Universidad de Valladolid

ESCUELA DE INGENIERÍAS INDUSTRIALES

DEPARTAMENTO DE INGENIERÍA QUÍMICA Y TECNOLOGÍA DEL

MEDIO AMBIENTE

TESIS DOCTORAL:

STUDY OF THE AUTOHYDROLYSIS PRETREATMENT OF SECONDARY SLUDGE AND ITS INFLUENCE ON THE ANAEROBIC DIGESTION

Andrea del Rosario Carvajal Guevara

Valladolid, Mayo 2012

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STUDY OF THE AUTOHYDROLYSIS PRETREATMENT OF SECONDARY SLUDGE AND ITS INFLUENCE ON THE ANAEROBIC DIGESTION

Presentada por Andrea del Rosario Carvajal Guevara para optar al grado de doctor por la Universidad de Valladolid

Dirigida por:

María del Mar Peña Miranda

UNIVERSIDAD DE VALLADOLID

ESCUELA DE INGENIERÍAS INDUSTRIALES

Secretaría

La presente tesis doctoral queda registrada en el folio Nº______

del correspondiente Libro de Registro con el Nº__________

Valladolid, a ______ de _______________ de 2012

Fdo. El encargado del Registro

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y Tecnología del Medio Ambiente

Universidad de Valladolid

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ANDREA DEL ROSARIO CARVAJAL GUEVARA ha realizado bajo su dirección el trabajo ***"Study of the autohydrolysis pretreatment of secondary sludge and its influence on the anaerobic digestion***", 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 expresan su conformidad con dicha presentación.

Valladolid a _____ de____________ de 2012.

María del Mar Peña Miranda

Reunido el tribunal que ha juzgado la tesis doctoral "***Study of the autohydrolysis pretreatment of secondary sludge and its influence on the anaerobic digestion***" presentada por Andrea del Rosario Carvajal Guevara y en cumplimiento con lo establecido por el Real Decreto 861/2010 (BOE 03.07.2010) ha acordado conceder por ________________________ la calificación de___________________.

Valladolid, a de de 2012

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AGRADICIMIENTOS

Al los proyectos de investigación *Cenit-Sostaqua* y *SCD 2007-00055 Novedar* por la financiación necesaria para el proyecto de investigación, también al programa de becas Erasmus-Mundus (External Cooperation Window for Chile – Lote 17), que concedió el financiamiento necesario para mi estadía en Universidad de Valladolid.

A mi directora de tesis doctoral María del Mar Peña Miranda, por todo el trabajo y tiempo que ha dedicado durante su dirección, por la confianza que ha depositado en mí y especialmente por su compañía y apoyo durante el desarrollo de la tesis.

A los integrantes del Grupo de Investigación Reconocido de Tecnología Ambiental, en especial a Sara, Fernando y María, por todas las dudas que ayudaron a solucionar y más importante por cada pregunta que me encargaron solucionar. A Araceli, Sara, Silvia y José María, los cuales entregan cada día un aporte fundamental para nuestro trabajo. También a todos los compañeros que están o han estado durante estos tres años de trabajo y que han hecho de cada día un momento más ameno, y mucho más emocionante. A Joao, Sergio, Javier, Iris, Raquel, Raúl, Ana, Lara, Jaime, Guzmán, María, Laura, Guillermo, Jose, Cynthia, Danilo, Katalin y Marta; imposible olvidar todas esas horas de laboratorio.

A los amigos: Elisa, Eva, Catarina, Israel, Alexandre, Ignacio, Raúl, Oscar, Javier, Jose, Laura, Vanessa y Alejandro; ustedes se convirtieron en mi familia española y se quedan con un pedacito de mi corazón. A los amigos que desde Chile me enviaron mucho cariño y no me olvidaron a pesar de la distancia: Cristina y Andrés, Rodrigo, Matías y Ángela, Christian, Claudia y Pato, Magdalena y Amory. También agradecer de forma especial a Carolyn, por enviarme lejos y luego ser el faro que me indica el camino.

A Daniel por cada día que hemos pasado juntos desde el principio de los tiempos… y hasta el final de los mismos. Te quiero!

A mi familia: Fernando, Mónica, Diana, Carlos y Marina, que me han acompañado a la distancia durante todo este tiempo y me han entregado esa ayuda incondicional cuando la necesitaba. También a mi familia adoptiva: Carmen, Jaime, Mónica y Mauricio, Loreto y Marcelo, gracias por su cariño y preocupación.

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ABSTRACT

The increase in the number of wastewater treatment plants and the more stringent quality requirement for the residue produced makes it necessary to improve the efficiency of Anaerobic Digestion of sludge. Different pretreatments of secondary sludge such as: thermal, mechanical, chemical or enzymatic, have shown important advances in the volatile solids removal and pathogen microorganisms elimination from the sludge, and they have also had a positive effect on biogas production; however, they are associated with high costs of operation.

This study was focused in the evaluation of the autohydrolysis pretreatment on secondary sludge, and the improvement of the anaerobic digestion. The work developed three main objectives: the study of the effect produced on the secondary sludge, the study of the effect produced on the anaerobic digestion and the description of the pretreatment mechanism.

The study of ***the effect produced on the secondary sludge*** showed the influence of the secondary sludge concentration and the aeration conditions during the pretreatment. The increase in the solids content produced as a consequence the increase in the solubilization of organic matter; moreover the presence of limited amount of air controlled the volatile fatty acids production and the consumption of organic matter. When the pretreatment was carried out at 55ºC during 12 hours, around 25 – 30% of the organic matter solubilization was reached (of the chemical oxygen demand), and also, around 5% of organic matter was consumed. On the other hand, the rheology behavior of the sludge was affected by the pretreatment, obtaining as a result the reduction of the flow resistance of the pretreated sludge. Therefore, the pumping and agitation requirement for high solid content was similar to the requirement of a low solid content without pretreatment.

The study of ***the effect produced on the anaerobic digestion*** showed the improvement of the process. The mesophilic anaerobic digestion of pretreated sludge showed that the methane productivity increased in approximately 20% on batch assays; on the other hand, no improvement was detected when the anaerobic digestion was carried out on thermophilic conditions. The study on continuous anaerobic digestion showed the feasibility of operates with high solid content secondary sludge as only feed, despite high concentration of ammonium obtained. Two anaerobic digesters, of 20L each one, were used

for the comparison of the pretreatment effect; when they were subjected to different operational conditions, some differences were obtained on their performance. The biogas production and methane productivity increased around 20% at 20d of SRT, 15% at 17d of SRT and 10% at 15d of SRT, however at 13d of SRT the difference become negligible.

The study of ***the description of the autohydrolysis pretreatment mechanism*** showed the increase in the enzymatic activity of two hydrolytic enzymes in the soluble phase (*amylase* and *protease*), while the total enzymatic activity decreased. On the other hand, the mesophilic population decreased and the thermophilic population grew sustainably during the pretreatment. Also, the comparison of different pretreatment temperatures showed a higher enzymatic stability and higher thermophilic population growth at 55ºC than at 40 or 70ºC. However, no direct relationship was observed between the thermophilic population growth and the increase in hydrolytic enzymes, and then the increase in the enzymatic activity observed during the first hours of pretreatment, was caused by the release of the enzymes from the exo-polymeric substances of the floc. Furthermore, the amount of actives thermophilic microorganisms after 9 hours of pretreatment, represented 3% of the total initial population in the raw sludge; therefore it is a small population to allow the quantification of the enzymes secreted, and also to produce significant organic matter consumption. Summarizing, the autohydrolysis pretreatment is the floc of secondary sludge destabilization due to a thermal effect, and then the released organic matter is hydrolyzed by the natives enzymes of the secondary sludge, which are relatively stable during 6 – 9 hours of pretreatment.

RESUMEN

El aumento del número de plantas de tratamiento de agua y las estrictas normas asociadas a la calidad de los residuos producidos, ha hecho necesario mejorar la eficiencia de la digestión anaerobia de fango. Los pre-tratamientos de fango secundario han mostrado importantes avances en la remoción de sólidos volátiles y en la eliminación de patógenos del fango, como también han mostrado efectos positivos en la producción de biogás. Sin embargo los pre-tratamientos de fango están asociados con altos costos de operación.

Este estudio se centró en la evaluación del pre-tratamiento de auto-hidrólisis en fango secundario, y la mejora de la digestión anaerobia. El trabajo desarrolla tres objetivos principales correspondientes al estudio del efecto producido por el pre-tratamiento de auto-hidrólisis en: el fango secundario, la digestión anaerobia y además presentar una propuesta de mecanismo del pre-tratamiento.

El estudio del ***efecto del pre-tratamiento en el fango secundario*** mostró la solubilización de la materia orgánica, el rendimiento se vio afectado por distintos factores: 1) el aumento de la concentración inicial de fango, aumentó la solubilización de materia orgánica, y 2) la cantidad limitada de aire disponible durante el pre-tratamiento controla la producción de ácidos grasos volátiles y el consumo de materia orgánica. Cuando el pre-tratamiento fue realizado a 55ºC y durante 12 horas se obtuvo entre 20 – 30% de solubilización de materia orgánica, y aproximadamente el 5% de consumo de materia orgánica. Por otra parte, la reología del fango también fue afectada por el pre-tratamiento, reduciendo su resistencia a fluir, por lo tanto el requerimiento energético asociado a bombeo o agitación de fango secundario con alto contenido de sólidos sería similar al requerimiento de un fango sin pre-tratamiento con bajo contenido de sólidos.

El estudio ***del efecto del pre-tratamiento en la digestión anaerobia*** mostró la mejora del proceso. La digestión anaerobia mesófila de fango pre-tratado mostró un aumento en la productividad de metano de aproximadamente 20% en ensayos en condiciones batch. Por otro lado no se obtuvo mejora cuando la digestión anaerobia fue realizad en condiciones termófilas.

La digestión anaerobia en operación continua, mostró la factibilidad de operar con fango secundario concentrado (8% de sólidos totales), a pesar de las altas concentraciones de amonio obtenidas como producto del contenido de compuesto nitrogenado en el fango. Se utilizaron dos digestores anaerobios de 20L cada uno para comparar el efecto del pre-tratamiento, obteniendo como resultado distintos rendimientos al ser sometidos a distintas condiciones operacionales. La producción de biogás y la productividad de metano aumentaron alrededor de 20% a 20d de tiempo de retención de sólidos, 15% a 15d de TRS y 10% a 15d de TRS, sin embargo la operación a 13d de TRS mostró una diferencia despreciable.

El estudio de la ***descripción del mecanismo del pre-tratamiento de auto-hidrólisis*** mostró un aumento en la cantidad de la actividad enzimática de dos enzimas en la fase soluble: *amilasa* y *proteasa*; mientras que la actividad enzimática total disminuyó durante el pre-tratamiento. Por otro lado, la población mesófila disminuyó, mientras la población termófila aumentaba durante el pre-tratamiento. La comparación del pre-tratamiento realizado a distintas temperaturas mostró una mayor actividad y estabilidad de las enzimas, como un mayor crecimiento de la población termófila, cuando el pre-tratamiento se realiza a 55ºC en vez de 40 o 70ºC. Sin embargo, no fue posible relacionar directamente el crecimiento de la población termófila con el aumento de las enzimas hidrolíticas, por lo tanto, el aumento observado de la actividad enzimática durante las primeras horas de pre-tratamiento, sería causada por la desorción de las enzimas desde la matriz exo-polimérica del flóculo. Adicionalmente, la cantidad de microorganismos activos termófilos luego de 9 horas de pre-tratamiento representan el 3% de la población inicial en el fango fresco, por lo tanto es una población muy pequeña como para permitir la cuantificación de las enzimas secretadas, o como para producir un consumo de materia orgánica significativo. En resumen, el pre-tratamiento de auto-hidrólisis consiste en la des-estabilización del floculo de fango secundario debido a un efecto térmico, luego la materia orgánica es hidrolizada por la acción de las enzimas presentes en el fango secundario, las cuales son relativamente estables durante 6 – 9 horas de pre-tratamiento.

THESIS OUTLINE

ESQUEMA DE LA TESIS

THESIS OUTLINE

The research presented in this thesis, entitled: "Study of the autohydrolysis pretreatment on secondary sludge and its influence on the anaerobic digestion" is divided as follows:

Chapter 1 presents the goal of this thesis project, and also lists the specific objectives of the different steps during the research.

The **Chapter 2** presents a general introduction into the thesis reasons. A brief overview of the sludge issue is presented, showing the production growth and the current legislation applied in the European Union and Spain. Also an introduction into the most common treatments of sludge are listed, and the presentation of the anaerobic digestion of sludge as a feasible solution, its advantages and disadvantages. Furthermore, different pretreatments of sludge to improve the efficiency of anaerobic digestion are presented.

Chapter 3 presents the autohydrolysis pretreatment of secondary sludge; this section focuses on the evaluation of oxygen presence and absence in the pretreatment and the evaluation of the initial sludge concentration, between 30 and 54 ($kg \cdot m^{-3}$) of total solids. The evaluation of the pretreatment effect was made through the solubilization and consumption of organic matter, the rheological behavior of the sludge and the anaerobic biodegradability. Finally, an energy assessment was performed trying to show the possible impact of the autohydrolysis pretreatment on the operational costs of the waste water treatment plant.

In **Chapter 4** the pretreatment was applied to secondary sludge with a high concentration of solids (8% of total solids). The increase of the solid content also increases the difficulty of air transference and the agitation requirement; therefore, the experimental system was changed. The evaluation of the pretreatment was made through the solubilization and consumption of organic matter again, comparing with the previous results obtained. However, the organic matter was also characterized by its proteins and carbohydrates content; also a bacterial viability test was performed. Finally, the thermophilic and mesophilic anaerobic biodegradability was compared.

In **Chapter 5** is presented the microbial activity and the enzyme behavior in the secondary sludge, as a fundamental tool for the autohydrolysis pretreatment mechanisms description. The pretreatment was carried out at three different temperatures (40, 55 and 70ºC), trying to isolate the thermal and the biologic-enzyme contribution for the hydrolysis of the organic matter. The pretreatment study was made through the quantification of two hydrolytic enzymes (*amylase* and *protease*), in both soluble and full sludge. The live heterotrophic bacteria at mesophilic and thermophilic conditions were measured, quantifying the decay and growth of the microbial population subjected to the pretreatment. Finally, the anaerobic biodegradability of separated phases: supernatant and suspended solids, was performed.

Chapter 6 shows the start-up and operation of two anaerobic digesters, 20L each of digestion volume, operated in parallel during 550 days. Both digesters were started-up using secondary sludge with high solid content as only feed. After, pretreated secondary sludge was used as feed for one of them; and the behavior of both digesters was compared at different operational conditions.

Finally, the **chapter 7** presents a summary of the research methodology and lists the main and specific conclusions obtained.

ESQUEMA DE LA TESIS

La investigación presentada en esta tesis, titulada: "Study of the autohydrolysis pretreatment on secondary sludge and its influence on the anaerobic digestion", se presenta de la siguiente forma:

El C**apitulo 1** presenta el objetivo principal y los objetivos específicos de este proyecto de tesis.

En el **Capitulo 2** se presenta la introducción general de la tesis. Se muestra una breve descripción del problema del aumento de la producción de fango y la legislación vigente tanto en la Unión Europa como en España. También se introducen algunos de los tratamientos de fangos más comunes y de forma más específica la digestión anaerobia, junto con sus ventajas y desventajas. Además, se presentan algunos de los pre-tratamientos de fango utilizado para mejorar la eficiencia de la digestión anaerobia.

El **Capitulo 3** presenta el pre-tratamiento de auto-hidrólisis de fango secundario. Esta sección está enfocada a la evaluación de la presencia y ausencia de oxigeno en el pre-tratamiento, también se estudió el efecto de la concentración inicial del fango, entre 30 y 54 ($kg \cdot m^{-3}$) de sólidos totales. Los efectos del pre-tratamiento se evaluaron cuantificando la solubilización y consumo de materia orgánica, el comportamiento reológico del fango y la biodegradabilidad anaerobia. Finalmente, se hizo una evaluación energética, intentando mostrar el posible impacto del pre-tratamiento de auto-hidrólisis en los costos operacionales de una estación depuradora de aguas residuales.

En el **capítulo 4** se aplicó el pre-tratamiento de auto-hidrólisis en fango secundario con alto contenido de sólidos totales (8%). Al aumentar el contenido de sólidos en el fango, la transferencia de aire y la agitación se hacen más difíciles, por lo que un nuevo dispositivo experimental fue necesario. Los efectos del pre-tratamiento se evaluaron a través de la solubilización y consumo de materia orgánica al igual que en el capítulo anterior, pero la materia orgánica fue caracterizada de forma específica, evaluando su contenido de proteínas y carbohidratos; también se estudio la viabilidad celular. Finalmente, se comparó el efecto del pre-tratamiento en digestión anaerobia mesófila y termófila.

En el **Capítulo 5** se presenta la actividad microbiana y el comportamiento de las enzimas en el fango secundario, como una herramienta fundamental para la descripción del mecanismo de hidrólisis del pre-tratamiento estudiado. El pre-tratamiento se realizó a distintas temperaturas (40, 55 y 70ºC), intentando aislar la contribución del efecto térmico y biológico-enzimático. Los efectos del pre-tratamiento se evaluaron a través de la cuantificación de dos enzimas hidrolíticas (*amilasa* y *proteasa*), en el fango y en el sobrenadante. Se midió la cantidad de bacterias heterotróficas vivas en condiciones mesófilas y termófilas, cuantificando el decaimiento o crecimiento de ambas poblaciones durante el pre-tratamiento. Finalmente, se evaluó la biodegradabilidad anaerobia de las distintas fases que componen el fango: sobrenadante y sólidos suspendidos.

El **Capítulo 6** muestra la puesta en marcha y operación de dos digestores anaerobios, de 20L de volumen cada uno, operados en paralelo durante 550 días. Los dos digestores fueron arrancados utilizando únicamente fango secundario concentrado como alimentación. Luego, se alimentó uno de los digestores con pre-tratado, comparando el efecto producido al ser sometidos a distintas condiciones de operación.

Finalmente, el C**apítulo 7** presenta un resumen de la metodología experimental utilizada e indica las conclusiones principales y específicas obtenidas.

CHAPTER 1

OBJECTIVES

OBJETIVOS

OBJECTIVES

The main objective of this thesis is the evaluation of the effect produced by the autohydrolysis pretreatment on secondary sludge, and its effect on the subsequent anaerobic digestion process. Additionally, try to clarify the possible mechanism involved in the hydrolysis of the sludge during the autohydrolysis pretreatment.

For this purpose the following specific objectives are developed in this study:

1) The study of the autohydrolysis pretreatment effect on the secondary sludge and its hydrolysis mechanisms:
    a. The study of the effect of the secondary sludge concentration
    b. The study of the presence and absence of air supplied during the pretreatment
    c. The study of the autohydrolysis pretreatment effect on the sludge rheology
    d. The study of the autohydrolysis pretreatment effect on the activity and viability of the microorganisms constituting the secondary sludge
    e. The study of the autohydrolysis pretreatment effect on the enzymatic activity of the secondary sludge, both in the soluble phase and in the total phase

2) The study of the autohydrolysis pretreatment effect on the anaerobic digestion of secondary sludge:
    a. The study of the effect produced by the different operational conditions of the autohydrolysis pretreatment on the anaerobic biodegradability of the secondary sludge
    b. The study of the effect produced on the anaerobic biodegradability of the pretreated secondary sludge, when the anaerobic digestion was carried out in mesophilic or thermophilic conditions.
    c. The study of the continuous anaerobic digestion of pretreated sludge and the with the digestion of non pretreated sludge.

OBJETIVOS

El objetivo principal de esta tesis es determinar el efecto provocado por el pre-tratamiento de auto-hidrólisis en el fango secundario y su efecto en el posterior proceso de digestión anaerobia. Adicionalmente, esclarecer los mecanismos que intervienen en la hidrólisis del fango secundario durante el pre-tratamiento de auto-hidrólisis.

Para obtener el objetivo general, se desarrollan los siguientes objetivos específicos:

1) El estudio del pre-tratamiento de auto-hidrólisis en el fango secundario y su mecanismo de hidrólisis:
    a. Estudiar el efecto de la concentración del fango
    b. Estudiar el efecto de la cantidad de aire disponible
    c. Estudiar el efecto del pre-tratamiento en la reología del fango
    d. Estudiar el efecto del pre-tratamiento de auto-hidrólisis en la actividad y viabilidad de los microorganismos que constituyen el fango secundario
    e. Estudiar el efecto del pre-tratamiento de auto-hidrólisis en la actividad enzimática del fango secundario, tanto en la fase soluble como en la fase total.

2) El estudio del pre-tratamiento de auto-hidrólisis en la digestión anaerobia de fango secundario:
    a. Estudiar los efectos producidos por las distintas condiciones operacionales del pre-tratamiento en la biodegradabilidad anaerobia del fango
    b. Estudiar el efecto producido en la biodegradabilidad anaerobia del fango pre-tratado, cuando la digestión anaerobia se realiza en condiciones mesófilas o termófilas.
    c. Estudiar la digestión anaerobia continua de fango secundario pre-tratado, y compararla con la digestión anaerobia continua de fango secundario sin pre-tratar.

CHAPTER 2

INTRODUCTION: SEWAGE SLUDGE PRODUCTION AND ITS MANAGEMENT ALTERNATIVES

ABSTRACT

This chapter is focused in the problem of the sewage sludge generation; it is a brief overview of the sludge generation and management. The current legislation in both, the European Union and Spain, are presented, and also the most used treatment methods for it stabilization. Specifically, the anaerobic digestion process is showed as one of the best alternatives for sewage sludge treatment, because the stabilization capacity to the sludge allows land application, and as a consequence the recovery of the residue in agriculture. Furthermore, biogas is produced during the treatment process, allowing energy recovery. Finally, the improvement of the anaerobic digestion of sludge through pretreatments application is described, presenting different techniques and its most important advantages, also the autohydrolysis pretreatment fundamentals are presented, introducing the pretreatment studied in this thesis document.

RESUMEN

En el siguiente capítulo se presenta la problemática asociada a la generación de fango en el tratamiento de aguas residuales. Se presenta la legislación vigente tanto en la Unión Europea como en España, y además los tratamientos más utilizados para la estabilización del fango. De forma específica, se presenta el proceso de digestión anaerobia, debido a que el producto generado permite su aprovechamiento en agricultura, y además durante el tratamiento se produce biogás, una fuente de energía renovable. Al finalizar, se describe la utilización de pre-tratamientos para mejorar el proceso de digestión anaerobia, presentando distintas técnicas utilizadas y sus ventajas, también se presentan las bases del pre-tratamiento de auto-hidrólisis, y así introducir el pre-tratamiento estudiado en esta tesis.

2.1. INTRODUCTION

The sewage sludge is an inevitable product of the waste water treatment process, the last decades have shown a significant improvement of the Waste Water Treatment Plants (WWTP) operation; probably caused by the stringent legislation associated to the management of urban and industrial waste. Moreover the numbers of WWTP have increased significantly due to the building of new installation in developing countries. As a result the sewage sludge production increases proportionally and therefore the managing of the produced sludge is one of the most demanding tasks for the waste water sector. Also, nowadays the treatment and disposal of the sludge has associated to the 50% of the WWTP operational costs (Pérez-Elvira et al. 2006), therefore the used treatment should not be only focused on the residue stabilization, but it also focused in the application of different techniques optimizing the associate costs and also allowing it use as a renewable energy source.

The increase of the sewage sludge production has been quantified, in the European Union, from 6.5 to 9.8 millions of tons of dry solids between 1992 and 2005, respectively (Kelessidis and Stasinakis 2012). In Spain, the sewage sludge production has increased from 853 to 1205 thousand of tons of dry matter between 2000 and 2009, respectively (Ministry of the Environment and Rural and Marine Affairs 2010).

2.2. LEGISLATION

Because all human activities cause environmental effects, and the direct relationship between quality of life and volume of waste generated, has become necessary to generate systems of control and prevention, as a consequence, the different organization have generated regulations and management mechanism to minimize the emission of waste.

In the European Union (EU), the basic concepts and definitions related to waste management such as waste, recycling and recovery, are described in the directive 2008/98/EC (European Parliament 2008). It describes some management principles, such as the management of the wastes should be done without damaging health or harming the environment, also indicates the implementation of waste management plans for each of the EU Member States. Furthermore, the hierarchy of the waste management is indicated, showing the priorities in legislation and policy as follow 2008/98/EC (European Parliament 2008):

- Prevention
- Preparing for re-use
- Recycling
- Other recovery, such as energy recovery, and
- Disposal.

The different wastes have been classified according to their hazard and toxicity levels, as a result the sewage sludge is considered as non hazardous wastes (Environmental Protection Agency 2002). Based on the use and recovery criteria, the sewage sludge utilization in agriculture have been encourage by the directive 86/278/EEC (Council Directive 1986), however its application without treatment is prohibited. The definition of treated sludge is any sludge that was subjected to some appropriate process that reduced its fermentability, and reducing the potential health risks from the residual pathogens. Also, limit values of heavy metal content was indicated for agricultural use in the sewage sludge (European Commission 2012).

Specifically, in the case of Spain, the previous legislation 10/1998 on Waste, and nowadays 22/2011 of Waste and Contaminated Soil, indicates the obligation to draw up national plans of waste management (Gobierno de España 2011). In fact, the Second National Plan of Sewage Sludge (II PNLD 2007-2015) approved in 2006, showed the necessity of improving the sewage sludge management. Its goal was the increase in sewage sludge recovery, mainly trending to increase its use as an agricultural fertilizer.

As a result of the environment law application, the sludge production in Spain grew from 853 to 1205 thousand of tons of dry matter, between 2000 and 2009, approximately 41% higher. And in 2009, of this amount 83% was used as a fertilizer in agriculture (Ministry of the Environment and Rural and Marine Affairs 2010).

2.3. SLUDGE GENERATION AND CHARACTERIZATION

Different sludge types are produced in the WWTPs as a consequence of the waste water treatment due to the application of different methods, such as mechanical, biological and physiochemical. Figure 2.1 showed a typical waste water treatment system.

The primary sludge is commonly obtained as a result of the primary settling operation, where high size compounds are removed from the waste water. On the contrary, the secondary sludge, also called waste activated sludge, is produced in the biologic treatment of the waste water, commonly in an aerated tank; after it is removed by the secondary settling operation. The most common mass balances of the organic matter removal in the WWTPs showed that the 1/3 of the influent BOD is removed in the primary settler, and after the microbial metabolisms, in the biologic treatment, transforms a half of the remaining organic matter into bacterial cells (Speece 2008), being this fraction the secondary sludge. Then, 2/3 of the inlet waste water organic matter content is transformed into sludge and needs to be stabilized.

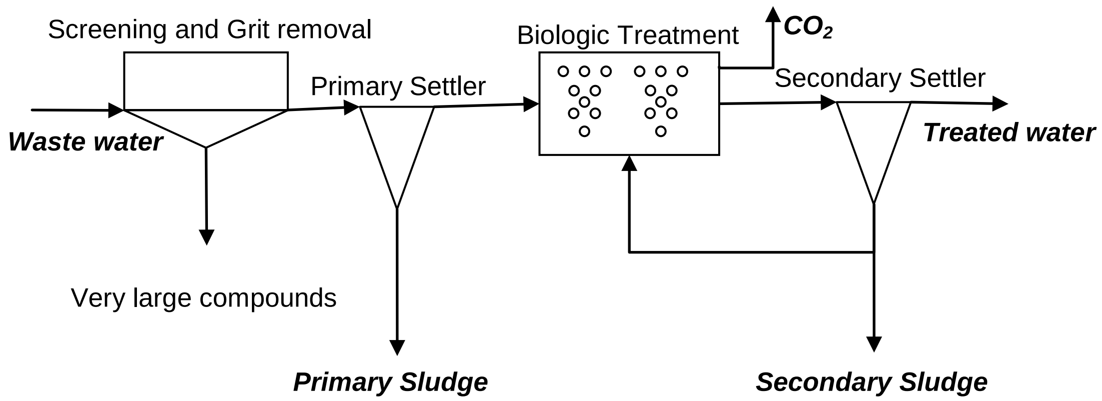

Figure 2.1. Simplified diagram of waste-water treatment system.

The main characteristics of the primary and secondary sludge are different. The solid content of the primary sludge can vary between 2 and 7%, and it can be dewatered rapidly because it is comprise of discrete particles. On the contrary, the secondary sludge, which is produced in the biological stage, contained lower content of solids, and also a lower dewaterability quality than the primary sludge. Table 2.1 presents some of the most important characteristics of both sludge types, and the table 2.2 presents a comparison between both size distributions.

Table 2.1. Comparative sludge characterization (Metcalf et al. 2003)

	Raw primary sludge	Raw secondary sludge
TS (%)	5 – 9	0.8 – 1.2
VS (% of TS)	60 – 80	59 – 88
Grease and fats (% of TS)	13 – 65	5 – 12
Protein (% of TS)	20 – 30	32 – 41
Nitrogen (% of TS)	1.5 – 4.0	2.4 – 5.0
Cellulose (% of TS)	8 – 15	

In addition to primary and secondary sludge, other wastes are produced in the waste water treatment, such as screening and grit, which are commonly sent to landfill, also if there is a tertiary treatment, some sludge production would allow. However, the quantity produced, of screening, grit and tertiary sludge, is significant lower than the production of primary and secondary sludge. Furthermore, other types of sludge can be produced such as chemical sludge; when some chemical is added improving the separation or promoting the precipitation and removal of hard-to-remove substances (Turovskiy and Mathai 2006). But this process is more typical for industrial waste water treatment than for urban WWTP.

Table 2.2. Particle size distribution (Turovskiy and Mathai 2006)

Sizes ranges (mm)	Raw primary sludge (%)	Raw secondary sludge (%)
> 7	5 – 20	-
1 – 7	9 – 33	-
> 3	-	0.4
1 – 3	-	1.6
0.2 – 1	27 – 44	8
< 0.2	23 – 40	90

As was mentioned before, due to its nature, the secondary sludge in more difficult to dewatering than the primary sludge, however both are high in organic matter content, presented in their ratios of VS to TS (Table 2.1). Also, both showed different response to some treatments, for example, when both sludge are subjected to ultrasound treatment, the secondary sludge showed greater decrease in particle size and increase in soluble organic compounds than the primary sludge (Mao et al. 2004). However, the primary sludge has shown higher degradation rates than the secondary, when the anaerobic digestion of both was compared at different conditions, higher methane production potential was obtained for the primary sludge (Gavala et al. 2003).

Both sludge types, primary and secondary, despite being different are composed by suspended solids, therefore for being subjected to biologic treatment needs, as first stage, reducing its size and transferring the organic matter into the soluble phase, as a consequence the hydrolysis is the limiting step of its biodegradation.

The more difficult biodegradation of secondary sludge than primary is mainly caused by the floc structure. The secondary sludge, besides being constituted by cells, is composed by exo-polymeric substances (EPS); also there is a contribution of non-biodegradable compounds in the inlet waste water, which remains attached to the floc. The EPS structure provides different properties to the floc such as flocculation, settling and dewaterability (Y. Liu and Fang 2003); in addition, this dead material corresponded to 80-95% of the total organic matter of the sludge (Nielsen et al. 2004). The EPS and microorganisms characterization in waste activated sludge depends of the type of biologic treatment, and even operating conditions such as solid retention time. In fact, the anaerobic digestion of secondary sludge produced at different solid retention times on the biologic treatment, showed that the specific gas production was inversely proportional to the solid retention time increase, that is to say, higher solid retention time in the biologic treatment reduced the biodegradability of the sludge under anaerobic conditions (Bolzonella et al. 2005). Furthermore, the unbiodegradable fraction of the secondary sludge, which is generated by the biologic process and also associated with the influent waste water content, and that can be quantified on aerobic process as a response of the activated sludge system, is also unbiodegradable under anaerobic conditions (Ekama et al. 2007).

2.4. SLUDGE TREATMENT

There are many different techniques for sewage sludge management, ones focused into the minimization of the sludge production in the biological stages (Yu Liu and Tay 2001). Others focused into the treatments of the sewage sludge, such as: alkaline stabilization, composting, thermal drying and incineration. However, the most common treatments used in European WWTPs are the aerobic and anaerobic digestion (Kelessidis and Stasinakis 2012).

Different techniques of treatment are applied to the sludge, mainly depending of the final disposition employed. Nowadays in Europe, the sludge reuse through the agricultural application appears to be the most used alternative for the sludge management (53% of produced sludge), followed by incineration (21%); and also seems to be the most adopted for the future application until 2020 (Fytili and Zabaniotou 2008; Kelessidis and Stasinakis 2012).

The waste water treatment reduces the organic matter content in the water, and also removed the pathogen microorganisms, transporting them to the sludge. In the case of the primary settling, transfer between 30 and 70%, and the activated sludge treatment reaches a reduction of 90 to 99% (Turovskiy and Mathai 2006). Those pathogen microorganisms, in common with the organic matter content of both sludge types, require stabilization for its valorization and disposal.

The main objective of the sludge treatment is to reduce the pathogen content, eliminate odors and finally, reduce or eliminate the putrefaction potential. All this parameters are directly related with the volatile solid content of the sludge; therefore, the treatment performed should reduce it content and/or provide a final product in which the microorganisms growth is not possible (Metcalf et al. 2003).

There are many different sludge treatments; the principal methods are briefly described below and, in addition, the main advantages and disadvantages are presented in the table 2.3:

- *Aerobic Digestion:* this process produces the organic matter decomposition by the aerobic microorganisms, and it is similar to the extended of the activated sludge process.
- *Anaerobic Digestion:* this biological process transforms the organic matter, at anaerobic conditions, to innocuous substaces and gaseous products, such as methane and carbon dioxide.

- *Alkaline Stabilization:* this process consists in the addition of alkaline material, usually lime. After, a high pH level is maintained during a period of time, producing the pathogen elimination.
- *Composting:* this process is an aerobic biothermal conversion of the organic matter in windrows or piles. The compost increases it temperature due to the microbial metabolisms, and it must be maintained at 40ºC at least 5 days, during this period of time a temperature of 55ºC during 4 hours as minimum should be maintained too.
- *Thermal drying and incineration:* both processes consists in the application of heat for water evaporation (thermal drying), and after the total destruction by the combustion of the organic solids (incineration). The dewatered sludge will ignite at 420 to 500ºC in the presence of oxygen. Commonly, this process is used when the valorization of the sludge is economically infeasible, or in some cases because of hygienic reasons.

2.5. ANAEROBIC DIGESTION OF SLUDGE

Comparing different sludge treatments, the anaerobic digestion appears to be one of the better alternatives, due to it allowed the sludge stabilization reducing the volatile solid content, eliminating pathogen microorganism, and also producing biogas, a renewable energy source. The use of the biogas as an energy source in the WWTP represents a significant impact in the economical balance. Furthermore, the sludge produced allows the reutilization through land application.

As was described above, the anaerobic digestion is the organic matter decomposition in the absence of oxygen. This process involves the interaction of a complex microorganism consortium conducting several series reactions; the rate of the global process is controlled by the slowest stage involved in the process.

Table 1.3. Main advantages and disadvantages comparative of some sludge treatments (Metcalf et al. 2003; Turovskiy and Mathai 2006).

Process	Advantages	Disadvantages
Aerobic digestion	- low capital cost - Odorless in the final product - Volatile solids removal slightly less than anaerobic digestion process - Easy operation - Good supernatant liquor quality - Safety operation.	- High operation costs - No biogas production - Reduced efficiency at cold weather - The performance is affected by the operational conditions.
Anaerobic digestion	- Methane gas is produced - Reduction of the 30 to 65% of the solids, reducing costs of the sludge disposal - Digested solids free of objectionable odors - High rate of pathogen elimination, higher for thermophilic anaerobic digestion.	- High capital costs - Large reactors are required, due to the high solids retention time - Microorganisms are sensitive to environment changes - Low quality of supernatants
Alkaline stabilization	- Low capital cost - Easy operation - Fast start-up and shut-down - Effective pathogen reduction - Reduce the odors - Improved dewaterability	- There is no mass reduction - Increased the transport and ultimate disposal costs - Lime handling requires operator attention - Possible harden during storage.
Composting	- The product is suitable for landscaping - The nitrogen content is released more slowly, so it is more consistent with plant uptake needs. - It is possible to produce class A biosolids - Simple operation and relatively simple mechanical equipment - It is possible to handle with different characteristics and peak of sludge production - In-vessel process require small areas	- Windrow and aerated static pile composting require large areas - Odor control is a common problem - The weather condition, such as ambient temperature, affects the process. - In-vessel reactors have limited flexibility to handle changing conditions of the sludge.
Thermal drying and incineration	- Reduction of the volume and mass by approximately 95% - Complete pathogens destruction - Toxins reduction or destruction - Potential energy recovery	- High capital and operation costs - Reduction of the beneficial uses of biosolids - Highly skilled operating staffs are required - Some solids residuals (ash) needs special disposal, such as hazardous waste. - The gaseous emissions require extensive treatment.

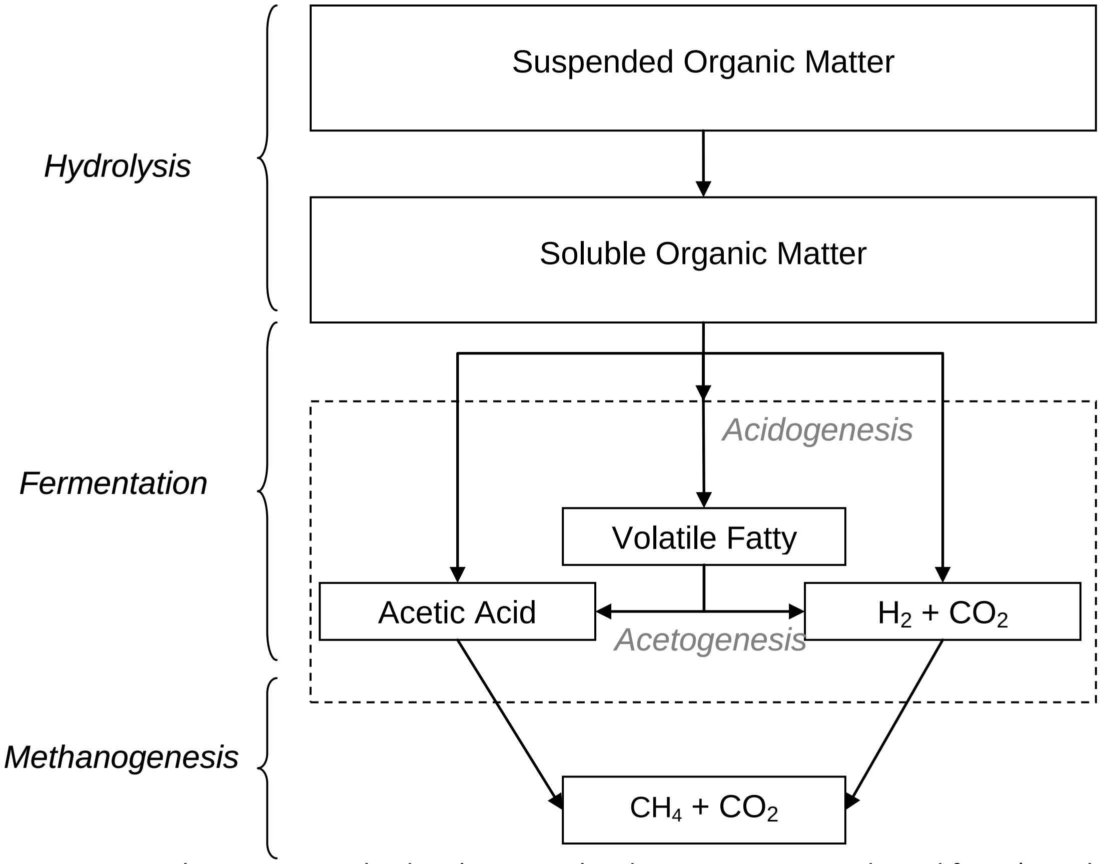

Figure 2.2. Serial reactions involved in the anaerobic digestion process. Adapted from (Appels et al. 2008; Metcalf et al. 2003)

The anaerobic digestion process can be described in three basic steps: hydrolysis, fermentation, which includes the acidogenesis and acetogensis processes, and methanogenesis.

- Hydrolysis: this stage corresponds with the solubilization and depolymerization of macromolecules, such as proteins, polysaccharides and lipids. This process is carried out by exo-enzymes, and has a fundamental importance in the anaerobic digestion of suspended organic matter, because the molecules generated must be capable to pass through the cell membrane in the subsequent process steps. This is the main reason because the hydrolysis is considered the limiting step of sludge anaerobic digestion (Appels et al. 2008).

- Fermentation: this stage includes the acidogenesis and actogenesis stages, where the soluble monomers produced in the previous stages are degraded into different products, depending of its nature and characteristics. The main products of acidogenesis stage are acetic acids and other shorts fatty acids, but also alcohols, hydrogen and carbon dioxide are produced. Some of these products can be used directly in the methanogenesis stage, but others need the subsequent transformation in the acetogenesis stage.

The acetogenesis stage transforms the intermediate products of the acidogenesis stage into acetate, carbon dioxide and hydrogen. But the reactions involved are endothermic, and therefore only at low partial pressure of hydrogen will be thermodynamically feasible.

- Methanogenesis: this is the final stage of the anaerobic digestion process, and the main products are methane and carbon dioxide. Two different groups of microorganisms are involved during this stage: the *aceticlastic methanogens*, transforming the acetate, and the *hydrogenotrophic archea*, using the hydrogen as the electron donor and the carbon dioxide as the electron acceptor, producing methane as a result, however, the about 72% of the methane produced is from acetate formation (Metcalf et al. 2003).

As a consequence, the relationship between the microorganisms involved in the acetogenesis and methanogenesis stages is symbiotic. The methanogenic population consumes the methabolic products of the acetogenic population, reducing the hydrogen partial pressure in the media.

As was mentioned before, the anaerobic process involves several biochemical reactions produced by different microorganisms producing synergic interactions. The performance of the anaerobic digestion is affected by environmental factors. Some of these factors are described in table 2.4.

2.6. PRETREATMENTS TO IMPROVE SLUDGE ANAEROBIC DIGESTION

The anaerobic digestion of sludge has associated different advantages, however, some disadvantages are observed too. Some of these disadvantages are: partial organic matter decomposition, large digestion volume, inhibitor presence low quality of the supernatant and high digestion time (Appels et al. 2008).

As a consequence, the improvement of the anaerobic digestion process of sludge has been studied, focused in the improvement of the hydrolysis stage (Carrere et al. 2010; Odegaard 2004)

Table 2.4. Some environmental factors affecting the anaerobic digestion process (Metcalf et al. 2003; Speece 2008; Turovskiy and Mathai 2006).

Environmental factor	Description	Suggested Value
Solid and hydraulic retention time (SRT)	Corresponded to the average time that the solids spent in the digester, it should provide sufficient time to allow significant volatile solid removal. The SRT is directly related to the extent of the hydrolysis, fermentation and methanogenesis reactions, due to it should provide enough time to allow the microorganisms growth.	14 days at 30ºC
Temperature	The temperature affects directly the metabolic activities of the microbial population, and also affects the gas transfer rates. The hydrolysis and substrate solubilization are affected by temperature. The stable temperature operation is one of the most important factors in the anaerobic digestion process, due to variation in temperature, adversely affect the microorganisms.	Mesophilic: 30–38ºC, 35ºC the most common value. Thermophilic: 50-57ºC, 55 the most common value.
pH and Alkalinity	The methanogens population is very sensitive to the pH variations. Therefore, calcium and ammonium bicarbonate are produced, providing a buffering source. The equilibrium between carbon dioxide and bicarbonate controls the pH in the anaerobic digestion process. The alkalinity content is proportional to the solids feed concentration.	Optimum pH ranged 6.8 to 7.2
Inhibitory compounds	Several compound can produce inhibitory effect on the anaerobic digestion, these compounds can be produced in the anaerobic digestion as a product, or be provided by feeding. Some of the most common inhibitory compounds are: ammonia, sodium and potassium, heavy metals, hydrogen and volatile fatty acids. The acclimation of many toxicants can be overcome after long exposure to different amounts of the inhibitor compound.	Moderate inhibitory concentration ($g \cdot L^{-1}$) Na^+:3.5-5.5 K^+: 2.5-4.5 $NH4^+$: 1.5-3.0

The sludge pretreatment apply different techniques in order to cause the disintegration of the organic matter. Some of these pretreatment techniques are: mechanical, chemical, thermal and hydrolytic enzymes (Bougrier et al. 2006; Climent et al. 2007; Dhar et al. 2012).

These pretreatments produced different effects on the anaerobic digestion, increasing the dewater-ability of the sludge (Neyens and Baeyens 2003), improving the pathogen elimination (Mayhew et al. 2002), and also increasing the biogas production. However, they are commonly associated to the high operation or capital costs, and also to the high complexity of the implementation (Pérez-Elvira et al. 2006). The results of some studies using different sludge pretreatments are presented in table 2.5.

- Mechanical: the sludge solubilization is promoted by the application of shear forces and mechanical compression, such as milling (Barjenbruch and Kopplow 2003; Climent et al. 2007).

- Chemical: the addition of chemical compounds that produces the sludge hydrolysis, such as acids or alkalis. Commonly, the addition of the chemical reactive is associated with an increase in temperature (Neyens et al. 2003; Vlyssides and Karlis 2004).

- Thermal: the sludge is subjected to the thermal energy effect, promoting the sludge destabilization and organic matter solubilization. Different temperatures ranges have been studied and classified in two main groups: low-thermal pretreatments (<100ºC) and thermal treatments (>100ºC). Commonly, the temperature of the thermal pretreatments ranged around 100 to 250ºC, however higher temperatures promoted the formation of recalcitrant compounds (Bougrier et al. 2007; Dwyer et al. 2008).

On the other hand, low thermal pretreatments have shown the ability of increase the solubilization of the sludge, however the hydrolysis mechanisms or an optimal operation conditions are not well known. Some authors have shown negligible effect on the anaerobic digestion performance, even though a high organic matter solubilization (Prorot et al. 2011), on the other hand, other studies have shown significant increase in methane production (Appels et al. 2010; Ferrer et al. 2008; Prorot et al. 2011). Moreover, the evaluation of a low-temperature pretreatment (70ºC) of primary and secondary sludge, followed by the mesophilic or thermophilic anaerobic digestion, showed that both temperature and duration of pretreatment should be chosen depending of the sludge type to pretreat (Gavala et al. 2003).

- Biologic-Enzymatic: this pretreatment can be divided in two groups, depending of the source of enzymes applied: the addition of commercial enzymes (Davidsson et al. 2007; Yang et al. 2010) or the utilization of some enzyme producing microorganism in the pretreatment (Hasegawa et al. 2000; Kim et al. 2002).

2.7. AUTOHYDROLYSIS OF SECONDARY SLUDGE

The secondary sludge is mainly composed by cells aggregation, the exo-polymeric substances have the objective to maintain the floc structure, and representing around 90% of the organic matter content (Nielsen et al. 2004). As a consequence, several studies have shown the presence of enzymes associated to these exo-polymeric substances (Frolund et al. 1995; Nabarlatz et al. 2010), some of them have been categorized as loosely bound and tightly bond, depending on the difficulty associated to release them from the support (Yu et al. 2007), even though several techniques have been used to extract and purify them (Nabarlatz et al. 2010; Nabarlatz et al. 2012).

In addition, some studies have shown the ability of the secondary sludge enzymes to hydrolyze the organic colloidal fraction of the waste water (Guellil et al. 2001), and also it is possible to stimulate the production of hydrolytic enzymes such as proteases by changing the environmental conditions of the sludge, such as temperature and oxygen quantity (Yan et al. 2008).

As a consequence, the ***autohydrolysis pretreatment*** is proposed a good alternative for anaerobic digestion of secondary sludge. It involves subjecting the secondary sludge to a temperature of 55ºC and a limited amount of oxygen in batch operation, so that the microorganism in the secondary sludge releases the hydrolytic enzymes contained in its own metabolic system. In consequence, the secondary sludge is hydrolyzed by the synergic effect produced by thermal solubilization and the released enzymes.

Table 2.5. Main results of some studies using different sludge pretreatments

Pretreatment	Description	Type of sludge	Solubilization	Biogas produced by the control	Improvement (%)	Reference
Mechanical	High pressure homogenizer	Mixed sludge (60:40)	-	350[3]	17	(Barjenbruch and Kopplow 2003)
Thermal	60min at (a) 80, (b) 90 y (c) 121°C				(a) 15, (b) 22, (c) 20	
Enzymatic	Commercial enzyme: *carbohydrase*				12	
Enzymatic	Pretreatment of mixed protease and glycosidic enzymes. Batch anaerobic digestion	Mixed (50:50)	-	437[1]	26	(Davidsson et al. 2007)
	Mixed protease and glycosidic enzymes added to the anaerobic digestion batch assay.		-	425[1]	14	
	Mixed enzymes added to continuous anaerobic digestion		-	398[1]	71	
Thermal	30min at 135°C	Secondary	34	194[2]	12	(Bougrier et al. 2007)
	15min at 190°C		46	217[2]	25	
Low Thermal	24h at 70°C	Mixed	-	220[1]	30	(Ferrer et al. 2008)
Dual Digestion	Continuous aerobic thermophilic pretreatment (1d of SRT) followed by mesophilic anaerobic digestion	Mixed (1:2)	-	480[1]	6	(Borowski and Szopa 2007)
Ultrasound	Three specific energy inputs: 1000, 5000 and 10000 ($kJ\cdot kg^{-1}$)	Secondary	10 – 35	325[3]	15 – 24	(Dhar et al. 2012)Dhar
Low Thermal	30min at 50, 70 and 90ºC		18 – 40		14 – 19	
Combined	Combination of previous conditions		25 – 40		19 – 30	
Biological	Thermophilic aerobic bacteria	Sterilized sludge	40	200	50	Hasegawa
Ultrasonic	Specific energy 6250 and 9350 ($kJ\cdot kg^{-1}$)	Secondary	15	300[2]	51 – 53	Bougrier, 2006
Thermal	Autoclave: 30min at 170 and 190ºC		19 – 21		8 – 25	
Ozone	Ozone dose of 0.1 and 0.16 ($g\cdot g^{-1}$)		40 – 50		59	

[1] $L_{CH_4}/kgSV_{in}$ [2] $L_{CH_4}/kgDQO_{in}$ [3] $L_{CH_4}/kgSSV_{in}$

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CHAPTER 3

AUTOHYDROLYSIS PRETREATMENT OF SECONDARY SLUDGE FOR ANAEROBIC DIGESTION, PRELIMINARY ASSESSMENT

ABSTRACT

This section presents the *autohydrolysis pretreatment*, which consists of subjecting the secondary sludge between 12 and 24 hours to a temperature of 55°C with a limited amount of oxygen in batch operation. This study focuses on the evaluation of the influence of oxygen presence in the pretreatment and the initial sludge concentration between 30 and 54 ($kg \cdot m^{-3}$) of total solids. The pretreatment produces a high solubilization of organic matter, increasing the fluidity of the sludge and improving the biogas production. The main results obtained showed that when autohydrolysis pretreatment was carried out over 12 hours, with a high solid concentration and microaerobic conditions, the solubilization of organic matter was increased by 40%, the methane productivity was improved by 23%, and the fluidity was also improved. Moreover, the energy assessment of the autohydrolysis pretreatment and anaerobic digestion system showed the energetic feasibility of this treatment method, because the increase in energy production covered the need for the extra energy required to carry out the process.

RESUMEN

Esta sección presenta el pre-tratamiento de auto-hidrólisis, el cual consiste en someter fango secundario durante un periodo de tiempo entre 12 y 24 horas a una temperatura de 55ºC, con una cantidad limitada de oxígeno en operación batch. Este estudio está centrado en la evaluación de la presencia de oxigeno durante el pre-tratamiento y el efecto de la concentración de sólidos. El pre-tratamiento produce la solubilización de la materia orgánica, aumentando la fluidez del fango y mejorando la producción de biogás. Los resultados principales mostraron que cuando el pre-tratamiento se realiza durante 12 horas y con una alta concentración de fango, se obtiene un factor de solubilización de 40%, y la productividad de metano aumenta un 23%. Además, la evaluación energética del pre-tratamiento de auto-hidrólisis y la digestión anaerobia de fango secundario mostró la factibilidad del método propuesto, debido a que el aumento de la energía producida cubre los requerimientos necesarios para el pre-tratamiento.

SIMBOLS

WAS	Waste activated sludge	WWTP	Waste water treatment plant
EPS	Exo-polymeric substances	P_{CH4}	Methane productivity ($m^3{}_{CH4}\cdot kg^{-1}{}_{VSfed}$)
COD_S	Dissolved chemical oxygen demand ($g_{O2}\cdot L^{-1}$)	τ	Shear stress ($Pa\cdot m^{-2}$)
COD_T	Total chemical oxygen demand ($g\cdot kg^{-1}$)	Υ	Shear rate
TS	Total solids ($g\cdot kg^{-1}$)	τ_0	Yield stress ($Pa\cdot m^{-2}$)
VS	Volatile solids ($g\cdot kg^{-1}$)	η_p	Plastic viscosity ($Pa\cdot m^{-2}\cdot s^{-1}$)
TOC	Total organic carbon ($g\cdot L^{-1}$)	n	Material parameter
VFA	Volatile fatty acids ($g\cdot L^{-1}$)	$\mathcal{F}$	Solubilization factor (%)
E_p	Thermal energy consumed in the treatment	E_{ad}	Heat added to anaerobic digestion
E_t	Electrical power consumed thickening stage	$E_{ad,\ elec}$	Electrical power produced
$E_{ad,\ h}$	Remaining heat recovered		

3.1. INTRODUCTION

The production of waste activated sludge (WAS) has increased over the last years because of the higher number of wastewater treatment plants (WWTP) and the requirements for better effluent quality. For example, Spain increased its sludge production by about 50% between 1997-2007 (Ministerio del Medio Ambiente 2007). Therefore, the reduction in sludge production has become an important challenge nowadays (Odegaard 2004; Wei et al. 2003).

Commonly, the sludge produced into the WWTP is treated, seeking to reduce the total volume. In addition, it is necessary to transform the putrescible organic matter into a stable residue or a reusable product. The most commonly used sludge treatment in WWTPs is anaerobic digestion (AD). This treatment reduces the solid content, eliminates pathogen microorganisms, and produces biogas, a renewable energy source. However, the AD of sludge has some disadvantages such as partial organic matter decomposition, large digestion volume, inhibitor presence, and the low quality of supernatant and high digestion time, making it necessary to improve its operation (Appels et al. 2008).

The limiting step of the AD of secondary sludge is hydrolysis. The hydrolysis stage includes the changing of insoluble macromolecules into soluble compounds, to make them available to the microorganisms involved in the AD. For the secondary sludge, the hydrolysis step involves breaking off the sludge floc, the cell wall disruption and degradation of extracellular polymeric substances (EPS) (Appels et al. 2008).

Many processes for the improvement of the reduction of WAS have been proposed, applying different techniques for the minimization of production or its disintegration. There are two principal strategies in the *sludge line techniques:* i) modification of usual anaerobic digesters or ii) application of pretreatments of sludge before the AD (Odegaard 2004; Pérez-Elvira et al. 2006).

The sludge pretreatments apply different techniques in order to cause the disintegration of WAS, prior to its AD of them. Some of these pretreatments are: mechanical, chemical, sonication, thermal and the utilization of hydrolytic enzymes (Bougrier et al. 2006; Carrere et al. 2010; Climent et al. 2007).

The biologic-enzymatic pretreatments include the possibility of applying commercial enzymes, which are added selectively in type and quantity (Davidsson et al. 2007). Also, it is possible to use an enzyme-producing microorganism that releases the hydrolytic enzymes during the hydrolysis step (Hasegawa et al. 2000). Finally, and as the proposed pretreatment in this work, comes the utilization of the inherent enzymatic activity of the secondary sludge waiting to be treated. The ability of the enzymes associated with the EPS extracted from the WAS to hydrolyze the organic colloidal fraction of the wastewater has been shown in some works (Burgess and Pletschke 2008; Guellil et al. 2001).

The *autohydrolysis pretreatment* is a biologic-enzymatic pretreatment; it involves subjecting the secondary sludge to a temperature of 55ºC and a limited amount of oxygen in batch operation, so that the microorganism in the secondary sludge releases the hydrolytic enzymes contained in its own metabolic system. In consequence, the secondary sludge is hydrolyzed by the effect of the released enzymes. Other researchers observed that when secondary sludge was subjected to environmental changes, such as an increase in temperature or a decrease of oxygen concentration, the production of hydrolytic enzymes was obtained (Yan et al. 2008). These hydrolytic enzymes of secondary sludge may be located in the soluble phase or in the sludge floc (Burgess and Pletschke 2008). In addition, other studies which applied a similar process called “enzymic hydrolysis”, obtained a high solubilization of organic matter, and showed an improved of dewaterability and pathogen elimination (Mayhew et al. 2002).

All pretreatments in literature have associated benefits and disadvantages, the most common of which are related to the high operation costs and/or with the high complexity of the implementation (Pérez-Elvira et al. 2006). The *autohydrolysis pretreatment* tries to uses synergic effects produced by the temperature applied and the hydrolytic enzymes released, thus obtaining a pretreatment with a simple operation process and low requirement of an external energy source. As a consequence, the product expected is a solubilized and hydrolyzed organic matter, with an anaerobic biodegradation potential improved, but without associated high costs.

The aim of this work is to study the effect of secondary sludge concentration and the presence and absence of oxygen supplied on the autohydrolysis pretreatment, and also to evaluate the effect produced during the subsequent anaerobic digestion of the WAS.

3.2. MATERIALS AND METHODS

3.2.1. Secondary Sludge

The secondary sludge used in this study came from the WWTP of Valladolid, Spain. The *activated sludge* process operates at 12 days of solid retention time and nutrient removal. This sludge was stored before experimentation less than 48 hours at 4°C. Two solid concentrations were studied (Table 3.1). The pre-thickening step was carried out using a laboratory centrifuge, operated for 5 minutes at 5000rpm. After that, a quantity of the supernatant was removed to achieve the desired concentration.

Table 3.1. Initial concentration of secondary sludge used

Sludge	TS ($kg \cdot m^{-3}$)	VS ($kg \cdot m^{-3}$)	COD_T ($kg \cdot m^{-3}$)	COD_S ($kg \cdot m^{-3}$)
Low	22.9 ± 0.7	18.1 ± 0.9	28.5 ± 0.5	0.26 ± 0.03
High	54.1 ± 0.7	40.9 ± 0.8	56.1 ± 0.5	0.35 ± 0.07

3.2.2. Experimental Procedure

3.2.2.1. Autohydrolysis Pretreatment

The pretreatment was carried out in batch conditions. The temperature was kept constant using a water bath at 55°C. The agitation was produced by magnetic stirring. Each flask of

500mL capacity was loaded with 200g of secondary sludge. All flasks were placed in the water bath at the same moment, and at the sample time three flasks were sacrificed.

Two aeration conditions were studied microaerobic and anaerobic. The microaerobic tests were conducted using open flasks. To avoid water evaporation, the top of the flasks were connected by perforated septum with long pipes as condensers, so that the presence of air in the gas chamber was ensured. The transfer of oxygen from the gas chamber to the sludge occurred by convection because of the agitation.

The anaerobic tests were carried out under absence of oxygen. Before the pretreatment, the flasks were closed and degassed. Helium gas was circulated in the gas chamber for 5 minutes, and after half an hour of pretreatment, the flasks were degassed again because of the increased pressure due to the higher temperature.

The samples were taken after 12 and 24 hours of pretreatment, and all the analyses were performed in triplicate flasks.

To compare the efficiency of the sludge hydrolysis during the pretreatment, the solubilization was evaluated using dimensionless ratios between soluble and total organic matter.

- *solubilization factor* ($\mathcal{F}$): represents the released soluble COD over the total COD measured in the sample (Wei et al. 2003).

$$\mathcal{F} = \frac{COD_S - (COD_S)_{t=0}}{COD_T} \tag{3.1}$$

- *final/initial* ratio: The concentration of soluble COD and soluble TOC was compared for each sample with the initial concentration.

$$\frac{COD_S}{COD_{S,0}}, \frac{TOC_S}{TOC_{S,0}} \tag{3.2}$$

3.2.2.2. Rheology

A rotational viscometer (Brookfield LV-DV+) was used for the determination of the rheological behavior of the WAS. Fresh sludge (without pretreatment) and secondary sludge subjected to 12 and 24 hours of *autohydrolysis pretreatment* in microaerobic conditions were compared.

This viscometer measured the apparent viscosity and the torque exerted on a cylindrical spindle immersed in a large volume of sample (700g of WAS). The measurement was made at several speeds; being applied upward and then downward to observe the hysteresis phenomenon. The temperature was constant (20ºC) and each experiment was performed in triplicate.

The data was fit to a Herschel-Bulkley model (*equation 3*), where: τ and γ represent the shear stress and the shear rate. The parameters τ_0 (yield stress), η_p (plastic viscosity) and n are called material parameters and they were determined and compared for each kind of sludge.

$$\tau = \tau_0 + \eta_p \cdot \gamma^n \qquad (3.3)$$

3.2.2.3. Anaerobic Biodegradability

The anaerobic biodegradability of the secondary sludge was evaluated by anaerobic mesophilic digestion test. The methane production of pretreated and non-pretreated sludge was compared.

The assays were carried out at 35ºC (±0.5ºC) in a thermostatic room and with orbital agitation (150rpm). Closed Pyrex bottles of 300mL were used as batch reactors with a gas chamber volume of 150mL. The initial ratio of pretreated sludge and anaerobic inoculum was 0.5 (volatile solid content of substrate per volatile solid content of inoculum). The inoculum was taken from an anaerobic digester operated for over a year in steady-state. At the beginning of the experiments, each bottle was degassed by circulating helium in the gas chamber. The biogas production was followed by pressure measurement using a pressure transmitter (IFM, 5mbar precision) for 25 days or until no biogas production occurred, also the composition of the biogas was measured by gases chromatography. Each experiment was done in triplicate.

The final results were presented as *methane productivity* ($\boldsymbol{P_{CH_4}}$), which is the volume of methane produced ($V^0_{CH_4}$) in normal pressure and temperature conditions (0ºC, 1bar), divided by the quantity of volatile solid fed ($\mathrm{VS_{fed}}$) into the assay.

$$\boldsymbol{P_{CH_4}} = \frac{V^0_{CH_4}}{\mathrm{VS_{fed}}}\left(\frac{m^3}{kg}\right) \tag{3.4}$$

3.2.3. Analytical Methods

Total solids (TS), volatile solids (VS) and chemical oxygen demand (total: COD_T and soluble: COD_S) were determined by Standard Methods (APHA et al. 2005). The soluble phase was obtained by centrifugation of the samples during 10min at 5000rpm; and after that, the supernatant was filtered by 0.45µm pore size.

Volatile fatty acid (VFA) production was measured by gas chromatography using GC HP 5890 Series II, with flame ionization detector (FID). Nitrogen was a carrier gas and hydrogen and air were oxidizing gases. The samples (5mL of filtered sample), were supplied with 100µL of sulfuric acid for carbon dioxide removal and stabilization. Finally 1.5mL was placed in a vial and 10µL was injected into the chromatograph.

Biogas composition was measured by sampling and injection in a gas chromatograph (Varian CP-3800 CG). The equipment included with a TCD and helium was a carrier gas (Díaz et al. 2010).

3.2.4. Energy Assessment

The energy required to pretreat and digest the secondary sludge was evaluated; those values were used to estimate the economic effect of the autohydrolysis pretreatment over the WWTP. The different energy types considered in this assessment were: the thermal energy consumed during the pretreatment (E_p), the heat applied to the anaerobic digestion (E_{ad}), and also the consumption of electrical power during the thickening stage (E_t). On the other hand, the recovered energy, obtained by burning the biogas in a cogeneration unit, was composed of: the electrical power produced ($E_{ad,elec}$) and the remaining heat recovered ($E_{ad,h}$).

The parameters used to calculate the different kinds of energies were follows: i) anaerobic digesters need 0.017MWh of heat per ton of sludge fed ($MWh{\cdot}t^{-1}$) (Yang et al. 2010), ii) electric power produced 0.0022MWh per cubic meter of biogas produced ($MWh \cdot m^{-3}$), iii) the remaining heat of the cogeneration unit measured 0.0035MWh per cubic meter of biogas produced ($MWh \cdot m^{-3}$) (Houdkova et al. 2008), iv) the sludge thickening power required was 2.2kWh per ton of raw sludge with an initial concentration of 5($kg \cdot m^{-3}$) of TS (Centrifugal extractor Pieralisi, Baby 1).

The energy applied to the pretreatment was calculated as being the difference between the energy required for heating and maintenance during the pretreatment, and the energy recovered from the pretreated sludge due to its temperature being higher than the raw sludge commonly used without pretreatment. The assumptions regarding the pretreatment energy balance were as follows: i) the heat applied to the sludge (Q) was calculated using the sludge heat capacity and assuming that the initial temperature was 23ºC (C_P=4.184$kJ \cdot kg^{-1} \cdot K^{-1}$), ii) the active heating occurred during the first hour, and the remaining time (maintenance) required the 5% of the heating value per hour of pretreatment, due to energy loss by the equipment, and iii) the 58% of the heating value could be recovered by the reduction of the feed temperature due to the occurrence of anaerobic digestion under mesophilic conditions (35ºC).

All these balances were performed for a hypothetical population of 500000 inhabitants and the performance of the anaerobic digestion plant used here corresponded to that of the secondary sludge under the experimental conditions presented in this study. Finally, the energy assessment presented did not take into account the investment and maintenance costs, and the pumping and mixing of energy types.

3.3. RESULTS AND DISCUSSION

3.3.1. Autohydrolysis Pretreatment

3.3.1.1. Solubilization of Organic Matter

The raw sludge used for all the experiments showed an initial concentration of soluble COD less than 0.5$kg \cdot m^{-3}$, which represents less than 1% of the total COD (Table 3.1). These initial values showed a small proportion of organic matter in the soluble phase. Nevertheless, and as a consequence of the authohydrolysis pretreatment, for all the conditions studied, soluble COD was increased considerably mainly during the first twelve hours of pretreatment. The concentration of soluble COD achieved was close to 10 and 20kg·m^{-3}, when the initial sludge concentrations were 23 and 54kg·m^{-3} of the total solids respectively. The comparison of the soluble COD concentration achieved for microaerobic and anaerobic conditions showed that in 24 hours of pretreatment no significant difference was obtained. However, in12 hours of pretreatment, a significant difference was obtained of 22% higher solubilization for the microaerobic condition and high initial concentration of solids (Figure 3.1-a).

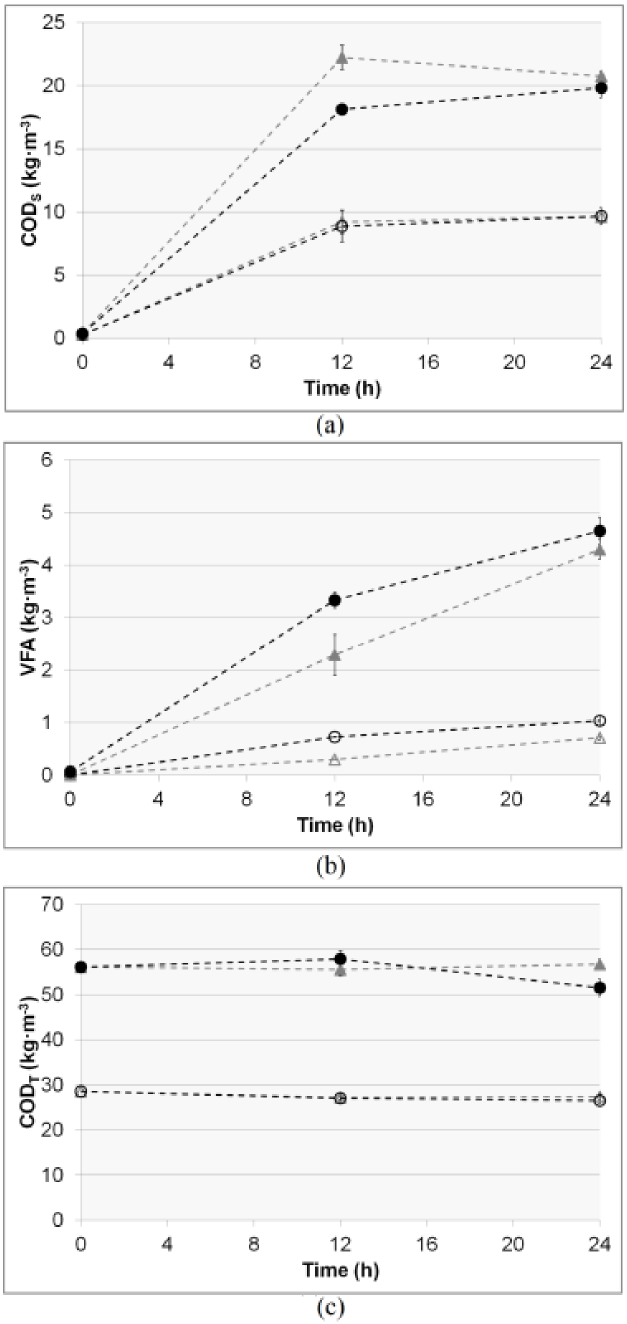


Figure 3.1. Concentration of a) soluble COD, b) VFAs and c) total COD, for microaerobic and anaerobic conditions during autohydrolysis pretreatment (Microaerobic 23kg·m^{-3}, Microaerobic 54kg·m^{-3}, Anaerobic 23kg·m^{-3}, Anaerobic 54kg·m^{-3}).

When the *solubilization factor* was compared, as shown in Table 2, it was noted that in assays with low initial concentration (23kg·m^{-3}) there was no significant difference between microaerobic and anaerobic conditions, and also, no significant difference of solubilization was observed between 12 and 24 hours of pretreatment. The maximum solubilization achieved was 35% (±2%) at 24 hours of pretreatment. In contrast, the results obtained for high concentration of solids was dissimilar. A significant difference was observed at 12 hours of pretreatment, and the maximum value of 39% (±2%) of solubilization was reached for the microaerobic condition. The behavior of COD or TOC in the soluble phase showed the same effect (Table 3.2), so this result showed that the microaerobic condition presents a higher solubilization than the anaerobic condition.

Table 3.2. Solubilization factors of secondary sludge subjected to autohydrolysis pretreatment

Condition	Time (h)	23 kg·m^{-3}			54 kg·m^{-3}		
		$\mathcal{F}$ (%)	$COD_S/COD_{S,0}$	$TOC_S/TOC_{S,0}$	$\mathcal{F}$ (%)	$COD_S/COD_{S,0}$	$TOC_S/TOC_{S,0}$
Microaerobic	0	0.9 ± 0.1	1	1	0.6 ± 0.1	1	1
	12	34 ± 4	36 ± 5	58 ± 13	40 ± 2	63 ± 13	78 ± 11
	24	35 ± 3	37 ± 5	65 ± 14	37 ± 1	59 ± 12	71 ± 10
Anaerobic	0	0.9 ± 0.1	1	1	0.6 ± 0.1	1	1
	12	33 ± 5	34 ± 6	54 ± 14	31 ± 1	51 ± 10	60 ± 8
	24	36 ± 2	37 ± 4	66 ± 14	38 ± 2	56 ± 12	67 ± 10

Literature has reported large and varied capacities for the solubilization of different pretreatments: comparison of these is not easy or trivial, due to the large spread of concentrations and the different types of sludge studied. The deflocculation of the WAS due to the increase in temperature was reported and measured, increasing the COD_S by 3-4 times with respect to the control levels in transient reactors subjected to the temperature shift from 30 to 45ºC (Morgan-Sagastume and Allen 2005). A study of low thermal pretreatment over a short period of time and with a low concentration of sludge (20min and TS=3.5g/L) showed the disintegration of the WAS between 50 and 95ºC, obtaining solubilization factors of around 2.5% and 18.6% respectively, with a linear behavior between them (Prorot et al. 2011), these values are considerably smaller that those obtained in this study when using the autohydrolysis pretreatment.

A comparative study of different WAS pretreatments subjected to sonication with a specific supplied energy of 6250($kJ \cdot kg^{-1}$) gave a solubilization of 15%, the ozonation pretreatment with a dose of 0.16 grams of ozone per gram of TS gave 25% solubilization ,and finally a thermal pretreatment of 170ºC during 90 minutes gave 45% of solubilization (Bougrier et al. 2006).

The *solubilization factor* reached by the *autohydrolysis pretreatment,* compared with others, has a relevant impact, because it shows the feasibility of achieving a high solubilization of organic matter with a low temperature pretreatment. Pretreatments which apply high temperature, required high energy source and are difficult to operate (Gavala et al. 2003), thus the reduction in the final temperature of the pretreatment shows the possibility of realizing pretreatments with good results and a lower requirement of external energy application.

It is probable that the high solubilization observed during the pretreatment was obtained because of the interaction between the increase of temperature and the release of hydrolytic enzymes from the intracellular content during the lysis, or even due to the release of extracellular enzymes attached to the extra- polymeric substances forming the floc. Some authors have observed the ability of some enzymes extracted from the WAS to hydrolyze the organic matter (Guellil et al. 2001). Others have observed the capacity of the activated sludge to produce and release hydrolytic enzymes from WAS subjected under environmental changes (Yan et al. 2008).

To summarize, the *solubilization factors* showed that with a high initial concentration of secondary sludge (54$kg \cdot m^{-3}$) and the microaerobic condition of autohydrolysis pretreatment it was possible to obtain a high solubilization of organic matter.

3.3.1.2. Production of VFA

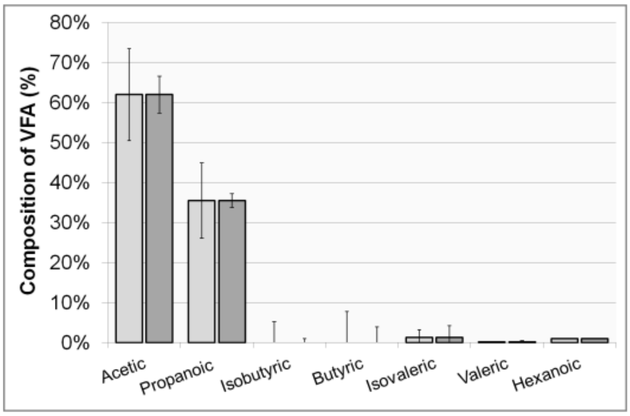
For both concentrations of secondary sludge studied, the anaerobic condition produced more VFA than the microaerobic condition (Figure 3.1-b). After 24 hours of pretreatment using a low concentration of solids (23$kg \cdot m^{-3}$ of TS) the production of VFA was 32% higher in the anaerobic condition than in the microaerobic one; however, for a high solid content (54$kg \cdot m^{-3}$ of TS) the difference was only 8%. When the concentration was 54$kg \cdot m^{-3}$ of TS, the agitation was difficult at the beginning of the experiment, because the viscosity of the sludge was higher than at low concentration (see rheology section). If the absence of air promotes the VFA production, and a high concentration of sludge can reduce the oxygen transfer, then the reduction in the difference detected between anaerobic and microaerobic production of VFA would be expected for a high solid concentration.

The VFA production based on the solubilization of COD showed a high level of production for a high solid content. When the solid content was $54 kg \cdot m^3$ the production of VFA was 21% and 24% of the soluble COD for the microaerobic and anaerobic conditions respectively. Instead, when the concentration of solid content was halved, the production of VFA was 7% and 11%. These results showed that the production of VFA was more significant when the concentration of solid content was high, and it increased in the anaerobic condition.

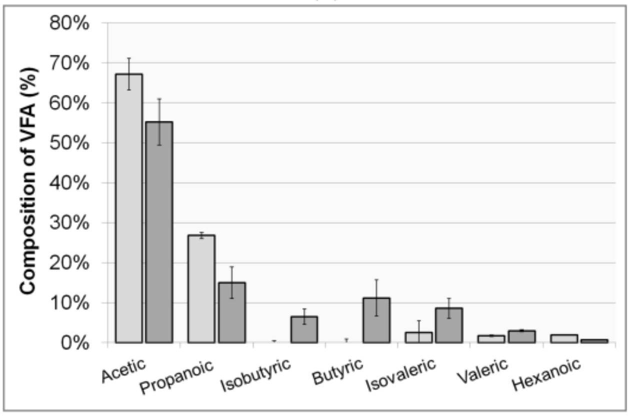
The VFA composition showed similar behavior between both aeration conditions during the first twelve hours of pretreatment (Figure 3.2). However, at 24 hours of autohydrolysis pretreatment the acetic acid content was seen to have increased under microaerobic conditions; however it decreased under anaerobic conditions. In addition, the presence of a significant concentration of other VFA was observed at 24 hours for anaerobic condition (Figure 3.2-b).

Previous works realized by this research group showed different compositions of VFAs according to the aeration conditions. When the aeration was forced applying external aeration (pumping air), the production of VFA was reduced (Carvajal et al. 2010). Also, other authors who applied thermal-pretreatment (low temperature) showed that time had an important effect on the VFA production depending on time (Ferrer et al. 2008).

As is well known, the solubility of oxygen is close to zero under this thermophilic condition (Borowski and Szopa 2007), however even when close to zero, the results obtained have shown that the presence and/or absence of air produces an effect upon the velocity of solubilization and the production and composition of VFA.



(a)



(b)

Figure 2. Composition of VFAs at a) 12 hours and b) 24 hours of autohydrolysis pretreatment and 54kg·m^{-3}(◻ microaerobic, ◼ anaerobic).

3.3.1.3. Total Chemical Oxygen Demand.

The variation of the total COD was smaller than 5% (±3%) during the *autohydrolysis pretreatment*, for both microaerobic and anaerobic conditions (Figure 1-c). This value can be interpreted as being the minimal consumption of organic matter during the pretreatment. This is relevant because the objective of any pretreatment is the solubilization of the organic matter; and thus, the biodegradation during the anaerobic digestion for energy recovery. In addition, this has proved to be the most important difference between this pretreatment and a dual digestion process. The dual digestion operation implies the reduction of organic matter in the first step, thus creating an energy gap in the whole process.

Previous studies by this group have shown a significant reduction of total COD, higher than 14% (±2%) at 24 hours of pretreatment, when experiments were carried out with external aeration (Carvajal et al. 2010). These results were the main reason why external aeration was discarded for this study, being focused on different solid concentrations.

It is probable that the presence of excess air during the pretreatment may promote the consumption of organic matter, since the WAS is mainly composed of aerobic microorganisms, thus the reduction of oxygen transfer stimulates the stress cell. However, as presented above, the complete absence of oxygen promotes the acidification of the system. The microaerobic aeration can provide a product with a large content of soluble organic matter, while also controlling acidification, and with a reduced consumption of the substrate for the subsequent anaerobic digestion.

3.3.2. Rheological Behavior

Previous studies have shown that both aerobic and anaerobic sludge possess Non-Newtonian behavior (Mu and Yu 2006; Pevere et al. 2006; Pevere et al. 2009). Also, one of the most popular models for description of sludge behavior is Herschel-Bulkley, because it is considered a generalization of other models (Baudez et al. 2004). The rheological behaviour of secondary sludge depends on different factors, such as solid content, particle size, sedimentation rate and drying capacity (Dentel 1997; Sanin 2002).

The data presented for high and low concentrations of secondary sludge (Figure 3.3) showed that the curves for pretreated sludge were located below the curves for non-pretreated sludge. This behavior means that the resistance to flow was decreased significantly due to the *autohydrolysis pretreatment*. It is also possible to observe the reduction of the hysteresis phenomena because of the pretreatment, for a low concentration of solids. For the non-pretreated sludge with a high concentration of solids (figure 3.3-b), the adjusted upward curve is not presented because the correlation of the data was very low. This result occurred because the high solid content and the structure of the sludge did not allow measurements to be reproduced in the viscometer during the experiments. However, the pretreatment was so effective that both upward and downward curves were obtained for the sludge after the pretreatment, and in addition, the comparison of those curves showed that the hysteresis phenomenon was decreased because of the pretreatment.

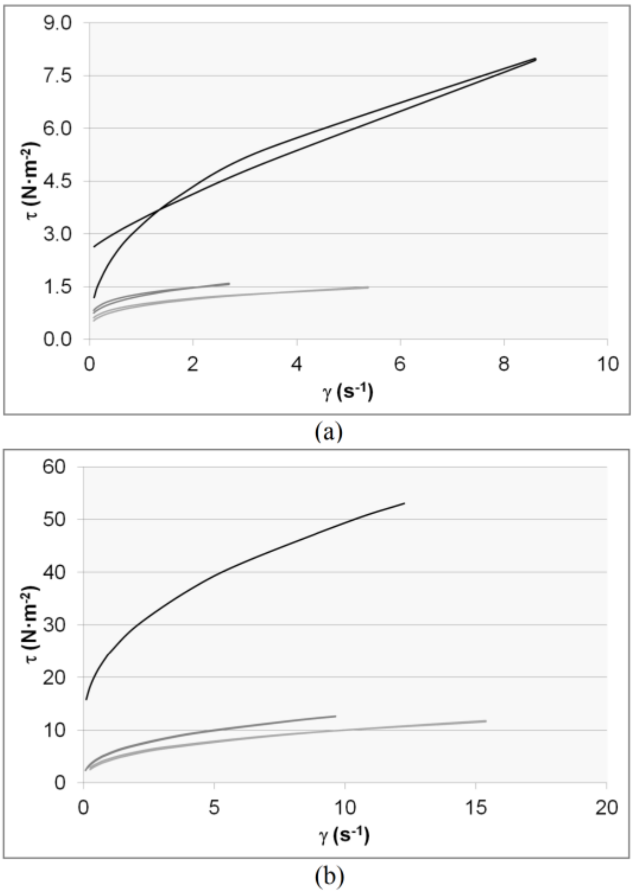


Figure 3.3. Herschel-Bulkley model obtained from experimental data of secondary sludge subjected to autohydrolysis pretreatment in microaerobic conditions and with a concentration of a) 23kg·m^{-3} and b) 54kg·m^{-3} (— (black line) without pretreatment, — (dark gray line) 12 hours and — (gray line) 24 hours of pretreatment).

The *material parameters* obtained for the studied sludge (Table 3.3) showed important variations due to the pretreatment. For sludge with a content of 23kg·m^{-3} (upward fit) the yield stress (τ_0) was reduced by 82%, which implies a reduction in the energy needed to produce the initial deformation, i.e. the movement of the sludge. In addition, the plastic viscosity (η_p) decreased by 10%. This result showed that the *autohydrolysis pretreatment* increased the fluency of the secondary sludge. For the sludge with high solid concentration, it was not possible to compare the behavior of the material parameters for the upward curve, because the correlation of non-pretreated sludge was very low. Instead, it was possible to observe an important increase in the plastic viscosity, compared to that seen in the low solid concentration, despite similar values of the n parameter. This effect can confirm the high viscosity of the sludge due to the concentration of solids.

Other studies have shown changes in the rheological behavior of sludge due to pretreatments, such as ultrasonication and fenton oxidation (Pham et al. 2010), and an increase in the fluency has also been shown.

Table 3.3. Parameters of adjusted rheological model of secondary sludge before and after autohydrolysis pretreatment.

		TS_0 = 23 ($kg \cdot m^{-3}$)				TS_0 = 54 ($kg \cdot m^{-3}$)			
Measure	Sludge	τ_0 ($Pa \cdot m^{-2}$)	η_p ($Pa \cdot m^{-2} \cdot s^{-1}$)	n	R^2	τ_0 ($Pa \cdot m^{-2}$)	η_p ($Pa \cdot m^{-2} \cdot s^{-1}$)	n	R^2
Upward	Without	2.52	0.910	0.829	0.959	*39.06*	*3.987*	*0.609*	*0.167*
	12 hour	0.42	0.816	0.367	0.996	-	5.67	0.345	0.991
	24 hours	0.18	0.761	0.319	0.998	-	4.65	0.335	0.988
Downward	Without	0.06	3.191	0.423	0.985	10.62	14.10	0.440	0.814
	12hours	0.11	1.183	0.205	0.996	-	5.50	0.364	0.987
	24hours	0.23	0.768	0.280	0.997	-	4.29	0.370	0.991

3.3.3. Anaerobic Biodegradability

The effect of the *autohydrolysis pretreatment* on the anaerobic biodegradability of the sludge was similar for both concentrations studied (Figure 3.4). The final productivity of methane was improved for the sludge that had been subjected to the pretreatment, but no significant difference was observed between the two times (12 and 24 hours).

For a low initial concentration of total solids, the productivity was almost equal over the first 5 days, however, after that point, it became higher for the pretreated sludge. At the end of the experiment, productivity had increased by 16% (±3%) compared with the non-pretreated sludge (Figure 4-a).

For a high initial concentration, at the beginning of the assay an inhibition effect was observed, where the production of methane was delayed for the pretreated sludge. This inhibition period was longer for the sludge subjected to 24 hours of pretreatment than to 12 hours. Nevertheless, the inhibition effect was overcome and the productivity was improved by 23% (±6%) at the end of the assay (Figure 4-b). The reduction in the initial productivity observed could be due to a high concentration of VFAs produced during the autohydrolysis pretreatment, or to the presence of certain inhibitory substances such as ammonia.

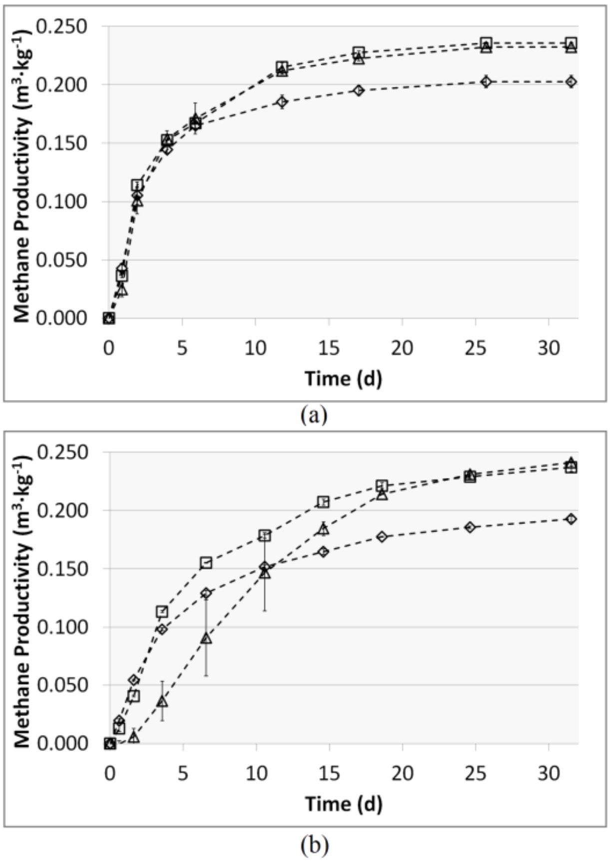


Figure 3.4. Methane productivity of the anaerobic biodegradation of secondary sludge subjected to autohydrolysis pretreatment with an initial total solid concentration of a) 23kg·m^{-3}.and b) 54kg·m^{-3} (-◇- without pretreatment, -□- 12 hours and -△- 24 hours of pretreatment).

Literature regarding the pretreatment effect on anaerobic digestion is plentiful, however the improvement of methane productivity values were scattered and in many cases did not coincide with the higher solubilization reached in the pretreatment step. Some studies on batch anaerobic digestion tests and thermal pretreatment at 190ºC for 90min increased 59% of the methane production of waste activated sludge (Bougrier et al. 2006), however the energy required to heat the sludge depended on the pretreatment temperature (Yang et al. 2010) and also it had associated high operation costs. In contrast, lower values were obtained when the pretreatment at 190ºC was carried out for 15 minutes, and 50 minutes to reach the desired temperature; in this case the improvement was the 25%, and the 12% at 135ºC, when it was digested in semi-continuous reactors; however, a long storage period was reported (Bougrier et al. 2007).

Other studies obtained improvements similar to the autohydrolysis pretreatment in this study: the low temperature pretreatment of secondary sludge at 70ºC for one day increased the net methane production by 20%. This study also showed the low effect of the pretreatment time on to the anaerobic digestion, because the increase of pretreatment time in 4 days improved the methane production by only 26% (Gavala et al. 2003). Furthermore, a biological pretreatment at 60ºC using thermophilic aerobic bacteria isolated from the secondary sludge increased the methane productivity 1.5 times (Hasegawa et al. 2000).

The analysis of biogas composition showed a significant difference in the methane content of pretreated and non-pretreated sludge. The comparison was made between the sixth and twentieth day, after stabilization of biogas composition. The box and whisker plot (Figure 11) showed the location of the methane composition data. For the pretreated sludge, 75% of the data was located above the 25% of the data for the non-pretreated sludge. The methane composition was increased by 3.5% (±1.5%) in the experiment with 24 hours of autohydrolysis pretreatment, from 65.0% to 68.5%. This value was obtained for sludge with a high solid content, and it was similar for the low concentration.

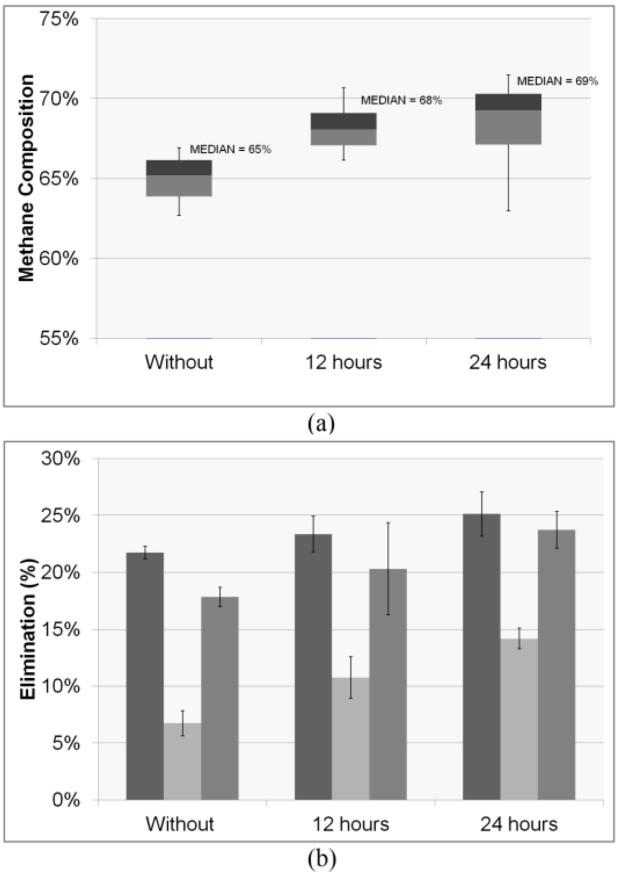


Figure6. 5. a) Box and Whisker plot of the methane composition of the biogas and b) The elimination of organic matter, during anaerobic biodegradability test (■ total COD, ■ TS, ■ VS). An initial total solid concentration of 54kg·m^{-3}was used in both cases.

The elimination of organic matter during anaerobic biodegradability assays expressed as total COD, total solids and volatile solids is shown in Figure 12. In all cases, the elimination was higher for pretreated sludge than for the sludge without pretreatment. This phenomenon explains the enhancement in methane productivity caused by the pretreatment; the production of methane was higher because the elimination of solids was higher too, i.e. it was possible to degrade more quantity of solids after the pretreatment.

3.3.4. Energy Assessment

The energy balance performed tries to show the possible impact of the autohydrolysis pretreatment on the cost or benefits of the WWTP, due to the anaerobic digestion improvement being necessarily related to a positive impact on the WWTP performance. The results presented in table 4 were estimated for a population of 500000 inhabitants, producing approximately $6.3 \cdot 10^{3}$ ($t \cdot d^{-1}$) of fresh secondary sludge from the settler, before the thickening step. The sludge concentration used as the basis for comparison was 23($kg \cdot m^{-3}$), a low concentration sludge. Due to the rheology of both forms, the low and high concentration pretreated sludge showed similar behavior, thus the pumping system could be quite similar and has therefore been excluded from this comparison.

The comparison of the pretreated and non-pretreated sludge at the same concentration showed an increase in the thermal energy required due to the autohydrolysis pretreatment; these requirements were directly related to the pretreatment time. In fact, the increase in methane production by the pretreatment was not enough to cover this extra energy requirement. Other studies have shown the increase in energy input required by thermal pretreatments of secondary sludge. These increases were related to the final pretreatment temperature, but also indicated that the heating costs could be lowered, increasing the sludge concentration fed by thickening (Yang et al. 2010).

On the other hand, the sludge with a high solid concentration reduced its thermal energy requirement significantly, due to the reduction of the feed, which was associated with the increase of the sludge concentration. When the pretreatment was carried out for 12 hours, the thermal energy produced from the burned biogas was sufficient, and even left over thermal energy for other requirements within the WWTP, but this was not so for 24 hours of pretreatment. Instead the electric consumption was increased due to the thickening step, and the difference between the electric power consumption and production was also increased by the pretreatment.

Clearly, the autohydrolysis pretreatment carried out over 24 hours did not represent any benefit for the system, because the energy requirement during this pretreatment was higher than for the 12-hour pretreatment, and, in addition, the improvement in methane production was negligible compared with the previous case.

Other comparative studies of pretreatments of WAS have shown the reduction of operational costs, for example, the ultrasound and ozonation pretreatments reduced operation costs by 44% and 22% respectively, and a reduction of 21% was also obtained by a thermal pretreatment at 90ºC (Salsabil et al. 2010).

All these results have shown that the energy requirement for the pretreatment can be covered by the extra energy produced during this process, and so the waste water treatment plant could benefit economically due to the autohydrolysis pretreatment. Another implication that should be noted is the possibility of reducing the size of the plant, and therefore also the foot-print of the system.

Table 4. Energy consumed and recovered from the autohydrolysis pretreatment and anaerobic digestion system, all results are presented in MW.

TS ($kg\cdot m^{-3}$)	Pretreatment Time (h)	*Thermal*			*Electric*	
		E_p	E_{ad}	$E_{ad,h}$	E_t	$E_{ad,elec}$
23	0	0.0	1.0	1.1	0.5	0.7
	12	2.1	1.0	1.3	0.5	0.8
	24	3.4	1.0	1.3	0.5	0.8
54	12	0.9	0.4	1.4	0.6	0.9
	24	1.5	0.4	1.4	0.6	0.9

3.4. CONCLUSIONS

The autohydrolysis pretreatment promotes the solubilization of the secondary sludge with a low consumption of organic matter content. For the two concentrations studied (23 and 54$kg\cdot m^{-3}$), the *solubilization factors* reached were higher than 30% after 12 hours of pretreatment (33 and 39% respectively), and in addition, the consumption of organic matter was less than 5%. The solubilization reached was similar to that obtained by other pretreatments with high energy requirements.

When the pretreatment was carried out in strictly anaerobic conditions instead of microaerobic conditions, a higher VFA concentration was obtained. This effect was less significant in the higher sludge concentration, probably due to the difficulty in oxygen transference.

The autohydrolysis pretreatment produced a significant change in the rheological behavior of secondary sludge. After the pretreatment, the flow resistance was significantly lower than the original.

The autohydrolysis pretreatment under microaerobic conditions improved the anaerobic digestion of secondary sludge. The methane productivity was increased by 23% after 12 hours of pretreatment.

The energy assessment showed the energetic feasibility of the autohydrolysis pretreatment and anaerobic digestion system. When the sludge concentration was increased and the pretreatment was carried out for 12 hours, the increase in energy production covered the extra energy required for the pretreatment, and therefore the WWTP could be benefit economically.

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CHAPTER 4

Evaluation of the effect of autohydrolysis pretreatment on high concentrated secondary sludge and its anaerobic digestion improvement

Abstract

This work presents the results obtained when autohydrolysis pretreatment is applied to secondary sludge with a high concentration of solids (8% of TS), and the subsequent effect on the anaerobic digestion of sludge. The anaerobic digestion was studied in thermophilic and mesophilic conditions.
The autohydrolysis pretreatment on concentrated secondary sludge produced a 30% of organic matter solubilization in 12 hours of pretreatment, the consumption of the organic matter was less than 5%, and the solubilization of proteins reached 25%. Microbial activity was detected by the consumption of carbohydrates and oxygen, and some population growth was observed after eight hours of pretreatment using live/dead ratio measurement. The anaerobic digestion of pretreated sludge shows an improvement of 20% on the methane productivity under mesophilic condition, but no improvement was detected under the thermophilic one.

Resumen

Esta sección presenta los resultados obtenidos cuando el pre-tratamiento de auto-hidrólisis se aplica a fango secundario con alta concentración de sólidos (8% of TS), y el efecto producido en la digestión anaerobia del fango pre-tratado. La digestión anaerobia se estudió en condiciones termófilas y mesófilas.
El pre-tratamiento de auto-hidrólisis de fango secundario concentrado produjo 30% de solubilización de la materia orgánica luego de 12 horas de pre-tratamiento, mientras el consumo de materia orgánica fue menor a 5%, además se obtuvo una solubilización del 25% de proteínas. Durante el pre-tratamiento se detectó actividad microbiana a través del consumo de carbohidratos y oxígeno, además se observó el crecimiento de una población microbiana luego de ocho horas de pre-tratamiento, midiendo la viabilidad de las bacterias en el fango. La digestión anaerobia del fango pre-tratado aumentó la productividad de metano en 20% para la condición mesófila, pero no se observó efecto en la condición termófila.

SIMBOLS

WAS	Waste activated sludge	WWTP	Waste water treatment plant
SS	Secondary slugde	*AD*	Anaerobic digestion
m-AD	Mesophilic anaerobic digestion	*t-AD*	Thermophilic anaerobic digestion
EPS	Exo-polymeric substances	P_{CH4}	Methane productivity ($m^3{}_{CH4}\cdot kg^{-1}{}_{VSfed}$)
COD_S	Dissolved chemical oxygen demand ($g_{O2}\cdot L^{-1}$)	*BD*	Biodegradability (%)
COD_T	Total chemical oxygen demand ($g\cdot kg^{-1}$)	P_∞	Methane productivity potential ($m^3{}_{CH4}\cdot kg^{-1}{}_{VSfed}$)
TS	Total solids ($g\cdot kg^{-1}$)	R_m	Maximum methane productivity rate($m^3{}_{CH4}\cdot kg^{-1}{}_{VSfed}\cdot d^{-1}$)
VS	Volatile solids ($g\cdot kg^{-1}$)	λ	Lag-phase time (d)
$\mathcal{F}$	Solubilization factor (%)	X	Microbial population ($CFU\cdot g^{-1}$)
Prot	Protein content ($g\cdot kg^{-1}$) or ($g\cdot L^{-1}$)	CHyd	Carbohydrate content ($g\cdot kg^{-1}$) or ($g\cdot L^{-1}$)

4.1. INTRODUCTION

The removal of organic and inorganic matter present in waste water produces sludge as the major waste in waste water treatment plants (WWTP). Primary sludge consists mainly of larger material, while secondary sludge (SS) is produced during biological waste water treatment, and consists of flocs of active microorganisms (0.5 grams of microorganisms per gram of eliminated organic matter).

Both types of sludge should be treated, in order to reducing the content of water and organic matter. The production of a stabilized and reusable solid is also sought after, principally in order to obtain “Biosolids Class A” (Metcalf and Eddy 2003a). The production of sludge has been growing over the last decades, for example, in Spain, the production of sludge grew by 50% between 1997 and 2007 (Ministerio de Medio Ambiente 2008).

Different alternatives have been proposed for sludge minimization. These alternatives can be classified into two types: *reducing production* and *elimination of sludge (Odegaard 2004; Pérez-Elvira et al. 2006)*. In the line of *reducing production,* several mechanisms exist, such as lysis-cryptic wroth, uncoupling metabolism, maintenance metabolism and bacterial predation. These strategies can increase both the total oxygen demand and the aeration costs, and it is also possible to produce an impact on the microbial community and influence the sludge dewatering and effluent quality (Wei et al. 2003).

Anaerobic digestion (AD) is the most common treatment for the *elimination of sludge*, because it is associated with the production of biogas, a renewable energy source. AD has some disadvantages such as a large digestion volume and a long digestion time, thus it is necessary to improve its operation (Appels et al. 2008). The limiting step in the AD of secondary sludge is hydrolysis, because it is necessary to break off the sludge floc and membrane cell. There are two possibilities for improving this process: modifying the digestion configuration (dual digestion) or introducing a pretreatment before anaerobic digestion (Pérez-Elvira et al. 2006). There are many pretreatments focused on different mechanisms e.g. mechanical, chemical, sonication, thermal and utilization of hydrolytic enzymes (Bougrier et al. 2006; Climent et al. 2007).

Pretreatments with hydrolytic enzymes include the possibility of using commercial enzymes (Davidsson et al. 2007; Q. Yang et al. 2010a), enzyme-producing microorganisms (Hasegawa et al. 1999; Kim et al. 2002) and the enzymatic system of sludge to be treated. It is possible to stimulate the production of hydrolytic enzymes such as proteases by changing the environmental conditions of the sludge such as temperature and oxygen quantity (Yan et al. 2008).

The improvement of AD by the application of SS pretreatments becomes more relevant when the treated sludge contains high concentration of solids, because it is necessary to improve fluidity and to ensure the biodegradability of this biosolid. The reduction of water content in sludge could be considered as an improvement too, because the total treatable volume is reduced, thus also reducing the digestion volume.

This work presents the autohydrolysis pretreatment of secondary sludge at 55°C over short periods of time, using a limited amount of oxygen, with the object of hydrolyzing the SS and improving the anaerobic digestion. If the pretreatment is carried out at 55ºC, anaerobic digestion can be realized at mesophilic conditions (35ºC), however the thermophilic condition (55ºC) appears to be a better option, seeking to avoid wasting the energy fed. Other studies of sludge pretreatment, using low temperature hydrolysis (70ºC), showed better results with thermophilic anaerobic digestion (Climent et al. 2007), however some authors showed that the best results depends on the ratio of primary/secondary sludge (Gavala et al. 2003).

The main objective of this study was to evaluate the effect of autohydrolysis pretreatment on secondary sludge with a high solid concentration. Mesophilic and thermophilic anaerobic digestion of the pretreated sludge was also carried out, in order to determine the best possible configuration for the autohydrolysis pretreatment system.

4.2. MATHERIALS AND METHODS

4.2.1. Sludge Characterization

The secondary sludge was taken from the WWTP of Valladolid, Spain. The sludge was concentrated in the WWTP by an industrial centrifugal extractor (Pieralisi, Baby). Afterwards, the concentrated secondary sludge was stored for less than 48h at 4°C before experimentation. In table 4.1, the concentrated sludge characteristics are presented.

Table 4.1. Characterization of the concentrated secondary sludge.

COD_T (g·kg^{-1})	COD_S (g·L^{-1})	TS (g·kg^{-1})	VS (% of TS)	$CHyd_T$ (% of VS)	$Prot_T$ (% of VS)
83.4 (±5.7)	3.0 (±0.2)	80.7 (±4.0)	76.4 (±5.4)	23.0 (±1.2)	52.4 (±2.7)

4.2.2. Experimental Procedure

4.2.2.1. Autohydrolysis Pretreatment

When the concentration of solids is increased (around to 8% of total solids), the difficulty of air transference and agitation is increased too. Therefore, the experimental system proposed in this work consists of a number of 2L closed bottles, each being ¼ full of concentrated sludge; thus allowing sufficient air presence in the gas chamber to provide oxygen for the microaerobic condition, and ensuring a high surface for air transfer through agitation by a roller system.

The pretreatment was carried out in batch conditions using 2-liter bottles, of 110mm diameter and 250mm height. Each bottle was loaded with 500g of concentrated sludge and closed with a rubber septum. It was then laid down horizontally in a roller bottle apparatus (Wheaton) inside a thermostatic chamber. The temperature of the chamber was kept constant at 55±0.5ºC with a convective flow of air and an electric resistor as heat source. All tests were performed in triplicate and the samples were taken at 0, 4, 8, 12 and 24 hours of pretreatment.

The consumption of oxygen was followed during the pretreatment. Bottle pressure was measured with a pressure transmitter (IFM, 5mbar precision) and the air composition was measured by sampling and subsequent gas chromatography analysis.

The efficiency of the sludge autohydrolysis pretreatment solubilization was compared using dimensionless ratios of soluble to total organic matter.

- *solubilization factor* ($\mathcal{F}$): This represents the organic matter released in the soluble phase over the total organic matter in the sample, and is commonly calculated for the COD content (Wei et al. 2003).

$$\mathcal{F} = \frac{COD_S-(COD_S)_{t=0}}{COD_T} \qquad (4.1)$$

- *final/initial* ratio: The concentration of soluble COD and soluble TOC was compared for each sample with the initial concentration.

4.2.2.2. Bacterial Viability Test

A LIVE/DEAD® BacLight™ bacterial viability kit (L13152, Molecular Probes, Invitrogen Detection Technologies) was used to determine the live/dead ratio. Each sample (1g of sludge) was put in a vial, and 1mL of 2X stock solution of LIVE/DEAD reagent mixture was added. The mixture was mixed and incubated at room temperature in the dark for 15 minutes. After that, 50µL of the stained bacterial suspension was trapped between a slide and a coverslip. The fluorescence was observed under a microscope (leica DM4000B Epifluerescence microscopy), and ten pairs of pictures of each slide were taken using both red and green filters.

Each pair of pictures was analyzed with daime® software, and the total area of green and red fluorescence was quantified (Daims et al. 2006). The proportion of live bacteria was calculated as being the area occupied by the live bacteria (green) divided by the total area (addition of live and dead bacteria).

4.2.2.3. Anaerobic Biodegradability

The anaerobic biodegradability of the pretreated and non-pretreated sludge was evaluated by anaerobic batch tests. The assays were performed under mesophilic conditions (m-AD) at 35ºC (±1°C), and also under thermophilic conditions (t-AD) at 55ºC (±1°C).

Both inocula were taken from two different bioreactors, the mesophilic one was operated at a steady state for over a year, and the thermophilic one for at least half a year. The initial ratio of organic matter to anaerobic inoculum was 0.5. At the beginning of the experiments, each bottle was degassed by circulating helium in the gas chamber. The biogas production was followed by pressure measurement using a pressure transmitter (IFM, 5mbar precision) for 25 days or until no biogas production occurred, while the composition of the biogas was also measured by gas chromatography. Each experiment was done in triplicate.

The final results were presented as *methane productivity* (P_{CH_4}), that is, the volume of methane produced under normal pressure and temperature conditions (0ºC, 1bar), divided by the mass of volatile secondary sludge solids fed into the assay.

The biodegradability of the sludge was calculated by dividing the methane productivity, expressed in COD value, by the theoretical maximum yield of methane $\left(350\left(m^3_{CH_4} \cdot kg^{-1}_{COD}\right)\right)$ (Mottet et al. 2010).

$$Biodegradability\ (\%) = \frac{P_{CH_4}\left(m^3_{CH_4} \cdot kg^{-1}_{COD}\right)}{350\left(m^3_{CH_4} \cdot kg^{-1}_{COD}\right)} \cdot 100 \qquad (4.2)$$

4.2.2.4. Analysis

Total solids (TS), volatile solids (VS) and chemical oxygen demand (total: COD_T & soluble: COD_S) were determined by Standard Methods (APHA et al. 2005). The soluble phase was obtained by centrifugation of the samples for 10min at 5000rpm, and afterwards the supernatant was filtered using a pore size of 0.45µm.

Biogas and air composition was measured by gas chromatography using Varian CP-3800 CG, helium was a carrier gas (Díaz et al. 2010).

Carbohydrate (CHyd) content was measured by Dubois's method (Dubois et al. 1956), using glucose as standard. Protein (Prot) content was measured by Lowry's method (Lowry et al. 1951), using casein as standard.

Soluble total organic carbon (TOC_S), inorganic carbon (IC_S) and total nitrogen (TN_S) were analyzed using a TOC-V_{CSH} analyzer (Shimadzu, Japan) coupled with a TN module (Shimadzu TNM-1, Tokyo, Japan) based on chemiluminesce detection.

4.2.2.5. Data Processing

The data was compared using statistic analysis software (STATGRAFICS Centurion ®). The comparison of different samples was made through the "ANOVA table"; and afterwards a "Multi Range Test" was performed to determine which means were significantly different from the others. All the analysis was done with a 95.0% of confidence level.

The experimental data of methane productivity was fitted using the modified Gompertz equation (Donoso-Bravo et al. 2010) applying a non-linear regression model to describe the relationship between the experimental data and the independent variable. The parameters: P_∞ "total methane productivity potential", R_m "maximum methane productivity rate" and λ "the lag-phase time" were determined for each sample studied.

$$P(t) = P_\infty \cdot exp\left[-\exp\left(\frac{R_m \cdot e}{P_\infty} \cdot (\lambda - t) + 1\right)\right] \qquad (4.3)$$

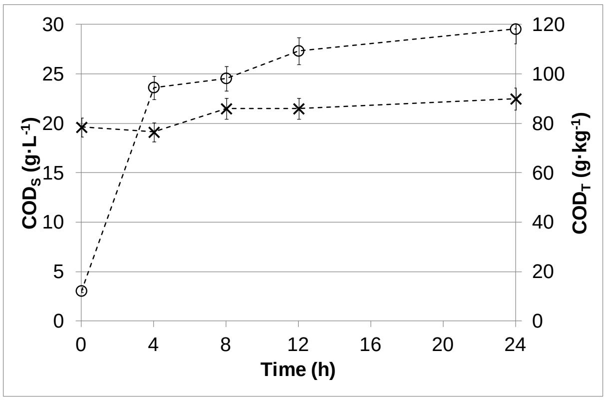
4.3. RESULTS AND DISCUSSION

4.3.1. Autohydrolysis Pretreatment

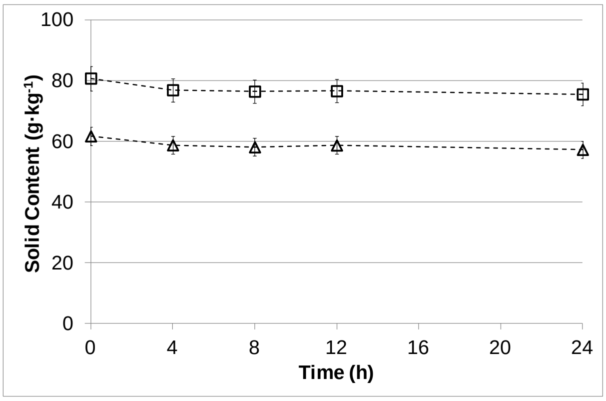
4.3.1.1. Solubilization of Organic Matter

The soluble chemical oxygen demand in the concentrated sludge was higher than the common values observed for the raw sludge without the thickening stage, but it represented just 4% of the total content of COD (table 1). The thickening stage increased the solid concentration 16 times, so therefore the existence of a stress effect on the sludge floc was expected. Nevertheless, the result obtained showed that the largest portion of the organic matter was located in the suspended phase.

As consequence of the pretreatment, the soluble COD increased greatly during the first four hours of autohydrolysis, after which it continued increasing at a slower rate (Figure 1-a). The solubilization factor of COD, which represents the organic matter transferred from the suspended phase to the soluble phase, grew to 25% (±2%) in the first four hours, and then increased to 32% (±3%) over 24 hours of pretreatment. Previous studies with a lower initial concentration of solids, around 3% of the TS, and a different experimental set-up, showed a similar solubilization factor due to the autohydrolysis pretreatment.



(a)



(b)

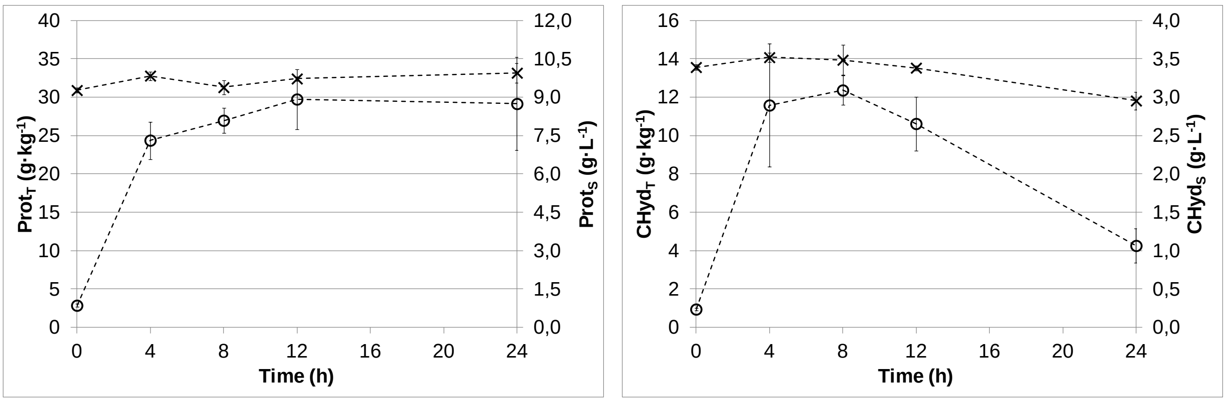
Figure 4.1. (a) COD content in soluble phase and sludge. (b) TS and VS content during autohydrolysis pretreatment.

The values of total COD showed a small variation during the assay, around 7%, but without a clearly tendency (Figure 4.1-a). This behavior was considered to be the experimental error of the analysis due to the difficulty in manipulating and measuring such a high concentration of sludge by conventional methods. This assumption was confirmed by the observation of the solid content behavior during the autohydrolysis pretreatment (Figure 4.1-b). Apparently, there was a small reduction in the solid content at the beginning of the assay, but the total variation was lower than 5% over the course of the experiments. In both cases, it was concluded that the consumption of organic matter during the autohydrolysis pretreatment was negligible in this experimental condition. The influence of the air supply on the autohydrolysis pretreatment was studied beforehand; and it was concluded that the extra air supply promoted an organic matter consumption of around 14%.

The negligible reduction of the organic matter content observed during the autohydrolysis pretreatment is an important issue for the secondary sludge pretreatment, as it allows the total organic matter to be available for the anaerobic digestion step, leading to a possible increase in biogas production. The consumption of organic matter during a pretreatment represents an energy gap in the system, and this was the main difference between the autohydrolysis pretreatment and other systems which applied biological pretreatment methods, since the latter obtained high reduction of the organic matter. Previous studies which worked with a dual digestion system treating mixed sludge with 70% of WAS, removed 14% of the volatile solid content, when the aerobic thermophilic pretreatment was operated at 1 day of hydraulic retention time (HRT) (Borowski and Szopa 2007); also, the hydrolytic-acidogenic anaerobic digestion stage (55ºC) applied to mixed sludge with 75% of WAS, showed volatile solid removal higher than 10% when it was operated with high solid content and 1 day of HRT (Ponsa et al. 2008). In both cases, the consumption of the easily biodegradable organic matter could be due to the increase and maintenance of the thermophilic population, even the acidogenic one, because of the continuous operation.

4.3.1.2. Characterization of organic matter

Information about the behavior of the autohydrolysis pretreatment is not based solely on the content of COD and VS, but can be complemented by information gained from other organic content, such as the nature of the hydrolyzed compounds and their influence over subsequent anaerobic digestion (Mottet et al. 2010). In addition, the sludge floc consists of an association of microorganisms, linked by exo-polymeric substances (EPS) which represents the majority of the organic matter content in the WAS (Nielsen et al. 2004). Therefore, the ability to destabilize and destroy the floc matrix will mark the success of the pretreatment.



(a) (b)

Figure 4.2. Concentration of a) proteins and b) carbohydrates during autohydrolysis pretreatment (the soluble phase and sludge).

As was shown in table 4.1, most of the waste activated sludge was composed of proteins, and its content remained constant during the course of the experiment (figure 4.2-a). Also, in the same way as the soluble COD, the protein concentration in the soluble phase was increased during the pretreatment. The maximum solubilization factor obtained was 25.2% (±3.7%) in 12 hours of pretreatment, and its concentration remained constant at the next measurement, taken at 24 hours.

On the other hand, the CHyd content in the sludge decreased after eight hours of pretreatment, achieving a total reduction of 14.8% (±4.3%) after 24 hours of pretreatment (figure 4.2-b). This apparently high reduction in carbohydrates was not so important considering the high solid concentration, in fact, this reduction was apparently negligible for the total organic matter content, being around 3% of VS. However, the consumption of CHyd could be indicative of some bacterial activity during the pretreatment. This effect was observed in the soluble phase concentration too: the maximum solubilization factor reached for Chyd was 20.5% (±1.8%) in 8 hours of pretreatment, and afterwards its concentration decreased, consuming a quantity proportional to the reduction observed in the total concentration (Figure 2-b). To summarize, after eight hours of the autohydrolysis pretreatment, the consumption of the easily biodegradable organic matter was observed.

4.3.1.3. Oxygen Consumption

The experimental set-up, 2L bottles lying in horizontal position, each one quarter full of concentrated sludge, allowed a thin layer of sludge to form. This experimental set-up was aiming for the formation of a high oxygen transfer surface from the gas phase to the liquid phase, because the oxygen solubility is very low at the operational temperature.

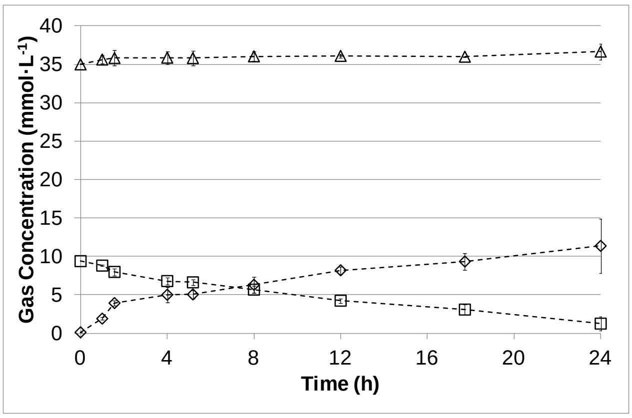


Figure 4.3. Gas concentration in the headspace during autohydrolysis pretreatment (-◇- CO_2, -□- O_2 and -△- N_2).

The nitrogen concentration in the headspace, presented in figure 3, showed a small increase at the beginning of the assay, during the first two hours; this period of time coincided with the initial heating stage, and after that point the concentration remained almost constant. This initial increase could be due to the desorption effect of the dissolved air in the sludge, which was released during the heating stage, and then after the thermal stabilization the concentration of nitrogen was stabilized.

In contrast with the nitrogen behavior, the concentration of oxygen and carbon dioxide in the headspace took place all through the pretreatment, revealing the existence of microbial activity requiring oxygen and releasing carbon dioxide. The rate of the oxygen consumption was almost constant throughout the pretreatment, and even showed a linear behavior. In addition, similar results were obtained for carbon dioxide production, but the effect during the first two hours was more significant than for the oxygen. The total rate of oxygen consumption during the autohydrolysis pretreatment was $1.02\ (mmol_{O_2} \cdot kg_{WAS}^{-1} \cdot h^{-1})$ and the production of carbon dioxide was $1.41(mmol_{CO_2} \cdot kg_{WAS}^{-1} \cdot h^{-1})$.

The consumption of CHyd during the autohydrolysis pretreatment, shown in figure 4.2, was $2(g_{CHyd} \cdot kg_{WAS}^{-1})$, which means a total consumption rate of $0.46(mmol_{C_6H_{12}O_6} \cdot kg_{WAS}^{-1} \cdot h^{-1})$. Therefore, only three moles of carbon were released as carbon dioxide, and this value coincide with the common value of organic matter degradation versus sludge production under aerobic conditions in the waste activated sludge process, in which half of the organic matter is released as carbon dioxide and the other half is used for cellular growth (Metcalf and Eddy 2003b). On the other hand, the oxygen consumed was 72% of the carbon dioxide produced, demonstrating the use of oxygen from the gas chamber. Although the oxygen concentration decreased during the autohydrolysis pretreatment, the minimum concentration in the gas chamber was $1.25(mmol_{O_2} \cdot L^{-1})$ at 24 hours, thus there was enough oxygen in the proposed experimental set-up to ensure the microaerobic condition.

4.3.1.4. Microbial Activity

The autohydrolysis pretreatment applied a temperature of 55ºC and a limited supply of oxygen with the object of provoking a synergic effect; firstly, the solubilization of organic matter by temperature increase, and secondly, the hydrolysis of the solubilized organic matter by the hydrolytic enzymes released from the WAS. Other studies have shown that environmental changes such as temperature increase and a low quantity of dissolved oxygen produces a stress condition called lysis-criptic growth condition, and in addition, the temperature increase promotes the manifestation of the thermophilic population (Yan et al. 2008).

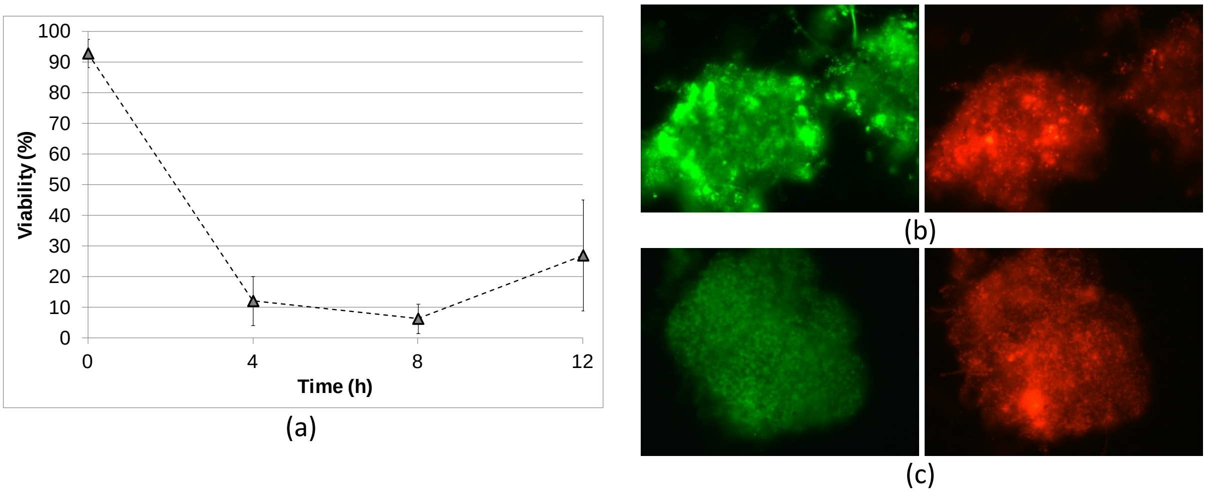


Figure 4.4. a) Percentage of live bacteria during autohydrolysis pretreatment. Photographs of live (green) and dead (red) bacteria; (b) non-pretreated sludge and (c) 8-hours pretreated sludge.

The photographs presented in figure 4.4 show the difference aspect of the sludge, before and after the pretreatment. The non-pretreated sludge (Figure 4.4-b) showed some brilliant cell agglomerations, because of the sludge floc stability. Also, the intensity of the green picture was higher than the red one. On the other hand, the pretreated sludge (Figure 4.4-c) showed a high quantity of free cells, although the association of cells was not easily observed, thus showing that the floc structure was destabilized; the intensity of both pictures was apparently similar.

The quantification of the live and dead ratio showed that the autohydrolysis pretreatment produced an important reduction in the live bacteria (Figure 4.4-a), which was reduced from 93% (±5%) to 12% (±8%) in the first four hours. However, after eight hours of pretreatment this behavior changed, and in 12 hours of pretreatment the live bacteria grew to 27% (±18%). Clearly, at the beginning of the assay, the growth rate was lower than the mortality one, and, after eight hours of pretreatment a microorganism population grew up adapting to the environmental change that it had undergone, probably a thermophilic population. In addition, these results could explain the consumption of carbohydrates and the production of carbon dioxide previously observed.

4.3.2. Anaerobic Biodegradability

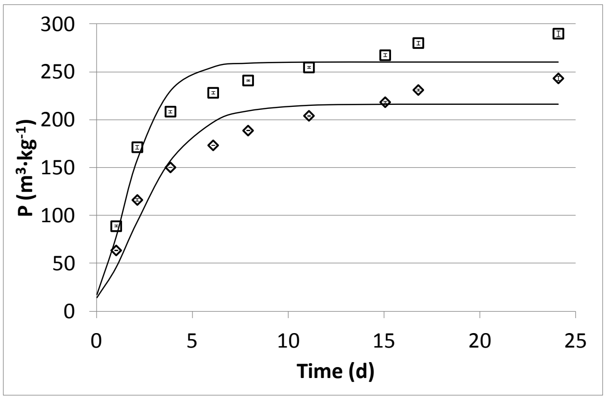
The ANOVA analysis performed to determine the methane productivity at the end of the assay (25th day) showed significant differences between the sludge studied: pretreated and non-pretreated anaerobically digested under mesophilic and thermophilic conditions $(F_{ratio} = 46.11)$. The specific comparison on the "Multiple Range Test" showed that the difference occurred between the pretreated sludge digested under mesophilic conditions and all the other condition, however, between these other condition, no difference was detected (data presented in Table 2). These results mean that the sludge subjected to the autohydrolysis pretreatment and then digested under anaerobic mesophilic conditions improved the methane productivity, but all the other conditions studied showed no significant difference.

Table 4.2. Experimental productivity of methane, its corresponding anaerobic biodegradability and the parameters of modified Gompertz equation.

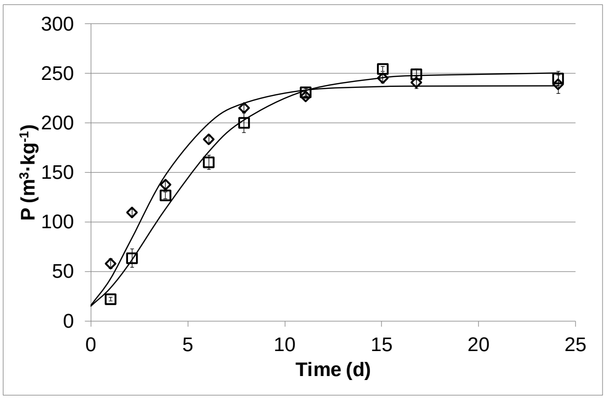
	m-AD		t-AD	
	Control	Pretreated	Control	Pretreated
$P_{25d}(m^3 \cdot kg^{-1})$	243.4 (±2.0)	290.1 (±2.8)	239.3 (±9.1)	245.1(±7.1)
$BD\ (\%)$	49.7 (±0.4)	59.2 (±0.6)	48.8 (±1.9)	50.0 (±1.5)
$P_{\infty}(m^3 \cdot kg^{-1})$	216.4 (±5.1)	260.5 (±4.7)	237.2 (±4.0)	250.6 (±3.7)
$R_m(m^3 \cdot kg^{-1} \cdot d^{-1})$	44.5 (±3.1)	77.1 (±4.9)	39.4 (±1.8)	30.2 (±1.7)
$\lambda(d)$	0	0	0	0.1 (±0.2)
R^2	0.890	0.901	0.956	0.986

The improvement in the methane productivity obtained between the pretreated and non-pretreated sludge subjected to m-AD was 19% (±1%), and as a consequence the biodegradability of the WAS was increased by 10% (table 4.2).

The improvement in biogas productivity obtained for the m-AD condition was similar to other pretreatments which used secondary sludge (Climent et al. 2007; Gavala et al. 2003). Also, the negligible effect of the autohydrolysis pretreatement on the t-AD had been observed before in other studies which applied low temperature (70ºC) pretreatment, suggesting that the improvement on the methane productivity in sludge digestion under t-AD was related to the primary sludge (Gavala et al. 2003).



(a)



(b)

Figure 4.5. a) m-AD and b) t-AD of secondary sludge: the continuous line represents the fitted Gompertz model (non-pretreated and 12-hours pretreated).

The negligible effect of the autohydrolysis pretreatment on the t-AD obtained by the statistic analysis can be observed in figure 4.5 too. Here it was observed that the autohydrolysis pretreatment had completely different effect over each anaerobic digestion condition studied. The values of the Gompertz parameters (table 2) show that the pretreatment does not affect only the biodegradability of the sludge. Although, it promotes an increase in the maximum methane productivity rate of the WAS subjected to m-AD, it does not further affect the t-AD.

For all conditions studied, the anaerobic digestion did not show any inhibition phenomena at the beginning of the test due to the presence of an inhibiting substance such as ammonia; only the hydrolyzed sludge in t-AD showed a small lag-phase time (λ).

On the other hand, some studies on batch anaerobic digestion tests and thermal pretreatment at 170ºC for 90min solubilized 40% of the organic matter and increased 59% of the methane production of waste activated sludge (Bougrier et al. 2006), however the energy required to heat the sludge depended on the pretreatment temperature (X. Y. Yang et al. 2010b) and also it had associated high operation costs.

Commonly, the biogas produced in the AD of sludge allows recovering energy by burning the biogas in a cogeneration unit. The recovered energy is composed of: the electrical power produced and the remaining heat recovered. The autohydrolysis pretreatment of WAS increased 20% the methane production, and so the power and the heat recovered. Previous work showed a positive gap of recovered heat when the pretreatment was carried out with a concentration of 5% of TS. The high sludge concentration used in this study reduced the thermal requirement of the autohydrolysis pretreatment due to the sludge flux reduction; therefore the pretreatment with 7% of solids implies a reduction of 30% of the heat requirement than these previous conditions, increasing the energy gap gained.

In conclusion, the energy requirement for the pretreatment can be covered by the extra energy produced during this process, increasing the electrical power production, and so the WWTP could benefit economically due to the autohydrolysis pretreatment. Another implication that should be noted is the possibility of reducing the size of the plant, and therefore also the foot-print of the system, due to the feasibility of work with high concentrated sludge.

4.4. CONCLUSIONS

The autohydrolysis pretreatment of secondary sludge with a high solid content (~8%) is a viable pretreatment in order to improve the anaerobic digestion of the sludge. The solubilization factor obtained reached 30% after 12 hours of pretreatment. The practically negligible consumption of organic matter showed that the pretreatment did not waste organic matter important to the subsequent energy recovery in the anaerobic digestion stage.

The experimental device allowed for the maintenance of oxygen, thus ensuring microaerobic conditions during the pretreatment without the need to ventilate the bottles. At the operational conditions the oxygen consumption was $1.02(mmol_{O_2} \cdot kg_{WAS}^{-1} \cdot h)$.

The maximum solubilization of protein (25%) was obtained after twelve hours of pretreatment and after that, it remained constant. However, the maximum solubilization of the carbohydrates (21%) was obtained after eight hours of pretreatment, followed by a reduction which showed the effect of microbial activity on the easily biodegradable substrate.

An important reduction in the live bacteria in the sludge was observed during the first four hours of pretreatment, nevertheless after eight hours this population had increased. This detected population could be formed by thermophilic bacteria.

Although the microbial activity was observed through the consumption of carbohydrates, the growth of the population, and the consumption of oxygen, this activity was slow enough to produce a negligible reduction of the total organic matter.

Autohydrolysis pretreatment presented a feasible way to improve the anaerobic digestion of secondary sludge under mesophilic conditions. The productivity of methane was improved by 20% during mesophilic anaerobic digestion and, as a consequence; the biodegradability of the sludge was increased in by 10%. Nevertheless, the autohydrolysis pretreatment did not improve the anaerobic digestion of the sludge when the digestion was carried out under thermophilic conditions.

The autohydrolysis pretreatment is energetically feasible, because the energy requirement for the pretreatment can be covered by the extra energy recovered during the AD process, increasing the electrical power production, and so the economical benefit to the WWTP.

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CHAPTER 5

Description of the autohydrolysis pretreatment mechanism in secondary sludge

ABSTRACT

In order to quantify the contribution of the thermal and biologic effect in the autohydrolysis pretreatment, the behavior of the microbial of thermophilic and mesophilic population, along with the enzymatic activity on the soluble and total sludge phases, were evaluated at three different temperatures of pre-treatment: 40, 55 and 70ºC.

The results obtained showed that only the 55ºC condition promoted the steady growth of thermophilic population during the whole pretreatment, and also this condition showed the highest enzymatic activity of *amylase* and *protease*, in both soluble and total phases. On the other hand, the 70ºC condition showed the fastest reduction of the mesophilic population while the thermophilic one increased only during the first three hours, and the 40ºC condition had a lesser effect on both factor studied, and as a consequence the lower organic matter solubilization. However, the relationship between the thermophilic population rise and the increase in the enzymatic activity was not obtained.

The anaerobic digestion of the full sludge increased the maximum rate of methane productivity for all temperatures conditions studied, probably due to the solubilization of the organic matter under thermal effect. However, only the pretreatment at 55ºC improved the biodegradability of the sludge. The anaerobic digestion of the soluble phase, which contains the easily released biodegradable organic matter, reached a biodegradability of 80%, while the anaerobic digestion of the suspended phase showed a reduction of its biodegradability at 40ºC and 70ºC pretreatment.

Summarizing, the release of the organic matter from the secondary sludge floc was produced by the thermal effect of the pre-treatment, however the improvement of the anaerobic digestion was because of the hydrolytic enzymes, which were natives of the secondary sludge and were stimulated due to the autohydrolysis pretreatment conditions.

RESUMEN

Con el objetivo de identificar las distintas contribuciones por los efectos térmicos y biológicos en el pre-tratamiento de auto-hidrólisis, se estudió el efecto provocado a tres temperaturas diferentes: 40, 55 y 70ºC; evaluando el comportamiento de: la población termófila y mesófila, junto con la actividad enzimática en fase soluble y total.

Los resultados obtenidos mostraron que sólo el pre-tratamiento a 55ºC provocó el crecimiento sostenido de la población termófila, además esta fue la condición donde se observó la mayor actividad enzimática de *amilasa* y *proteasa*. Por otro lado, el pre-tratamiento a 70ºC mostró la reducción más rápida de población mesófila, pero la población termófila sólo creció durante las primeras tres horas. El pre-tratamiento a 40ºC tuvo el menor efecto sobre las dos factores estudiados, y por lo tanto la menor solubilización de materia orgánica. Sin embargo, no fue posible establecer una relación entre el crecimiento de la población termófila y el aumento de la actividad enzimática.

Todas las temperaturas estudiadas mostraron un amento de la velocidad máxima de productividad de metano durante la digestión anaerobia del fango pre-tratado, probablemente por la solubilización de materia orgánica debido a un efecto térmico. Sin embargo, sólo la condición de 55ºC aumentó la biodegradabilidad del fango. La digestión anaerobia de las fases solubles, que contenían materia orgánica fácilmente biodegradable, obtuvieron una biodegradabilidad de 80%, mientras la biodegradabilidad de los sólidos suspendidos disminuyó cuando el pre-tratamiento se realizó a 40 y 70ºC.

En resumen, la desestabilización del flóculo de fango secundario fue producto del efecto térmico del pre-tratamiento, sin embargo la mejora de la digestión anaerobia se obtuvo gracias al efecto de las enzimas hidrolíticas, las que son nativas del fango secundario y se estimulan debido a las condiciones del pre-tratamiento de auto-hidrólisis.

SIMBOLS

WAS	Waste activated sludge	WWTP	Waste water treatment plant
EPS	Exo-polymeric substances	P_{CH4}	Methane productivity ($m^3_{CH4} \cdot kg^{-1}_{VSfed}$)
COD_S	Dissolved chemical oxygen demand ($g_{O2} \cdot L^{-1}$)	BD	Biodegradability (%)
COD_T	Total chemical oxygen demand ($g \cdot kg^{-1}$)	P_∞	Methane productivity potential ($m^3_{CH4} \cdot kg^{-1}_{VSfed}$)
TS	Total solids ($g \cdot kg^{-1}$)	R_m	Maximum methane productivity rate($m^3_{CH4} \cdot kg^{-1}_{VSfed} \cdot d^{-1}$)
VS	Volatile solids ($g \cdot kg^{-1}$)	λ	Lag-phase time (d)
$\mathcal{F}$	Solubilization factor (%)	X	Microbial population ($CFU \cdot g^{-1}$)
a	Enzymatic activity ($U \cdot mL^{-1}$) / ($U \cdot g^{-1}$)		

5.1. INTRODUCTION

Thermal pretreatments have been shown to have an important effect on the improvement of the anaerobic digestion of waste activated sludge (WAS), but they also have a high operation cost because of the energy applied. From this point of view, a reduction of the temperature used for pretreatment could have a big impact on the waste water treatment plant economy, and also on the complexity of the pretreatment process (X. Y. Yang et al. 2010b).

Commonly, WAS pretreatments need to apply the energy required to destabilize the floc structure, and simultaneously break the cell wall, thus releasing the intracellular contents. Some studies have showed that low temperature pretreatments, ranging from 50 to 100ºC, cannot provide enough energy for this porpoise (Prorot et al. 2011). On the other hand, studies showed that the main contain of this organic matter is not the live cells, but the exopolimeric substances (EPS), forming around 80% of the organic matter (Nielsen et al. 2004). Therefore, the most important requirement is to transform the large compounds, such as carbohydrates and proteins, into small compounds, such as glucose and amino acids, and this is why some pretreatments at low temperature have shown possibilities of improving the subsequent anaerobic biodegradability of the secondary sludge (Climent et al. 2007). However, the hydrolysis mechanism of these pretreatments is not well known.

Usually, the effect produced by thermal pretreatments is evaluated through the solubilization factor (soluble chemical oxygen demand increase), and this parameter has even been correlated to the improvement in anaerobic biodegradation (Bougrier et al. 2008). Nevertheless, for pretreatment at low temperature, the effect produced on/by the microbial activity or viability has been named as a fundamental tool in the description of the pretreatment mechanisms (Prorot et al. 2011). From this point of view, some studies have shown an increase in the thermophilic population in the WAS when it was subjected to heat-treatment for the reduction of excess sludge, even though a protease-secreting bacteria was identified (Yan et al. 2008). This microbial population could affect the hydrolysis of the sludge solubilized during low temperature pretreatments, but the best condition has not been well established. Some studies have shown that pretreatment at 70ºC improved the methane produced by thermophilic anaerobic digestion of mixed sludge (Ferrer et al. 2008); while at 40ºC, deflocculation of the WAS was observed (Morgan-Sagastume and Allen 2005); and finally, autohydrolysis pretreatment at 55ºC showed a good improvement in methane production for the mesophilic anaerobic digestion of WAS. For this temperature range, between 40ºC and 70ºC, it is not well known whether the main effect produced is due to the temperature and/or the microbial activity.

The objective of this work is to clarify whether the main contributor to the secondary sludge solubilization during the autohydrolysis pretreatment is the thermal effect, or the microbial activity (enzymatic activity). For this purpose, the autohydrolysis pretreatment of secondary sludge was studied at 40, 55 and 70ºC. For each temperature tested, the microbial contribution was determined through the activity of two enzymes, and the quantification of the thermophilic and the mesophilic populations. The subsequent mesophilic anaerobic digestion was carried out separately for each phase, both the soluble one, and the suspended one, in order to determine how the pretreatment affected to the biodegradability of each phase, and in order to determine the best operational conditions.

5.2. EXPERIMENTAL METHODS

5.2.1. Autohydrolysis pretreatment

The waste activated sludge was taken from the WWTP of Valladolid, Spain, and it was concentrated by an industrial centrifugal extractor (Pieralisi, Baby). The concentrated sludge was stored less than 48h at 4°C before experimentation. Table 5.1 presents the characterization of the sludge.

Table 5.1. Raw sludge characterization.

COD_T (g·kg^{-1})	COD_S (% of COD_T)	TS (g·kg^{-1})	VS (% of TS)	CHydT (% of VS)	$Prot_T$ (% of VS)
83.5 ± 4.2	1.8 ± 0.2	78.4 ± 3.9	73.6 ± 3.0	23.0 ± 1.2	52.4 ± 2.7

The pretreatment was carried out in batch, using 2L bottles loaded with 500g of concentrated sludge and closed with a rubber septum. These were laid horizontally in a roller bottle apparatus (Wheaton) inside a thermostatic chamber. Three pretreatment temperatures were studied: 40ºC, 55ºC and 70ºC. The temperature of the chamber was kept constant, using a convective flow of air and an electric resistor as the heat source. All tests were performed in triplicate. The samples were collected at 3, 6, 9, 15 and 24 hours of pretreatment, and were centrifuged for 10min at 5000rpm to separate the supernatant and the suspended phase. All parameters were analyzed in the supernatant (soluble phase), suspended phase (solid phase) and in both phases together (full sludge sample).

To compare the efficiency of the autohydrolysis pretreatment, the solubilization factor ($\mathcal{F}$) was evaluated.

5.2.2. Anaerobic biodegradability

The anaerobic biodegradability of the pretreated sludge was evaluated in a mesophilic anaerobic digestion batch test (35ºC (±1°C)). The tests were carried out for each phase separately, the soluble phase and suspended phase and also for the full sludge.

The inoculum was taken from an anaerobic digester that operated at a steady state for at least six months. The assay was carried out in 300mL Pyrex bottles with a gas chamber of 175mL. The inoculum concentration in the bottles was 5g·L^{-1}, and the sludge was added to achieve the ratio of 0.5 between the COD content of the sludge and the VS content of the anaerobic inoculum. At the beginning of the experiments, each bottle was degassed by circulating helium in the gas chamber. The biogas production was followed by pressure measurements using a pressure transmitter (IFM, 5mbar precision) for 25 days; the composition of the biogas was also measured by gas chromatography. Each experiment was done in triplicate.

The final results were presented as *methane productivity* (P_{CH_4}), which is the volume of methane produced at normal pressure and temperature conditions (0ºC, 1bar), divided by the quantity of COD of secondary sludge fed into the assay.

The biodegradability (*BD*) of the samples was calculated, dividing the experimental methane productivity by the theoretical maximum yield of methane ($350m^3_{CH4}\cdot kg^{-1}_{COD}$) (Mottet et al. 2010).

$$BD(\%) = \frac{P_{CH_4}\left(m^3_{CH_4}\cdot kg^{-1}_{COD}\right)}{350\left(m^3_{CH_4}\cdot kg^{-1}_{COD}\right)}\cdot 100 \qquad (5.1)$$

5.2.3. Analysis

Total solids (TS), *volatile solids* (VS) and *chemical oxygen demand* (total: COD_T & soluble: COD_S) were determined by Standard Methods (APHA et al. 2005). The soluble phase was obtained by centrifugation of the samples for 10min at 5000rpm, and afterwards, the supernatant was filtered by 0.45µm pore size.

Biogas composition was measured by gas chromatography using Varian CP-3800 CG, and helium as a carrier gas (Díaz et al. 2010).

The enzymatic activity analysis was done immediately after taking the samples to the full sludge and the soluble phase. The measurement of the activity was done at the same temperature as the pretreatment. *Amylase activity* was measured using a modified Miller method (Miller 1959). One unit of amylase activity was defined as the amount of enzyme which releases 1µmol of glucose equivalent per minute using starch as substrate.

Protease activity was measured using a modified Lowry method (Lowry et al. 1951). One unit of protease activity was defined as the amount of enzyme which releases 1µmol of l-tyrosine equivalent per minute using casein as a substrate.

Total live heterotrophic bacteria (TLHB) were measured by standard serial dilution and plating on R2A agar (APHA et al. 2005). The mesophilic and thermophilic populations in the sludge were quantified by plates incubated at 30ºC and 60ºC respectively. The plates incubated at 30ºC were counted after 48hours; and those at 60ºC were counted after 12 hours. The results are presented as the number of colony forming units (CFU) per gram of sludge.

5.2.4. Data Processing

The data was compared using statistic analysis software (STATGRAFICS Centurion ®). The comparison of different samples was done by the “ANOVA table”; after this, a “Multi Range Test” was performed to determine which means were significantly different from each others. All the analysis was done with a confidence level of 95.0%.

The experimental data of methane productivity was fitted using the modified Gompertz equation (Donoso-Bravo et al. 2010), applying a non-linear regression model to describe the relationship between the experimental data and the independent variable. The parameters P_∞ (total methane productivity potential), R_m (maximum methane productivity rate) and λ (the lag-phase time) were determined for each digested phase.

$$P_{CH4}(t) = P_\infty \cdot exp\left[-\exp\left(\frac{R_m \cdot e^1}{P_\infty} \cdot (\lambda - t) + 1\right)\right] \qquad (5.2)$$

5.3. RESULTS AND DISCUSSION

5.3.1. Solubilization and Supernatant factor

Commonly, the effectiveness of a pretreatment is evaluated by the transference of the organic matter from the suspended phase to the soluble phase through the solubilization factor. Nevertheless, the use of only this single parameter is not adequate in order to evaluate the effect of a pretreatment (Prorot et al. 2011). Other effects could include not only the solubilization of large compounds, but also their break down of it into small organic molecules such as glucose and amino acids, through the hydrolysis processes. This phenomenon is observed in numerous thermal pretreatments which were seen to present optimum values of solubilization under certain operational conditions, however the improvement of anaerobic biodegradability did not occur at the maximum temperatures studied (Carrere et al. 2010).

Figure 5.1 shows the evolution of the soluble COD for the autohydrolysis pretreatment realized at 40, 55 and 70ºC. The results showed that the solubilization of organic matter was directly affected by the temperature: the maximum value and rate of solubilization increased when the temperature was increased. The solubilization factors achieved were 15% at 40ºC after 24 hours, 23% at 55ºC after 9 hours and 26% at 70ºC after 6 hours of pretreatment. After that time, the solubilization increase was negligible, and, in fact, a reduction in the soluble COD was achieved after 15 hours at 70ºC. The difference observed in the higher solubilization factor between the experiments at 55 and 70ºC was 3% (±1%), and was negligible for similar pretreatment times; even the initial solubilization rate was similar at these temperatures; nevertheless the difference between the experiments at 40 and 55ºC was 8% (±1%), more than double the previous value, and almost triple the time was needed.

Previous studies have shown the effect produced by pretreatments on the rheology behavior of the sludge (Pham et al. 2010). As a consequence of the pretreatment, the floc destabilization took place and the fluency of the sludge was considerable improved, this effect was observed when considering the feasibility of separating the soluble and suspended phases from the sludge when it was subjected to the different temperature conditions. The amount of supernatant obtained from the raw sludge was 24.5% (±0.7%); however, after the pretreatment the supernatant was increased to 33.8% (±0.7%), 35.8% (±0.2%), and 37.8% (±0.5%) when the sludge was subjected to 24, 9 and 6 hours and 40, 55 and 70ºC respectively. This increase in the amount of supernatant, and the fluency changes previously observed, were due to the floc destabilization. Therefore, effects observed included not only the increase in the soluble organic matter, but also the structure destabilization of the floc and the release of the water entrapped in it. Previous studies have observed similar behavior on the floc destabilization when a thermal pretreatment was applied to secondary sludge (Bougrier et al. 2008).

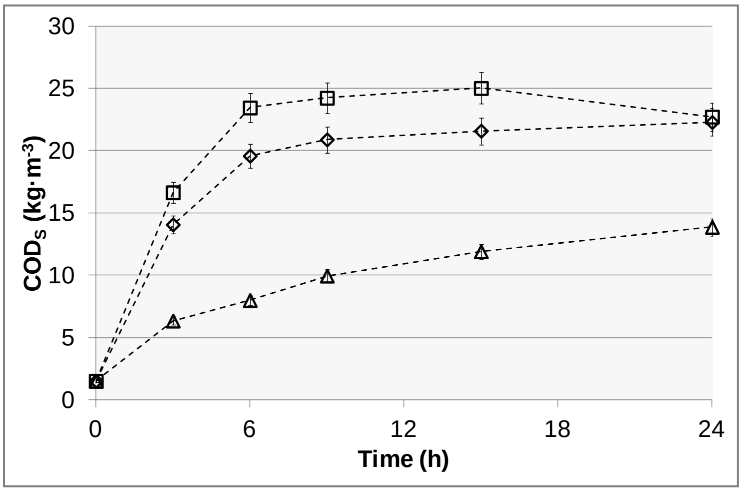


Figure 5.1. Soluble COD content in the soluble phase during autohydrolysis pretreatment at different temperatures (△ 40ºC, ◇ 55ºC and □ 70ºC).

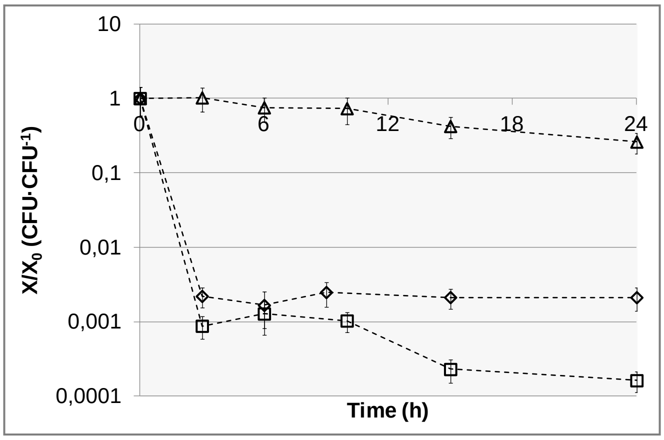
5.3.2. Microbial Population

The initial population detected in the raw sludge showed $7.0 \cdot 10^8$ ($\pm 2.1 \cdot 10^8$) $CFU \cdot g^{-1}$ and $4.4 \cdot 10^4$ ($\pm 0.7 \cdot 10^4$) $CFU \cdot g^{-1}$ of *mesophilic* and *thermophilic population* respectively. These values mean that the initial *thermophilic population* was less than 0.01% of the total population. When the sludge was subjected to pretreatment at different temperatures, the *mesophilic population* was reduced under all the conditions studied, but the rate of death was different at each temperature (Figure 5.2-a). The highest rate was obtained at the highest temperature (70ºC): only 0.10% (±0.03%) of the original population was alive after 10 hours of pretreatment, and it continued decreasing. On the other hand, the pretreatment at 40ºC showed the lowest rate of death: 73% (±29%) of the original population was alive after 10 hours of pretreatment. However, the pretreatment at 55ºC caused a population reduction over the first 10 hours similar to that obtained at 70ºC: 0.25% (±0.09%) of the population was alive after 9 hours of pretreatment, and it remained almost constant at this level during the rest of the experiment.

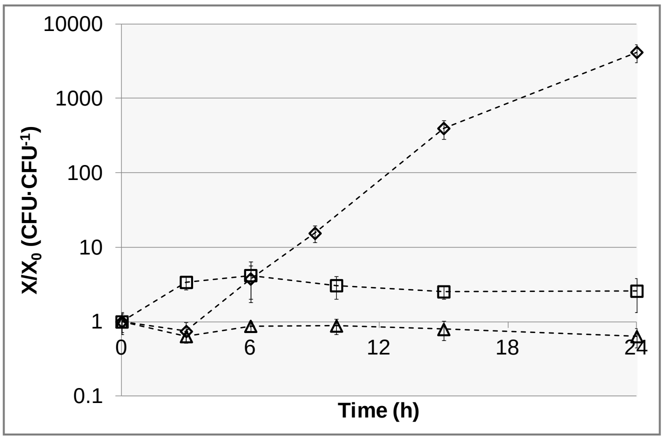
The resistance to temperature changes depends on the type of microorganism and its capacity to endure an environmental change, also the microbial activity diminution have been related with the reduction of the flocculation capacity on WAS (Coello Oviedo et al. 2003). The lowest temperature applied (40ºC) showed a defloculation effect i.e. the floc of WAS lost its structure, but the activity of the sludge and the viability of the cells was not completely eliminated. These results, obtained at 40ºC, mean that the cells were not disrupted, and that the low solubilization factor obtained was due to the EPS solubilization by thermal effect, but not due to the microbial activity. Clearly, a higher temperature should have a stronger effect on the mesophilic population, but the difference in the death rate between the pretreatment at 70 and 55ºC was not so significant in the first ten hours, representing 0.15% of the initial population. The utilization of treatment at 55ºC such as a pasteurization method of secondary sludge was described in previous studies, and the main result was the elimination of the pathogen population in the raw sludge (Le 2007).

The *thermophilic population* behavior was also different for each temperature studied too (Figure 5.2-b). During the pretreatment at 40ºC, the *thermophilic population* was reduced, the alive microorganisms representing 88% (±20%) of the initial population after 10 hours of pretreatment. Therefore, this condition did not encourage the growth of the *thermophilic population*. On the other hand, the pretreatment at 70ºC tripled its *thermophilic population* during the first six hours, but after that it remained constant; therefore, this higher temperature produced a strong initial effect over this population, but it was not maintained over time. Finally, the pretreatment at 55ºC reduced the population during the first three hours, but after that point it grew sustainably over time, showing this to be a good condition for optimal growth of the *thermophilic population*, which have shown the ability to secrete hydrolytic enzymes in previous studies (Hasegawa et al. 2000). However, the *thermophilic population* measured after 15 hours of pretreatment at 55ºC represented only 3% of the total population in the raw sludge; therefore this population was quite small compared with the original one, so the low and almost negligible organic matter consumption detected in previous studies could be due to its nutrients requirement, and it was lower than for the original sludge.

Clearly, all temperatures studied were shown to have some effect on the microbial viability of the WAS, but only the pretreatment at 55ºC produced the desired effect. The pretreatment at 55ºC reduced the *mesophilic population* in ranges similar to the higher temperatures, causing the deflocculation and the increase in the *thermophilic population* more than the other temperatures studied, and, probably, the cellular lysis. This *thermophilic population* increase during the first 10 hours may be sufficient to produce the hydrolysis of the organic matter, but not high enough to cause a significant consumption of the substrate.



(a)



(b)

Figure 5.2. Variation of (a) mesophilic and (b) thermophilic population when secondary sludge was subjected to temperatures of 40, 55 and 70ºC.

5.3.3. Enzymatic Activities

The measurement of the enzymatic activity in the full samples of raw sludge showed the different potential of the two enzymes studied at different temperatures (Figure 5.3-a and 5.3-b).The highest enzyme activity measured for *amylase* was at 55ºC, the next one was at 40ºC, being 58% (±3%) of the previous value, and the lowest one was at 70ºC, being 13% (±1%) of the 55ºC value, probably because of denaturation under this temperature; although the existence of some enzymes, which can withstand higher temperatures, depending of the structure differences allowing a conformational stability and enzymatic activity (Daniel 1996).

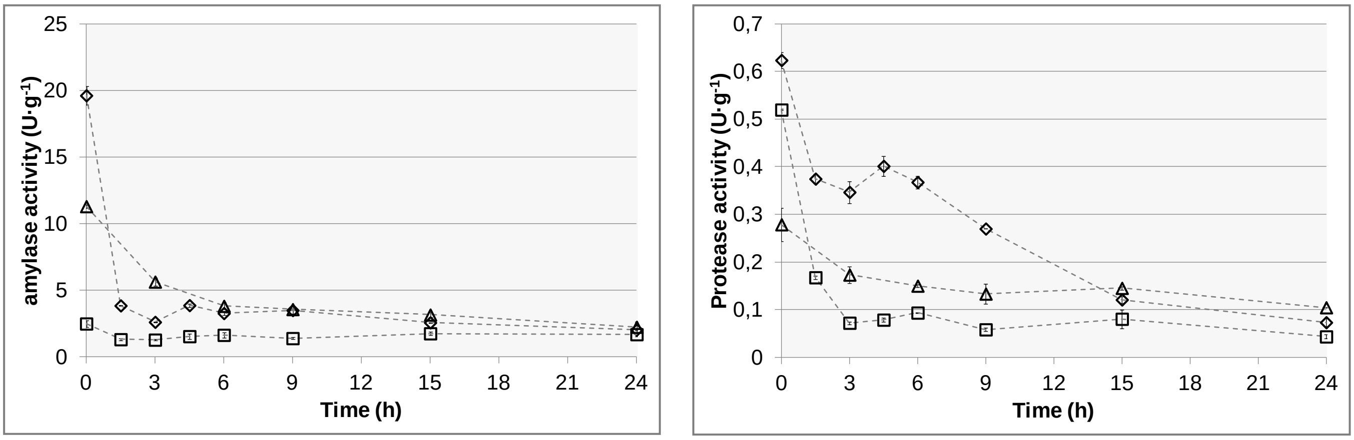
As a consequence of the low temperature pretreatment, the enzymatic activity of *amylase* decreased during the experiments at 40 and 55ºC. After 15 hours of pretreatment, the values reached were almost the same as the 70ºC one, which remained virtually constant all throughout the experiment. This behavior showed that despite a high potential of the *amylase*, it was not thermostable; also, the inactivation rate of the enzyme was higher for the 55ºC condition than for the 40ºC one.

In the case of *protease*, the highest enzymatic activity in the full samples of the raw sludge was also at 55ºC, the next one was at 70ºC, being 83% (±2%) of the 55ºC value, and the lowest one was at 40ºC condition, with an initial value of 45% (±6%) of the 55ºC value.

In the same way, as consequence of the low temperature pretreatment, the enzymatic activity of *protease* decreased. The highest reduction rate was for the 70ºC condition, showing a deactivation effect during the first three hours. The 40ºC experiment showed a constant deactivation rate during the first 6 hours, and afterwards it remained almost constant. On the other hand, the 55ºC experiment showed a 40% reduction in the activity after the first hour, but it remained constant for the next five hours of pretreatment; showing a higher potential activity than the other temperatures studied. As with amylase, after 15 hours of pretreatment, the enzymatic activities were almost the same for the three temperatures. Although, the results showed the deactivation of the enzyme due to the temperature, between 1.5 and 6 hours of autohydrolysis pretreatment at 55ºC, the enzymatic activity of *protease* remained high and constant.

All results obtained showed that the autohydrolysis pretreatment at 55ºC presented higher enzymatic activity potential of *amylase* and *protease* than the others temperatures studied for the full samples of the raw sludge; and a deactivation effect was also demonstrated, due to the temperature and depending on time. The enzymatic activity of *protease* was more stable to the temperature than the *amylase*, as it remained constant for a long period of time during the autohydrolysis pretreatment. This effect is quite important considering that the main content of the WAS was protein, as indicated in the characterization of the raw sludge in the Table 5.1.

Other important issue to remark is the high enzymatic potential contained in the secondary sludge. Some enzymatic pretreatments of secondary sludge, which added commercial enzymes, obtained similar results in solubilization of organic matter, and furthermore the enzymatic activities added to these assays were similar to the enzymatic potential measured in this study. In particular, the addition of 6% of a mixture in ratio 1:3 of protease and a-amylase, possessed an equivalent activity of 0.62 ($U \cdot mL^{-1}$) and 2.41 ($U \cdot mL^{-1}$) respectively (Q. Yang et al. 2010a).



(a) (b)

Figure 5.3. Enzymatic activity of the full sludge sample during autohydrolysis pretreatment (a) *amylase* and (b) *protease* (40, 55 and 70ºC).

Many authors have indicated that the concentration of free enzymes in the soluble phase of the WAS was negligible, because the enzymes were commonly attached to the sludge floc, either to the EPS and/or to the cell wall (Frolund et al. 1995; G. H. Yu et al. 2007). In this study, the presence of free enzymes in the soluble phase of the raw sludge was detected, probably due to the high concentration of sludge studied. After the thickening step the solids concentration of the sludge value was 16 times higher than the original one; however, the values detected in the soluble phase were lower than 2% of the total enzymatic activity in the raw sludge. These results showed that, in all cases, the majority of the enzymatic activity was located in the floc of the raw sludge, as expected.

Table 5.2. Percentage of enzymatic activity in the soluble phase (a_{S0}) related to the total phase (a_{T0}), of raw sludge.

	$(a_{S0}/a_{T0})_{40ºC}$ (%)	$(a_{S0}/a_{T0})_{55ºC}$ (%)	$(a_{S0}/a_{T0})_{70ºC}$ (%)
Amylase	0.50 ± 0.01	0.14 ± 0.04	1.71 ± 0.32
Protease	0.72 ± 0.13	0.40 ± 0.21	0.39 ± 1.08

As a consequence of the autohydrolysis pretreatment, the activity in the soluble phase of both enzymes studied increased during the first hour of pretreatment (Figure 5.4-a and 5.4-b). Previous studies have indicated that the amylase enzymes were mainly located in the loosely bound EPS fraction (LB-EPS) which represented the easily exchanged substances with the bulk solution; a few of both enzymes were located in the tightly bound EPS fraction (TB-EPS) which represented the substances that were more difficult to exchange, however the *protease* was mainly located in the pellet and thus associated to the cell wall (G. H. Yu et al. 2007). Therefore the increase in the soluble phase of both enzymes studied would be possible because the deflocculation and cellular lysis of the sludge promotes both the release of the enzymes associated to the EPS and also the release of the enzymes associated to the cell wall. Other researchers have obtained similar results applying other techniques such as ultrasonic pretreatment, to improve the aerobic digestion of secondary sludge (G.-H. Yu et al. 2008).

During the autohydrolysis pretreatment, the maximum value of enzymatic activity of *amylase* in the soluble phase was obtained for the 55ºC condition (Figure 5.4-a): it increased in the first hour of pretreatment, and remained almost constant for the next six hours. The other two conditions studied showed increased the enzymatic activity too, but the values were lower and remained constant during the whole study. After six hours, the enzymatic activity of the 55ºC condition decreased, reaching similar values to those obtained for the other conditions studied after 24 hours of pretreatment.

The enzyme activity of *protease* in the soluble phase increased at a constant rate during the first three hours of autohydrolysis pretreatment at 55ºC (Figure 5.4-b), it remained almost constant for the next six hours, and after that time it decreased, becoming negligible after 15 hours. The other conditions studied showed increased its enzymatic activity, but at a lower rate.

If it was assumed that the floc rupture was more significant at 70ºC, then one would also expected the maximum enzyme activity because of the release of the deflocculated sludge; however, this temperature increased the initial enzymatic activity, but it only represented around 60% of the enzymatic activity in the soluble phase reached at 55ºC after three hours of pretreatment (maximum value for both enzymes studied). These results showed an enzyme deactivation when the pretreatment was carried out at 70ºC , probably because of the temperature effect, and hence it was not wrong to assume that most of the hydrolysis of the organic matter under these conditions would be due to a thermal effect and not an enzymatic one. In the case of the 40ºC experiment, the soluble enzyme activity showed a slow growth rate, reaching its maximum values at 15 hours of pretreatment.

To summarize, the pretreatment at 55ºC was demonstrated as the best condition for release of both enzymes studied, *amylase* and *protease*, from the sludge floc to the soluble phase; also, it was even possible to maintain high enzymatic activity between the third and the ninth hour of pretreatment in the soluble phase. However, it was not possible to observe a relationship between the emerging thermophilic population, and the increased enzyme activity detected in the soluble phase during the autohydrolysis pretreatment.

Studies have indicated the ability of the thermophilic population to secrete hydrolytic enzymes such as proteases (Yan et al. 2008), however in this work it has not been possible to associate the growth of the thermophilic population with the behavior of the enzyme activity evaluated.

From the results obtained in this study it is possible to conclude that the high enzymatic activity detected and maintained during the assay at 55ºC, can produce the sludge hydrolysis during the pretreatment. This is due to the synergic effect produced by the deflocculation of the floc, releasing the active enzymes detected in the raw sludge, and also its non-deactivation of its due to the appropriate temperature pretreatment.

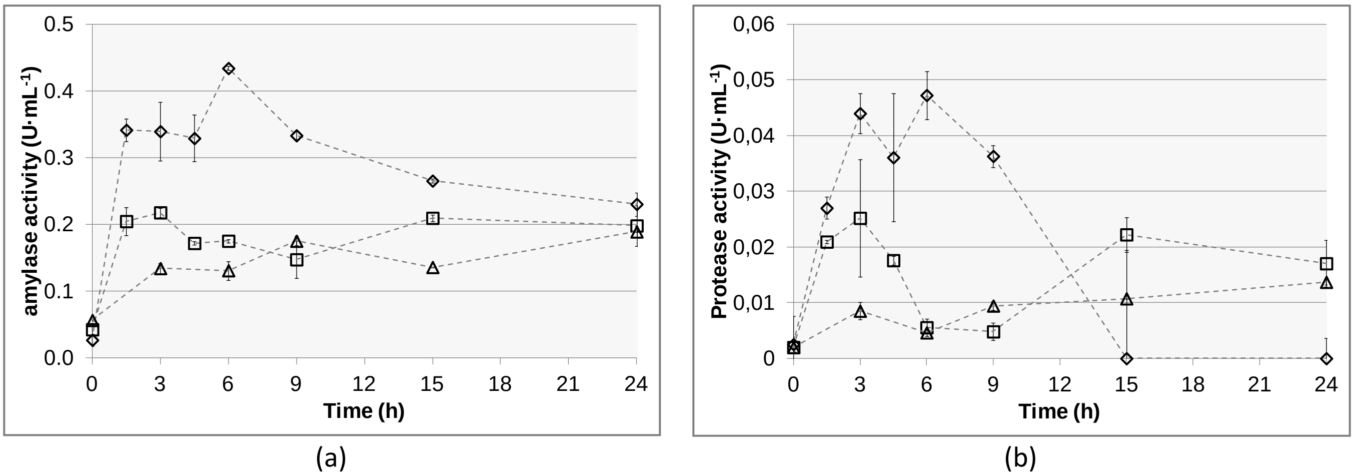


Figure 5.4. Enzymatic activity in the soluble phase of waste activated sludge during autohydrolysis pretreatment (c) *amylase* and (d) *protease* (△ 40, ◇55 and □ 70ºC).

5.3.4. Anaerobic Biodegradability

Previous studies with the autohydrolysis pretreatment (55ºC) have shown the improvement of the anaerobic biodegradability of the WAS subjected to 12 hours of pretreatment. The results obtained in this study showed a significant effect on the methane production of the full sludge samples at the three temperatures studied (Figure 5.5). The maximum rate of methane productivity (R_m) was doubled for all the conditions studied compared with the non-pretreated sludge, showing that when the organic matter was subjected to a low temperature pretreatment, then it was more easily biodegradable under anaerobic conditions, improving the digestion velocity. However, only the pretreatment at 55ºC showed an improvement in the biodegradability of the sludge (*BD*), i.e. only this condition produced the increase of the methane production per gram of WAS fed (productivity) at the end of the assay.

Considering the results obtained in this work, it is possible to indicate that the increase in the productivity rate (R_m) is made possible by the solubilization of the organic matter due to a thermal effect, which has been observed for all the conditions studied; however, the increase of the anaerobic biodegradability (*BD*) could be an effect of the enzymatic hydrolysis of the organic matter by the hydrolytic enzymes of the WAS that, in this case, produced smaller biodegradable compounds without inhibitory effect. These results showed, that the contribution of the thermal effect produced on the sludge by the pretreatment at low temperature was not enough to improve the anaerobic digestion of the WAS, as was observed by other authors using different pretreatment conditions at a low temperature (Prorot et al. 2011). Therefore, the experimental condition of the autohydrolysis pretreatment, such as the pretreatment time and oxygen supply, was the key to the improvement in the anaerobic digestion, because it ensured the enzyme action on the solubilized organic matter, promoting the synergic effect expected of a thermal-biological pretreatment.

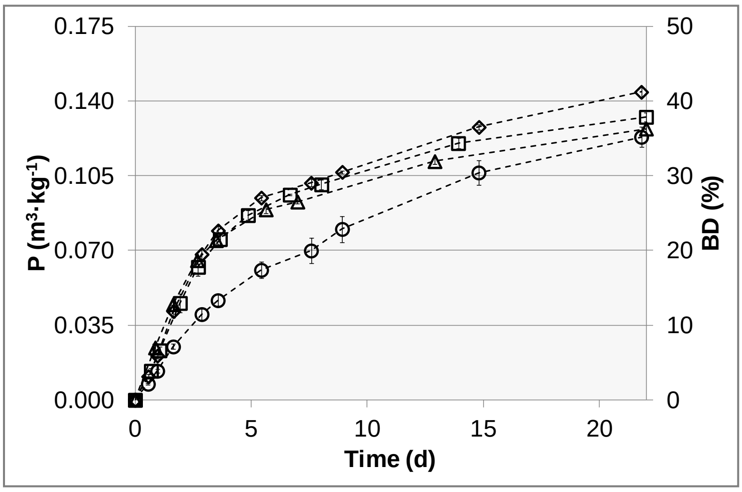


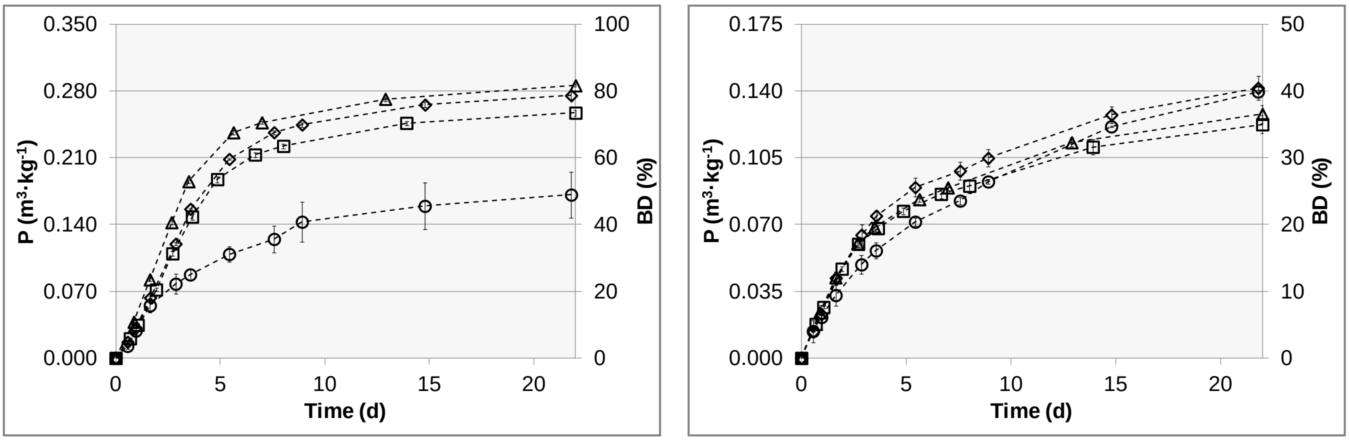
Figure 5.5. Anaerobic biodegradability and its corresponding parameters fitted to the Gompertz model, showing full sludge samples of non-pretreated, and pretreated sludge at different temperatures: 40, 55, 70ºC.

Table 5.3. Corresponding parameters fitted to the Gompertz model of full sludge.

	Control	40ºC	55ºC	70ºC
$\boldsymbol{P_{20}}$ ($m^3 \cdot kg^{-1}$)	0.123 ± 0.005	0.127 ± 0.003	0.144 ± 0.002	0.133 ± 0.002
$\boldsymbol{BD}$ (%)	35.2 ± 1.4	36.3 ± 0.8	41.2 ± 0.6	37.9 ± 0.6
$\boldsymbol{P_\infty}$($m^3 \cdot kg^{-1}$)	0.119 ± 0.004	0.113 ± 0.003	0.126 ± 0.003	0.120 ± 0.003
$\boldsymbol{R_m}$($m^3 \cdot kg^{-1} \cdot d^{-1}$)	0.010 ± 0.000	0.022 ± 0.001	0.021 ± 0.001	0.020 ± 0.001
λ (d^{-1})	0	0	0	0
R^2	0.9648	0.9459	0.9566	0.9665

The anaerobic digestion of the separate phases showed that, in the same way as the full sludge samples, the maximum rate of the methane production was increased for all the conditions studied and for both phases, the soluble and the suspended, when it was compared with the non-pretreated sludge (Figure 5.6-a and 5.6-b). However, no statistical differences were found between the suspended phases pretreated at the three temperatures. The comparison between suspended and soluble maximum rate values showed that the suspended ones were smaller than the soluble ones, obviously due to the substrate nature (suspended/soluble). All these results showed that the higher value of the maximum rate parameter (R_m) was obtained for the 40ºC condition, which was not expected, because the highest values of the enzymatic activity measured occurred at 55ºC and so this temperature should have been the fastest one. Clearly, the 40ºC pretreatment released the easiest biodegradable fraction, but on the other hand, the quantity of the released organic matter was the lower one too, probably because this condition did not achieve an important effect on the microbial population.

The comparison of the biodegradability revealed significant effects for all the conditions studied (Figure 5.6). The soluble phase biodegradability was increased around 25%, but the least improvement was obtained at 70ºC, where the highest content of organic matter was present; so it is probable that the thermal effect at this temperature released some non biodegradable compounds. On the other hand, the biodegradability of the suspended phase pretreated at 40 and 70ºC were decreased in comparison with the non-pretreated and also with the 55ºC pretreated one. This result showed the main effect between the pretreatment conditions, because the destabilization and rupture of the floc should not cause a reduction in its biodegradability. This effect would be due to the enzymes, detected only at the 55ºC pretreatment, that could produce a selective rupture of the large size molecules bounds.



(a) (b)

Figure 5.6. Anaerobic biodegradability and its corresponding parameters of (a) soluble phase and (b) suspended phase. Samples of non-pretreated sludge, and sludge pretreated at different temperatures: 40, 55, 70ºC.

Table 5.4. Corresponding parameters fitted to the Gompertz model of full sludge.

	Soluble Phase				Suspended Phase			
	Control	40ºC	55ºC	70ºC	Control	40ºC	55ºC	70ºC
$\boldsymbol{P_{20}}$ ($m^3 \cdot kg^{-1}$)	0.171 ± 0.024	0.276 ± 0.001	0.275 ± 0.004	0.257 ± 0.003	0.140 ± 0.001	0.118 ± 0.005	0.141 ± 0.006	0.122 ± 0.005
$\boldsymbol{BD}$ (%)	48.9 ± 6.9	78.7 ± 0.4	78.6 ± 1.0	73.4 ± 0.9	39.9 ± 2.8	33.7 ± 1.3	40.4 ± 1.8	35.0 ± 1.3
$\boldsymbol{P_\infty}$ ($m^3 \cdot kg^{-1} \cdot d^{-1}$)	0.160 ± 0.007	0.273 ± 0.003	0.264 ± 0.002	0.247 ± 0.002	0.132 ± 0.005	0.116 ± 0.004	0.126 ± 0.004	0.109 ± 0.003
$\boldsymbol{R_m}$ ($m^3 \cdot kg^{-1}$)	0.022 ± 0.002	0.058 ± 0.002	0.049 ± 0.002	0.045 ± 0.001	0.013 ± 0.001	0.019 ± 0.001	0.019 ± 0.001	0.019 ± 0.001
λ (d^{-1})	0	0.26 ± 0.09	0.46 ± 0.07	0.38 ± 0.07	0	0	0	0
R^2	0.9464	0.9939	0.996	0.9953	0.9486	0.9392	0.9507	0.9406

5.4. CONCLUSIONS

As a consequence of the pretreatment at a low temperature, a reduction in the total microbial population was observed, this being almost complete at 55 and 70ºC. The highest death rate occurred at 70ºC. On the other hand, only the pretreatment at 55ºC showed the growth of the thermophilic population during the entire pretreatment.

Most of the enzymes *amylase* and *protease* were associated with the suspended phase, and neither were thermostable; their enzymatic activities decreased during the pretreatment at the three low temperatures tested. Nevertheless, the enzymatic activities of *amylase* and *protease* increased notably in the soluble phase as a consequence of the autohydrolysis pretreatment during the first hours, reaching the highest activity at 55ºC.

The pretreatment of secondary sludge at low temperatures (40, 55 and 70ºC) showed different potentials for the solubilization of organic matter, depending on the temperature. This solubilization process was a combination of the synergic effect of the temperature, and enzyme activity.

The anaerobic digestion of the pretreated sludge (full sludge, suspended phase and soluble phase) increased the maximum rate of methane production for all the temperatures studied, but only the sludge subjected to pretreatment at 55ºC improved its biodegradability (full sludge samples). This could be due to the lower biodegradability of the organic matter released into the soluble phase at 70ºC, compared against the biodegradability of the compounds obtained at 55ºC. In addition, the suspended phases of the sludge subjected to pretreatment at 40 and 70ºC showed a reduction in its biodegradability, demonstrating the negative effect of this pretreatment condition over the organic matter.

The autohydrolysis pretreatment of secondary sludge at 55ºC showed the highest enzymatic activity potential; it also increased the soluble enzyme activity and promoted the expression of the *thermophilic population* in a way that none of the other conditions studied did. Therefore, the release of the organic matter from the secondary sludge floc was produced by the thermal effect of the pre-treatment, however the improvement of the anaerobic digestion was because of the hydrolytic enzymes, which were natives of the secondary sludge and were stimulated due to the autohydrolysis pretreatment conditions.

5.5. REFERENCES

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Chapter 6

Continuous anaerobic digestion of high concentrated secondary sludge and the effect produced by the autohydrolysis pretreatment

ABSTRACT

The autohydrolysis effect on the continuous anaerobic digestion of secondary sludge was studied. For this purpose two anaerobic digesters (20L each one), was compared working at different SRT.

Both digesters were started-up at the same conditions and with non-pretreated secondary sludge, with high sludge concentration (8% of total solids). The acclimation of the inoculum requirement was evaluated, obtaining as a result 150 days of non steady-state operation. Also, a mixing condition of the digester, operating with high solid content, was evaluated. The residence time distribution was performed through a pulse injection of LiCl. As a result, the good mixing condition of the anaerobic digester was observed, however a possible canalization of the feed was obtained.

After the steady-state, both digesters were compared, obtaining a negligible difference between the operations of both (95% of certainty). The study of the autohydrolysis pretreatment was evaluated comparing the performance of digesters, one was fed with raw and the other with pre-treated sludge during 300 days. As a result, the methane production increased 20% at 20d of solid retention time, and when the solid retention time was reduced the difference between both digesters decreased, reaching an improvement of 15% and 10% at 17 and 15 days of solid retention time respectively, also at 13d the difference became negligible. Therefore, the autohydrolysis pretreatment of high concentrated secondary sludge increased the methane productivity 20%, respect to the untreated sludge, when de anaerobic digestion was carried out at 20 days of solid retention time.

RESUMEN

En esta sección se estudió el efecto del pre-tratamiento de auto-hidrólisis del fango secundario en la digestión anaerobia continua. Se comparó la operación de dos digestores anaerobios (20L cada uno), operando a distintos tiempos de retención de sólidos.

El arranque de ambos digestores fue realizado en las mismas condiciones, usando fango sin pre-tratar con alto contenido de sólidos (8% de sólidos totales). Se estudió la adaptación del inóculo anaerobio, obteniendo como resultado 150 días de operación en estado no estacionario. Además se estudió la calidad de agitación de uno de los digestores operando con alto contenido de sólidos. Se obtuvo la distribución del tiempo de residencia usando la inyección en pulso de un trazador (LiCl). Como resultado, se obtuvo una buena condición de agitación del digestor anaerobio, sin embargo una posible canalización fue obtenida. Se comparó el rendimiento de ambos digestores operando en estado estacionario, obteniendo una diferencia nula entre ambos (95% de certeza).

Luego, se estudió el pre-tratamiento de auto-hidrólisis, comparando la respuesta de ambos digestores al operar con distintas alimentaciones, uno con fango sin pre-tratar y el otro con fango pre-tratado. Se obtuvo un aumento en la productividad de metano de 20% cuando los digestores operaron a 20 días de retención de sólidos, disminuyendo la diferencia según se diminuía el tiempo de retención de los sólidos, obteniendo 15% y 10% a 17 y 15 días respectivamente; la diferencia se hizo insignificante cuando la retención de sólidos se redujo a 13 días. Por lo tanto, el pre-tratamiento de auto-hidrólisis de fango secundario con alto contenido de sólidos aumentó la productividad de metano en 20%, respecto al fango no pre-tratado, cuando la digestión anaerobia se operó a 20 días de retención de sólidos.

SIMBOLS

AD	Anaerobic digestion	WWTP	Waste water treatment plant
Alk_R	Alkalinity ratio (dimensionless)	Alk_T	Total alkalinity ($g·L^{-1}$)
COD_T	Total chemical oxygen demand ($g·kg^{-1}$)	N-TKN	Total nitrogen measured as Kjeldahl ($g·kg^{-1}$)
COD_S	Dissolved chemical oxygen demand ($g_{O2}·L^{-1}$)	$N\text{-}NH_4^+$	Soluble nitrogen as ammonia ($g·L^{-1}$)
OLR	Organic loading rate ($kg_{VSfeed}·m^{-3}{}_{digester}·d^{-1}$)	VPR_b	Volumetric production rate of biogas ($m^3{}_{biogas}·m^{-3}{}_{digester}·d^{-1}$)
ORR	Organic removal rate ($kg_{VSremoved}·m^{-3}{}_{digester}·d^{-1}$)	E_ϑ	Dimensionless concentration of the tracer
P	Methane productivity ($m^3{}_{CH4}·kg^{-1}{}_{VSfed}$)	ϑ	Dimensionless time
HRT	Hydraulic retention time	b	Fraction of volume increased in the mixing zone
SRT	Solid retention time (d)	d	Fraction of volume increased in the mixing zone
TS	Total solids ($g·kg^{-1}$)	n	Number of tank in series
VS	Volatile solids ($g·kg^{-1}$)	λ	Delay time (d)

6.1. INTRODUCTION

The sludge management has associated around 50% of the operational cost of the waste water treatment plant (WWTP), therefore its reduction has become an important challenge nowadays (Odegaard 2004; Wei et al. 2003).

The sludge treatment seek to reduce the content of water and organic matter, also to transform the putrescible organic matter into a stabilized and reusable product, ideally a “Biosolids Class A” (Metcalf and Eddy 2003).

The anaerobic digestion (AD) is the most used sludge treatment, due to it offers several advantages, including: biogas production, high reduction of the solid content, and the elimination of pathogen microorganisms. Commonly, the produced biogas is exploited in the WWTP, firstly converting into electricity with an associate efficiency, and secondly as a heat source for the WWTP facilities (Turovskiy and Mathai 2006).

On the other hand, the AD has associated some disadvantages such as partial organic matter decomposition, large digestion volume, inhibitor presence, and the low quality of supernatant and high digestion time, making it necessary to improve its operation (Appels et al. 2008; de la Rubia et al. 2006).

The AD process mainly consists of three major steps, associated to different chemical and biochemical reactions: hydrolysis, fermentation (also known as acidogenesis) and methanogenesis. The hydrolysis includes transforming macromolecules such as fats, proteins and complex carbohydrates in soluble and simpler compounds such as fatty acids, amino acids and sugars, and it is considered as the limiting step for the AD of secondary sludge (Appels et al. 2008).

Some environmental factors affects the process stability and efficiency, the most important factors are: solid retention time (SRT), hydraulic retention time (HRT), temperature, alkalinity (Alk), pH, presence of inhibitory substances and the availability of nutrients and trace metals.

The SRT is one of the most important variables in the anaerobic digestion process; it controls directly the three major steps of the anaerobic process, affecting the amount of available time to metabolize the volatile solids. There is a minimum SRT for each reaction, therefore it controls the microbial population in the digester (Lee et al. 2011).

The presence of inhibitory compounds in the feed, such as heavy metals, and also as a result of the microbial metabolism, such as ammonia, may affect and also cause the failure of the anaerobic digestion process. However, metals in trace amount are necessary for cell synthesis and also the ammonia is produced during the anaerobic digestion of the proteins and urea. As all toxic compounds, their effects are determined in function of content and time of exposure.

The mixing condition allows the use of the total volume of the digester, without dead zones. Also it improves the contact between the raw feed with the microbial population into the digester. However, the mixing can reduce the proximity of the different populations with coupled metabolisms reducing the anaerobic digestion performance (Speece 2008).

The study of the mixing quality allows take some decisions in order to achieve maximum process efficiency (Smith et al. 1993), reducing the non-uniformity of the digester volume, the presence of some dead volume and also avoiding the existence of some feed by-pass into digesters.

There are many studies focused on accelerating the sludge hydrolysis by different strategies in order to cause the disintegration of secondary sludge, some of these techniques are the application of pretreatments such as: mechanical, chemical, sonication, thermal and the utilization of hydrolytic enzymes (Bougrier et al. 2006; Carrere et al. 2010; Climent et al. 2007).

All pretreatments in literature have associated benefits and disadvantages, the most common of which are related to the high operation costs and/or with the high complexity of the implementation (Pérez-Elvira et al. 2006).

As was shown in previous chapters, the autohydrolysis pretreatment is a biologic-thermal pretreatment that uses the inherent enzymatic activity of the secondary sludge. It involves subjecting the secondary sludge to 55ºC during nine hours and a limited amount of oxygen in batch operation. This experimental condition produces the floc destabilization and releases the enzymes attached to this structure and also from cell wall, producing the organic matter hydrolysis.

The studies of the previous chapters presented the effect of the authohydrolysis pretreatment on the anaerobic digestion. The pretreatment have been studied on different operational conditions, and also the effect on thermophilic and meshophilic anaerobic digestion was compared. However, all the studies of autohydrolysis pretreatment effect were performed on batch anaerobic digestion assays, therefore the effect produced on a continuous operation have not been studied yet.

6.2. OBJECTIVES

The main objective of this section was to evaluate the effect of the autohydrolysis pretreatment of secondary sludge on its continuous anaerobic digestion. For this porpoise two twin anaerobic digesters were operated in parallel and under the same operational conditions.

This global objective was studied through the evaluation of the specific objectives, and different operation stages:

- The study of the start-up behavior of the two anaerobic digesters using secondary sludge with high solids concentration as feed.
- The comparison of both digesters performances after reached the steady-state, evaluating parameters as biogas production and solid elimination. The study of residence time distribution, evaluating the mixing quality in the anaerobic digester with high solid content.
- The study of the autohydrolysis pretreatment effect on the continuous anaerobic digestion on secondary sludge. In order to carried out this objective, the feed of one digester is switched to pretreated sludge; the effect is evaluated at different operational conditions.

6.3. MATHERIALS AND METHODS

6.3.1. Secondary Sludge

The secondary sludge was delivered ones a week from the WWTP of Valladolid, Spain, and stored at 4ºC before experimentation. The sludge was thickened in the WWTP by an industrial centrifugal extractor (Pieralisi, Baby) without the addition of polyelectrolyte. The main characteristics of the concentrated sludge varied in function of the WWTP operation and are presented in the table 6.1, which presents the average value plus its standard deviation, and also the minimum and the maximum value of each parameter measured during this study.

All data collected for the sludge characterization were analyzed for each parameter in the periods of digesters operation, finding some outside of normal disturbances that can produce interference in the normal digesters operation. This statistic analysis showed a normal distribution of the most of data (95% of confidence); also it was possible to identify some particular points significantly distant from the normality. Those points were identified, marked, and the corresponded periods of its presence were not considered for the comparison between both digesters operation.

Table 6.1. Feed sludge characterization for each period of digesters operation (Average ± standard deviation (maximum – minimum)).

	Start-up Nº1 (30d)	Start-up Nº2 and Steady-state (206d)	Pretreatment Effect (345d)	
			D1	D2
TS ($g \cdot kg^{-1}$)	88.2 ± 7.9 (76.2 – 97.8)	71.5 ± 10.0 (36.0 – 90.0)	65.9 ± 10.1 (38.5 – 85.2)	65.2 ± 9.7 (38.5 – 85.0)
VS ($g \cdot kg^{-1}$)	67.6 ± 5.6 (58.9 – 74.0)	54.8 ± 7.1 (27.8 – 65.8)	50.5 ± 7.2 (29.7 – 62.3)	49.9 ± 6.9 (29.7 – 61.9)
COD_T ($g \cdot kg^{-1}$)	100.8 ± 8.0 (88.4 – 110.2)	81.0 ± 11.5 (39.0 – 100.5)	*75.8 ± 11.8 (44.6 – 106.5)	74.8 ± 10.8 (43.8 – 106.5)
COD_S ($kg \cdot m^{-3}$)	6.4 ± 1.7 (3.2 – 7.9)	2.3 ± 0.9 (0.4 – 4.2)	*1.7 ± 0.9 (0.4 – 4.0)	19.9 ± 3.0 (12.1 – 26.4)
NKT ($g \cdot kg^{-1}$)	6.8 ± 0.7 (5.9 – 7.4)	5.6 ± 0.4 (4.8 – 6.9)	*5.1 ± 0.7 (3.0 – 6.4)	5.1 ± 0.7 (3.0 – 6.2)
NH_4^+ (g/L)	0.3 ± 0.1 (0.1 – 0.5)	0.2 ± 0.1 (0.1 – 0.3)	*0.2 ± 0.1 (0.1 – 0.4)	0.4 ± 0.2 (0.1 – 0.9)

6.3.1. Autohydrolysis Pretreatment

The pretreatment was carried out in batch conditions using 2-liter bottles loaded with 500g of concentrated sludge and closed with a rubber septum during 10 hours. It was then laid down horizontally in a roller bottle apparatus (Wheaton) inside a thermostatic chamber. The temperature of the chamber was kept constant at 55±0.5ºC with a convective flow of air and an electric resistor as heat source. The pretreatment was carried out ones a week, the same day that it was sent from the WWTP, so both fresh and pretreated sludge were stored under 4ºC during one week and no more, avoiding decomposition.

6.3.1. Digesters set-up

The continuous anaerobic digestion was performed in two anaerobic digesters with a working volume of 20L each one (30L total volume, 100cm high and 20cm diameter), ran at the same time. The digesters temperature was kept constant at 35ºC (±1ºC) with an electric resistor located around the reactor transferring heat through the walls and controlled by PID. Each reactor was mixed with solids recirculation provided by a Bredel peristaltic pump, and the feeding was performed manually first, and automatically after, with a Whatson-Marlow peristaltic pump, the figure 6.1 shows the digesters diagram. The feed was replaced every day by a new bottle, avoiding the digestion or decomposition in the feeding tank.

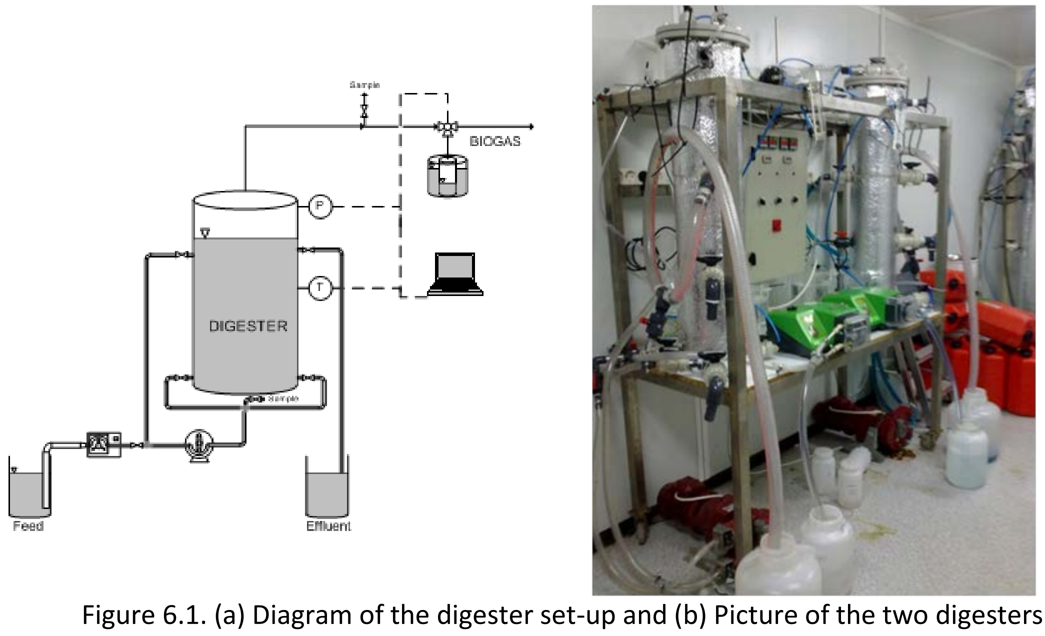


Figure 6.1. (a) Diagram of the digester set-up and (b) Picture of the two digesters.

The digesters were monitored online: pressure, temperature and biogas production. The biogas was conduced using a tygon tubing, and it production was measured by liquid displacement in an inverted cylinder; an electro valve released the biogas contained when it reaches a volume of 125 (±5)mL. Biogas composition was measured ones a day by gas chromatography using a Varian CP-3800, helium was a carrier gas (Díaz et al. 2010)

6.3.2. Operational conditions

Both digesters were inoculated (100% inoculum) from a full-scale reactor operating with mixed sludge in the WWTP of Valladolid. The inoculum was thickened until de desired concentration before being placed into de digesters and the initial conditions were: $N\text{-}NH_4^+$ 0.4($g{\cdot}L^{-1}$), COD_S 3 ($g{\cdot}L^{-1}$), COD_T 45 ($g{\cdot}kg^{-1}$), VS 30 ($g{\cdot}kg^{-1}$). The feed at high SRT was performed in semi-batch condition, both digesters were feeding manually two times per day (approximately the first 100 days), after that period, the feed was performed by a peristaltic pump eight times per day.

After the start-up period, both digesters were operated at 20d of SRT reaching the steady-state, and comparing the behavior of both. After this period, the feed of one of the digesters was switched from raw sludge to pretreated sludge (D2). In order to add randomness to the system, a coin was used to select the digester operating with pretreated sludge. Both digesters (D1 and D2) were operated at different SRTs, comparing its performance; the operation description is given below.

6.3.3. Experimental analysis

Samples of the feed and effluent were taken and analyzed two times per week. Total solids (TS), volatile solids (VS), chemical oxygen demand (total: COD_T & soluble: COD_S), ammonia (NH_4^+) and total Kjendal Nitrogen (TKN) were determined by Standard Methods (APHA et al. 2005). The soluble phase was obtained by centrifugation of the samples for 10min at 5000rpm, and afterwards the supernatant was filtered using a pore size of 0.45µm. The total and partial alkalinity, and also the VFA content were measured by titration of the soluble phase (APHA et al. 2005).

6.3.4. Hydrodynamic behavior

A residence time distribution experiment was performed for digester D1, a pulse of tracer injection was made; 25mL of 24.43 ($g \cdot L^{-1}$) LiCl, equivalent to 100 (mg) of lithium, was injected into the digester. The Li^+ content was measured through to the full sludge samples by atomic absorption spectroscopy (PU-9400) at 670.8nm of wavelength and air-acetylene flame ($1.2 L \cdot min^{-1}$). Samples were taken in the effluent and also in the recirculation line during 40 days, corresponding to twice the solids residence time.

Even the Li^+ showed low adsorption and also negligible inhibition effect to the experimental condition used in this study (Anderson et al. 1991), the samples were previously exposed to a microwave digestion, releasing some possible adsorbed Li^+.

The exit age distribution of the tracer was obtained from the experimental data, composed by the dimensionless exit concentration ($E_\vartheta = C(t) / C_0$), versus the dimensionless time ($\theta = t / t_{med}$) (Levenspiel 1999). The fit of the data was performed through the minimization of the sum of the differences between experimental and calculated data, using SOLVER® a tool of EXCEL® software.

6.3.5. Comparisons and statistical analysis

All data presented have been analyzed using statistic analysis software (STATGRAFICS Centurion ®), and all the analysis was done with a 95% of confidence level.

For the sludge characterization, the central tendency, the variability and the shape of the data was studied by evaluating the values of the standardized skewness and the standardized kurtosis parameters for each group of data. All these values were between the ranges -2 to +2 indicating a normal behavior for the characterization of the sludge.

The comparison of the digesters behavior, biogas and methane productivity and also solids removal, was performed by a means comparison using a t-test, the variances of each data group was previously compared by an F-test.

6.3.6. Operational strategy description

The results presented in this document are mainly divided in two sections:

1) The start-up of the two anaerobic digesters fed with secondary sludge. This section includes the comparison of the steady-state behavior of both digesters. Also, the residence time distribution assay.

The second section shows the operation with different sludge types, D1 was fed with raw sludge and D2 was fed with pretreated secondary sludge. Both digesters behavior were compared at different SRT operation: 20, 17, 15 and 13 days.

Figure 6.2 presents a summary diagram of the different operational conditions assayed during the 550 days of the study. The half-time and stabilization period, between 206th and 320th days, corresponded to a period of time where the experimental operation was stopped and re-started.

The results will be presented in three groups of complementary figures:

1) The operational conditions, including SRT, OLR and ORR, and finally the different ratios of biogas production.
2) The evolution of the full sludge characteristic of both feed and effluent.
3) The characterization of the soluble phase of both feed and effluent.

To avoid excessive graphs, some results are attached at the end of this chapter.

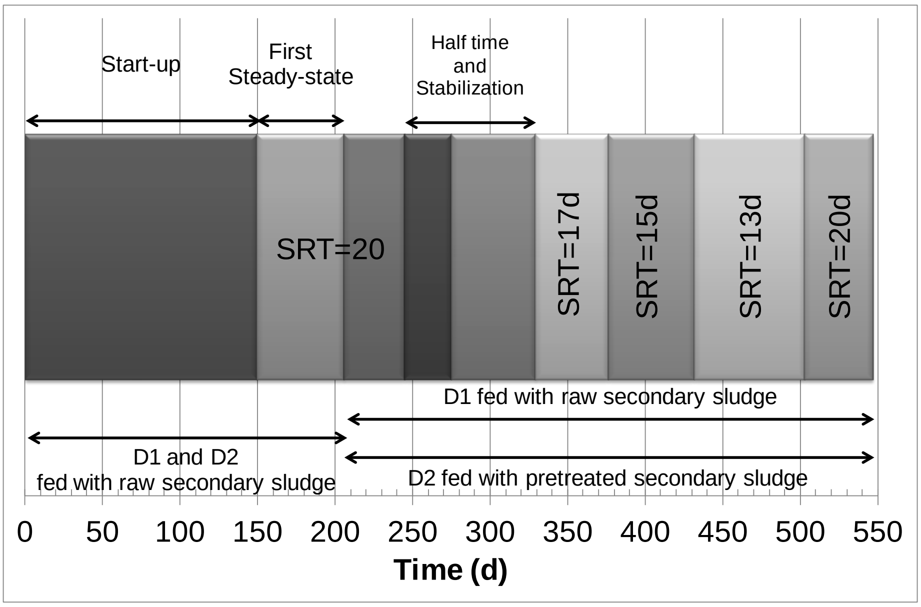


Figure 6.2. Summary diagram of operational conditions assayed during 550 days of digesters operation.

6.4. RESULTS AND DISCUSSION

6.4.1. Start-up of high-solid content anaerobic digesters

6.4.1.1. Start-up Nº1

The start-up Nº1 assumed that the inoculums coming from the WWTP can operate with the secondary sludge as only feed, or a low acclimation time was required. This period began the operation at 70d of SRT, which was reduced at 35d during the first five days of operation; after, it condition was maintained until digester stabilization.

The operation at 35d of SRT should not be a stressful condition for inoculum which comes from an anaerobic digester of a WWTP, due to its common operational condition is around 20 to 25 days of SRT. On the other hand, the OLR used in the WWTP was lower than the used during this operation, because of the solid content. In fact, the SRT applied during this start-up operation corresponded to the increase on OLR from 1 to 2 ($kg \cdot m^{-3} \cdot d^{-1}$), due to the high solids content of the secondary sludge used as feed.

As a consequence of the OLR increase, the COD_S increased gradually during the start-up period. The 22th operation day, the methane composition decreased significantly, and also, the VFAs and the Alk_R increased quickly; both are common signs of the anaerobic digestion process inhibition (Chen et al. 2008). The corrective action used was stopped the feeding, at 25th day of operation, seeking to recover the digester. As a result, the composition of the methane increased four days after, but the VFAs content continued increasing, showing the destabilization of the anaerobic digestion process. The table 6.3 presents the characterization of the effluent during the star-up period.

Probably, the main responsible of the process inhibition during the start-up, was the increase in the $N\text{-}NH_4^+$ content, its concentration tripled in both digesters in a period of 20 days of operation. Ammonia content can be higher and hence reached toxic levels, when the anaerobic digestion of highly concentrated sludge was performed (Turovskiy and Mathai 2006). However, the anaerobic digestion has been carried out with high ammonia concentrations in previous studies, but a high acclimation time is required to adapt the microbial population (Calli et al. 2005; Chen et al. 2008). On the other hand, the high concentration of ammonia reached was expected, because it is a product of the proteins and urea anaerobic digestion, and in addition, proteins are the most content of the secondary sludge (Nielsen et al. 2004).

Table 6.3. Effluent characterization during the Star-up Nº1.

Time (d)	COD_S ($g·L^{-1}$)		VFA ($g·L^{-1}$)		Alk_T ($g·L^{-1}$)		Alk_R		$N\text{-}NH_4^+$ ($g·L^{-1}$)		CH_4 (%)	
	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2
2	1.2	1.2	0.5	0.5	-	-	-	-	-	-	64.2	65.8
4	2.1	3.4	0.5	0.3	-	-	-	-	0.7	0.8	65.5	64.3
8	3.4	3.9	0.5	0.9	-	-	-	-	0.9	0.9	66.7	62.9
11	4.6	5.3	0.7	0.7	5.5	6.1	0.18	0.18	1.0	1.3	63.8	61.8
15	5.6	6.5	1.2	1.5	5.5	6.6	0.18	0.17	-	-	60.8	60.8
18	6.5	7.5	0.9	1.2	6.7	6.9	0.18	0.19	1.8	2.0	61.8	61.8
22	8.5	9.7	1.7	2.3	7.4	7.6	0.22	0.22	-	-	53.5	51.8
25	14.2	14.8	4.5	3.9	8.7	8.7	0.37	0.37	2.6	2.7	45.1	41.8
29	15.2	17.3	5.2	6.0	8.9	9.4	0.38	0.42	2.8	3.0	55.3	47.8
30	15.4	17.1	5.1	5.8	12.8	13.2	0.40	0.42	-	-	58.3	47.8

6.4.1.2. Start-up Nº2

To avoid the inhibition obtained in the first start-up attempt, by the high concentration of ammonia reached and therefore the accumulation of VFAs, the second start-up rate was slower than the previous one. This new start-up, with a new inoculum, provided higher acclimation time to the microbial population, and then the SRT was reduced gradually causing an equivalent increase of the OLR from 0.25 to 2.5 ($kg·m^{-3}·d^{-1}$), this period took around 150 days, and any of the operational conditions with highs SRT were maintained during larges period of time, due to they are not interesting at full scale operation. After, the operation at 20d of SRT was maintained during 50 days, comparing the behavior of both digesters.

The results presented in figure 6.3 was obtained for D2 operation, the corresponding figures of D1 are attached in the appendix of figures.

The evolution of the digester operation, showed the increase of the OLR in function of the SRT reduction. It is possible to note that the fluctuation caused by the solid content variation, due to the thickening stage described in table 6.1, was absorbed by the digesters operation. The biogas production increased proportional to the feeding increase, reaching values of 1 cubic meter of biogas per cubic meter of digestion volume per day ($1.0m^3 \cdot m^{-3} \cdot d^{-1}$), when the OLR was around 3 ($kg \cdot m^{-3} \cdot d^{-1}$). Even though, the biogas and methane production may appear to be small, both digesters were only fed with secondary sludge, and the biodegradation of this substrate have shown lower biogas production than the primary sludge (Gavala et al. 2003).

The SRT reduction was mainly controlled by the evolution of the methane productivity and the alkalinity ratio, these ratios can show the destabilization of the equilibrium between fermentation and methanogenesis on the anaerobic digestion process, trough a dissimilar change on its usual value (Speece 2008).

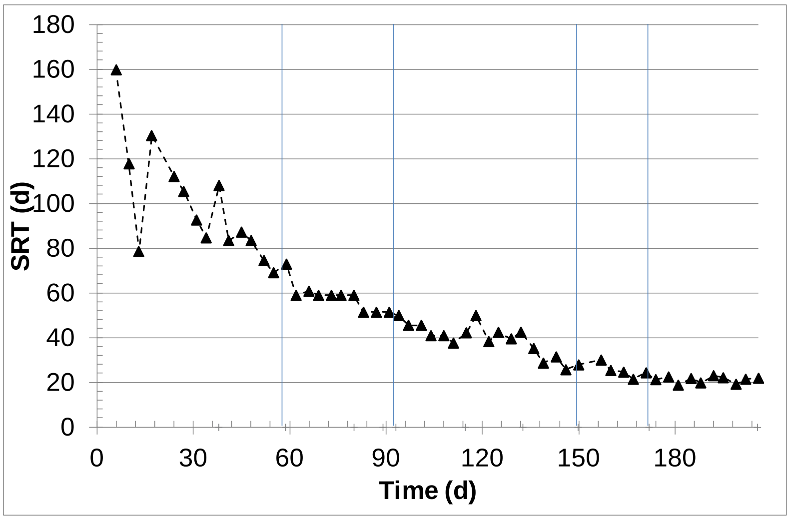
The biogas and methane productivities showed some variations during the operation; its values were quite higher at the beginning of the operation when the SRT was high. The highest reduction of the productivity, comparing with the previous period, were observed around the 60^{th} and 80^{th} days, corresponding with an OLR of 1 ($kg \cdot m^{-3} \cdot d^{-1}$). During this period, the productivity decreased (averages values) around 17% and 13% for D1 and D2 respectively.

Simultaneously with the decrease in biogas and methane productivity, higher levels of VFAs and also a significant variation in the Alk_R was observed, but more significant for D1. Therefore, the digester D1 was subjected to a starvation period during one week, and as a consequence of this period, the productivity increased and stabilized around 0.35 and 0.20 ($m^{-3} \cdot kg^{-1}$) for biogas and methane respectively. This values obtained are similar for both digesters (D1 and D2), and also similar to predicted values for secondary sludge anaerobic digestion, in the bibliography (Kepp and Solheim 2000).

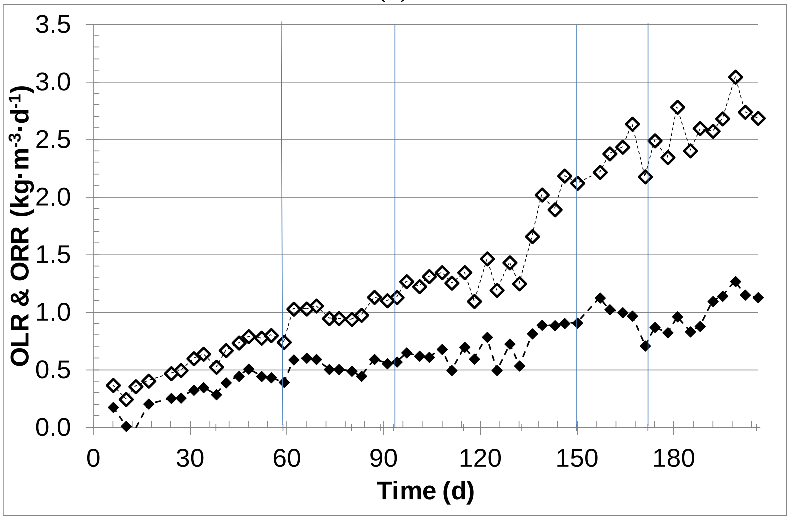
The ratio between ORR and OLR represents the volatile solids removal by the process. This ratio was higher than 55% at the beginning of the operation (between 1^{st} and 69^{th} days) due to the high SRT; after, it decreased stabilizing around 50% when the SRT was around 40d and 30d. The SRT showed significant effect on the anaerobic digestion performance, due to it controlled the microbial population into the anaerobic digesters (Lee et al. 2011). In addition, the anaerobic digestion of secondary sludge has associated low solids removal efficiencies. When the SRT was lower than 25 days, the ratio between ORR and OLR decreased, reaching 40% of VS removal. However, this average value corresponding with typical values of volatile solids elimination on anaerobic digestion of secondary sludge (Coelho et al. 2011).

The VS and COD_T content in the feed and effluent are presented in the figure 6.4 for the digester D2, the corresponding figures of the digester D1 are presented in the appendix of figures. The ratio between feed and effluent content showed the removal ability of the anaerobic digestion process, both ratios were proportional. As mentioned before, the elimination of organic matter was higher at the beginning of the operation, for longer SRTs.

The data enclosed in circles, 10^{th} and 13^{th} days, corresponded to the data which have been identified as points significantly distant from the normality. Both data corresponded to the second week of operation, and that values corresponded to a failure in the thickening operation in the WWTP. As a consequence of the low solid content in the feed, the digesters solid content decreased. However, during the whole start-up process, the VS content increased, recovering the original inoculated value, around 30 ($g \cdot kg^{-1}$).



(a)



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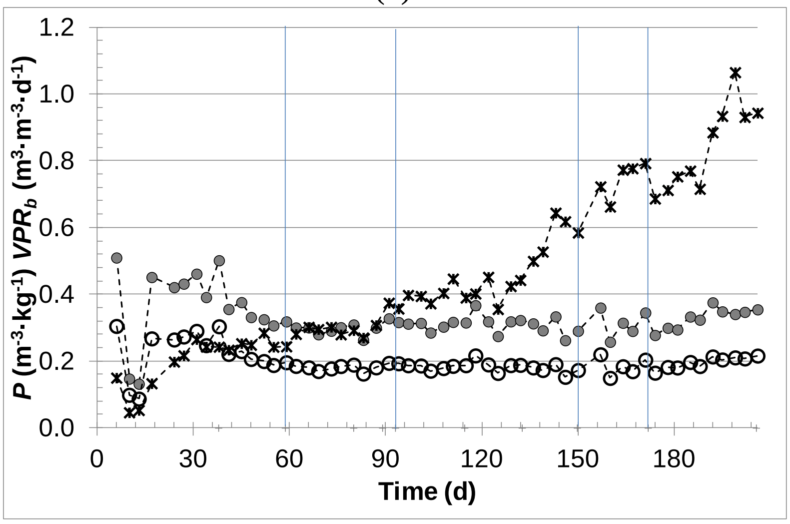


(c)

Figure 6.3. Operational conditions of digester D2 during the start-up period: a) ▲ SRT, b) ◇ OLR and ◆ ORR, and c) productivity of ● biogas, ⊖ methane and ✱ VPR_b.

The anaerobic digestion of nitrogenous organic compounds have associated ammonia production, therefore the N-TKN content, in both feed and effluent, should remain constant. The behavior of the N-TKN showed a significant gap between feed and effluent at the beginning of the operation, and so it increased during the start-up period reaching similar values between feed and effluent.

The N-TKN content increased lineally in the digesters between 1st and 80th days, corresponding with the first wash-up, or volume renewal, of both digester; also coinciding with the destabilization of the anaerobic digestion process detected (starvation period for D1). The second renewal of the digester content was reached at the 140th day of operation, and as a consequence, the next measurement at 150th day of operation, showed a non-statistic difference between feed and effluent content of N-TKN content.

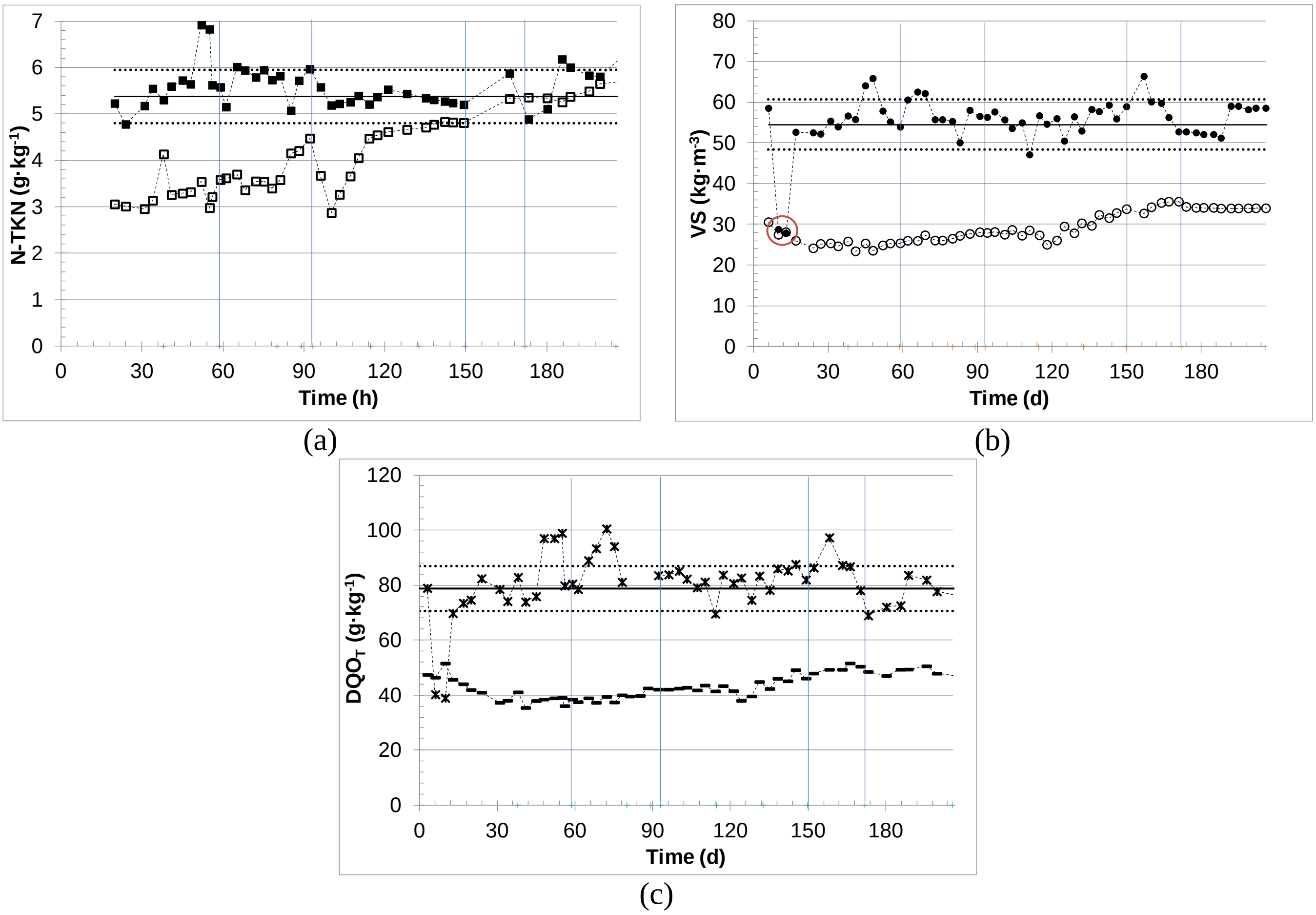


Figure 6.4. Physico-chemical characterization of feed and effluent full sludge samples during the start-up period of D2. a) N-TKN (■ feed, □ effluent), b) VS (● feed, ○ effluent) and c) COD_T (✱ feed, ▬ effluent).

The figures 6.5 y 6.6 presents the characterization of the soluble phase of both digesters. All the parameter studied in the soluble phase, increased during the start-up period, becoming significantly higher than the inoculated ones. The low quality of the supernatant has been indicated as one of the disadvantages of the anaerobic digestion of sludge (Appels et al. 2008).

The first 60^{th} days of operation, the COD_S and the VFA remained almost constant for digester D1, but it increased slowly for digester D2. On the contrary, the $N\text{-}NH_4^+$ and Alk_T increased progressively for both digesters. The relationship between the $N\text{-}NH_4^+$ and Alk_T content is directly proportional, due to the $N\text{-}NH_4^+$ is one of the compounds that provides alkalinity in the anaerobic digestion (Gerardi 2003). Despite, the $N\text{-}NH_4^+$ content reached values of 1.5 ($g{\cdot}L^{-1}$), the ratio between partial and total alkalinity remained almost constant and ranged between 0.18 and 0.22. Even though, the alkalinity ratio is not the best anaerobic digester stability indicator, its negligible variation during the first operational period, showed that the fermentation-methanogenesis equilibrium was well balanced, and thus with an inhibition absence (Speece 2008).

From day 60^{th}, corresponding with an OLR of 1 ($kg{\cdot}m^{-3}{\cdot}d^{-1}$), the COD_S increased lineally on D1 (figure 6.5). Also the VFA content showed a similar behavior, increasing from 1.5 to 2.3 ($g{\cdot}L^{-1}$) in 6 days, causing as a response, the increase of the Alk_R from 0.22 to 0.29. Simultaneously, the ammonia concentration increased lineally, reaching 1.8 ($g{\cdot}L^{-1}$) of $N\text{-}NH_4^+$. All these observations, simultaneously with the methane productivity reduction, suggested the destabilization of the AD process, therefore the feeding was stopped during one week, coinciding with the reduction of the Alk_R to 0.25.

Apparently, the starvation period gave enough time to acclimate the microbial population to the new environmental conditions in digester D1, and although the $N\text{-}NH_4^+$ and COD_S continued increasing during the next 60 days, the Alk_R was stabilized around 0.2 for the remained operation.

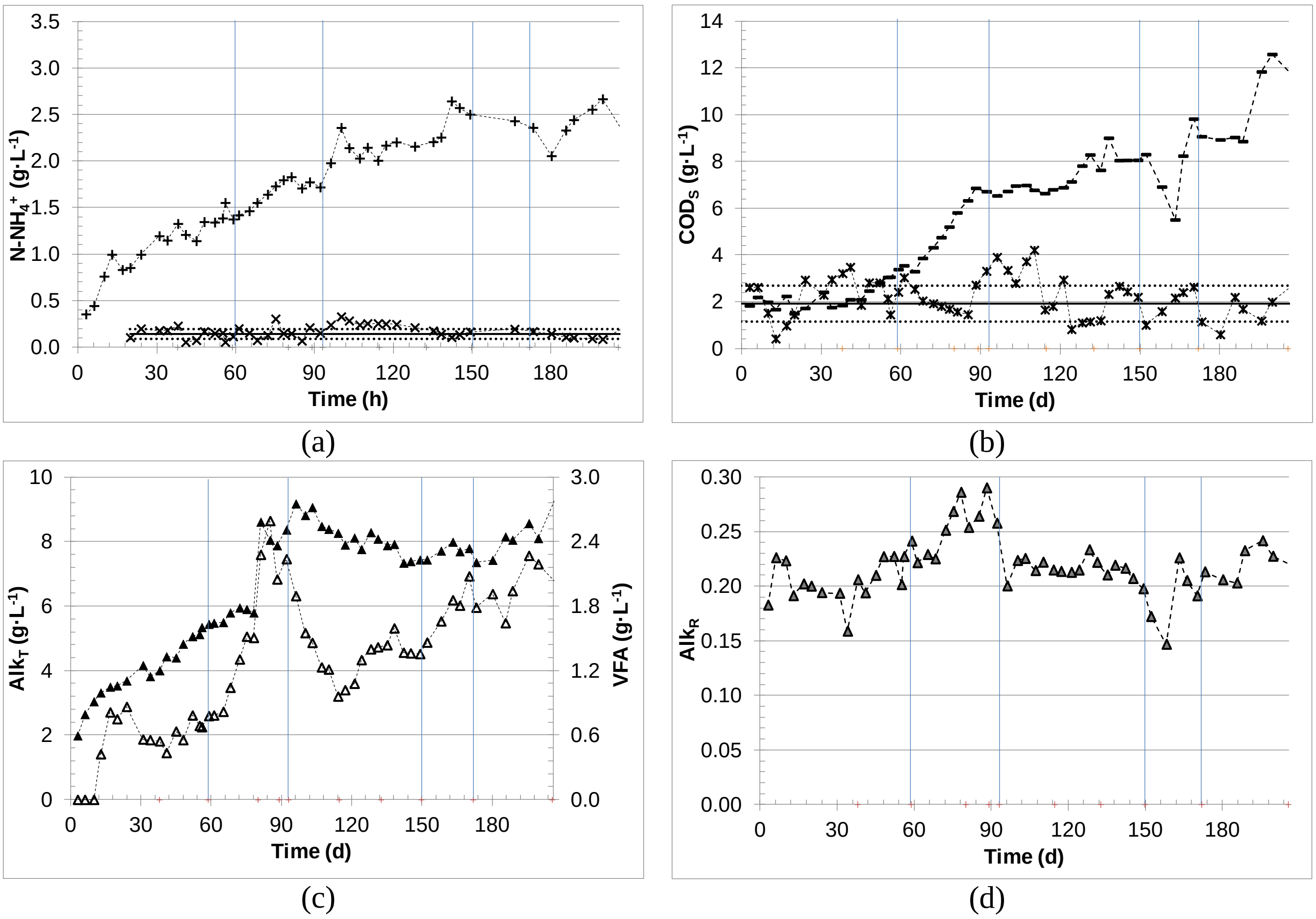


Figure 6.5. Physico-chemical characterization of feed and effluent soluble phase samples during the start-up period of digester D1: a) $N\text{-}NH_4^+$ (✕-feed, +-effluent), b) COD_S (✱-feed, -▬-effluent), c) ▲-Alk_T and △-VFAs, and d) -▲-Alk_R.

As for the digester D1, after the 60th day of operation, the COD_S increased lineally on D2 (figure 6.6). However, the main difference between both digesters was observed in the VFAs content, its content increased, but the maximum value reached was 1.8 ($g\cdot L^{-1}$), and as a result the Alk_R not exceed a value of 0.26. Even though the observed behavior for both digesters was the same in tendency, the maximum values reached by the digester D2 was not as high; therefore the feeding did not stop.

On the other hand, the $N\text{-}NH_4^+$ content remained constant between 40th and 80th days of operation in digester D2, around 1.5 ($g\cdot L^{-1}$), showing a delay in the increase of its concentration respect to the digester D1. This response could explain the lower destabilization on the anaerobic digestion process comparing with D1. The VFA and COD_S increased, due to the OLR increase, but the lower $N\text{-}NH_4^+$ content in D2 allowed the stabilization of it.

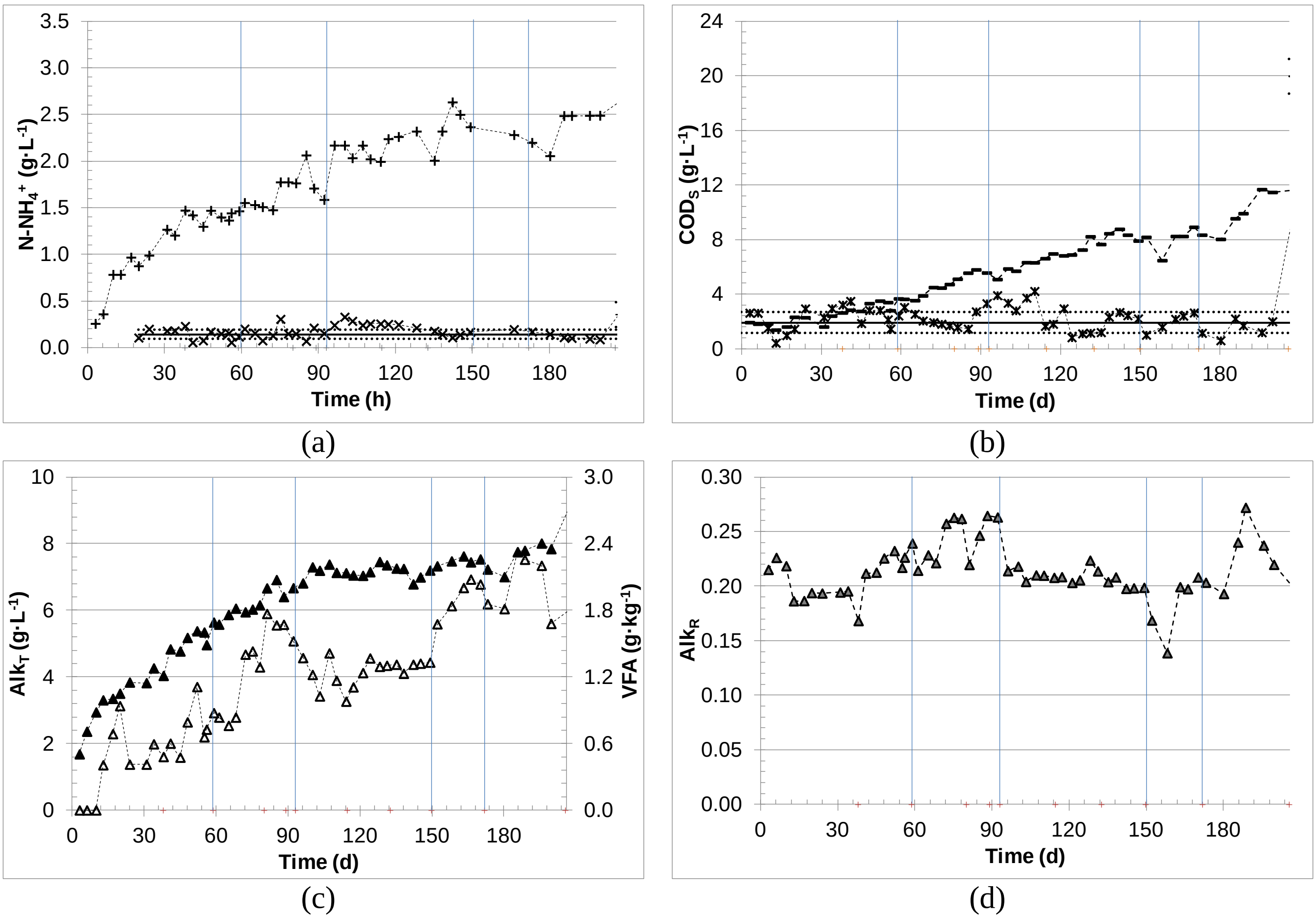


Figure 6.6. Physico-chemical characterization of feed and effluent soluble phase samples during the start-up period of digester D2: a) NH_4^+ (feed, effluent), b) COD_S (feed, effluent), c) Alk_T and VFAs, and d) Alk_R.

The two previous sections showed the digesters behavior during the start-up, and both digesters responded equally to the stimulus. As well the comparison of the digesters performance at steady-state was evaluated between the 150th and 206th days of operation at 23 and 20d of SRT. The evaluation of the steady-state after the 150th day of operation was used due to the nitrogen balance showed the stabilization between feed and effluent content.

Tables 6.4 and 6.5 show the average operational conditions of both digesters. These averages values were calculated using the all data collected in each period.

The comparison of the data collected after the 150th day of operation, with the same SRT of operation for both digesters, showed that both volumetric production of biogas (VPR_b) and methane productivities (P_{CH4}) matched with a 95% of significance, i.e. there was no significant different between both data groups.

Table 6.4. Average operational conditions of digester D1 during the start-up and first-steady state.

Days	Δt (d)	SRT (d)	OLR ($kg \cdot m^{-3} \cdot d^{-1}$)	VPR_b ($m^3 \cdot m^{-3} \cdot d^{-1}$)	P_{CH4} ($m^3 \cdot kg^{-1}$)
0 - 38	38	108 ±26	0.47 ±0.14	0.18 ±0.07	0.254 ±0.101
39 - 59	20	78 ±7	0.75 ±0.05	0.24 ±0.03	0.202 ±0.028
60 - 80	20	59 ±1	0.99 ±0.05	0.28 ±0.02	0.167 ±0.012
81 - 89	8	*	*	0.15 ±0.04	*
90 - 93	3	85 ±11	0.66 ±0.08	0.13 ±0.02	0.142 ±0.010
94 - 115	21	59 ±10	0.94 ±0.17	0.27 ±0.04	0.178 ±0.022
116 - 133	17	48 ±4	1.12 ±0.12	0.35 ±0.04	0.178 ±0.017
134 - 150	16	37 ±7	1.60 ±0.26	0.53 ±0.11	0.196 ±0.029
151 - 172	**21**	**23 ±3**	**2.41 ±0.14**	**0.75 ±0.13**	**0.191 ±0.028**
173 - 206	**33**	**21 ±1**	**2.77 ±0.16**	**0.90 ±0.10**	**0.201 ±0.018**

* Starvation period.

The evolution on the organic content into the digesters showed a fully stabilization too. The VS and COD_T contents did not show any variation during the period without operational changes. In addition, the behavior of N-TKN showed the balance of the organic nitrogen into the digesters, due to no significant difference obtained between feed and effluent content.

The evolution of the physic-chemical parameters in the soluble phase (figures 6.5. and 6.6), during the steady state, showed a stabilization of all parameters measured too, even though some oscillation was observed, due to the VS content oscillation in the feed. The $N\text{-}NH_4^+$ and the Alk_T content stabilized around 2.5 and 8 ($g \cdot L^{-1}$) respectively, and the COD_S oscillated between 9 and 12 ($g \cdot L^{-1}$). The Alk_R increased twice, reaching a value of 0.25, but corresponding with the maximum OLR and COD_S reached.

As a final comparison of the steady state period, it is possible to conclude that any inhibition of the anaerobic digestion process was observed, the biogas production rate, the biogas and methane productivity, and all parameters evaluated both in the soluble phase as in the total one, have no statistical difference between both digesters.

Table 6.5. Average operational conditions of digester D1 during the start-up and first-steady state.

Days	Δt (d)	SRT (d)	OLR ($kg \cdot m^{-3} \cdot d^{-1}$)	VPR_b ($m^3 \cdot m^{-3} \cdot d^{-1}$)	P_{CH4} ($m^3 \cdot kg^{-1}$)
0 - 38	38	110 ±25	0.45 ±0.13	0.18 ±0.09	0.246 ±0.105
39 - 59	20	78 ±7	0.75 ±0.05	0.25 ±0.03	0.207 ±0.021
60 - 80	20	59 ±1	0.99 ±0.05	0.29 ±0.02	0.181 ±0.009
81 - 93	12	51 ±1	1.08 ±0.07	0.33 ±0.04	0.183 ±0.015
94 - 115	21	42 ±3	1.29 ±0.05	0.40 ±0.03	0.183 ±0.009
116 - 133	17	42 ±5	1.29 ±0.14	0.42 ±0.05	0.188 ±0.023
134 - 150	16	30 ±4	1.98 ±0.21	0.59 ±0.10	0.175 ±0.030
151 - 172	**21**	**23 ±3**	**2.43 ±0.19**	**0.74 ±0.15**	**0.191 ±0.037**
173 - 206	**33**	**21 ±1**	**2.72 ±0.17**	**0.88 ±0.12**	**0.198 ±0.018**

6.4.1.3. Hydrodynamic behavior of high solid content anaerobic digester

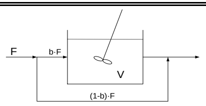
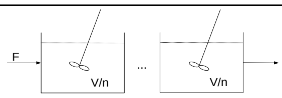
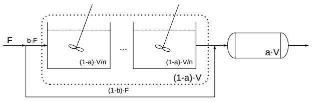
The hydrodynamic behavior of vessels are commonly described through the RTD (residence time distribution), which described the age of the particles distribution in a closed vessel (E).

$$E(t) = \frac{C(t)}{\int_0^\infty C(t)\,dt} \qquad (6.1)$$

The comparison of the age distribution with theoretical curves allows the description of the digester behavior (Levenspiel 1999). The most common comparison method is fitting the experimental data to various combinations of compartments models. However, new models have been described with the same porpoise (Terashima et al. 2009).

With the objective of determine the degree of mixing in the anaerobic digester, the characteristics parameters of different compartment models, presented in the table 6.6, were determined: *b* as the fraction of fluid entering to the mixing zone, *n* as the number of tanks in series, and λ as the delay time of the system.

Table 6.6. Compartment models used for data fitting (Capela et al. 2009; Levenspiel 1999)

Compartment model	Representation	Model
By-pass		$E_\theta = b^2 \cdot \exp(-b \cdot \theta)$
Tanks in series		$E_\theta = \dfrac{n^n \cdot \theta^{n-1}}{\Gamma(n)} \cdot \exp(-n \cdot \theta)$
Gamma distribution with by-pass		$E_\theta = \dfrac{(n \cdot b)^n \cdot \theta^{n-1}}{\left(1 - \lambda/t_{med}\right)^n \cdot \Gamma(n)} \cdot \exp\left(\dfrac{-n \cdot b}{1 - \lambda/t_{med}} \cdot \theta\right)$

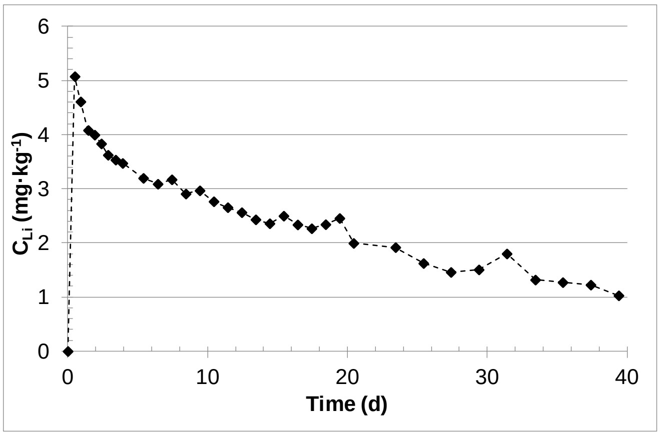
As a response of the solid content increase in the feed, the solid content into de anaerobic digesters increased, therefore the operational conditions such as mixing, pumping and heat transfer can be affected (Jolis 2008; Martin 2000). In consequence, the operation can be converted in an inefficient operation due to the viscosity effect.

Previous studies showed the effect produced by secondary sludge pretreatment on the rheological behavior, reducing the viscosity and so the energy requirement for pumping and mixing (Pham et al. 2010). A good digester mixing condition allows the maximization of the use of the digester volume, and also, ensures the contact between the raw feed with the microbial population into the digester.

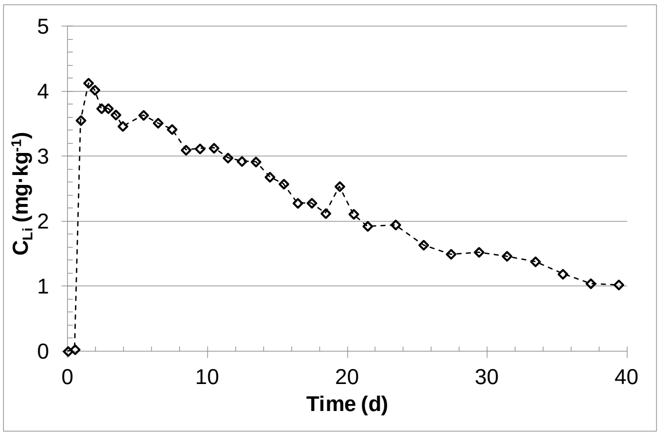
The figure 6.7 presents the lithium content in both samples points, the recirculation and the effluent, during the assay. While, the recirculation reached the maximum concentration in the first sample at 0.5th day, on the contrary, the effluent showed a delay in its content increase, reaching the maximum value one day later than the recirculation one. In addition, the maximum lithium content reached was different for both samples point, while the value reached in the recirculation was the maximum theoretical value expected, coinciding with the amount of Li^+ injected; the effluent point was 1 ($mg \cdot kg^{-1}$) lower than the previous one.

The delay observed in the effluent point, can be caused by the effluent line in the digester, due to the effluent sludge exited through a line-like "O", which provided a watermark to the digester. Clearly, in the effluent line, the sludge showed plug-like displacement, and its volume around 500mL, should corresponds to this delay.

The maximum content reached in the recirculation line showed that the volume of digester was quite similar to the theoretical volume, and also showed a low possibility of a large portion of dead volume.



(a)



(b)

Figure 6.7. Concentration of lithium measured in (a) the recirculation and (b) the effluent samples point, during the residence time distribution experiment.

The mean of the residence time was obtained using the equation 6.2, which is specifically obtained thought mass balances for a pulse experiment. The table 6.7 shows the values obtained for both samples points, the value obtained was around 18% lower than the theoretical.

$$t_{med} = \frac{\int_0^\infty t \cdot C(t)dt}{\int_0^\infty C(t)dt} \cong \frac{\sum_i ti \cdot C_i \cdot \Delta t_i}{\sum_i C_i \cdot \Delta t_i} = \frac{V}{Q} \qquad (6.2)$$

The obtained values of the residence time were lower than the expected, and they showed the possible existence of a stagnant volume (dead zone) into the digester, but on the other hand, the maximum Li^+ content reached showed the opposite information. Other studies have shown inconsistency and inaccuracy of point indices, despite of its ease of use (Smith et al. 1993). Furthermore, the Li^+ recovered during all the experiment was only the 89.7%, and hence the approximation made in the calculation assumed that 100% of tracer is recovered. On the other hand, it is possible a reduction of the real digester volume due to the presence of gas, which would not be strange in an anaerobic digester, then around 18% of the total volume, would be used by gas.

Table 6.7. Experimental mean of residence time distribution.

Sample point	$t_{med, exp}$ (d)	Li^{+} quantified
Effluent	16.7	91.2%
Recirculation	16.4	89.7%

The parameters fitted to each compartment model is presented in table 6.8., the figures are presented in the appendix of figures. The age distribution fitted to different compartments models showed a different behavior than that observed previously. The evaluation of the stagnant zone not provided the expected adjustment; the best value for the calculated parameter was one, i.e. with a negligible stagnant volume. When constrains, which maintaining the parameters values between cero and one, were suspended, the calculated parameter was greater than the unity, a physically impossible value. In conclusion, the age distribution behavior rejected the presence of a stagnant zone.

The tanks in series model showed different values for both samples points. For the recirculation point, the value was closer to one and clearly, the number of thanks increased due to the presence of the effluent line named before.

The presence of canalization into the digester was observed trough the evaluation of the by-pass model. The effluent showed that 13% of the raw sludge was canalized, but only 9% for the recirculation point. The presence of a by-pass into the anaerobic digester showed an important operation problem, due to the production of the Class A sludge not be possible, and as a consequence, a longer SRT will be required for efficient pathogen destruction (Speece 2008).

Finally, the comparison of the gamma distribution with by-pass, which is the mixture of the tanks in series and the by-pass models, with the addition a piston flow reactor at the end of the system, showed the best data fit. This model confirmed the results obtained before: there was a delay time, being more significant for the effluent line, and equivalent to 225mL approximately, and also a canalization have been observed, being equivalent to 30% of the total feed.

The results obtained showed a possible canalization into the digesters, also showed a closely continuous stirred-tank reactor behavior, with a deviation caused by the effluent line in the digester. Considering, that the digester have been working with a TS content higher than 60 ($g \cdot L^{-1}$), and only with sludge recirculation as agitation system, the result obtained was good enough. And for the future operation increasing the agitation velocity, should decrease the mixing departure due to the canalization observed.

Table 6.8. Characteristics parameters of the compartment models used for samples points, Recirculation (Rec) and effluent (Eff).

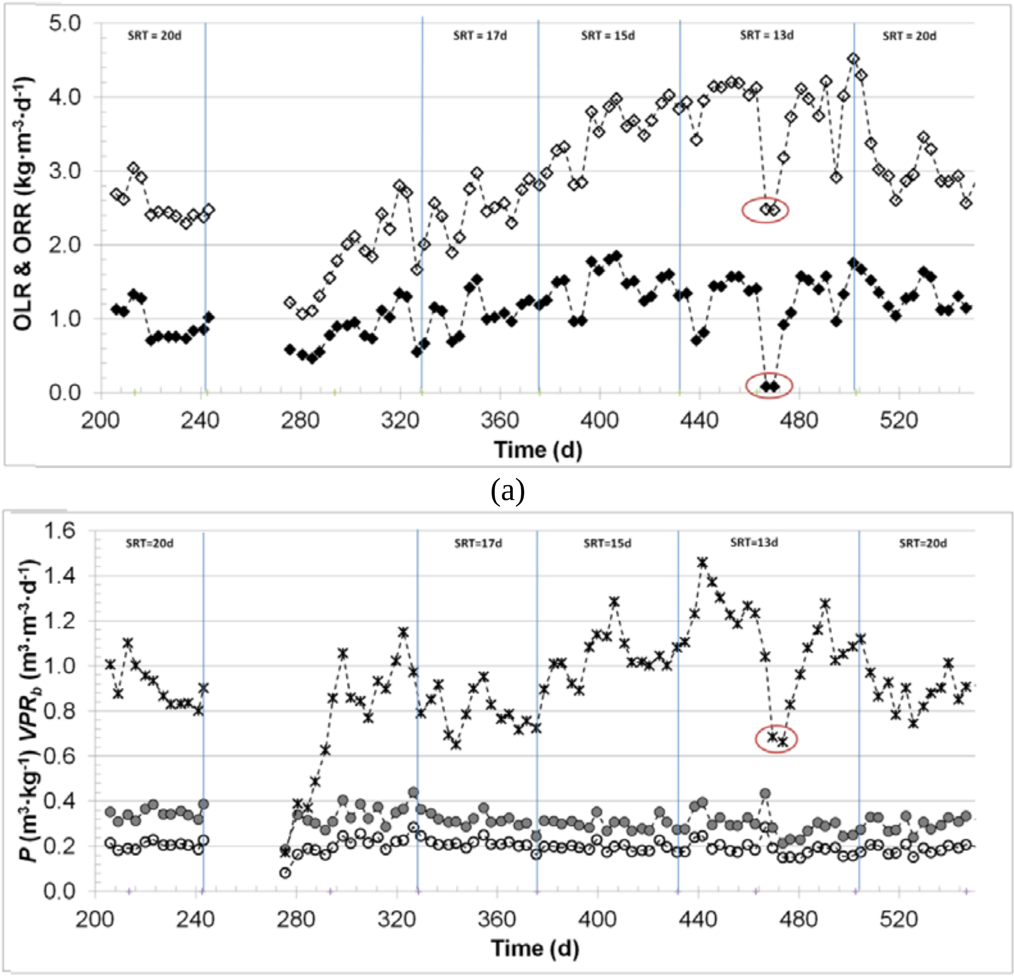
Compartment model		Rec	(RSS)	Eff	(RSS)
Stagnant zone	*d*	1.28	(0.81)	1.43	(1.32)
Tanks in series	*n*	1.05	(0.37)	1.34	(0.37)
By-pass	*b*	0.91	(1.53)	0.87	(1.53)
Gamma distribution with by-pass	*λ*	0.98		4.51	
	b	0.48	(0.02)	0.70	(0.36)
	n	0.88		1.29	

6.4.2. Autohydrolysis pretreatment effect on continuous anaerobic digestion

The main objective of operate two twin digesters in parallel corresponds to the feasibility of realize a comparison during long operation time, an important requirement considering the high duplication time of the anaerobic population, and so as a consequence for the anaerobic digester stabilization (Gerardi 2003).

The comparison of the two anaerobic digesters in continuous operation at different SRT was evaluated. The feed of digester D2 was switched to pretreated sludge in the 206th day of operation. After 40 days of operation (246th) the two digesters were stopped during 30 days and restarted in 40 days. During the half-time period, both digesters were agitated continuously and the temperature reduced until 20ºC reducing the microbial activity in the starvation period. The start over was performed in two steps, the first 10 days both digesters were operated at 40d of SRT, and after the feeding was increased progressively until reached the 17d of SRT condition, around the 20th day both digester should operate at 20-25d of SRT. The 10th day of operation, the agitation pump of digester D2 presented a malfunction, causing the accidental release of 5 liters of digester biomass and an increase in temperature. As a consequence of the volume reduction, the restart of digester D2 was performed slower than the digester D1. After, both digesters worked synchronized.

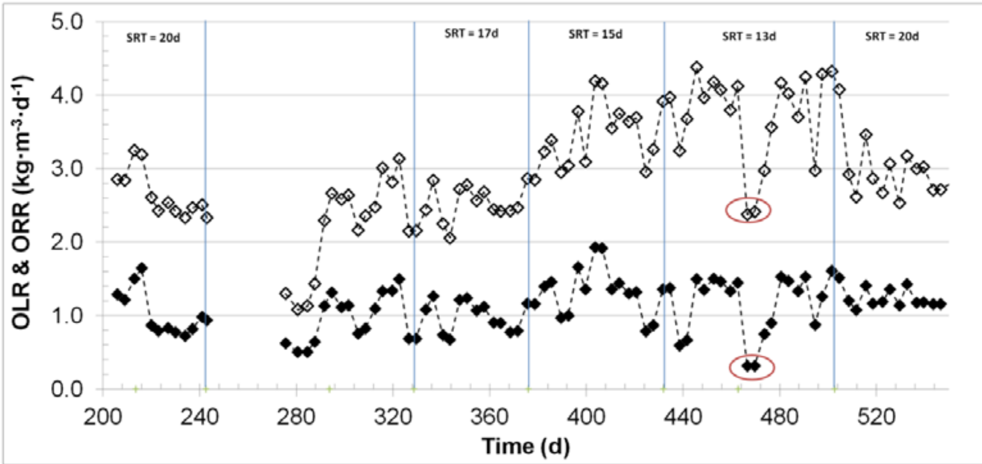
To compare the digesters behavior during the operation, every condition studied was divided in two periods, the first one considered as stabilization, equivalent to one SRT, and the second one considered as the steady state. An additional stabilization period was added at half of the operation period at 13d of SRT, due to a significant reduction of the sludge content because a malfunction of the thickening.



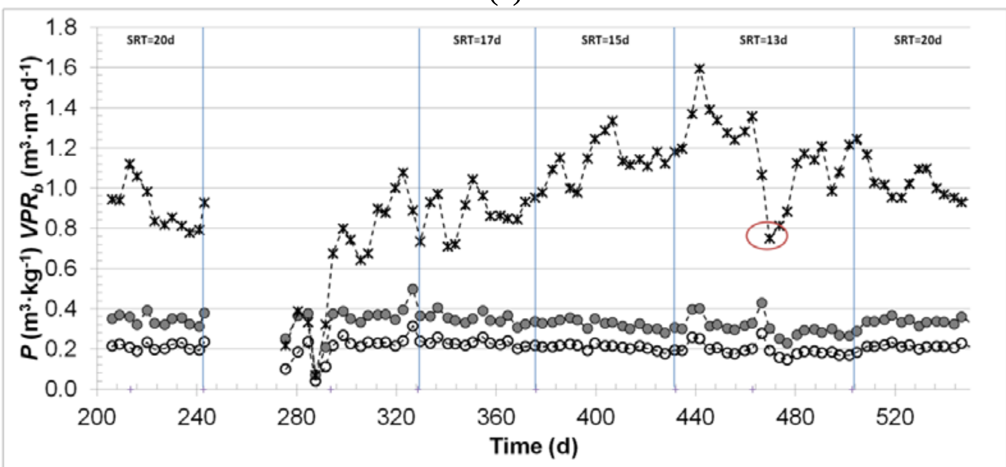
(b)

Figure 6.8. Operational conditions of D1 working with raw sludge: a) ◇ OLR and ◆ ORR, and b) productivity of ● biogas, ○ methane and ✱ VPR_b.

Figure 6.8 and 6.9 presents the operational conditions of the digesters during each stage. As a consequence of the SRT reduction from 20 to 13d of SRT, the OLR increased from 2.5 to 4 ($kg \cdot m^{-3} \cdot d^{-1}$), corresponding to the upper limit of recommended values for anaerobic digestion of sludge treatment (Metcalf et al. 2003). The high OLR reached during the experimentation, showed the feasibility of operate in a stable condition, when concentrated secondary sludge was used as only feed on the anaerobic digestion process. Other studies have also shown the feasibility of operate at high OLR, with a thickened feed as high as possible reducing the water content of the sludge (Duan et al. 2012). The application of a dewatering process with the aid of a high molecular flocculants based on polyacrylamide was used to a mixture of primary, secondary and tertiary sludge, reaching sludge content between 8 to 12 (%) of TS, and as result the anaerobic digesters were operated at 15d of SRT with an OLR of 4 (kg·m-3·d-1) (Nges and Liu 2010). These experimental values were not much higher than the presented in this work, considering that no polyelectrolyte was added, and only secondary sludge was used, which contain lower solids content than the primary sludge.



(a)



(b)

Figure 6.9. Operational conditions of D2 working with pre-treated sludge: a) ◇ OLR and ◆ ORR, and b) productivity of ● biogas, ⊖ methane and ✱ VPR_b.

Table 6.9 shows the average and standard deviation values for the steady state period obtained for both digesters in each condition studied. When the SRT was reduced from 17 to 15 days, the OLR increased for both digesters, showing, as a response, the increase of the

ORR and the VPR_b too. The increase of the removal capacity and the biogas production by the digesters, due to the SRT reduction, showed the digester adaptation trough the different operational conditions (de la Rubia et al. 2006).

The performance of both digesters showed similar behavior, but with different intensities, always the digester D2 showed higher values of removal and biogas production than digester D1. The maximum ORR reached was 1.36 and 1.50 ($kg \cdot m^{-3} \cdot d^{-1}$) for D1 and D2 respectively at 15d of SRT, corresponding with a difference of 10% between them. On the contrary, when the SRT was reduced from 15 to 13d, the VPR_b increased, as was described above, but the ORR decreased for both digesters, showing a reduction of the removal capacity, and therefore a probable destabilization of the anaerobic digestion process; however, the difference of the ORR between them was maintained. Previous studies of the SRT effect on the anaerobic digestion showed the no proportional increase of the OLR and ORR, when the OLR was higher than 4 ($kg \cdot m^{-3} \cdot d^{-1}$) (Lee et al. 2011).

At 467th day, the feed solid content decreased to 30 ($kg \cdot m^{-3}$) of VS, due to the malfunction of the thickening operation in the WWTP. This operational problem was used to evaluate the effect produced in the anaerobic digester process, when a stress dilution condition was produced. The diluted feed was maintained during one week, and after the regular operation was restored in the next step. After the dilution step, both digesters recovered its previous operation condition apparently, however some differences were observed at steady state: the ORR increased but the biogas production decreased; the new operational conditions reached were similar to the representative values obtained at 15 days of SRT. Even though, the removal capacity was increased, the biogas production decreased due to the probably wash-out of some active population. Also, the difference observed between both digesters was reduced, reaching no significant difference between both.

Previous sections in this document showed the autohydrolysis pretreatment improvement on the anaerobic digestion of secondary sludge, but on batch assays, obtaining a methane productivity increase around 20%. The comparison between both digesters operation, showed that the methane productivity was always higher for the digester operated with pretreated secondary sludge than the non-pretreated sludge; but the methane productivity decreased for both digesters when the SRT decreased. The difference of methane productivity between both digesters were 20% at 20d of SRT, 14% at 17d of SRT, 10% at 15d of SRT, and finally become negligible at 13d of SRT. On the other hand, when the SRT returned to 20d, with an OLR of 3 ($kg \cdot m^{-3} \cdot d^{-1}$), the methane productivity increased for both digesters, obtaining a difference of 20% between them.

Table 6.9 present the methane yield, i.e. the methane produced in terms of COD removed, and all values obtained were similar to the stoichiometric ratio ($0.35 m^3 \cdot kg^{-1}$), with the exception of the last value for D1 (20d of SRT), where is probably an indicator of a reduced methanogenic activity.

Other studies which applied continuous or semi-continuous anaerobic digestion after different types of sludge pretreatments obtained a wide range of outcomes about methane productivity improvement, the table 6.10 showed some of its results.

Table 6.10. Sludge pretreatments applied to continuous mesophilic anaerobic digestion.

Sludge type	Pretreatment and anaerobic digestion conditions	Main outcomes	Reference
Mixed sludge (60% suplus + 40% primary)	Mechanical: high pressure homogenizer at 600bar (HPH) Thermal: different temperatures 80 , 90 and 120ºC during 60min Enzymatic: *carbohydrase*	Thermal pretreatment superseded the foam. Methane production increased: 20% thermal, 12% enzyme and 17% HPH.	(Barjenbruch and Kopplow 2003)
Mixed sludge (1:2 / primary/WAS)	Aerobic thermophilic pretreatment: supplied with 0.6Lpm of air	2% of VS reduction improvement. 6% of biogas increase production.	(Borowski and Szopa 2007)
WAS	Microwave (MW) Two-stage anaerobic digestion Microwave + two-stage digestion	40% of biogas improvement for MW and two-stage. 56% of biogas improvement for microwave plus two-stage digestion.	(Coelho et al. 2011)

Table 6.9. Operational conditions at steady-state of both digesters.

Days	SRT (d)		OLR ($kg\cdot m^{-3}\cdot d^{-1}$)		ORR ($kg\cdot m^{-3}\cdot d^{-1}$)		VPR_b ($m^3\cdot m^{-3}$)		P_{CH4} ($m^3\cdot kg^{-1}$)		Y_{CH4} ($m^3\cdot kg^{-1}$)	
Δt (d)	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2
215 – 243 (28)	20 ±1	20 ±1	2.54 ±0.26	2.46 ±0.28	0.93 ±0.28	0.86 ±0.18	0.86 0.07	0.88 0.08	0.200 ±0.019	0.220 ±0.015	0.373 ±0.078	0.380 ±0.090
309 – 329 (20)*	19 ±1	23 ±2	2.66 ±0.39	2.27 ±0.46	1.13 ±0.32	1.01 ±0.31	0.96 0.14	0.92 ±0.12	0.226 ±0.041	0.232 ±0.033	0.375 ±0.113	0.361 ±0.083
346 – 376 (30)	17 ±1	17 ±0	2.60 ±0.17	2.66 ±0.22	1.02 ±0.18	1.18 ±0.20	0.81 0.09	0.92 ±0.09	0.203 ±0.018	0.231 ±0.022	0.358 ±0.058	0.358 ±0.060
391 – 432 (41)	15 ±1	15 ±1	3.59 ±0.42	3.69 ±0.32	1.36 ±0.36	1.50 ±0.26	1.08 0.10	1.17 ±0.09	0.193 ±0.024	0.213 ±0.028	0.330 ±0.066	0.356 ±0.074
445 – 463 (18)	13 ±1	13 ±1	4.08 ±0.20	3.92 ±0.25	1.22 ±0.32	1.35 ±0.26	1.24 0.05	1.30 ±0.04	0.186 ±0.020	0.191 ±0.019	0.345 ±0.027	0.346 ±0.025
483 – 503 (20)	13 ±1	13 ±1	3.96 ±0.48	3.93 ±0.51	1.37 ±0.25	1.45 ±0.25	1.11 0.11	1.16 ±0.09	0.169 ±0.023	0.180 ±0.019	0.335 ±0.060	0.332 ±0.048
524 – 547 (23)	20 ±3	19 ±2	2.85 ±0.22	2.96 ±0.26	1.22 ±0.10	1.30 ±0.19	0.87 0.08	1.00 ±0.07	0.182 ±0.026	0.218 ±0.022	0.288 ±0.049	0.341 ±0.052

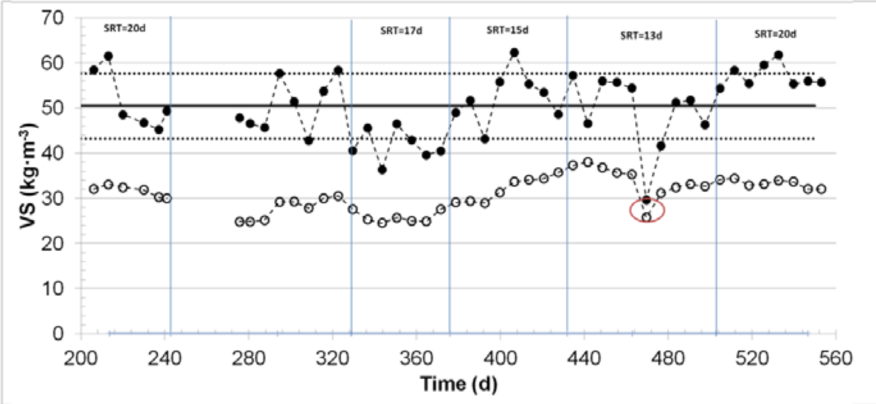
* No steady-state

The full sludge characterization presented in figure 6.10 showed the evolution of the digesters content during each operational period. As was commented before, the sludge solids content during the operation at 17d of SRT was lower than normal values, but the whole period showed a similar operation condition. In the period at 15d of SRT, the solids content increased significantly during the operation, therefore, with the objective of ensure the representatively of the data and also to prevent a possible results alteration, the operation was maintained during 56 days, 4.7 times the SRT.

The significant reduction of organic matter content at 13 days of SRT was after 18 days of steady state operation, equivalent to 1.4 times the evaluated SRT; for this reason the operation was repeated during a similar period of time (20 days at steady state).

Despite the operational conditions showed variations, the data was characterized as a normal distribution, as was shown before. The only exception corresponded to de lower solid content detected in the feed at 13 days of SRT. During this period, the content of organic matter in the feed was lower than inside of the digesters, then the dilution and as a consequence the microbial population loss should occur. But, despite the biogas production decreased and also the reduction of the solid content into the digesters, the solids removal continued at similar levels before the event.

During the whole experimentation period, the N-TKN content in feed and effluent oscillated around 5 ($g \cdot kg^{-1}$), showing the stabilization of the nitrogen content into both digesters.



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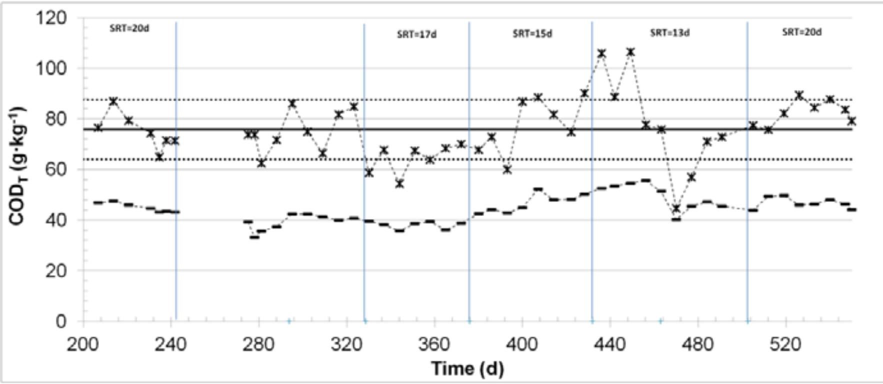


(b)

Figure 6.10. Physico-chemical characterization of feed and effluent full sludge samples of D1: a) VS (-●- feed, -○-effluent) and c) COD_T (-✱- feed, -▬- effluent).

The removal of VS and COD was proportional, and ranged between 34% and 42% at 13 and 20d of SRT respectively for the digester D1, and ranged 35.5% to 44.0% at 13 and 20 days of SRT respectively for D2 (table 6.11). Those values showed that the organic matter elimination was always higher for the digester fed with pretreated sludge. However, the difference between both digesters elimination was small. Other studies of continuous anaerobic digestion which applied some sludge pretreatments showed a wide range of outcomes in this regard, particular results are presented in table 6.10.

The treatment of secondary sludge has associated low capacities of organic matter removal, due to its composition. Even though the sludge is composed by cellular associations (floc), some studies have shown that the most is composed by dead materials such as the exopolimeric substances (EPS), between 80 to 95% (Nielsen et al. 2004). Also, the secondary sludge biodegradability has been predicted trough its biochemical characterization, such as carbohydrates and protein content, and two macroscopic parameters (Mottet et al. 2010), however many studies have shown its low biodegradability associated, ranged 20 to 50% (Speece 2008). Even the comparison with primary sludge showed less than half of methane potential (Gavala et al. 2003). On the other hand, it was showed that the unbiodegradable fraction, originated by the activates sludge endogenous process and also present in the influent wastewater, was non-biodegradable in both aerobic and anaerobic conditions (Ekama et al. 2007).

Table 6.11. Removal efficiencies on steady state.

Days Δt (d)	SRT (d)	VS_e (%)	
		D1	D2
215 – 243 (28)	20	36.7 ±3.3	35.1 ±3.6
346 – 376 (30)	17	38.8 ±5.5	44.4 ±4.9
391 – 432 (41)	15	37.2 ±7.2	40.6 ±5.3
445 – 463 (18)	13	35.1 ±0.9	35.5 ±1.7
483 – 503 (20)	13	34.0 ±4.0	36.3 ±2.7
524 – 547 (23)	20	42.8 ±2.6	43.9 ±3.5

The comparison between raw and pretreated sludge as “pared samples”, during this operation period, showed that the $N\text{-}NH_4^+$ and COD_S were always higher for the pretreated sludge, due to the effect produced by the authydrolysis pretreatment. The figure 6.11 shows the variation of the soluble phase characterization after the pretreatment of autohydrolysis, and corresponding to the feed of the digester D2. The average solubilization factor during this study was **22.5%,** corresponding with the organic matter released from the suspended phase to the soluble one. Also, the $N\text{-}NH_4^+$ content doubled after the pretreatment.

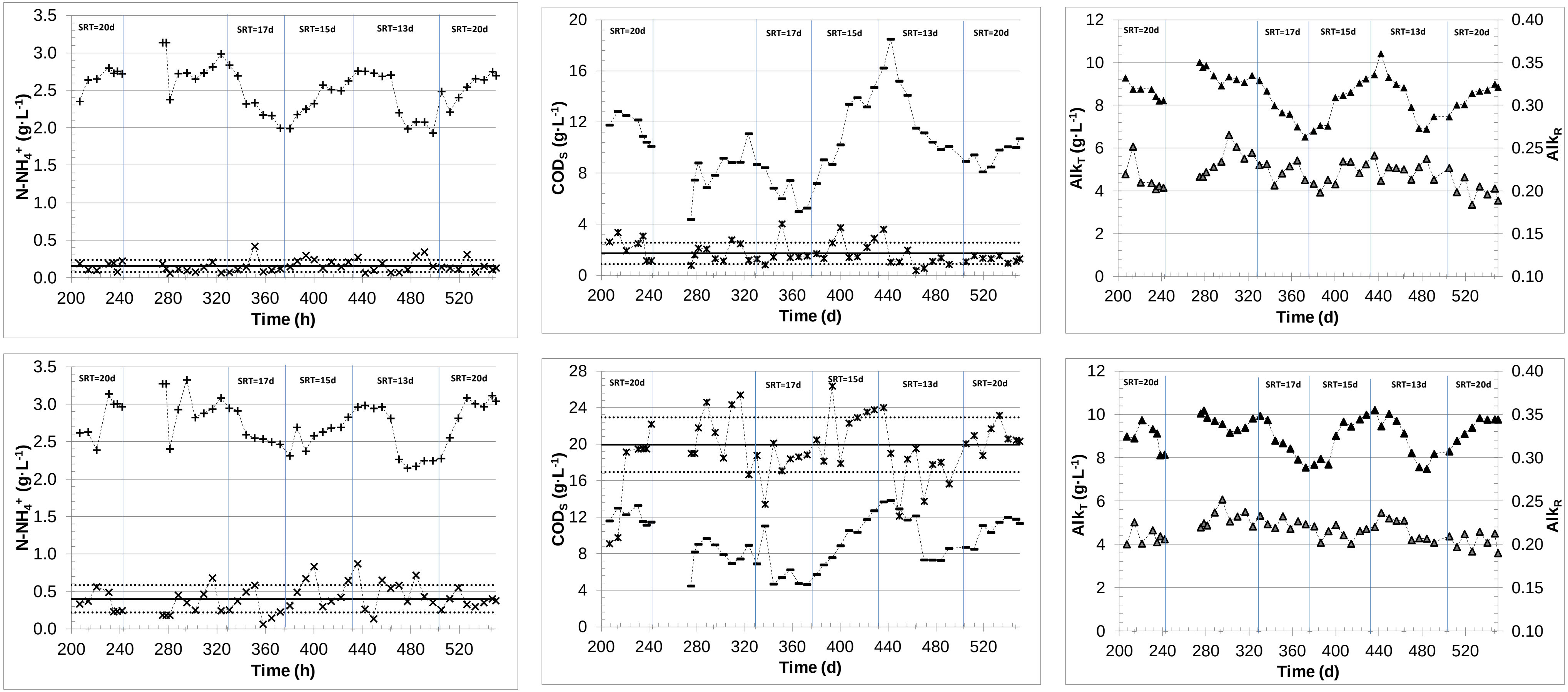
Figure 6.11 presents the evolution of the soluble phase of both digesters during each operational condition. All the parameters measured varied significantly during the different periods of operation. The $N\text{-}NH_4^+$ in the effluent was ranged between 2 and 3.5 (g·L-1), while the COD_S was between 4 and 20 (g·L-1). Both parameters decreased during the operational condition at 17d of SRT, and after showed a significant increase during the operational condition at 15d of SRT, actually the same way behaved the Alk_T, ranged between 6.5 and 10

($g \cdot L^{-1}$). This proportional relationship between the parameter measured in the soluble phase are not surprising, either the anaerobic and the aerobic degradation of nitrogenous organic compounds produced a proportional fraction of alkalinity (Speece 2008). On the other hand, the alkalinity ratio that showed the destabilization of the anaerobic digestion process, only exceeded the value of 0.25 during the start-up period, and during the whole operation was ranged between 0.2 and 0.25, showing that even the ORR decreased at 13d of SRT, the anaerobic digestion process was correctly balanced.

During all the stages evaluated, the average levels for Alk_T and NH_4^+ content, at the steady state, were higher for the digester operated with pretreated secondary sludge than for control digester, one probably reason is that the pretreated sludge has higher NH_4^+ content than the non-pretreated sludge. Therefore, the raw secondary sludge have 0.2 ($g \cdot L^{-1}$) of NH_4^+ content, and the pretreated sludge doubled its content, being its difference ($0.2 g \cdot L^{-1}$) the virtual difference that distinguishes the two digesters. On the other hand, the COD_S content was always lower for the D2 than D1, showing a higher removal capacity on the soluble phase to pretreated sludge.

Table 6.12. Characterization of the soluble phase on steady state condition.

Days Δt (d)	SRT (d)	Alk T ($g \cdot L^{-1}$)		N-NH_4^+ ($g \cdot L^{-1}$)		COD_S ($g \cdot L^{-1}$)	
		D1	D2	D1	D2	D1	D2
346 – 376 (30)	17	9.2 ±0.2	9.5 ±0.3	2.8 ±0.1	3.0 ±0.1	9.6 ±1.3	7.8 ±1.0
391 – 432 (41)	15	7.2 ±0.5	8.2 ±0.5	2.2 ±0.1	2.5 ±0.0	5.9 ±1.1	5.3 ±0.7
445 – 463 (18)	13	8.8 ±0.4	9.6 ±0.4	2.5 ±0.1	2.7 ±0.1	13.1 ±1.7	10.9 ±1.5
483 – 503 (20)	13	9.0 ±0.2	9.6 ±0.5	2.7 ±0.0	2.9 ±0.1	13.6 ±1.9	12.3 ±0.6
524 – 547 (23)	20	7.3 ±0.3	8.0 ±0.4	2.0 ±0.1	2.2 ±0.0	9.6 ±0.6	8.2 ±0.8



(a) (b) (c)

Figure 6.11. Physico-chemical characterization of feed and effluent of soluble phase samples, D1 in the top and D2 in the bottom : a) N-NH$_4^+$ (×-feed, +-effluent), b) COD$_S$ (✱-feed, ‒-effluent), c) ▲-Alk$_T$ and △-Alk$_R$.

6.5. CONCLUSIONS

Despite, the increase in the concentration of inhibitory products in the digesters due to the high solid content of the secondary sludge, the start-up showed the feasibility of the anaerobic inoculum adaptation; however the acclimation time took around 150 days. After, both digesters were operated at steady-state, obtaining a volumetric production rate of biogas of 1 ($m^3 \cdot m^{-3} \cdot d^{-1}$) when the SRT was 20d.

The study of the residence time distribution showed the good mixing condition of the anaerobic digester operated with high solids content and mixed by solids recirculation; however a possible canalization into the digester was detected.

The autohydrolysis pretreatment improved the anaerobic digestion of secondary sludge; the methane productivity increased 20% when both anaerobic digesters worked at 20 days of SRT. When the SRT was reduced, the difference between both decreased, reaching 14% and 10% at 17 and 15 days of SRT respectively, also the difference was negligible at 13d of SRT. On the other hand, the ORR increased 10% due to the pretreatment effect, reaching the corresponding values of 1.36 and 1.5 ($kg \cdot m^{-3} \cdot d^{-1}$) for D1 and D2 respectively at 15 days of SRT.

The autohydrolysis pre-treatment affected the supernatant quality of the digested secondary sludge, the COD_S content decreased in the supernatant of the pre-treated sludge, on the other hand, the $N\text{-}NH_4^+$ content increased in the pre-treated digested sludge, working between 2.5 and 3.2 ($g \cdot L^{-1}$), without an inhibition effect.

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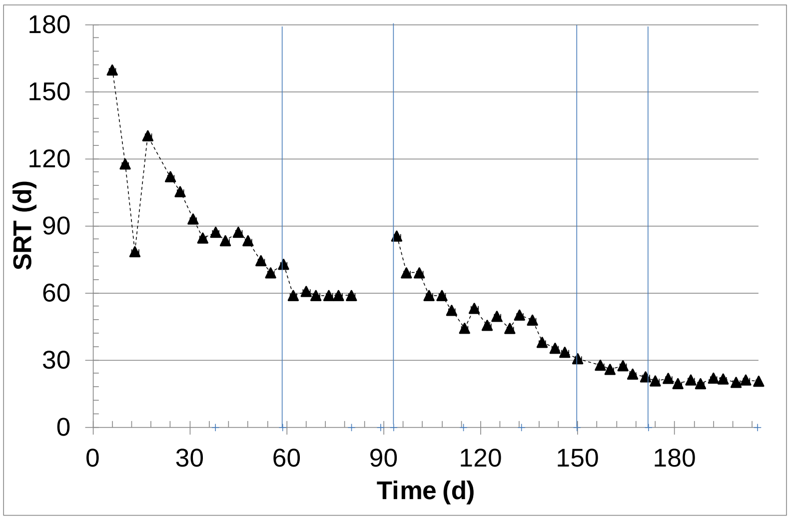
FIGURES APPENDIX



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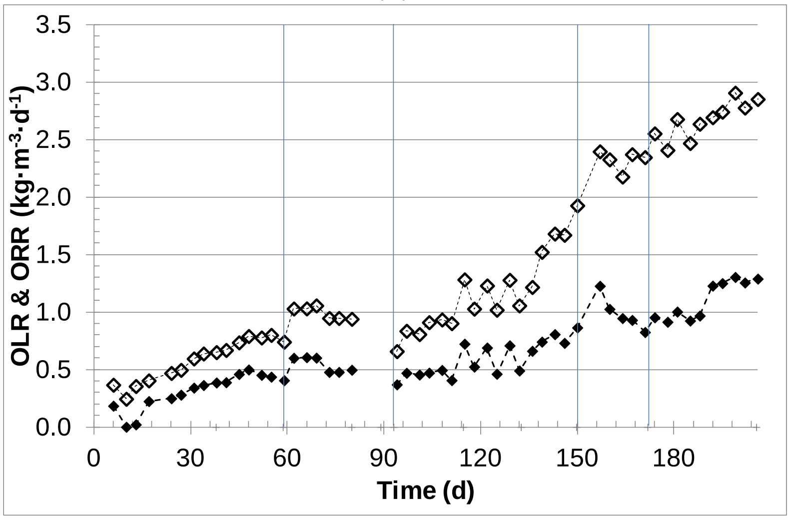

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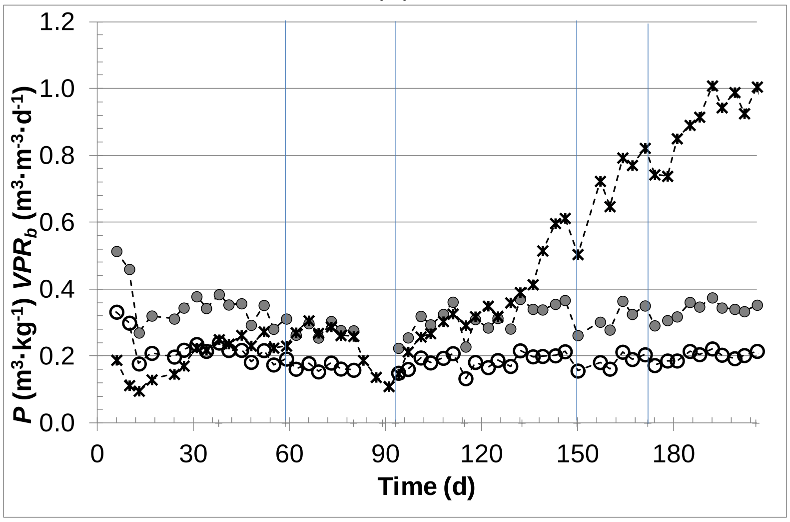
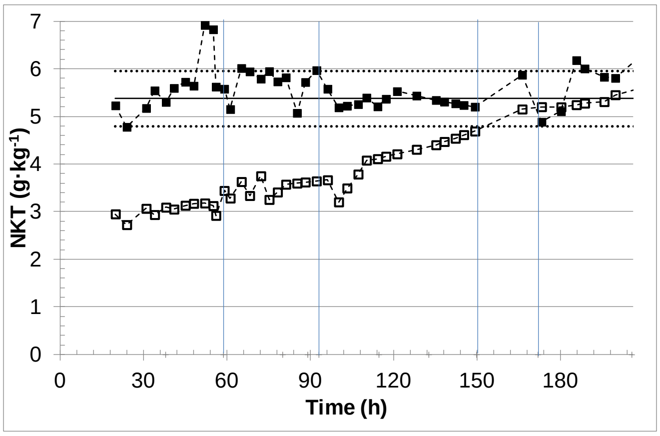


(c)

Figure 6.12. Operational conditions of digester D1 during the start-up period: a) ▲ SRT, b) ◇ OLR and ◆ ORR, and c) productivity of ● biogas, ○ methane and ✱ VPR_b.



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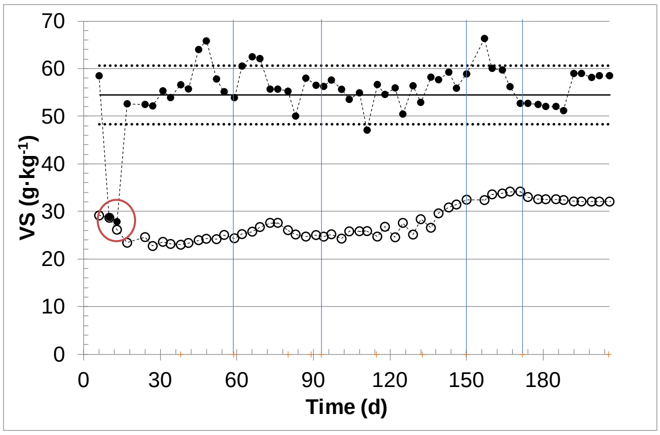

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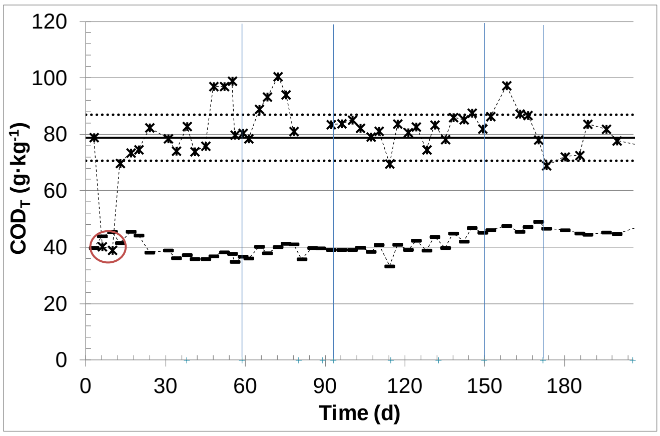


(c)

Figure 6.13. Physico-chemical characterization of feed and effluent full sludge samples during the start-up period of D1. a) NKT (■- feed, □ effluent), b) VS (●- feed, ○effluent) and c) COD_T (✱- feed, -▬- effluent).

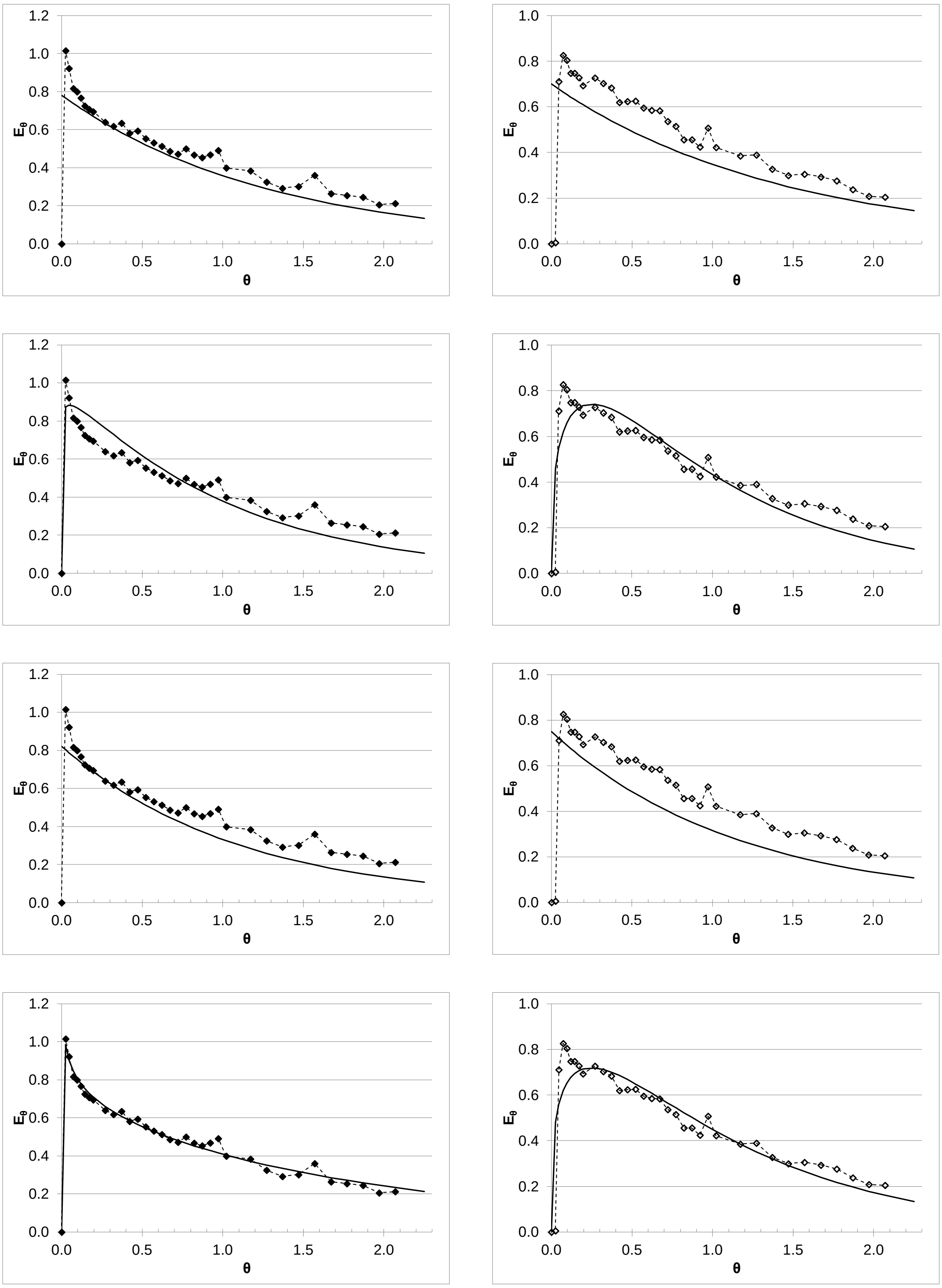
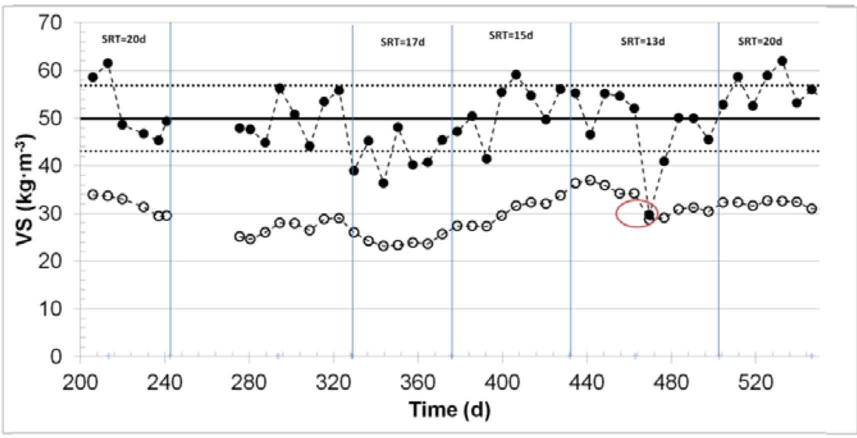


Figure 6.13. Comparison between the experimental age distribution measured in: (-◆-, left) recirculation and (-◇-, right) effluent and the different (—) fitted models: stagnant, tanks in series, by-pass and gamma distribution with by-pass, respectively from top to bottom.



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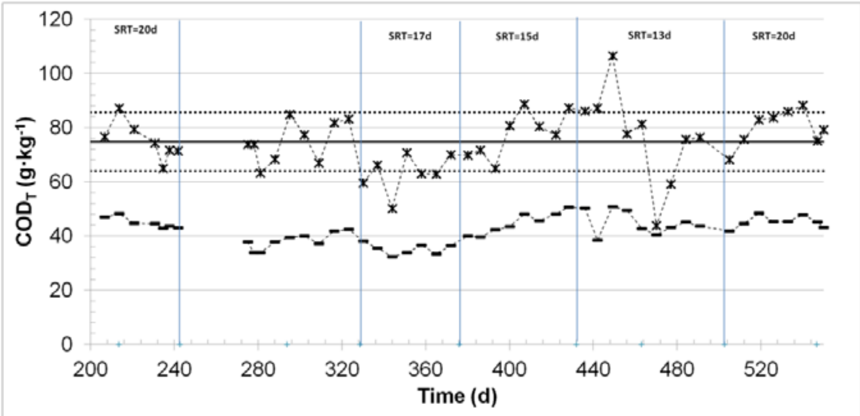

(b)

Figure 6.14. Physico-chemical characterization of feed and effluent full sludge samples of D2:

a) VS (-●- feed, -○-effluent) and c) COD_T (-✱- feed, -▬- effluent).

CHAPTER 7

CONCLUSIONS

CONCLUSIONES

CONCLUSIONS

From the results described in this document, the main conclusion obtained was that the autohydrolysis pretreatment of secondary sludge improved the anaerobic digestion, increasing the methane productivity approximately 20%.

From the specific objectives the conclusions obtained were:

- The study of the autohydrolysis pretreatment with different solids concentration showed the increase in the solubilization factor between 33 to 39%, when the sludge concentration increased from 23 to 54 ($kg \cdot m^{-3}$) of total solids, and the consumption of organic matter was less than 5%. Additionally, the autohydrolysis pre-treatment of secondary sludge with high solid content (~8%) obtained a solubilization factor of 30% after 12 hours of pre-treatment, using a different device with closed bottles, and also practically negligible consumption of organic matter.
- When the pretreatment was carried out in strictly anaerobic conditions instead of microaerobic conditions, a higher volatile fatty acids concentration was obtained. This effect was less significant in the higher sludge concentration, probably due to the difficulty in oxygen transference.
- The second experimental device allowed for the maintenance of oxygen, thus ensuring microaerobic conditions during the pretreatment. At the operational conditions the oxygen consumption was $1.02(mmol_{O_2} \cdot kg_{WAS}^{-1} \cdot h)$.
- The autohydrolysis pretreatment produced a significant change in the rheological behavior of secondary sludge. After the pretreatment, the flow resistance was significantly lower than the original.
- The autohydrolysis pretreatment under microaerobic conditions improved the anaerobic digestion of secondary sludge. The methane productivity was increased by 23% after 12 hours of pretreatment when the assays were performed with a solid content of 54 ($kg \cdot m^{-3}$) of total solids. Additionally, the productivity of methane was improved by 20% during mesophilic anaerobic digestion, when the solid content was ~8%, nevertheless, the autohydrolysis pretreatment did not improve the anaerobic digestion of the sludge when the digestion was carried out under thermophilic conditions.

- The evaluation of the microbial viability showed an important reduction in the live bacteria during the first four hours of pretreatment; nevertheless after eight hours this population had increased. When the population behavior was evaluated through the heterotrophic live bacteria, the mesophilic population decreased being virtually completely eliminated. On the other hand, the thermophilic population growth during the pretreatment.
- The evaluation of the enzymatic activity on the secondary sludge showed that most of the enzymes were associated with the suspended phase in the raw sludge, however as a consequence of the autohydrolysis pretreatment, the enzymatic activities of *amylase* and *protease* increased significantly in the soluble phase.
- The comparison of the pre-treatment effect carried out under different temperatures showed that the higher stability of enzyme activity and also the higher growth of thermophilic population were obtained at 55ºC. Furthermore, the effect of the pretreatment at different temperatures on the anaerobic biodegradability, improved the anaerobic digestion of the secondary sludge, while the other conditions showed negligible effects.
- The proposed mechanism was that release of the organic matter from the secondary sludge floc was produced by the thermal effect of the pre-treatment, however the improvement of the anaerobic digestion was because of the hydrolytic enzymes, which were natives of the secondary sludge and were stimulated due to the autohydrolysis pretreatment conditions.
- The start-up of the two anaerobic digesters working with concentrated secondary sludge showed the feasibility of the inoculums adaptation, however a high acclimation time was required to adapt the microbial population.
- The study of the residence time distribution into the anaerobic digester, working with high solid content secondary sludge and mixed by solids recirculation, showed a good mixing condition; however a possible canalization into the digester was detected.
- The autohydrolysis pretreatment improved the continuous anaerobic digestion of secondary sludge. The methane productivity increased 20% when two anaerobic digesters were compared at 20 days of SRT. When the SRT was reduced, the difference between both decreased, reaching 14% and 10% at 17 and 15 days of SRT respectively, also the difference was negligible at 13d of SRT.

CONCLUSIONES

Luego de los resultados presentados en este documento, se obtiene como conclusión general que el pre-tratamiento de auto-hidrólisis, del fango secundario, mejora la digestión anaerobia, aumentando la productividad de metano en aproximadamente 20%

De los objetivos específicos planteados se obtiene como conclusiones:

- El pre-tratamiento de auto-hidrólisis promueve la solubilización de la materia orgánica del fango secundario. Al aumentar la concentración inicial de sólido desde 23 a 54 ($kg \cdot m^{-3}$) de sólidos totales, el factor solubilización aumentó desde 33 a 39% y su consumo de materia orgánica fue menor al 5% de la materia orgánica total. Adicionalmente, el pre-tratamiento de auto-hidrólisis en fango secundario con un alto contenido se sólidos (~8%) obtuvo una solubilización de 30% luego de 12 horas de pre-tratamiento, usando un nuevo sistema con botellas cerradas. Consistentemente, el consumo de materia orgánica también fue despreciable.
- La presencia de oxígeno durante el pre-tratamiento de auto-hidrólisis controla la producción de ácidos grasos volátiles, cuando el pre-tratamiento se realizó en ausencia de oxígeno, la producción fue mayor. Sin embargo, este efecto fue menos significativo al aumentar concentración de fango, probablemente debido a una mayor dificultad a la transferencia de oxígeno desde el gas a la fase soluble.
- El segundo sistema experimental para el pre-tratamiento de auto-hidrólisis permitió la transferencia de oxígeno desde el gas a la fase soluble, la velocidad de consumo fe $1.02(mmol_{O_2} \cdot kg_{WAS}^{-1} \cdot h)$ para las condiciones experimentales utilizadas.
- El pre-tratamiento de auto-hidrólisis produjo un cambio significativo en el comportamiento reológico del fango secundario. La resistencia a fluir del fango pre-tratado fue significativamente menor a la del fango sin pre-tratar.
- El pre-tratamiento de auto-hidrólisis en condiciones micro-aerobias mejoró la digestión anaerobia del fango secundario. La productividad de metano aumentó un 23% luego de 12 horas de pre-tratamiento, para fango con 54 ($kg \cdot m^{-3}$) de sólidos totales. Adicionalmente, la productividad de metano aumentó en 20% en la digestión anaerobia mesófila (contenido de sólidos ~8%), sin embargo, el pre-tratamiento de autohydrolysis no mejoró la digestión anaerobia termófila del fango secundario.

- La viabilidad microbiana, evaluada con el ratio bacterias vivas/muertas, mostro una importante reducción debido al pre-tratamiento de auto-hidrólisis, sin embargo luego de ocho horas, aumentó nuevamente. El estudio de la población microbiana usando el conteo de bacterias heterotróficas vivas, mostró la reducción de la población mesófila, siendo virtualmente eliminada, debido al pre-tratamiento de auto-hidrólisis, por el contrario, la población termófila creció de forma sostenida durante el pre-tratamiento.
- El estudio de la actividad enzimática del fango secundario mostro que la mayoría de las enzimas se encuentran adheridas al flóculo del fango secundario, sin embargo, el pre-tratamiento de auto-hidrólisis provocó el aumento significativo de la actividad enzimática de *amilasa* y *proteasa* en la fase soluble.
- El estudio del pre-tratamiento a distintas temperaturas mostró que la mayor estabilidad de las enzimas, y el mayor aumento de la población de microorganismos termófilos ocurría a 55ºC. Además, la digestión anaerobia del fango pre-tratado a distintas temperaturas, mejoró la digestión anaerobia del fango secundario tratado a 55ºC, mientras que las otras condiciones estudiadas mostraron efectos despreciables.
- El mecanismo de hidrólisis propuesto consistió en que la materia orgánica suspendida es desorbida del flóculo debido a un efecto térmico, sin embargo la mejora de la digestión anaerobia se obtiene debido a la acción de las enzimas hidrolíticas presentes en el fango, que además son estimuladas por el pre-tratamiento de auto-hidrólisis.
- El arranque de los dos digestores anaerobios operando con fango secundario concentrado permitieron la adaptación del inóculo, sin embargo el tiempo de aclimatación requerido para adaptar la población microbiana fue algo.
- El estudio de la distribución del tiempo de residencia en uno de los digestores operando con fango secundario concentrado y con recirculación de biomasa, mostró buenas condiciones de agitación, sin embargo una posible canalización de la alimentación fue detectada.
- El pre-tratamiento de auto-hidrólisis mejoró la digestión anaerobia del fango secundario. La productividad de metano aumentó en 20% cuando ambos digestores operaron con 20 días de tiempo de retención de sólidos.