., Du, Y., Feng, H., Hu, L. F., Shen, D. S. & Long, Y. Y. 2015. Sulfide Oxidation And Nitrate Reduction For Potential Mitigation Of H2s In Landfills. *Biodegradation,* 26**,** 115-26.

Gostelow, P., Parsons, S. & Stuetz, R. 2001. Odour Measurements For Sewage Treatment Works. *Water Research,* 35**,** 579-597.

Greben, H., Sigama, N., Radebe, V. & Wilsenach, J. An Environment-Friendly Technology For Biological Sulphate And Sulphide Removal From Acid

Mine Drainage. International Mine Water Conference, Pretoria, South Africa, 2009. 478-485.

Hernández, J., Lafuente, J., Prado, Ó. J. & Gabriel, D. 2012. Simultaneous Removal Of H2s, Nh3, And Ethyl Mercaptan In Biotrickling Filters Packed With Poplar Wood And Polyurethane Foam: Impact Of Ph During Startup And Crossed Effects Evaluation. *Water, Air, & Soil Pollution,* 223**,** 3485-3497.

Janssen, A., Lettinga, G. & De Keizer, A. 1999. Removal Of Hydrogen Sulphide From Wastewater And Waste Gases By Biological Conversion To Elemental Sulphur: Colloidal And Interfacial Aspects Of Biologically Produced Sulphur Particles. *Colloids And Surfaces A: Physicochemical And Engineering Aspects,* 151**,** 389-397.

Kim, H., Lee, H., Choi, E., Choi, I., Shin, T., Im, H. & Ahn, S. 2014. Characterization Of Odor Emission From Alternating Aerobic And Anoxic Activated Sludge Systems Using Real-Time Total Reduced Sulfur Analyzer. *Chemosphere,* 117**,** 394-401.

Kim, H., Mcavoy, T., Anderson, J. & Hao, O. 2000. Control Of An Alternating Aerobic–Anoxic Activated Sludge System—Part 2: Optimization Using A Linearized Model. *Control Engineering Practice,* 8**,** 279-289.

Lateef, A., Nawaz Chaudhry, M. & Ilyas, S. 2013. Biological Treatment Of Dairy Wastewater Using Activated Sludge. *Scienceasia,* 39**,** 179.

Metcalf, E. E. & Eddy, H. 2003. Wastewater Engineer Treatment Disposal, Reuse. *New York: Mcgraw*.

Munoz, R., Sivret, E. C., Parcsi, G., Lebrero, R., Wang, X., Suffet, I. H. & Stuetz, R. M. 2010. Monitoring Techniques For Odour Abatement Assessment. *Water Res,* 44**,** 5129-49.

Munz, G., Mannucci, A., Arreola-Vargas, J., Alatriste-Mondragon, F., Giaccherini, F. & Mori, G. 2015. Nitrite And Nitrate As Electron Acceptors For Biological Sulphide Oxidation. *Water Science And Technology*, 72**,** 593-599.

WHO 2011. *Guidelines For Drinking-Water Quality,* Geneva: World Health Organization.

Van Der Werf, G. R., Randerson, J. T., Giglio, L., Collatz, G., Mu, M., Kasibhatla, P. S., Morton, D. C., Defries, R., Jin, Y. V. & Van Leeuwen, T. T. 2010. Global Fire Emissions And The Contribution Of Deforestation, Savanna, Forest, Agricultural, And Peat Fires (1997–2009). *Atmospheric Chemistry And Physics,* 10**,** 11707-11735.

Yuan, W. & Bandosz, T. J. 2007. Removal Of Hydrogen Sulfide From Biogas On Sludge-Derived Adsorbents. *Fuel,* 86**,** 2736-2746.

Zhang, L., De Schryver, P., De Gusseme, B., De Muynck, W., Boon, N. & Verstraete, W. 2008. Chemical And Biological Technologies For Hydrogen Sulfide Emission Control In Sewer Systems: A Review. *Water Res,* 42**,** 1-12.

Zub, S., Kurissoo, T., Menert, A. & Blonskaja, V. 2008. Combined Biological Treatment Of High-Sulphate Wastewater From Yeast Production. *Water And Environment Journal*, 22**,** 274-286.

Appendix

Table 1-Calibration of Thermo Scientific Orion Dual Star pH/ISE Dual Channel Benchtop Meter

Volume (VPb) (0.1MPb)	Ec(mv)	Comments	standard	fd	Cs*fd	mv
0.2	845.9		P1	0.005	0.576	-759.2
0.4	842.9		P2	0.01	1.152	-777.4
0.6	840.8		P3	0.03	2.88	-790.1
0.8	837.2		P4	0.05	5.76	-799.5
1	833.7		P5	0.10	11.52	-808.8
1.2	830					
1.4	825.2					
1.6	818.4					
1.8	799.7	Nearest volume				
2	682.9					
Concentration of Sulphur (Cs)=(VPb*0.1*Mpb*1000)/Vstand *Volume of lead *Molar mass of lead (MPb) -32g/mol *Volume of standard solution used (50ml)		115.2mgS/L	Cs*fd values are used for the calibration			

Table 2a-Daily reactor monitoring start up 1

ID	**Elpased time (h)**	Oxygen (mg/L)			**Temp (^{0}C)**	**Aeration flowrate (ml/s)**	Flowrate(L/d)
		$O_{2\text{-Anaerobic}}$	**$O_{2\text{-anoxic}}$**	**$O_{2\text{-Aerobic}}$**			**Effluent (L/d)**
1	0	UNDER STUDY		2.4	14.8	50	
2	24			2.5	14.8	50	8.5
3	24			2.3	15.6	52	8.5
4	19			2.2	15.7	55	6.5
5	24			2	16.4	50	7.5
6	24			2.1	15.6	55	8.5
7	27			2.2	16.5	50	9.5
8	24			2.2	16.5	50	8
9	24			2.4	18.6	55	8.5
10	18			2.2	18.4	55	6.5
11	24			2.3	18.5	50	8
12	30			2.3	17.6	55	9
13	18			2.2	17.56	50	6.5
14	30			2.2	18.5	55	9.5
15	24			2.2	17.6	55	8.5
16	24	1.6	2.7	2.2	16.8	50	8
17	18	1.4	2.5	2.1	18.4	55	6.5
18	24	1.2	1.8	2.1	17.5	50	8
19	24	1.2	1.6	2.2	19.5	50	8
20	24	0.9	1.4	2.2	18.6	50	7.5
21	24	0.9	1.2	2.3	22.5	50	7.5

22	26	0.9	1.2	2.2	22.7	50	8.5
23	24	0.8	1.1	2.2	21.4	50	8
24	24	0.9	1.1	2.2	22.3	50	8
25	26	0.8	1.1	2.1	22.7	50	8.5
26	24	0.8	1.1	2.1	23.4	50	8
27	24	0.8	1.1	2.1	22.8	50	8
28	24	0.8	1.2	2.1	22.4	50	8
29	24	0.7	0.9	2.2	22.5	49	8.5
30	24	0.7	0.9	2.3	22.7	50	8
31	24	0.8	1	2.1	22.9	50	8.5
32	24	0.8	0.9	2.2	22.7	51	8.5
33	24	0.6	0.9	2.1	22.9	50	8.5
34	24	0.5	0.9	2.1	22.8	50	8.5
35	24	0.5	0.8	2	22.7	51	8
36	24	0.6	0.8	2.3	20.5	50	8.5
37	24	0.6	0.8	2.2	20.9	48	8
38	24	0.7	0.8	2.3	15.7	50	8
39	24	0.7	0.8	2.2	15.6	51	8
40	24	0.5	0.8	2.2	18.5	50	8.5
41	24	0.6	0.8	2.1	19.5	51	8.5
42	24	0.6	0.7	2.1	19.6	50	8
43	24	0.6	0.8	2.2	20.1	50	8
44	24	0.6	0.7	2.1	22.2	50	8

ID		Oxygen (mg/L)	**Temp (^{0}C)**		Flowrate(L/d)

	Elpased time (h)	$O_{2\text{-Anaerobic}}$	$O_{2\text{-}}$ anoxic	$O_{2\text{-}}$ Aerobic		Aeration flowrate (ml/s)	Effluent (L/d)
1	0			2.4	14.8	50	
2	24			2.5	14.8	50	8.5
3	24			2.3	15.6	52	8.5
4	19			2.2	15.7	55	6.5
5	24			2	16.4	50	7.5
6	24			2.1	15.6	55	8.5
7	27			2.2	16.5	50	9.5
8	24	UNDER STUDY		2.2	16.5	50	8
9	24			2.4	18.6	55	8.5
10	18			2.2	18.4	55	6.5
11	24			2.3	18.5	50	8
12	30			2.3	17.6	55	9
13	18			2.2	17.56	50	6.5
14	30			2.2	18.5	55	9.5
15	24			2.2	17.6	55	8.5
16	24	1.6	2.7	2.2	16.8	50	8
17	18	1.4	2.5	2.1	18.4	55	6.5
18	24	1.2	1.8	2.1	17.5	50	8
19	24	1.2	1.6	2.2	19.5	50	8
20	24	0.9	1.4	2.2	18.6	50	7.5
21	24	0.9	1.2	2.3	22.5	50	7.5
22	26	0.9	1.2	2.2	22.7	50	8.5
23	24	0.8	1.1	2.2	21.4	50	8
24	24	0.9	1.1	2.2	22.3	50	8

25	26	0.8	1.1	2.1	22.7	50	8.5
26	24	0.8	1.1	2.1	23.4	50	8
27	24	0.8	1.1	2.1	22.8	50	8
28	24	0.8	1.2	2.1	22.4	50	8
29	24	0.7	0.9	2.2	22.5	49	8.5
30	24	0.7	0.9	2.3	22.7	50	8
31	24	0.8	1	2.1	22.9	50	8.5
32	24	0.8	0.9	2.2	22.7	51	8.5
33	24	0.6	0.9	2.1	22.9	50	8.5
34	24	0.5	0.9	2.1	22.8	50	8.5
35	24	0.5	0.8	2	22.7	51	8
36	24	0.6	0.8	2.3	20.5	50	8.5
37	24	0.6	0.8	2.2	20.9	48	8
38	24	0.7	0.8	2.3	15.7	50	8
39	24	0.7	0.8	2.2	15.6	51	8
40	24	0.5	0.8	2.2	18.5	50	8.5
41	24	0.6	0.8	2.1	19.5	51	8.5
42	24	0.6	0.7	2.1	19.6	50	8
43	24	0.6	0.8	2.2	20.1	50	8
44	24	0.6	0.7	2.1	22.2	50	8

Table 2b-Daily reactor monitoring start up 2

ID	**Elpased time (h)**	Oxygen (mg/L)			**Temp (^{0}C)**	**Aeration flowrate (ml/s)**	Flowrate(L/d)
		$O_{2\text{-Anaerobic}}$	**$O_{2\text{-}}$ anoxic**	**$O_{2\text{-}}$ Aerobic**			**Effluent (L/d)**

1	24	0.8	1.1	2.4	22.3	50	9
2	24	0.8	1	2.3	21.4	50	9
3	24	0.6	0.8	2.1	20.2	51	9
4	24	0.6	0.9	2.1	21.2	50	9
5	24	0.5	0.8	2.2	22.1	50	8.5
6	24	0.5	0.8	2.3	22.3	50	8.5
7	24	0.5	0.9	2.2	22.3	52	9
8	24	0.6	0.7	1.9	22.4	55	9
9	24	0.5	0.8	1.9	22.3	60	8
10	24	0.5	0.8	2.1	23.5	60	9
11	24	0.5	0.9	2.1	24.2	60	8.5
12	24	0.5	0.9	2.1	18.6	60	8.5
13	24	0.5	0.7	2.1	24.5	70	10
14	24	0.4	0.8	2.3	22.3	70	9
15	24	0.4	0.7	2.4	23.4	65	9.5
16	24	0.3	0.8	2.2	25.6	60	10
17	24	0.3	0.9	1.8	26.3	70	9
18	24	0.3	0.7	1.9	24.4	65	10.5
19	24	0.4	0.7	2.1	23.5	60	10.5
20	24	0.4	0.5	2.1	22.4	60	9
21	24	0.5	0.6	2	22.4	60	8
22	24	0.4	0.5	2.3	22.5	60	8.5
23	24	0.5	0.6	2.1	22.5	60	8.5
24	24	0.6	0.6	2.3	22.4	60	8.5
25	24	0.4	0.5	2.2	23.4	60	8.5
26	24	0.4	0.5	2.1	22.2	60	8.5

27	24	0.4	0.6	2.2	22.1	60	8.5
28	24	0.5	0.7	2.1	22.1	60	8.5
29	24	0.5	0.6	2.2	22.1	60	9
30	24	0.5	0.6	2.1	22.2	60	8.5
31	24	0.5	0.6	2.2	22.3	60	8.5
32	24	0.4	0.6	2.3	22.4	60	8.5
33	24	0.5	0.6	2.2	22.1	60	8.5
34	24	0.4	0.7	2.1	22.1	60	8.5
35	24	0.5	0.6	2.2	22.1	60	8.5
36	24	0.5	0.5	2.1	21.4	60	8.5
37	24	0.5	0.5	2.2	20.5	60	8.5
38	24	0.5	0.6	2.1	22.6	60	8.5
39	24	0.4	0.5	2.2	23.4	60	8.5
40	24	0.4	0.5	2.1	24.2	60	8.5

Table 3-TSS and VSS Monitoring

ID	TSS(g/L)						VS(g/L)					SRT	
1	Sludge(BE0)	Anaerobic (BE2)	Anoxic (BE3)	Aerobic (BE4	Settler (BE5)	Effluent	Anaerobic	Anoxic (BE3)	Aerobic (BE4)	Settler (BE5	Effluent	productivity (g/d)	Volume of purge (ml/d)
2				1.5	5.38				1.26	4.46		1.935	359.6654275
3				1.1	8.6				0.9	7.1		1.419	165
4				1.48	1.85				1.26	1.62		1.9092	1032
5		1.43	1.18	0.92	8.73	0.05	1.21	1.02	0.8	7.3	0.05	1.3284	152.1649485
6		1.49	1.34	1.38	5	0.05	1.26	1.2	1.2	4.54	0.05	1.7848	356.96
7	3.52	1.49	1.34	1.38	5.2	0.05	1.28	1.2	1.2	4.54	0.05	1.7848	343.2307692
8		1.1	0.6	0.29	5.27	0.08	1.02	0.53	0.27	4.67	0.08	0.5712	108.3870968
9		0.43	0.41	0.44	5.23	0.08	0.39	0.37	0.39	4.73	0.07	0.5581	106.7112811
10		0.58	0.71	0.45	2.72	0.05	0.55	0.65	0.43	2.5	0.05	0.6689	245.9191176
11		0.73	0.54	0.4	6.23	0.07	0.73	0.52	0.37	5.67	0.07	0.6	96.3081862
12		0.49	0.53	0.2	6.63	0.04	0.49	0.53	0.2	6.17	0.05	0.3877	58.47662142
13		0.75	0.55	0.55	3.8	0.09	0.69	0.53	0.29	4.7	0.09	0.7375	194.0789474
							Reactor restart						
1		2.6	1.14	0.94	12.3	0.1	1.98	0.9	0.8	9.37	0.05	1.499	121.8699187
2		2.08	1.78	1.72	10.8	0.11	1.72	1.44	1.38	8.8	0.1	2.2854	211.6111111
3		1.74	1.54	0.94	6.83	0.13	1.38	1.16	0.74	5.67	0.13	1.4866	217.6573939
4		1.5	0.9	0.77	15	0.05	1.26	0.7	0.62	12.13	0.01	1.1306	75.37333333
5	4.4	0.9	0.82	0.87	10.33	0.09	0.76	0.71	0.7	8.23	0.09	1.113	107.7444337
6		2.66	1.62	1.96	10.33	0.11	2.24	1.42	1.6	8.33	0.1	2.5346	245.3630203
7		2.08	0.68	0.49	6.13	0.06	1.82	0.59	0.41	4.97	0.06	0.906	147.7977162
8		1.28	0.66	0.26	3.67	0.08	1.16	0.58	0.24	3.07	0.08	0.5862	159.7275204
9		1.6	0.74	0.43	5.1	0.06	1.44	0.66	0.38	4.27	0.07	0.8022	157.2941176
10		3	2	0.39	3.92	0.04	2.44	1.66	0.31	3.24	0.04	1.3032	332.4489796

Table 4a -Weekly Monitoring

	TN(mg/L)					dilution factor	TOC (mg/L)					TC(mg/L)					IC(mg/L)				
	Feed (BE1)	Anaerobic (BE2)	Anoxic (BE3)	Aerobic (BE4)	Effluent (BE6)		Feed (BE1)	Anaerobic (BE2	Anoxic (BE3)	Aerobic (BE4)	Effluent (BE6)	Feed (BE1)	Anaerobic (BE2)	Anoxic (BE3)	Aerobic (BE4)	Effluent (BE6)	Feed (BE1)	Anaerobic (BE2)	Anoxic (BE3)	Aerobic (BE4)	Effluent (BE6)
1	44.09	10.54	11.63	14.29			203.82	43.74	20.916	21.429		381.6	172.47	130.35	166.89		177.69	128.7	109.44	145.47	
2	31.26	12.13	11.94	9.556	9.91		72.03	19.767	9.378	6.102	13.41	254.88	196.38	171.72	122.52	181.62	182.85	152.64	162.33	116.4	168.21
3	32.96	18.32	16.49	15.95	12.56	3	229.77	37.74	33.51	24.378	30	387.9	210	199.89	201.708	212.25	158.13	172.26	166.38	177.33	182.25
4	42.15	19.5	10.07	14.13	11.43		179.52	25.158	24.1158	19.275	17.367	381	163.8	170.82	171.3	183.6	202.05	138.72	128.73	154.02	164.58
5	39.41	37.52	30.54	30.78	29.27		112.29	42.12	41.73	43.53	31.53	303	199.8	184.74	213.12	261.33	192.96	157.68	152.01	171.39	187.8
6	42.55	30.05	30.99	28.86	28.64		134.4	51.99	50.13	57.27	42.99	302.4	226.08	212.43	222.99	207.78	167.88	174.09	162.3	165.72	164.79
7	41.35	34.18	21.93	23.07	12.8		159	65.01	64.8	32.7	40.71	349.2	229.2	238.8	241.8	226.2	207.96	180.36	177.63	186.3	172.8
8																					
9	40.38	29.66	29.52	20.21	29.26		180.42	61.59	67.86	32.1	31.35	354.6	203.4	212.7	226.8	170.1	187.05	160.95	163.5	174.96	163.8
10	40.63	32.1	26.21	27.31	31.43		71.82	36.9	30.95	30.41	21.45	383.4	288.21	250.92	253.56	250.56	186.21	177.42	158.07	162.12	167.91
11	37.74	32.55	32.57	32.12	36.67		98.97	28.94	23.41	24.7	19.94	256.83	260.25	253.2	262.8	242.19	175.2	173.73	182.07	182.37	157.83
12	30.47	28.34	29.42	27.17	29.81		144.56	28.62	32.91	27.29	30.75	294.72	294.72	242.88	269.07	241.02	217.86	214.26	234.36	213.75	206.13
13	37.78	33.77	30.69	31.06	31.94		154.4	56.09	39.08	40.77	37.71	338.8	299.2	273.5	280	283.41	242.3	243.1	234.4	243.1	184.4
14	34.5	27.95	26.01	34.82	33.66		105.3	40.1	30.28	62.42	39.85	299.5	231.2	245.5	232.6	296.6	256.7	183.1	207.2	237.1	186.7

	NH_4^+(mg/L)					NO_2 (mg/L)					NO_3/N_3 (mg/L)					SO_4 (mg/L)					Cl (mg/L)				
	Feed (BE1)	Anaerobic (BE2)	Anoxic (BE3)	Aerobic (BE4)	Effluent (BE6)	Feed (BE1)	Anaerobic (BE2	Anoxic (BE3)	Aerobic (BE4)	Effluent (BE6)	Feed (BE1)	Anaerobic (BE2)	Anoxic (BE3)	Aerobic (BE4)	Effluent (BE6)	Feed (BE1)	Anaerobic (BE2)	Anoxic (BE3	Aerobic (BE4)	Effluent (BE6)	Feed (BE1)	Anaerobic (BE2)	Anoxic (BE3)	Aerobic (BE4)	Effluent (BE6)
1		5.55	4.75	5.19			-	-		-	-	-	-	-	-	-	31.57	22.01	27.51	-	-	39.54	31.58	30.73	-
2	8.92	7.08	4.49	4.84	6.59		4.78	5.8	5.41	-	-	-	7.37	7.35	-	-	8.38	-	7.39	-	-	15.5	17.21	15.31	-
3	1	8.2	8.23	8.5	5.82	-	-	46.75	30.46	1.72	-	-	-	-	-	2.88	2.85	4.55	4.18	3.28	14.97	15.33	15.35	16.11	16.77
4	<1	2.15	<1	<1	2.23		5.18	5.75	8.99	4.31	-	6.68	15.89	111.25	23.7	17.31	3.07	-	210.54	50.58	14.25	14.41	15.01	18.55	16.74
5	6.67	9.26	8.84	9.5	13.5		-	-	-	-	-	-	-	-	-	3.91	-	-	-	3.25	14.23	17.19	17.73	19.11	17.96
6	10	11.9	10.8	8.5	9.6																				
7	9.63	4.07	7.22	6.23	7.34																				
8																									
9	9.05	15.7	13.9	12.9	12.3											9.45	-	-	6.88	-	17.19	21.59	16.47	19.72	16.94
10	6.04	44.6	29.2	36	10											33.25	-	-	-	-	11.2	13.16	11.91	13.64	11.53
11	3.18	4.05	4.06	4.45	5.33											8.54	-	-	6.8	-	13.92	16.32	14.28	14.76	12.92
12	24.1	32.1	25.9	25.1	27.1											35.77	27.73	20.71	24.37	20.48	31.75	29.19	32.99	36.33	35.94
13	30.8	25.4	30.7	22.2	28.6					3.16						35.77	19.33	17.58	17.7	22.21	42.13	43.13	42.92	42.43	38.9
14	30.8	24.1	34	22	18.5					-	-	1.53	-	-	-	29	25.65	8.87	16.05	24.54	42		36	36	38

Table 5-Kinectics Tap and synthetic wastewater

Time (h)	Tap		SWW		Scale up (fd*results)			
0	8.18	7.59	7.33	7.08	16.36	15.18	14.66	14.16
2	7.95	7.52	6.51	6.38	15.90	15.04	13.02	12.76
4	7.72	7.50	5.09	4.98	15.44	15.00	10.18	9.96
6	7.33	7.08	4.31	4.06	14.66	14.16	8.62	8.12
8	7.27	7.22	3.55	3.42	14.54	14.44	7.10	6.84
10	7.48	7.19	3.30	3.08	14.96	14.38	6.60	6.16

Table 6-Kinetics different S/N and S/B ratios

SWW (1)	SWW (2)	SWW + 2g (1)	SWW + 2g (2)	SWW + 8g (1)	SWW + 8g (2)
7.41	7.37	6.33	6.9	8.86	7.93
7.56	7.3	6.62	6.98	6.96	6.9
7.03	6.06	5.78	5.93	6.06	5.61
6.53	5.46	6.23	6.6	6.39	6.14
6.28	4.73	6.19	6.33	6.02	5.92
6.49	4.11	6.09	6.23	5.97	5.68
Sludge addition and nitrate control					
SWW (1)	SWW (2)	SWW + 0.5g (1)	SWW + 0.5g (2)	SWW + 0.1g (1)	SWW + 0.1g (2)
8.98	8.92	6.5	6.45	4.11	11.8
7.69	8.42	6.47	6.97	4.71	11.7
7.85	8.38	6.34	6.76	4.21	11.8
7.41	8.17	6.28	6.62	3.65	11.7
7.16	7.96	6.1	6.38	3.43	11.9
7.13	7.8	5.96	6.05	3.39	11.7
Evaluating Nitrate					
A 1(No nitrate)	A2 (No nitrate)	B1 (0.15 g nitrate)	B2 (0.15 g nitrate)	C1 (0.45 g nitrate	C2 (0.45 g nitrate
5.83	5.71	4.79	5.15	4.77	5
5.44	5.09	4.74	5.14	4.71	4.71
5.45	5.05	4.83	5.21	4.94	5.58
5.56	4.81	4.73	5.25	4.98	5.57
5.58	4.83	5.03	5.35	5.08	5.48
5.82	5.23	4.98	5.18	4.82	5.26

Table 7-Adaptation of nitrate-Sulphide kinetics

Days	Nitrate (g/L)	**S-2 accumulation (g/L)**	**Bottles (S-2 mg/L)**		**Ions**				
			A	B	Cl (mg/L)	NO2 (mg/	NO3 (mg/	PO4 (mg/L)	SO4 (mg/L)
0	1.44	0.45	0.0838	0.0864					
4	2.88	0.9	0.0002	0.0092	66.95	-	469.57	205.6	122.23
8	4.32	1.35	0.0099	0.0285	71.5	-		200.62	91.51
12	5.76	1.8	0.0032	0.0076	45.3	3.16	106.89		57.31