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INNOVATION FOR SUSTAINABLE LIVESTOCK

Libro de Actas

II Congreso Español de Gestión Integral de Deyecciones Ganaderas

Barcelona, 9-10 de Junio 2010

International Workshop on Anaerobic Digestion of Slaughterhouse Waste

Barcelona, 11 de Junio 2010

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International Workshop on Anaerobic Digestion of Slaughterhouse Waste

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PRESENTATION

An animal by-product (ABP) is defined as any product of animal origin not intended for human consumption for health and safety reasons (CE, 2002). Around 50% of the body mass of animals entering slaughterhouse is not destined for human consumption (Woodgate and van der Veen, 2004). In the past few decades, the normal treatment of ABPs has been to turn them into meat meal (through a rendering process), separating the fats and leaving the meal for use in animal feed. The rendering industry in Europe are producing around $1.5 \cdot 10^6$ tones of fats and $3 \cdot 10^6$ tones of proteins (Woodgate and van der Veen, 2004). In recent years, because of BSE (Bovine Spongiform Encephalopathy) the economical value of these materials has reduced substantially and in many cases have to be disposed off as a waste (EC, 2002). Further modification of European Parliament and of the Council Regulations about disposal and uses of animal by-products, allows biogas transformation if approved pre-treatments depending on the by-product category (according to BSE risk) are applied (pasteurization, high pressure and temperature or alkaline hydrolysis).

Slaughterhouse wastes are characterized by its high organic content, mainly composed by proteins and fats. Few references are available on quantification, characteristics and anaerobic potential of animal by-products and waste from slaughterhouses. Tritt and Schuchardt (1992) and Edström *et al.* (2003) reported the first reviews of the material flows and possible treatment strategies in German and Swedish piggery and cattle slaughterhouses, respectively. Salminen and Rintala (2002) reported quantities and anaerobic biodegradabilities of waste produced in Finnish poultry slaughterhouses. Recently, Hejnfelt and Angelidaki (2009) and Palatsi (2010) have characterized individual fractions of Danish and Spanish animal by-products, respectively, determining its potential methane yields. The effects of the regulated thermal pre-treatments on bioavailability, biodegradability and biogas potentials have been reported also (Luste *et al.*, 2008; Rodríguez-Abalde *et al.*, 2009).

Because of their composition, high fat and protein content, slaughterhouse waste and animal by-products can be considered a good substrate for anaerobic digestion plants, according to the high potential methane yield. However, slow hydrolysis rates and inhibitory process have been reported. Furthermore, lipids can cause biomass flotation and wash-out, and long-chain fatty acids (LCFA) are produced during lipids hydrolysis by extracellular lipases. Those intermediate are well described as inhibitory species (Alves *et al.*, 2001; Pereira *et al.*, 2005). Also, acidogenesis of amino acids must be coupled with methanogenic microorganisms activity (Miron *et al.*, 2000) which can be negatively affected by ammonia released during this step, since NH_3 is a known inhibitor of acetoclastic microorganisms. For all those reasons, and due to the difficulties of its digestion as unique substrate, large experiences with anaerobic digestion of slaughterhouse by-products (mainly rumen, stomach or intestinal content and sludge from slaughterhouse wastewater treatment plants) consist on their co-digestion with other industrial, agricultural or domestic waste, as a suitable substrate for centralized biogas plants (Angelidaki and Ellegard, 2003; Resh *et al.*, 2006).

More experiences are reported in literature about the anaerobic treatment of slaughterhouse wastewaters. The increased atomisation of carcass dressing and incorporation of washing at every meat-processing stage have increased water consumption in slaughterhouse facilities, and consequently the treatment requirements. Wastewaters from meat-processing industry are normally submitted to a primary treatment producing sludge, generally suitable for anaerobic digestion. Some slaughterhouse wastewater treatment plants have a secondary anaerobic reactor, usually based on UASB or EGSB systems, due to the high organic content of these wastewaters.

Although the reported difficulties in slaughterhouse waste treatment, such as hardly degradable substrate, high organic content, ammonia and LCFA inhibitory phenomena or possible biomass wash-out, some strategies were remarkable. Most of them are based on adapting anaerobic biomass to efficiently degrade these substrates. Addition of adsorbents to protect biomass (Palatsi *et al.*, 2010), the application of feeding patterns like pulse feeding (Cavaleiro *et al.*, 2008) or the addition of easily degradable substrates (Kuang *et al.*, 2006) were demonstrated to achieve that objective. To recover inhibited reactors by LCFA is feasible adopting the appropriate strategy (Palatsi *et al.*, 2009).

Nowadays, many new biogas projects plants in Spain are planning to use slaughterhouse waste to increase energy production and to ensure the economical feasibility of these facilities, mainly in pig farms where current regulations in Spain are promoting biogas production as method for greenhouse gas emissions mitigation. The energy advantages of using ABPs as co-substrates are usually known, but less is known about difficulties that overloading will cause, or the effect of thermal pretreatments, following current regulations, on anaerobic digestion yields.

GIRO Technological Centre researchers have been working in the recent years on the characterization of anaerobic digestion of slaughterhouse waste and its anaerobic biodegradability, on the analysis of its variations depending on the thermal pre-treatment applied, on the study of the dynamics of proteins and lipids during the anaerobic digestion process, on the inhibition phenomena related to LCFA and on methods to prevent this inhibition, or to recover inhibited reactors, and on the characterization of co-digestion of ABP and livestock manure. These studies have been done in the framework of the following projects: BIOESUCA (2004-2005), financed by the Spanish company ABANTIA and the Spanish Ministry of Science and Innovation (MICINN); CAD/CRAI (2004-2007), financed by MICINN; NIREC (2006-2008), financed by European Commission; and the projects on progress PROBIOGAS (2007-2011) and OPA_LAP (2007-2010) financed also by MICINN. Results indicate that it is necessary to tend to design new reactor configurations, or new operational strategies, in order to overcome the limitations imposed by ammonia or LCFA inhibition.

This Workshop organization was an objective of the OPA_LAP project, Optimization of Anaerobic digestion of Lipids from Animal by-Products, focussed on the study of the dynamics of lipids and LCFA, the characterization of LCFA inhibition phenomena and to study methods to prevent this inhibition, including co-digestion practices. In this project, research team at GIRO is collaborating with the group of Dr. Madalena Alves, from University of Minho (Portugal). In the framework of this project, the initial aim of the Workshop was to diffuse results related to lipids dynamics, but it was finally considered that this objective could better achieved in the context of an global overview of opportunities, limitations and challenges of biological energy production from ABP by anaerobic digestion.

The objective of the Workshop is to launch research and applied work related to anaerobic digestion of ABP, to discuss their impact and to guide new studies and facilities for the future. This is a great opportunity to present the restrictions and the challenges of the anaerobic digestion, either from a basic research studies or from full-scale applications point of view. So, this workshop is addressed to researchers, technology agents and industrials. Invited leading international experts will contribute with their lectures, enclosed in this proceedings book, to have a global overview and to descend to the details with the desired scientific rigor.

This Workshop is organized in the framework of EXPOAVIGA - ECOFARM animal farming exhibition and in the framework of the II Spanish Congress on Integral Management of Manure. ECOFARM activities are aiming to build an atmosphere where scientist, industrial suppliers,

consultancy and engineering companies and farmers exchange knowledge and experiences. A desirable objective is to achieve that scientists contaminate farming and related activities sector with their rigorous approach to problems and solutions, and that farmers and related industrials contaminate laboratories with their requirements and inquietudes. We hope that this Workshop will contribute to this aim and that the exchange of experiences and discussion will conclude with results benefiting all participants.

The organizing committee, constituted by Belén Fernández (Workshop Secretary), Jordi Palatsi (Congress Secretary), Francesc Prenafeta (Congress Scientific Committee Secretary), August Bonmatí (Book proceedings edition coordinator) and Anna Ramon (Communication and diffusion) knowledge the support of EXPOAVIGA and entities collaborating in the organization of ECOFARM activities, and the support of Spanish Ministry of Science and Innovation and the Spanish Ministry of Environment and Agriculture. We specially knowledge and thank the lecturers who have accepted our invitation to contribute to the success of this meeting.

Xavier Flotats
President of the Organizing Committee
Director of GIRO Technological Centre

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Anaerobic Digestion of Complex Organic Waste: Perspectives and Opportunities.

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Abstract

In the last 20 years, applications of anaerobic digestion have expanded significantly to new areas within both wastewater treatment, and renewable energy production. This has produced a shift to wastewaters and feed materials outside the normal comfort zone of anaerobic designers and practitioners. In this paper, emerging trends in reactor design are highlighted, as well as measurement of material degradability properties. The classic designs for anaerobic systems are either high-rate anaerobic systems, for moderate strength industrial wastewaters, and mixed digesters for liquid phase (1%-10%) liquid phase solids digestion. Applications of high-rate anaerobic systems have now expanded to dilute wastewaters, while there are new designs (e.g., solid phase leach bed digesters) available for high-solids applications. However, for design to be effective, knowledge is required regarding material degradation rate and extent, particularly for non-standard materials. This can be acquired by batch testing, which is accurate and low cost. However, there are questions over its applicability to performance in continuous reactors. Previous results are reviewed to show that when hydrolysis is rate limiting (e.g., in activated sludge), the batch test is a reasonable, if conservative estimate of performance in full-scale. In complex materials, however, where there is both rapidly and slowly degradable material, hydrolysis rate coefficient was at least 10 times higher in the continuous system (compared to the batch test), and degradability 20% higher. There is therefore a need to modify the batch test specifically for more complex materials.

Keywords

Anaerobic; Batch test; Hydrolysis; Modelling; Reactor design.

INTRODUCTION

Anaerobic digestion has been expanding into new areas within both wastewater treatment, and for production of renewable energy from organic solids. Classic applications for anaerobic digestion have included:

- Relatively well defined, relatively dilute organic solids, such as sewage sludges and manures.
- Highly degradable carbohydrate based soluble wastewaters such as cannery and brewery effluent.

In the past 20 years, these applications have expanded significantly into a wide range of complex materials, such that anaerobic digestion is an applicable technology through most of the wastewater treatment train, and is a leading contender for conversion of low quality organic material to methane. As a biochemical process, anaerobic technologies are noteworthy in that the underlying processes are relatively well understood, as well as their influence on the underlying processes. In addition, modes of failure are well known, and the reasons behind them can be linked to basic physicochemical processes (Batstone and Jensen, 2010; Speece, 2008). Despite this, compared to aerobic processes, anaerobic processes are often designed and operated on very basic principles, including retention time and mass-volumetric loading. This is partly because they are relatively robust and inexpensive to overdesign, but partly also to avoid “risks” associated with optimal design.

On classic applications, anaerobic digestion is an easy default option, as it is known to work well, and is robust. Material will either feed in as a composite mixture of solids (top level substrate in Figure 1),

or as a single soluble intermediate (lower elements on Figure 1). The system is either hydrolysis limited for composites, or acetoclastis limited for simple substrates. Complex material however, can have multiple feeds, with inhibitors such as ammonia and long chain fatty acids that can cause the system to be simultaneously hydrolysis, and acetoclastis limited. In addition, solids levels are often outside the normal selection ranges; either very high (in the case of solid waste and manures), or very low (in the case of domestic sewage). This calls for a more flexible method of process selection and feed material assessment.

This paper aims to assess these two topics in particular that can cause these barriers, of selection of appropriate technology, beyond the simple bimodal choices of mixed and high-rate digesters, and assessment of unusual feed types for feasibility analysis in anaerobic digestion applications.

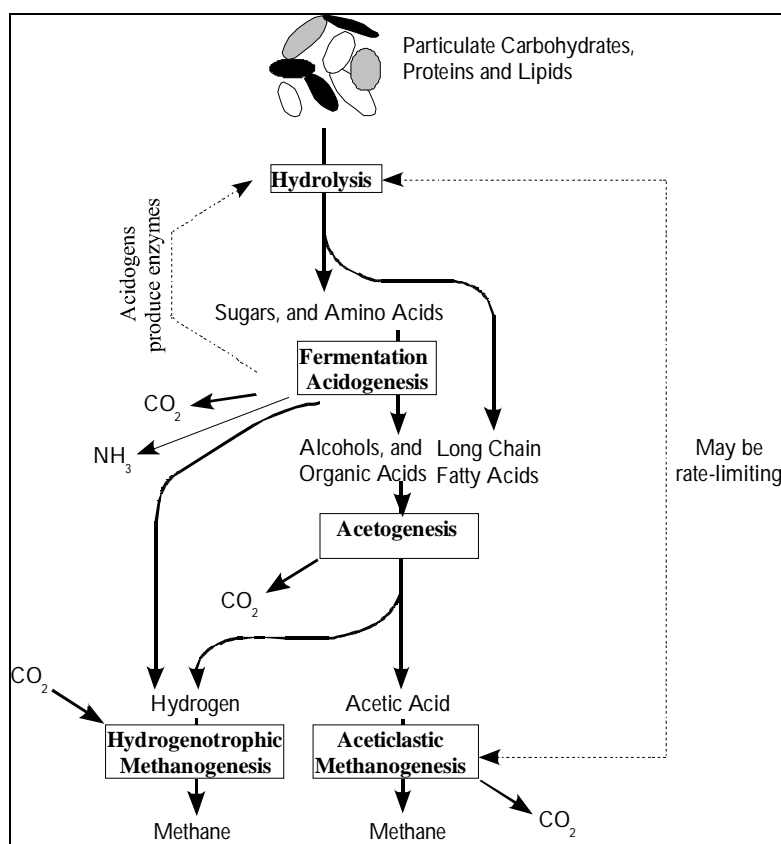


Figure 1. The Anaerobic Digestion Process (Batstone and Jensen, 2010).

TECHNOLOGY

Implementation of anaerobic digestion needs to address the two key issues of (a) maintaining sufficient retention time to allow for hydrolysis of particulate substrates, and (b) providing beneficial conditions for acetoclastic methanogenesis, including maintaining pH above 7.0. Technologies are split among wastewater treatment technologies, which need to focus on goal (b), with extended sludge retention times, but limited liquid retention times, and those which need to focus on goal (a), with extended solids retention times (Figure 2).

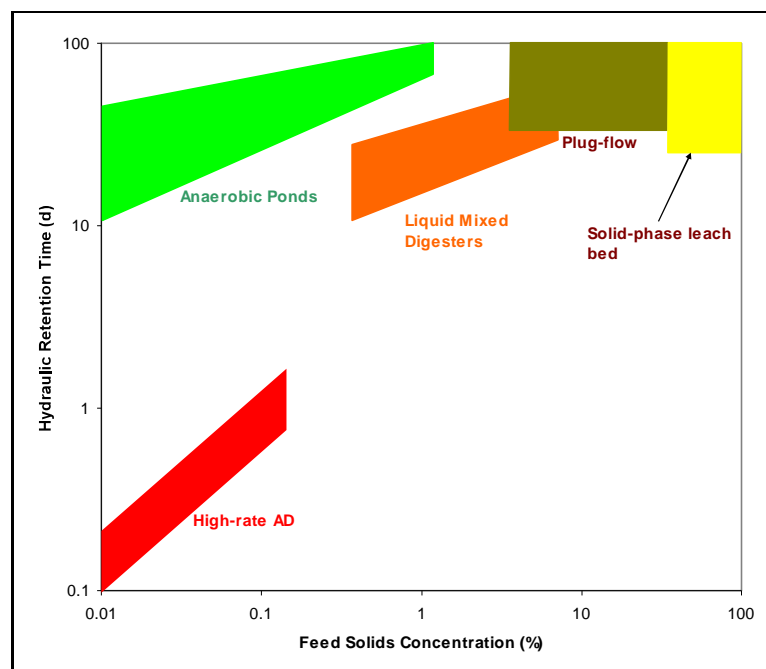


Figure 2. Anaerobic Treatment Technologies.

Treatment technologies are summarised in Table 1, and include:

High-rate anaerobic digestion. High rate anaerobic digesters normally operate with extended solids retention time, and short hydraulic retention times, by integrating solids retention within the main digester. The most common type is an upflow anaerobic sludge blanket (UASB) reactor, which relies on a naturally forming granular sludge blanket (particle size 1mm), through which the liquid percolates. They require a low solids feed, with relatively high amounts of soluble feed material, and are most often used for domestic sewage treatment, as well as industrial wastewaters (van Lier 2008). Hydraulic retention times are normally short with <48 hours, while solids retention times can be very long.

Anaerobic ponds. These are a low capital cost option, but tie up land, and require desludging approximately 10 years, which can be excessively expensive (\$200/dry tonne). Overall costs are heavily driven by solids loading. Methane capture is relatively poor. Because of the large volumes, correction under failure can be extremely expensive or impractical.

Liquid mixed digester. These operate as a fully mixed system, with either gas recirculation, or mechanical mixing. Because they need to be mixed, the maximum in-reactor solids concentration is approx. 6%. Costs are relatively high.

Liquid plug flow. These operate as a semi-solid liquid (10%-20%) in a long polyethylene tube. Material is loaded at the front of the digester, and passes through to product at the end. As it is not mixed, contact with biomass is poor.

Solid phase (leach bed). This is similar to an engineered, high-rate landfill, where material is loaded in a reactor, tumbler, or baskets, and leachate liquid is circulated through the reactor. It can be in either batch (where the system is reacted until no more methane is produced), or continuously (where material is continually added, and spent material removed). The latter is considerably more expensive.

Table 1. Anaerobic digestion technologies.

| Technology | Principle | Advantages | Disadvantages |
|-----------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| High rate/UASB | Mainly liquid wastewater flows upwards through a granular bed. | Low footprint, low capital cost, very stable, produces good effluent. | Intolerant to solids. |
| Anaerobic pond | Large retention time mixed vessel. | Low capital cost | Very high footprint. Must be desludged. Methane capture poor. Can produce odors. |
| Mixed Tank | Dilution to 3-6%, and continuous feed in mixed tank. Retention of 20 days. Used in many industries | Established tech Easy to control Continuous gas production | Poor volumetric loading rate Expensive tanks Need dilution liquid Liquid (not solid) residue |
| Liquid plug-flow (RCM) | Dilution to 15%, and feed through a liquid plug-flow reactor | Very high loading rates. Continuous gas production. | Need dilution liquid. Poor contact with active biomass. Liquid residue. |
| Batch solid phase | Fill and react in a solid phase reactor. Can be an engineered landfill (but must be properly sealed). System is loaded, enclosed, and leachate/inoculum circulated intermittently. | Can be very cheap. Very high loading rates. Good gas conversion due to retention of active biomass. Easy to control via leachate. No milling required. | Non-continuous system (gas flow changes in quality and flow over time). Can be difficult to seal (gas seals). Needs loading and unloading |
| Continuous dry solid phase (plug-flow) | Continuous feed of solid phase through a system. Recirculation of leachate around solid phase. | Continuous gas and residue production. Do not need dilution liquid Very good loading rates. | Extremely high capital costs, and only really practical at very large scale. Very complicated mechanical system. Potential solids handling issues. |

There are a couple of observations that can be made from this figure, and the accompanying text:

- Well known technologies that are widely applied in their target range (e.g., 2 g/L-20 g/L COD for high-rate technologies) can be applied well outside this range.
- There are a wide range of alternative technologies available, particularly at higher levels that are very compelling compared to alternative. As an example, solid-phase leach bed digestion (solid phase digestion coupled with high-rate digestion), with a 20 day retention time is an extremely compelling alternative to high-cost alternatives such as incineration.

MATERIALS PROPERTIES

Material property assessment is critical to proper design of anaerobic processes. They determine both overall feasibility of a process, as well as performance for a given loading rate. For well known materials such as activated and primary sludge, and highly soluble carbohydrate based wastewaters, the material properties are well known. However even these processes are normally built with a significant

overdesign factor to account for variation in wastewater properties. For complex wastewaters, the risk is significantly higher, representing a barrier to implementation. Research and case studies are invaluable, but there is little substitute for testing on the actual wastewater. The most widely used test of materials properties is the modified biochemical methane potential (BMP) test (Angelidaki *et al.*, 2006). On hydrolysis limited substrates, this can provide both degradability extent (f_d), and 1st order hydrolysis coefficient (k_{hyd}). These are the two key properties necessary for feasibility and design in mixed digesters. It is less useful for highly soluble wastewaters, but a modified activity test can be used in these cases.

The BMP test involves placing substrate with inoculum (normally in a 50% volume ratio), and measuring methane production over time. Fitting of a first order kinetic curve allows for estimation of the two parameters. Estimation of the two parameters should be simultaneous, and consider fully parameter correlation and uncertainty, using a repeatable objective function such as sum of squared errors (RSS). The 95% uncertainty limits of the correlated parameter set can then be estimated by an F distribution in F as has been previously described (Batstone *et al.*, 2009). This gives quantifiable estimates of the quantity and uncertainty in both rate and extent of degradation.

The test is relatively straightforward, low cost, and accurate. However, there are a number of questions in relation to full-scale design and analysis:

- How representative is the batch test of expected performance in full-scale?
- How does batch test applicability change when applied to complex materials?

Here, we will compare batch test vs continuous testing on two scenarios – waste activated sludge – a well defined composite material, and thermally hydrolysed waste activated sludge, a complex material.

The waste activated sludge tested was tested in both batch, as well as a two-stage laboratory scale mesophilic digester similar to Ge *et al.*, (2009). Results are further reported in Batstone *et al.*, (2010b). The results are shown in Figure 3. This shows a two-parameter confidence space for degradability (x -axis), and rate coefficient (y -axis). All parameter sets within the space are statistically identical for that experiment.

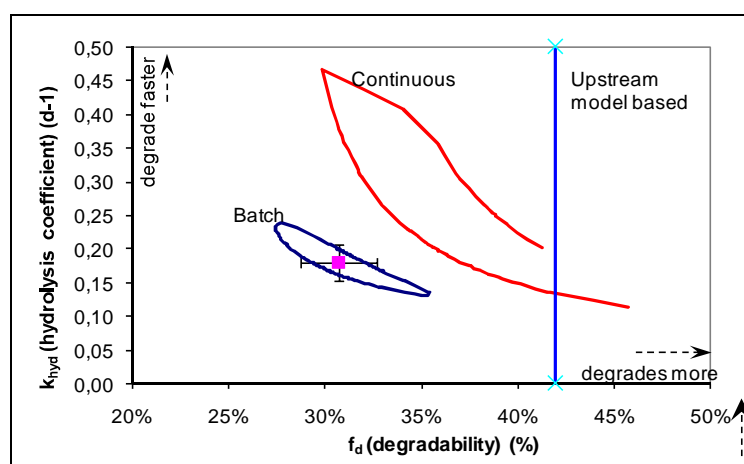


Figure 3. Comparison of confidence regions, indicating confidence in degradability (f_d), and hydrolysis coefficient (k_{hyd}) for a batch test, continuous reactor analysis, and using an upstream model to determine activated sludge degradability. From Batstone *et al.*, (2010b), used with permission.

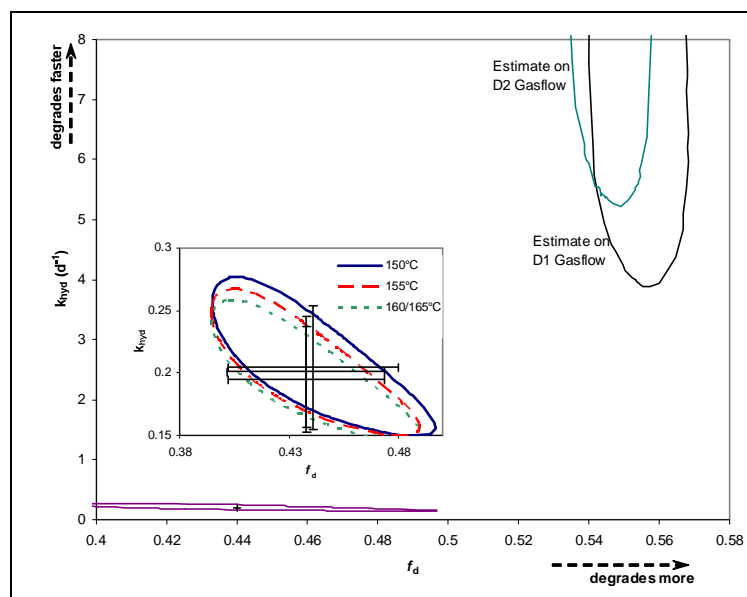


Figure 4. Comparison of confidence regions, indicating confidence in degradability (f_d), and hydrolysis coefficient (k_{hyd}) for a batch test, continuous reactor analysis, and using an upstream model to determine waste activated sludge degradability. Modified from Batstone *et al.*, (2009).

The main conclusion is that the batch test (a) far more accurate than the continuous test, with a much smaller confidence region, and that (b) it is slightly conservative compared to the continuous test with a lower median degradability and rate coefficient. It is however, a reasonable, if conservative representation of the performance that would be expected in the full-scale system.

The same analysis done on thermally hydrolysed waste activated sludge is shown in Figure 4, comparing a batch test to estimates at full scale (from Batstone *et al.*, 2009). This indicates that (a) hydrolysis rate as measured in the full-scale systems is dramatically (over an order of magnitude) higher, and (b) that degradability is considerably higher (>20%).

The reason that the batch test is a poor representation on the complex thermally hydrolysed material as compared to the digester is likely that the thermally hydrolysed material has both a highly degradable, and slowly degradable fraction. In the batch test, methane production rate is controlled by the microbial activity rate, and the slowly degradable material, whilst in the full-scale system, dynamics are controlled by the rapidly degradable material. Therefore, as material complexity increases, a batch test becomes a poorer predictor of performance that would be expected in a full-scale process.

CONCLUSIONS

In this paper, we have raised a number of emerging issues that may enhance or limit applicability of anaerobic processes to complex wastewaters and wastes. Anaerobic reactor technologies have emerged substantially from the previously available high-rate systems (applicable to medium strength industrial wastewater), and mixed digesters (applicable to liquid phase solids digestion). There are now a wider range of reactors available in both highly concentrated and dilute applications. In particular, solid phase leach bed reactors can be applied as an alternative to other organic solids disposal options.

A continuing issue is prediction of degradability and rate of materials, particularly for those which have been limited in application previously. Batch testing is an effective and low cost method for testing speed and extent of anaerobic digestion. Our analysis has shown that batch testing is slightly conservative, but representative of hydrolysis controlled substrates such as activated sludge. However, it is not representative of complex materials where both rapidly and slowly degradable materials simultaneously exist.

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Use of animal by-products of slaughterhouses for the production of biogas. Legal aspects and characterisation

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Abstract

Slaughterhouses are one of the most important sources of animal by-products. These materials are regulated due to public and animal health reasons. The regulation classifies by-products according to their risk and specifies which uses are allowed for each one. Biogas production is an option for the valorisation of animal by-products coming from slaughtered houses, but these materials are also demanded for other purposes as pet food production. Potential changes in feed regulations could also threaten the profitability of biogas production.

Keywords

Animal by-products; biogas production; slaughterhouse.

INTRODUCTION

Animal by-products not intended for human consumption (ABP) are a potential source of risks to public and animal health, as past crises have shown: outbreaks of foot-and-mouth disease, the spread of transmissible spongiform encephalopathy or the presence of dioxins in feeding stuff. These crises were linked in their origin to improper use or management of certain ABP, resulting in a reintroduction of these products in the feed chain. These crises had also a strong adverse impact on society, by their impact on the socioeconomic situation of the farmers and of the industrial sectors concerned, as well as on consumer confidence in the safety of food and products of animal origin. Environment has been also affected due to problems related with the disposal of ABP, as well as regarding biodiversity.

In 2002 the European Parliament and the Council laid down Community health rules (Regulation (EC) n° 1774/2002) concerning ABP. These rules, based on scientific advice, were aimed at protecting the safety of the food and feed chain by way of classifying ABP into three categories according to their potential risk and setting for each category specific conditions for collection, transport, handling, treatment, transformation, processing, storage, placing on the market, distribution, use or disposal.

In 2009 a new ABP regulation has been approved (Regulation (EC) n° 1069/2009), which will start to apply in 2011 (Reg. 1069/2009). This regulation, which will repeal the former one, tries to improve it, clarifying its objectives, considering scientific and technical progress since 2002, reducing administrative burden and being more proportionate to the real level of risks arising from the different ABP.

In Spain, the ABP regulation was implemented by *Real Decreto 1429/2003, de 21 de noviembre, por el que se regulan las condiciones de aplicación de la normativa comunitaria en materia de subproductos de origen animal no destinados al consumo humano*. This Decree, among other measures, creates the *Comisión Nacional de subproductos animales no destinados al consumo humano*, in which all competent authorities concerned by this regulation are represented. As a first step, the *Comisión*

published a white book on ABP (MAPA, 2007), a comprehensive analysis from different points of view of the production and management of ABP in Spain. The white book gathers the works of eleven working groups dedicated each one to a specific part of the management chain of ABP. Working group nº 3 was responsible for the analysis of ABP generated in slaughterhouses.

This communication will try to characterize, under the point of view of the ABP regulation, what animal by-products are generated in slaughterhouses; which requisites have been set for their disposal by anaerobic digestion; and which other possibilities for their use and management are allowed by the regulation that could threaten their potential use for the production of biogas.

MATERIALS AND METHODS

The review of the legal requisites required for the production of biogas from ABP have been worded considering the current legal framework (Reg. 1774/2002) as well as the one which will apply from march 2011 (Reg. 1069/2009).

The same documents have been used to review other possible ways for the management of those ABP, as well as figures on the use of ABP for the production of pet food, as presented by ANFACC in the seminar “*Jornadas SANDACH*” which took place last 29-30 April 2010 (Guadalajara, Spain).

The estimation of the amounts of ABP generated in slaughterhouses has been made using the average quantities per slaughtered animal of different species included in the final report of the Working Group nº3 on ABP of slaughterhouses, during the elaboration of the white book. These figures have been applied to the number of animals slaughtered in Spain in 2008, according to the statistical information elaborated by the *Ministerio de Medio Ambiente, y Medio Rural y Marino* of Spain (ANFAAC, 2008).

RESULTS

Processing requirements for the production of biogas

Under Reg. 1774/2002, ABP must receive a treatment before being used in a biogas reactor for the production of biogas. The processing requirements depend on the category and type of ABP to be used. This regulation classifies the by-products in three categories, according to their potential risk for human and animal health, and specifically for the food and feed chain. Category 1 is for those materials presenting maximum risk, and category 3 for those presenting minimum potential risk. Category 3 materials come mostly from slaughterhouses, food industry, food retailers and kitchen waste.

Category 1 materials cannot be used for the production of biogas, unless using the high pressure hydrolysis process (annex III of Reg. 92/2005), which requires prior processing of the raw material using method 1 (133°C, 3bar, 20 minutes), a treatment of the defatted material at a minimum temperature of 220°C for at least 20 minutes at an absolute pressure of at least 25 bar. The resulting material is then mixed with water and fermented in a biogas reactor and the biogas shall be combusted rapidly in the same plant at a minimum temperature of 900 °C followed by rapid chilling. Digestion residues must be disposed of as material of Category 1.

Category 2 materials must be processed prior to their use in a biogas reactor, in an authorised processing plant using pressure sterilisation, also called “method 1” (133°C, 3bar, 20 minutes). However, the following category 2 ABP do not require any treatment before being introduced in the reactor: manure, digestive tract content separated from the digestive tract, milk and colostrums.

Category 3 materials must be submitted to a pasteurisation/higienization (70°C, 60 minutes, and 12 mm of maximum particle size entering the unit). Milk, milk products and colostrums of category 3 does not require this higienization.

Biogas plants must be equipped with a pasteurisation/higienization unit which cannot be by-passed, and with installations for monitoring temperature against time, recording devices for this parameters and a safety system preventing insufficient heating. The pasteurisation unit is not compulsory if it transforms only material that has been processed in other plant or material which does not require pasteurisation.

The competent authority may authorise the use of other standardised process parameters. The applicant must demonstrate that such parameters ensure minimising of biological risks. The demonstration must include a validation carried out according to the procedure specified in Reg. 1774/2002 (Annex VI, chapter II.C.13a). Generally speaking, this validation includes a complete identification and analysis of possible risks; a risk assessment evaluating how processing conditions are achieved under normal and atypical circumstances; a full definition of processing conditions; a measure of the reduction of viability/infectivity using endogenous indicator organisms or test organism or virus; and a control programme monitoring the functioning of the process.

Digestion residues can be used as fertilisers and soil amendments if they do not come from category 1 raw material.

In the framework of the new ABP regulation, requirements for the production of biogas from ABP are still under discussion between the experts of the European Commission, the member states and the European Food Safety Agency (EFSA).

Characterization of ABP generated in slaughterhouses

In slaughterhouses a variety of ABP are generated during its operations. It is difficult to calculate global quantities or amounts per category of ABP, for it depends on a large number of variables, as the species of origin, the size of the slaughterhouse, the season of the year or the preferences of the customers (for instance, the size of the carcasses or their presentation), which may vary significantly between regions. Table 1 includes a list of ABP that can arise in slaughterhouses.

Considering the processing requirements for biogas production, these raw materials can be classified in the following groups: ABP not suitable for biogas production; ABP requiring prior processing (method 1); ABP requiring only pasteurization/higienization; and ABP not requiring treatment (Table 2).

The white book on ABP includes an estimation of the average amount of by-products obtained by animal slaughtered of different species. Applying these values to the number of animals slaughtered in Spain (2008), an estimation of the total amount of ABP produced in slaughterhouses is obtained (Tables 3a, b, c and d).

According to the groups made in Table 2 regarding process requirements, the amounts of slaughterhouse ABP produced in 2008 in Spain are estimated in Table 4.

ABP requiring only pasteurization are probably the most interesting group for the production of biogas in slaughterhouses, as they do not require being prior processed in a processing plant, and are next to 79% of the total amount of ABP in slaughterhouses.

However these are precisely the ABP with have more possible uses allowed by ABP Regulation, and with the exception of some of them (fur or feathers) with specific management problems, they are demanded for the production of processed animal protein and feeding stuff. For instance, ANFACC (ANFAAC, 2008) estimates that an average of 1.500.000 tons of ABP (from slaughterhouses and rendering plants) is used in Spain every year for the production of pet food. This means that there is an active market and strong demand for category 3 material originated in slaughterhouses.

Table 1. Types of ABP in slaughterhouses and categorization.

| By-product | Category |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| Hides and skins Fur, feathers Stomachs Intestines not SRM Other viscera (lungs, liver, spleens, etc.) Bones, horns, hooves, etc. Legs, heads, necks (poultry) Fat Blood | 3 |
| Digestive tract content and manure from transport or boxes Viscera rejected for human consumption Viscera and parts of carcasses rejected for human consumption due to the presence of residues of veterinary drugs and contaminants exceeding the permitted levels Parts of carcasses rejected for human consumption Animals dead during transport or while waiting for slaughtering Manure from transport or boxes Material collected when treating waste water in slaughterhouses in which SRM is not removed | 2 |
| Specified risk material (SRM) Viscera and parts of carcasses rejected for human consumption due to the presence of certain prohibited substances and residues or environmental contaminants or residues Material collected when treating waste water in slaughterhouses in which SRM is removed | 1 |

Furthermore, there is a debate at UE level reconsidering the prohibition of feeding farm animals with meat and bone meal. Should this prohibition be lifted, the demand for processed animal protein will significantly raise.

Other problem that may arise is the management of digestion residues. They can be a good quality fertiliser, due to the nature of the raw material used, but in case the market couldn't admit all the quantities produced, the operators should face extra costs disposing of these residues.

Table2. Processing requirements for the use of slaughterhouse ABP for biogas production.

| Processing requirements | BY-PRODUCT |
|-------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ABP not suitable for biogas production | Specified risk material Viscera and parts of carcasses rejected for human consumption due to the presence of certain prohibited substances and residues or environmental contaminants or residues Material collected when treating waste water in slaughterhouses in which ERM is removed |
| ABP requiring prior processing (method 1) | Viscera and parts of carcasses rejected for human consumption due to the presence of residues of veterinary drugs and contaminants exceeding the permitted levels Animals dead during transport or while waiting for slaughtering Material collected when treating waste water in slaughterhouses in which ERM is not removed |
| ABP requiring only pasteurization/higienization | Fat Blood Stomachs Intestines not SRM Other viscera (lungs, liver, spleens, etc.) Bones, horns, hooves, etc. Fur, feathers Legs, heads (poultry) Hides and skins |
| ABP not requiring treatment | Manure from transport or boxes Digestive tract content |

Table 3.a. Production of ABP in slaughterhouses –Ruminants.

| | Bovine < 12 months | | Bovine > 12 months | | Ovine | | Caprine | |
|---------------------------------------------------|------------------------------|--------------------|------------------------------|--------------------|--------------------|--------------------|--------------------|-------------------|
| Average weight (carcass) | 230 kg | | 325 kg | | 13,5 kg | | 13,5 kg | |
| N° animals slaughtered | 945.434 | | 1.494.971 | | 12.555.904 | | 1.154.508 | |
| By-product | kg/ carcass | Total 2008 | kg/ carcass | Total 2008 | kg/ carcass | Total 2008 | kg/ carcass | Total 2008 |
| Hides and skins | 35 | 33.090.190 | 42 | 62.788.782 | 2,5 | 31.389.760 | 2,5 | 2.886.270 |
| Fur | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Stomach | 0 | 0 | 0 | 0 | 0,2 | 2.511.181 | 0,2 | 230.902 |
| Other viscera | 3,25 | 3.072.661 | 5,25 | 7.848.598 | 0,2 | 2.511.181 | 0,2 | 230.902 |
| Bones, hornes, hooves, etc. | 20 | 18.908.680 | 35 | 52.323.985 | 0,4 | 5.022.362 | 0,4 | 461.803 |
| Fat | 30 | 28.363.020 | 50 | 74.748.550 | 0,25 | 3.138.976 | 0,25 | 288.627 |
| Blood not destined for human consumption | 18,5 | 17.490.529 | 18,5 | 27.656.964 | 3 | 37.667.712 | 3 | 3.463.524 |
| SRM | 22,5 | 21.272.265 | 35 | 52.323.985 | 0,3 | 3.766.771 | 0,3 | 346.352 |
| Digestive tract content | 30 | 28.363.020 | 50 | 74.748.550 | 1 | 12.555.904 | 1 | 1.154.508 |
| Viscera rejected for human consumption | 2,75 | 2.599.944 | 2,75 | 4.111.170 | 0,2 | 2.511.181 | 0,2 | 230.902 |
| Parts of carcasses rejected for human consumption | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TOTAL | 162 | 153.160.309 | 238,50 | 356.550.584 | 8,05 | 101.075.028 | 8,05 | 9.293.790 |

Table 3.b. Production of ABP in slaughterhouses –Mammals non ruminants.

| | Pigs | | Equidae | | Rabbits | |
|---------------------------------------------------|--------------------|------------------------|--------------------|------------------------|--------------------|------------------------|
| Average weight per carcass | 80 kg | | 240 kg | | 1,2 kg | |
| N° animals slaughtered | 41.395.592 | | 30.563 | | 42.501.220 | |
| By-product | kg/ carcass | Total 2008 (kg) | kg/ carcass | Total 2008 (kg) | kg/ carcass | Total 2008 (kg) |
| Hides and skins | 0 | 0 | 20 | 611.260 | 0,2 | 8.500.244 |
| Fur | 1,2 | 49674710 | 0 | 0 | 0 | 0 |
| Stomach | 0,6 | 24837355 | 0 | 0 | 0 | 0 |
| Intestines | 0 | 0 | 0 | 0 | 0,377 | 16.022.960 |
| Other viscera | 2,8 | 115907658 | 0 | 0 | 0 | 0 |
| Bones, hooves, etc. | 0,108 | 4470724 | 26 | 794.638 | 0,098 | 4.165.120 |
| Fat | 2,76 | 114251834 | 15 | 458.445 | 0 | 0 |
| Blood not destined for human consumption | 3 | 124186776 | 26,6 | 812.976 | 0,045 | 1.912.555 |
| SRM | 0 | 0 | 0 | 0 | 0 | 0 |
| Digestive tract content | 3 | 124186776 | 10 | 305.630 | 0 | 0 |
| Viscera rejected for human consumption | 0,25 | 10348898 | 55 | 1.680.965 | 0 | 0 |
| Parts of carcasses rejected for human consumption | 0 | 0 | 0 | 0 | 0,029 | 1.232.535 |
| TOTAL | 13,718 | 567.864.731 | 152,6 | 4.663.914 | 0,749 | 31.833.414 |

Table 3.c. Total production of ABP in slaughterhouses (mammals). (Table 3.a and 3.b).

| By-product | TOTAL (Kg) |
|---------------------------------------------------|----------------------|
| Hides and skins | 139.266.506 |
| Fur | 49.674.710 |
| Stomach | 27.579.438 |
| Intestines | 16.022.960 |
| Other viscera | 129.571.000 |
| Bones, horns, hooves, etc. | 86.147.312 |
| Fat | 221.249.452 |
| Blood not destined for human consumption | 213.191.036 |
| SRM | 77.709.373 |
| Digestive tract content | 241.314.388 |
| Viscera rejected for human consumption | 21.483.060 |
| Parts of carcasses rejected for human consumption | 1.232.535 |
| TOTAL | 1.224.441.770 |

Table 3.d. Production of ABP in slaughterhouses –Poultry. Note: Only broilers and laying hens have been considered.

| | BROILERS | | LAYING HENS | | |
|-----------------------------------|-------------------------|------------------------|--------------------|------------------------|--------------------|
| Average weight per carcass | 2,350 kg | | 2 kg | | |
| Animals slaughtered (2008) | 579.541.100 | | 36.850.400 | | |
| By-product | ABP /carcass (g) | Total 2008 (kg) | g/carcass | Total 2008 (kg) | TOTAL (kg) |
| Manure | 7,99 | 4.630.533 | 29 | 1.068.662 | 5.699.195 |
| Dead animals in transport | 23,5 | 13.619.216 | 20 | 737.008 | 14.356.224 |
| Blood | 94 | 54.476.863 | 80 | 2.948.032 | 57.424.895 |
| Feathers | 164,5 | 95.334.511 | 172 | 6.338.269 | 101.672.780 |
| Other viscera | 117,5 | 68.096.079 | 266 | 9.802.206 | 77.898.285 |
| Legs | 94 | 54.476.863 | 60 | 2.211.024 | 56.687.887 |
| Necks and heads | 188 | 108.953.727 | 112 | 4.127.245 | 113.080.972 |
| Liver, gizzard, fat | 110,45 | 64.010.314 | 176 | 6.485.670 | 70.495.984 |
| TOTAL | 799,94 | 463.598.106 | 915,2 | 33.718.116 | 497.316.222 |

CONCLUSIONS

The use of animal by-products in slaughterhouses for biogas production in annexed plants it's an interesting alternative for the management of ABP, as it means a treatment in place, avoiding extra costs of collection and transport, and allowing an energetic valorisation of this materials.

However, there are several threatens to the profitability of this projects: The ABP more suitable for biogas production (as they could be pre-treated in place) are also demanded by other sectors. Other ABP may be not so profitable, for them need to be processed by pressure sterilisation prior to their use in the biogas plant. The demand for ABP of category 3 could increase significantly if the feed ban were revised by the European Union, because the production of feeding stuff could potentially be a more profitable way for the management of ABP than the biogas production. The operators will have to find a way to eliminate digestion residues if the market does not admit all this residues as fertilisers, and this may mean extra cost or less income.

Table 4. ABP produced in Spain (2008), classified according to process requirements.

| | BY-PRODUCT | MAMMALS | POULTRY | TOTAL |
|-------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|--------------------|---------------|
| ABP not suitable for biogas production | Specified risk material | 77.709.373 | | 77.709.373 |
| | Viscera rejected for human consumption and parts of carcasses rejected for human consumption due to the presence of certain prohibited substances and residues or environmental contaminants or residues | | | |
| | Material collected when treating waste water in slaughterhouses in which ERM is removed | | | |
| ABP requiring prior processing (method 1) | Viscera rejected for human consumption and parts of carcasses rejected for human consumption due to the presence of residues of veterinary drugs and contaminants exceeding the permitted levels | 22.715.595 | | 37.071.819 |
| | Animals dead during transport or while waiting for slaughtering | | 14.356.224 | |
| | Material collected when treating waste water in slaughterhouses in which ERM is not removed | | | |
| | Fat | 221.249.452 | | |
| | Blood | 213.191.036 | 57.424.895 | |
| ABP requiring only pasteurization/hygiene | Stomachs | 27.579.438 | 70.495.984 | 1.359.963.217 |
| | intestines | 16.022.960 | | |
| | Other viscera (lungs, liver, spleens...) | 129.571.000 | 77.898.285 | |
| | Bones, horns, hooves... | 86.147.312 | | |
| | Fur, feathers | 49.674.710 | 101.672.780 | |
| | Legs, heads, necks (poultry) | | 169.768.859 | |
| | Hides and skins | 139.266.506 | | |
| | Manure from transport or boxes | | 5.699.195 | |
| | Digestive tract content | 241.314.388 | | |
| | TOTAL | 1.224.441.770 | 497.316.222 | |
| ABP not requiring treatment | | | 247.013.583 | |

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Anaerobic co-digestion of treated slaughterhouse wastes

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Abstract

Co-digestion of solid pasteurized slaughterhouse pig waste (PSW) with pig manure (PM), and in a final step with residual glycerine from biodiesel production (in order to increase C/N ratio), was studied in a mesophilic continuous stirred reactor. The reactor operation was divided in 5 periods, with an organic loading rate (OLR) between 2.5-3.2 kgCOD·m⁻³d⁻¹ and a hydraulic retention time (HRT) between 20-33 days. Significant differences between period 1 and the rest were observed and also positive differences related to methane potential were found in period 5 (higher C/N ratio). Inhibition processes due to ammonia and/or VFA concentration were not observed in any period. The biodegradability of every waste in mesophilic batch assays was previously assessed. It was started up (period 1) with an adapted inoculum to high ammonium concentration (2.7 gNH₄⁺/L) and was fed with PM alone at 20 days of HRT, with a methane yield of 3.63 m³CH₄·t⁻¹. The addition of PSW in period 2, increased the methane yield till 9.73 m³CH₄·t⁻¹, while the increment of the HRT from 20 to 33 days, the methane yield improved till 13.61 m³CH₄·t⁻¹. In the period 4 and 5, with 33 days of HRT, glycerine was added to increase the C/N ratio (8.0-10.3), reaching a maximum yield of 18.66 m³CH₄·t⁻¹.

Keywords

Anaerobic co-digestion; glycerine; low C/N ratio; pasteurized slaughterhouse waste.

INTRODUCTION

Pig industry (farm and slaughterhouses) is one of the most important economic activities, being the 4th in Spain (Blancafort, 2008; MAPA, 2009). This sector, due to the high production quantities, has associated a high generation of wastes, which belong to the so-called animal by-products (ABP). Depending on the animal, a 25-40% of the animal weight will become a waste, since only the 68%, 62-75%, 54-60% and 52-58% of the weight of a chicken, a pig, a cow or a goat/sheep, respectively, is destined to human consume (MEMO/04/107). Daily in the slaughterhouses, different types of wastes are obtained (blood, bones, hoofs, rumens content, stomachs or intestines) as well as manure from the delivery hall (Álvarez and Liden, 2008). In the European Community, the ABP are regulated by specific regulations (1774/2002/EC, modified by the 92/2005) which divide these materials in 3 categories, depending on their dangerous for human and animal health and for the environment. It also includes anaerobic digestion as a valorisation method, but after applying higienization methods (pasteurization is the less restrictive and is applied to category 3 wastes: 70° during 60 minutes with a particle size < 12 mm).

ABP are characterized by the high organic content and biological oxygen demand, but a low carbon/nitrogen ratio. They are composed by proteins, lipids and carbohydrates, mineral salts, vitamins and water in different proportions, depending on the slaughterhouse specialization and centralization, and if one or more types of animals are processed in the same facility (Madrid, 1999; Álvarez and Liden, 2008). Slaughterhouse wastes also have a high theoretical methane potential, but during their fermentation in anaerobic digesters, an increase in the ammonia concentration, due to protein decomposition, or LCFA concentration, due to fat degradation, could inhibit the process (Salminen and

Rintala, 1999; Chen *et al.*, 2008; Galbraith *et al.*, 1971; Hanaki *et al.*, 1981; Koster and Cramer, 1987; Hwu *et al.*, 1996). Although it is generally accepted that the unionized form of ammonia causes inhibition of the anaerobic microorganisms (Angelidaki and Ahring, 1993; Hansen *et al.*, 1998), the inhibitory concentration values are different in bibliography: from low values as 0.10-0.14 gNH₃ L⁻¹ (Poggi-Varaldo *et al.*, 1991; Webb and Hawkes, 1985) to higher ones, as 0.7-1.1 gNH₃ L⁻¹ (Angelidaki and Ahring, 1993; Hansen *et al.*, 1998). Floating scum and foam also can be produced due to fat concentration (Broughton *et al.*, 1998; Cuetos *et al.*, 2008). Moreover, the degradation of high LCFA concentrations could cause an accumulation of unionized VFA, which are inhibitory to anaerobic microorganisms too. For this reason, an exhaustive control of the ammonia, VFA, pH and alkalinity should be done to avoid inhibitions.

If the control of the anaerobic process is suitable, a progressive acclimatization of the bacteria to an ammonia rich medium is possible (Angelidaki and Ahring, 1993; Edström *et al.*, 2003) and also with the high fats and LCFA concentration (Broughton *et al.*, 1998).

A good option to treat these slaughterhouse wastes is the co-digestion with another waste with a less total solid concentration for diluting it, as pig manure. Pig manure although has a low biogas production potential due to the poor organic matter content has an important buffer capacity and contribute with a wide nutrients variety that are necessary for the anaerobic microorganisms growing (Angelidaki *et al.*, 1997). Co-digestion is considered as one strategy to improve the biogas production using the pig manure as substrate (Hartmann *et al.*, 2006), another are thermal pre-treatments (Bonmatí *et al.*, 2001) or even the increase of the anaerobic digestion temperature (Angelidaki *et al.*, 1993). There is a lot of positive results about the co-digestion of this waste plus substrates with very different characteristics, as sludge from wastewater treatment plants (Flotats *et al.*, 1999), vegetal wastes (Dar and Tandon, 1987; Trujillo *et al.*, 1993), wastes from dairy industry (Gavala *et al.*, 1996; Desai and Madamwar, 1994), wastes from the fish cannery or sludge from the beer industry (Callaghan *et al.*, 1999).

Despite of possible inhibitions, good results have been also found related to the co-digestion of ABP, reaching high yields of about 0.7-0.86 m³biogas·kgVS⁻¹ and 0.8-1.0 m³biogas·kgVS⁻¹ (Edstrom *et al.*, 2003, Murto *et al.*, 2004) and specific methane yields from 0.52-0.55 m³CH₄·kg VS⁻¹ added depending of the mixture tested (Salminen and Rintala, 2002).

The objective of this work was to select the mixture with pig manure that enhances the anaerobic digestion of pasteurized slaughterhouse pig waste, at different operation conditions of hydraulic retention time (HRT), organic loading rate (OLR) and also with glycerine supplementation.

MATERIAL AND METHODS

Raw materials

Pasteurized slaughterhouse waste (PSW) of category 3, pig manure (PM) and glycerine (G) were used as substrates. Slaughterhouse waste was collected in a pig slaughterhouse facility located in Barcelona (Spain) and it was composed by a mixture of internal organs (kidney, lungs, livers and hearts, reproductive organs and piggery fatty wastes). All the slaughterhouse fractions were minced (4 mm maximum particle size), mixed and frozen. Thermal pretreatment (pasteurization: 70° during 60 minutes) was applied in the GIRO laboratory by triplicate and the pretreated samples (PSW) were lyophilized before characterization in order to improve their homogeneity. The PM was obtained from a mesophilic anaerobic digestion plant located in Lleida (Spain), where pig manure is the principal substrate. During the continuous reactor experiment, the collected fresh PM (14 different samples) had

different characteristics, due to seasonal variations and to the animal type. Residual glycerin was collected from a biodiesel plant of Barcelona (Spain).

Analytical methods

Total and volatile solids (TS, VS), pH, alkalinity, total and ammonia nitrogen (TN, TAN), volatile fatty acids (VFA) and soluble chemical oxygen demand (COD_s) of the three wastes were measured according to Standard Methods (APHA, AWA, WEF, 2005). Total carbon and nitrogen were determined by elemental analysis (Leco, USA). The total chemical oxygen demand (COD_t) was also determined by elemental analysis using the empiric formulas of each sample (Angelidaki *et al.*, 2009). Proteins were calculated by multiplying the organic nitrogen by $6.25 \text{ g protein gNorg}^{-1}$ (Gelegenis *et al.*, 2007). The fat content was analyzed by the SoxtecTM 2050 extraction equipment (Foss, Denmark) according to recommendations of n-hexane extractable material (HEM) for sludge, sediment and solid samples (EPA 2005, Method 9071B). Volatile fatty acids (VFA) were determined with a CP-3800 gas chromatograph (Varian, USA), according to Campos *et al.* (2008). Methane (CH_4) content was determined by gas chromatography according to Angelidaki *et al.* (2009).

Thermal pretreatments

Pasteurization at 70°C during and 60 minutes was performed by triplicate with 500 g of solid slaughterhouse pig waste in a high pressure and temperature autoclave of 2 L (working volume), with maximum operational conditions of 232°C and 151 bars (Iberfluid Instruments, Spain).

Anaerobic batch experiments

The anaerobic biodegradability (AB; % COD_t initial) of wastes was determined according to Campos *et al.* (2008) and Soto *et al.* (1993). Anaerobically digested sewage sludge from a mesophilic anaerobic digester of a WWTP was used as inoculum. It was characterized by its volatile suspended solids (VSS) and methanogenic activity. AB was determined by triplicate in glass flasks of 1200 mL, which were filled with 500 g of a solution composed for inoculum, macro and micronutrients, substrate and $NaHCO_3$ as a buffer. Three flasks without substrate were performed as blanks to obtain the methane production of the residual COD, although the inoculum was maintained in an incubation chamber (35°C) during 7 days to reduce the amount of this residual COD. The initial inoculum and substrate concentrations were 5 gVSS L^{-1} and $5 \text{ gCOD}_t \text{ L}^{-1}$, respectively. The pH was adjusted to neutrality using HCl or NaOH. The flasks were continuously shaken (100 rpm) during incubation at 35°C for 30 days. The time course of methane production was followed, sampling the head space periodically. The gas volume was normalized to 35°C and 1 atmosphere.

Experimental set up

A 6 L mesophilic continuous stirred reactor was used in this work due to its simplicity (Gavala *et al.*, 1999). It was inoculated with a mixture of 4 L mesophilic sludge from an anaerobic digestion plant of pig manure (Lleida, Spain) and 1 L mesophilic sludge of the digester of a WWTP (Barcelona, Spain). The stirrer and pump of its feed were temporized to work simultaneously 4 times per day. The biogas flow was measured by displacement Ritter flow-meter, after a silica bed (to lose water vapor) and a filter (to avoid particles in the gas).

The operational values were fixed at a hydraulic residence time (HRT) of 20-30 days, and an organic loading rate (OLR) of $2\text{-}3 \text{ kgCOD}_t\text{-m}^{-3}\text{d}^{-1}$. For each experimental condition, the reactor was continuously operated at least for two consecutive HTRs to achieved steady-state conditions. Reactor performance was monitored by daily measured (inlet and outlet flows, biogas flow and temperature) or twice a week measured parameters (pH, alkalinity, NH_4^+ , VFA, biogas composition and effluent COD_t).

Statistical data analyses

Statistical data analysis was carried out with the software MatLab (The Mathwork, USA), one-way analysis of variance and Tukey-Kramer test were applied to compare the average values.

RESULTS

Raw materials characterization

The 3 substrates used in this work have very different characteristics, especially CODt, TS and C/N ratio (Table 1). The C/N ratio was 5.7 and 14.1 gC.gN⁻¹ for the pig wastes, while residual glycerine had a very high value (587.5 gC.gN⁻¹). The organic matter of PWS was 1318.0 gCODt.kg⁻¹ and 1517.0 gCODt.kg⁻¹ for G. PM had an average value for 14 samples of 45.4 gCODt.kg⁻¹, with 34.7 and 54.9 gCODt.kg⁻¹ as minimum and maximum values, respectively.

Volatile solids were very different between the three substrates, having the pig manure the lowest value and being perfect to dilute the mixtures. The total nitrogen content was high in PM and PSW: 3.2 and 26.0 gNT.kg⁻¹, respectively, being organic nitrogen the predominant form in the PSW. The N was almost inexistent in glycerine.

Table 1. Initial characterization of wastes. Nomenclature: PM- pig manure (average of 14 different samples), PSW - pasteurized pig waste, G – glycerine, nd- not detected.

| Parameter | PM | PSW | G |
|-------------------------------------------------------|-----------|------------|-------------|
| TS (g.kg ⁻¹) | 36.7±10.2 | 551.7±3.1 | 926.1±0.1 |
| VS (g.kg ⁻¹) | 26.0 ±8.3 | 542.5±2.1 | 924.4±1.2 |
| IS (g.kg ⁻¹) | 1.2 | 0.9 | 2.0 |
| C/N (g/g) | 5.7 | 14.1 | 587.5 |
| CODt (g.kg ⁻¹) | 45.4±7.1 | 1318.0* | 1517.0±12.9 |
| CODs (g.kg ⁻¹) | - | 175.4±7.5 | - |
| N- NH ₄ ⁺ (g.kg ⁻¹) | 2.7±0.4 | 1.9±0.0 | nd |
| Protein (g.kg ⁻¹) | 3.1 | 156.7 | nd |
| VFA (gCOD.L ⁻¹) | 8.3±4.9 | 2.5±0.1 | - |
| Fat (g.kg ⁻¹) | - | 363.4 ±0.6 | - |
| SO ₄ ²⁻ (g.kg ⁻¹) | - | - | 1.7±0.1 |

*Calculated value from elemental analysis.

Biodegradability of raw materials

First of all, anaerobic biodegradability (AB) of substrates was studied (Table 2). Neither lag phase nor acidification was observed in all assays after 30 days (*data not shown*). The PM had the lower AB, compared to PSW and G (41.0, 94.3 and 65.3 %CODt respectively), with a non-biodegradable fraction of 49.0% COD_i (being 5.7 % and 34.7 %CODt for PSW and G, respectively).

PSW had the highest methane potential yield (476.33 m³CH₄.t⁻¹ or 0.88 m³CH₄.kgVS⁻¹), while PM had the lowest (5.99 m³CH₄.t⁻¹ or 0.20 m³CH₄.kgVS⁻¹). PSW potential is higher than 0.23-0.62 m³CH₄.kgVS⁻¹ reported by Hefjinfelt *et al.* (2009). PM potential is lower than 0.36-0.52 m³CH₄.kgVS⁻¹ (Moller *et al.*, 2004; Grebrezgabher *et al.*, 2009) or 16 m³CH₄.t⁻¹ (Bernet *et al.*, 2009), probably due to slowly degradable lignocellulosic materials (Moller *et al.*, 2004) or different particle sizes or distribution during manure storage (Rodriguez *et al.*, 2002) or even manure management (Palatsi *et al.*

2004). Residual glycerine had a potential of $201.93 \text{ m}^3\text{CH}_4.\text{t}^{-1}$ (or $0.35 \text{ m}^3\text{CH}_4.\text{kgVS}^{-1}$), less than $1.295 \text{ m}^3\text{CH}_4.\text{t}^{-1}$ reported by Amon *et al.* (2006), due to the presence of sulphate or other components from biodiesel production.

Table 2. Anaerobic biodegradability of wastes. Nomenclature: PM- pig manure, PSW- pasteurized pig waste and G- glycerine.

| Parameter | PM | PSW | G |
|------------------------------------------|------------|--------------|---------------|
| AB (%CODt) | 41.0± 0.70 | 94.3± 3.00 | 65.3± 4.80 |
| Yields: | | | |
| $\text{m}^3\text{CH}_4.\text{kgVS}^{-1}$ | 0.20± 0.00 | 0.88± 0.00 | 0.35± 0.03 |
| $\text{m}^3\text{CH}_4.\text{t}^{-1}$ | 5.99± 0.13 | 476.33± 7.24 | 201.93± 29.34 |

Continuous reactor experiment

The continuous experiment was 85 weeks, divided in 3 steps. Table 4 gives the feed composition and the main operational / control parameters. The first step (periods 1 and 2) began with a feed composed by 100:0:0 (%VS) and 93:7:0 (%VS) PM:PSW:G mixtures, both with 20-22 days of HRT. The second step (periods 3) consisted in an increment of the HRT till 33 days and a feed composed by a 64:36:0 (%VS) PM:PSW:G mixture. The third step (periods 4 and 5) was carried out to quantify the specific CH_4 yield after the supplementation of glycerine, without changing the HRT: 34:50:16 and 35:47:18 (%VS) PM:PSW:G mixtures. The OLR was between 2.5-3.2 $\text{kgCOD}.\text{m}^{-3}\text{d}^{-1}$, except in period 1 with $0.8 \text{ kgCOD}.\text{m}^{-3}\text{d}^{-1}$.

Table 4. Operation and control parameters during the continuous co-digestion with different feeding mixtures. Nomenclature: PM-pig manure, PSW pasteurized pig waste, G-glycerine.

| Period | P1 | P2 | P3 | P4 | P5 |
|-------------------------------------------------------------|---------|--------|---------|----------|----------|
| PM:PSW:G (%VS _{in}) | 100:0:0 | 93:7:0 | 64:36:0 | 34:50:16 | 35:47:18 |
| C/N influent ($\text{g}.\text{g}^{-1}$) | 6.3 | 6.1 | 5.9 | 8.0 | 10.3 |
| HRT (d) | 22 | 20 | 33 | 33 | 32 |
| OLR ($\text{kgCOD}.\text{m}^{-3}\text{d}^{-1}$) | 0.8 | 3.0 | 2.6 | 2.5 | 3.2 |
| COD degrad. (%) | 30% | 48% | 44% | 51% | 55% |
| CH_4 (%v/v) | 65% | 73% | 73% | 71% | 71% |
| Yields: | | | | | |
| $\text{m}^3\text{CH}_4.\text{m}^{-3}\text{d}^{-1}$ | 0.22 | 0.47 | 0.39 | 0.48 | 0.64 |
| $\text{m}^3\text{CH}_4.\text{kgVS}_{in}^{-1}$ | 0.13 | 0.34 | 0.41 | 0.38 | 0.38 |
| $\text{m}^3\text{CH}_4.\text{t}^{-1}$ | 3.63 | 9.73 | 13.61 | 15.12 | 18.66 |
| Effluent: | | | | | |
| N-NH ₄ ⁺ ($\text{g}.\text{L}^{-1}$) | 1.7 | 2.8 | 3.4 | 2.7 | 2.5 |
| N-NH ₃ ($\text{g}.\text{L}^{-1}$) | 0.1 | 0.3 | 0.4 | 0.2 | 0.3 |
| VFA (% CODt) | 1.5% | 3.6% | 2.8% | 4.2% | 1.9% |
| Alkalinity ($\text{g CaCO}_3.\text{L}^{-1}$) | 0.19 | 0.19 | 0.22 | 0.22 | 0.24 |

Step 1: Periods 1(100% PM) and 2 (mixture PM:PSW:G= 93:7:0)

The period 1 was operated feeding only PM. The steady-state was achieved quickly due to the previous adaptation of the inoculum to this type of substrate. The COD degradation was 30% and the yield was $3.63 \text{ m}^3\text{CH}_4.\text{t}^{-1}$ ($0.22 \text{ m}^3\text{CH}_4.\text{m}^{-3}\text{d}^{-1}$). Biogas composition was 65% v/v and the effluent ammonia and

VFA concentration were 0.1 g L^{-1} y $1.5\% \text{ COD}_{\text{tin}}$, respectively. The low yield of pig manure was due to the low organic matter concentration. The addition of a 7% VS of PSW at the beginning of period 2 caused an OLR increase from 0.8 to $3.0 \text{ kgCOD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$, improved biogas production till $9.73 \text{ m}^3 \text{CH}_4 \cdot \text{t}^{-1}$ ($0.47 \text{ m}^3 \text{CH}_4 \cdot \text{m}^{-3} \cdot \text{d}^{-1}$). The CH_4 also grew until 73% v/v. So, in period 2, improved methane yields: 114% ($\text{m}^3 \text{CH}_4 \cdot \text{m}^{-3} \cdot \text{d}^{-1}$) and 168% ($\text{m}^3 \text{CH}_4 \cdot \text{t}^{-1}$) respect to period 1. Although ammonia and VFA concentrations in the effluent increased ($0.3 \text{ gNH}_3 \cdot \text{L}^{-1}$ and 3.6 % COD_{tin} of VFA), there was not instability in the system.

Step 2: Period 3 (mixture PM:PSW:G = 64:36:0)

In this period, the HRT was increased from 20 to 33 days to avoid possible problems due to the increase of PSW concentration from 7 to 36 % VS. Higher HRT were used to facilitate the adaptation of biomass in manure digesters (Viswanath and Nand, 1994, Salminen and Rintala, 2002) or codigestion of sewage sludge and manure (Murto *et al.*, 2004). An increment of the biogas production was observed respect to pig manure digestion, achieving values of $13.61 \text{ m}^3 \text{CH}_4 \cdot \text{t}^{-1}$ and $0.39 \text{ m}^3 \text{CH}_4 \cdot \text{m}^{-3} \cdot \text{d}^{-1}$. With an ammonia concentration of $0.4 \text{ g} \cdot \text{L}^{-1}$ due to the PM characteristics, a little decrease in COD degradation (from 48% to 44%) and methane yields were observed compared to period 2, but there was no VFA accumulation (2.8 % COD_{tin}).

Step 3: Periods 4 (mixture PM:PSW:G = 34:50:16) and 5 (mixture PM:PSW:G = 35:47:18)

Due to the low ratio C/N of PW and PSW, glycerine was added for increasing C/N ratio of feeding. ORL were different (2.5 and $3.2 \text{ kgCOD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$) although the substrate percentage was almost the same, by the different volatile solid content in pig manure. Amon *et al* (2006) also used glycerine like carbon supplementation, adding 6%, which produced and increase from 0.57 - $0.68 \text{ m}^3 \text{CH}_4 \cdot \text{kgVS}^{-1}$ in the mixture of pig manure and maize silage.

Volumetric yields and COD degradation improved from 0.22 to $0.48 \text{ m}^3 \text{CH}_4 \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ and $0.54 \text{ m}^3 \text{CH}_4 \cdot \text{m}^{-3} \cdot \text{d}^{-1}$, and from 30 % to 51% and 55% COD comparing periods 4 and 5 with 1. After the glycerine addition, there was an increase in VFA concentration (4.2% COD_{tin}) but in last period, with the similar G percentage, it decreased due to the microorganisms' degradation (1.9% COD_{tin}). Methane yield achieved $15.12 \text{ m}^3 \text{CH}_4 \cdot \text{t}^{-1}$ and $18.66 \text{ m}^3 \text{CH}_4 \cdot \text{t}^{-1}$, which represents an increment of 316.5% and 414.05% respect to period 1. Biogas composition in periods 4 and 5 was 71% v/v CH_4 . Ammonia concentrations in period 4 and 5 (0.2 and $0.3 \text{ g NH}_4^+ \cdot \text{L}^{-1}$ respectively) were lower than in 3.

Periods 3 and 4 had the same HRT (33 days) and similar OLR, but different C/N ratio due to the glycerine addition in period 4. This different composition improved the yields of the period 4: 23% ($\text{m}^3 \text{CH}_4 \cdot \text{m}^{-3} \cdot \text{d}^{-1}$) and 11% ($\text{m}^3 \text{CH}_4 \cdot \text{t}^{-1}$) respect to period 3. In period 3, the bigger N-NH_4^+ concentration was found, but the VFA was low (2.8% COD), comparing to periods 2 and 4.

Angelidaki and Ahring (1993) observed that the growing rate of methanogenics decreased a 20% with concentrations of $0.7 \text{ gNH}_3 \cdot \text{L}^{-1}$ and Poggi-Varaldo *et al.* (1991) also demonstrated that the acetoclastic methanogens growth rates were very sensitive to the concentrations of free ammonia below about $0.10 \text{ gNH}_3 \cdot \text{L}^{-1}$. Respect to pig manure, Hansen *et al.* (1998), observed inhibition from $1.1 \text{ gNH}_3 \cdot \text{L}^{-1}$ in pig manure due to the long adaptation of the inoculums used.

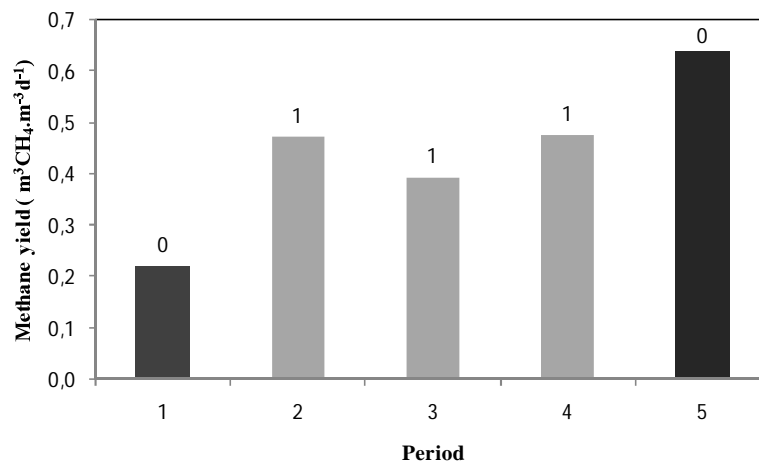


Figure 1. Methane yield (m³CH₄.m⁻³.d⁻¹) and differences between periods, where: 1 - not significant difference and 0 - significant difference.

The main difference between periods 4 and 5 was the dilution of feeding that permitted maintains the substrates percentages varying only the OLR (2.5 and 3.2 kgCOD.m⁻³.d⁻¹). There were increases in yields of period 5 respect to 4: m³CH₄.m⁻³.d⁻¹ (33%), m³CH₄.kgCOD_{in}⁻¹ (6%) and m³CH₄.t⁻¹ (23%), except in m³CH₄.m⁻³.d⁻¹ that remain equal. In period 5, the pH and alkalinity reached the highest values (8 and 0.24 g CaCO₃/L, respectively) meanwhile VFA had a very low, similar to period 1 (only PM). The yields obtained (m³CH₄.kgVS⁻¹) in period 2, 3, 4 and 5 (Figure 1) were slightly bigger than other referenced in bibliography as the co-digestion of animal by-products with pig manures and fruit (0.27-0.35 m³CH₄.kgVS⁻¹; Alvarez *et al.*, 2008) or even ABP and sludges (0.38-0.43 m³CH₄.kgSV⁻¹; Luste *et al.*, 2010).

CONCLUSIONS

Anaerobic digestibility of solid pasteurized slaughterhouse waste is determined by the high solid content due to the protein and fat concentration. Co-digestion with pig manure gave good results, also improved by adding glycerine as carbon source. Batch assays of pig manure and solid pasteurized slaughterhouse did not show a lag phase or inhibition processes when were carried out previously. The same behavior happened with the three mixtures, showing that an increment of VS due to PSW was proportional to bigger methane yields, as was expected. The best results related to methane production potential were obtained with the highest C/N periods, 4 and 5, both with glycerin addition, achieving in period 5: 18.66 m³CH₄.t⁻¹, which represents an increment of 414.0, 246.0 and 139.1% respect to PM alone or PM+PSW in period 2 and PM+PSW in period 3, respectively.

High ammonia values were obtained in period 3, due to high PM ammonia value but inhibition processes were not observed in all the experiments.

Significant differences in the methane yield (m³CH₄.m⁻³.d⁻¹), comparing pig manure anaerobic digestion and co-digestion of solid pasteurized slaughterhouse waste, pig manure plus glycerine (with the higher OLR), with the rest of periods, have been found.

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Lipids and LCFA inhibition: key parameter in anaerobic digestion process

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Abstract

Wastewaters, particularly those from food processing industries, contain considerable amounts of long-chain fatty acids (LCFA). These compounds, resulting from the hydrolysis of lipids, are potentially attractive for biogas production because of their high potential methane yield. Yet, removal of lipids from the wastewaters prior to anaerobic treatment is rather standard, which implies the loss of their energetic value. Reasons for this procedure are, basically, related with the recurrent reports on the alleged toxic/inhibitory effect of LCFA towards methanogenic activity as well as with problems of sludge flotation and washout during the treatment of LCFA-rich wastewater in high rate anaerobic reactors. In the past years, important advances have been made within the anaerobic digestion of lipids/LCFA-based effluents, a main subject of research at our research group. It was found that anaerobic sludge remained metabolically active after extended contact with LCFA, contradicting the bactericidal or permanent toxic effects of LCFA, previously reported. The “floating” sludge was able to produce high amounts of methane from the biomass-associated LCFA accumulated during a continuous reactor operation and transport limitations phenomena were identified as an important factor behind the observed transitory microbial LCFA inhibition. It was demonstrated that the observed LCFA inhibition, either metabolic and/or physical, is reversible in the range of LCFA content between 1000 and 5000 mgCOD-LCFA gVSS⁻¹. From our research it became clear that lipids and LCFA-rich anaerobic wastewater treatment can be feasible with energy recovery. This opened new scenarios for the anaerobic treatment of wastewater with high lipid content.

Keywords

Inhibition; LCFA; methanogenic activity; transport limitations.

INTRODUCTION

The concept “waste-to-energy” is nowadays on the world agenda. In this framework, biogas plants, as a core of sustainable multifunctional facilities, combining nutrients and water recycling with energy production are, undoubtedly, the most versatile and flexible options for a sustainable processing of any kind of biodegradable organic matter. Waste lipids are ideal potential substrates for biogas production, since they have a twofold theoretical methane yield when compared to proteins or carbohydrates. Lipids are LCFA bonded to glycerol, alcohols or other groups by an ester or ether linkage. Fats and oils are a subgroup of lipids that have the alcohol groups esterified with fatty acids, predominantly in the form of triglycerides. These compounds are the most abundant family of lipids present in domestic sewage and industrial effluents. Wool scouring and olive mill processes can generate effluents with lipids concentrations in the range of 5 to 25 g L⁻¹ (Beccari *et al.*, 1998; Becker *et al.*, 1999). Lower concentrations were detected in a sunflower oil mill wastewater, with LCFA concentrations ranging from 0.2 to 1.3 g L⁻¹ (Saatci *et al.*, 2003). Total lipids concentrations in a dairy wastewater were reported to vary from 0.9-2.0 g L⁻¹ (Kim *et al.*, 2004b). Slaughterhouses can produce effluents with a total fat matter between 0.35 and 0.52 g L⁻¹ (Sayed *et al.*, 1988). In domestic sewage, lipids represent generally 20 to 25% of the total organic matter, with concentrations ranging from 40 to 100 mg L⁻¹ (Quémeneur and Marty, 1994).

In general, hydrolysis of fats and oils to glycerol and LCFA proceeds rapidly in anaerobic digestion processes, resulting in the accumulation of LCFA in the wastewater (Hanaki *et al.*, 1981; Angelidaki and Ahring, 1992). Palmitic acid and oleic acid are the most abundant saturated and unsaturated LCFA present in domestic and industrial wastewaters.

Drawbacks of anaerobic treatment of lipids/LCFA-rich wastewaters

Research on the application of anaerobic technology to treat wastewaters containing lipids/LCFA has been emerging in the past 25 years. Two main problems specifically related to the treatment of these effluents were identified: (1) sludge flotation and washout due to the adsorption of lipids/LCFA onto the biomass and (2) inhibition of acetogenic bacteria and methanogenic archaea by LCFA.

Sludge flotation and washout due to the adsorption of fatty matter onto the biomass is widely reported in the literature. Samson *et al.* (1985) referred the treatment failure of an industrial scale UASB reactor treating milk fat, due to sludge flotation. Hawkes *et al.* (1995) observed poor biomass retention in four different reactors treating ice-cream wastewater with high fat content. Rinzema *et al.* (1989; 1993) tested the treatability of LCFA-containing wastewaters in UASB reactors. When the reactors were overloaded a severe washout caused by flotation was observed. Sam-Soon *et al.* (1991) used a UASB reactor to study oleic acid degradation and reported that the original inoculated granules suffered from disintegration and encapsulation by a gelatinous and whitish mass. Hwu *et al.* (1998) showed that the specific LCFA organic load necessary to induce complete sludge flotation ($0.203 \text{ g COD g VSS}^{-1} \text{ day}^{-1}$) corresponded to an LCFA concentration of $263 \text{ mg LCFA L}^{-1}$, which was far below the minimum inhibitory concentration ($401 \text{ mg LCFA L}^{-1}$) of methanogenesis. This suggested that deterioration of the UASB process by LCFA adsorption and consequent sludge washout are likely to occur prior to inhibition of the methanogenic archaea by the LCFA.

An acute toxic effect of LCFA on methanogenic and acetogenic microorganisms is generally documented (Hanaki *et al.*, 1981; Koster and Cramer, 1987; Angelidaki and Ahring, 1992; Rinzema *et al.*, 1994; Lalman and Bagley, 2000; Lalman and Bagley, 2001; Lalman and Bagley, 2002). Both, acetoclastic and hydrogenotrophic methanogens, are affected by LCFA, although acetoclastic methanogens are apparently more affected by the presence of these compounds (Hanaki *et al.*, 1981; Hwu and Lettinga, 1997; Alves *et al.*, 2001; Lalman and Bagley, 2001). Inhibitory effects of unsaturated LCFA are reported to be more severe than those of saturated LCFA (Lalman and Bagley, 2002). According to some authors, the mechanism of LCFA toxicity seems to be related to the adsorption of the surface active acids onto the cell wall, which affects its transport and/or protective functions (Demeyer and Henderickx, 1967; Galbraith *et al.*, 1971; Rinzema, 1988).

For many years it was believed that high rate treatment of lipid-rich effluents was not possible. Hwu (1997) tried to enhance the anaerobic treatment of wastewater containing oleic acid and found a higher susceptibility of suspended sludge than granular sludge to LCFA toxicity. This observation in batch assays, though interesting, was of little practical relevance, since granular sludge was not structurally stable when LCFA were present.

New concepts on LCFA inhibition and degradation

Though LCFA are difficult to degrade compounds, microbial injury due to intensive (high concentration) and extended (long time) contact between anaerobic sludge and LCFA was found to be less severe than could be expected (Alves *et al.*, 2001; Pereira *et al.*, 2002; 2004; 2005). When performing a routine assessment of the specific methanogenic activity (SMA) of sludge collected from a continuous reactor fed with oleic acid, we observed a surprisingly high methane production in the blank vials, where no external substrate was added (Alves *et al.*, 2001). The observed methane

production resulted from the degradation of substrate that accumulated onto the sludge during the reactor's operation, contradicting the previous conclusions about permanent LCFA inhibition.

A clear evidence of LCFA inhibition reversibility was provided by an experiment where the SMA of sludge samples, containing a biomass-associated LCFA content between 1000 and 5000 mg COD-LCFA gVSS⁻¹, was determined before, and after the conversion of the embedded LCFA to methane. In general, the loaded sludge had no quantifiable activity except with H₂ as substrate. However, after the degradation of the biomass-associated LCFA, a significant increase of the SMA was observed for the selected substrates (Pereira *et al.*, 2004).

The low SMA measured before the depletion of the biomass-associated LCFA, may result from a strong effect of transport (diffusion) limitations imposed by the LCFA layer surrounding the cells, which could hamper the access of the added substrates, as well as the subsequent biogas release. This is reinforced by the fact that H₂, the smallest molecule used as substrate, was easily mineralized to methane. Transport limitations phenomena may also be responsible for the observed lag phases that previously have been ascribed to mechanisms of cell wall damage and bactericidal effects.

Table 1. Specific methanogenic activities exhibited by three different sludges, before and after the conversion to methane of the biomass-associated LCFA (adapted from Pereira *et al.*, 2004).

| Sludge specific LCFA content (mg COD-LCFA.g VSS ⁻¹) | Specific methanogenic activity (mg COD-CH ₄ gVSS ⁻¹ day ⁻¹) | | | |
|--------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|--------|---------------------------------|----------|
| | Acetate | | H ₂ /CO ₂ | |
| | Before | After | Before | After |
| 1221±144 | 143±29 | 326±13 | 1462±94 | 1670±81 |
| 2838±63 | 0 | 579±4 | 1218±1 | 2817±146 |
| 4571±257 | 0 | 533±95 | 401±21 | 2709±38 |

The role of transport limitation effects

The accumulation of LCFA on microbial cells and aggregates has two distinct effects. (i) the purely physical barrier that hinders the access of substrates to the cells as well as the transfer of microbial products and (ii) the physiological consequence of this substrate privation on microbial metabolism.

The inhibition due to transport limitation/metabolic effects was further studied by Pereira *et al.* (2005). Two anaerobic reactors (EGSB with external settler) were individually fed with oleic and palmitic acids, in order to promote the accumulation of LCFA onto the sludge. Palmitic acid was the main LCFA that accumulated in both sludges. The way of palmitic acid accumulation was different in the oleic and in the palmitic acid fed reactors. When oleic acid was fed, the biomass-associated LCFA (83% as palmitic acid) were mainly adsorbed and entrapped in the sludge that became “encapsulated” by a LCFA layer. However, when palmitic acid was fed, the biomass-associated LCFA (the totality as palmitic acid) was mainly precipitated in white spots like precipitates in between the sludge, which remained “non-encapsulated”. Total LCFA content of 4570±257 and of 5200±9 mgCODgVSS⁻¹ were determined in the oleate and palmitate fed sludges, respectively. The SMA was assessed for both sludges (Figure 1).

The obtained results evidenced the strong effect of diffusion limitations imposed by the LCFA layer accumulated onto the sludge. The “non-encapsulated” sludge exhibited a considerable initial methanogenic activity on all the tested substrates, with the single exception of butyrate. However, for the “encapsulated” sludge only methane production from ethanol and H₂/CO₂ was detected during the first 400 hours. Lag phases up to 1200 hours were detected preceding the initial methane production

from the other tested substrates. Hydrogen (H_2), the smallest substrate tested, was the first to be transformed into methane, suggesting a faster transport of this molecule through the LCFA layer than the observed for the other substrates. In the case of ethanol, methane production was also observed, likely because this substrate dissolved the accumulated LCFA, overcoming, to some extent, substrate and product diffusion limitations. In the “non-encapsulated” sludge, the accumulated LCFA was in a loosely association with the cells, and thus mass transfer limitations are not expected to occur in a similar extent as in the “encapsulated” one.

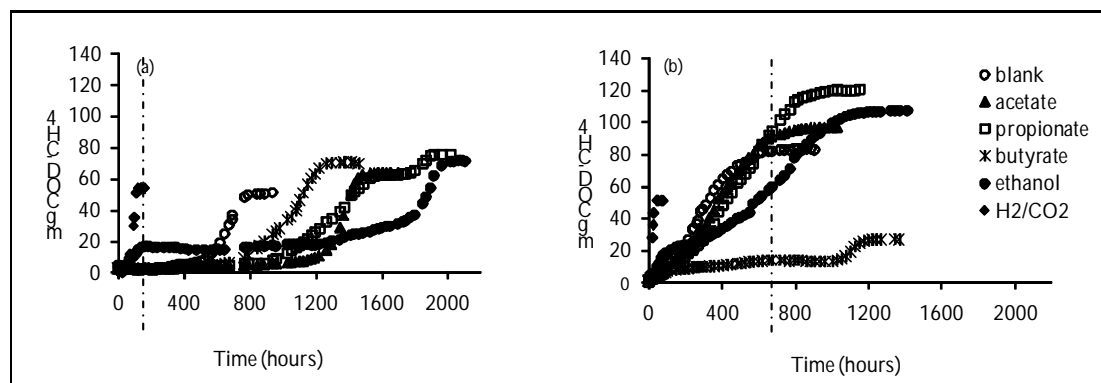


Figure 1. Prolonged monitoring of methane production during the methanogenic activity batch experiments: (a) “encapsulated” sludge containing 4570 ± 257 mgCOD.gVSS⁻¹ of biomass-associated LCFA (83% palmitic acid), (b) “non-encapsulated” sludge containing 5200 ± 9 mgCOD.gVSS⁻¹ of biomass-associated LCFA (100% palmitic acid).

The “encapsulated” sludge used in this experiment represents a situation of severe LCFA load where a lag phase as high as 500 hours was found to precede the initial methane production from the degradation of the biomass-associated LCFA (Fig1.a). This fact can be overcome when avoiding excessive accumulation of LCFA into the sludge, thus lowering the time required for its degradation. The conditions that allow optimal conversion to methane of the LCFA associated to the sludge were assessed and a specific content of about 1000 mg COD-LCFA gVSS⁻¹ was found to be the optimal for an efficient methane production rate (about 250 mg COD-CH₄ gVSS⁻¹day⁻¹) and no lag phase observed (Pereira *et al.*, 2004).

CONCLUSIONS

The anaerobic treatment of wastewater with high lipid content is possible, providing the correct reactor design and feeding strategies are applied (see Picavet *et al.*, this seminar).

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Strategies to prevent or overcome LCFA inhibition: Adsorption as key process

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Abstract

Anaerobic treatment of lipidic substrate can be limited by long-chain fatty acids (LCFA) inhibition. Physical adsorption of LCFA, that can hinder or limit cell nutrients transport, has been proposed as the main inhibitory mechanism. By mean of specific batch test and semi-continuous manure based reactors, the adsorption process of LCFA, the effect of adsorption over LCFA inhibition, and the possibility of introduce competitive additives, as prevention or recovery strategy, was investigated. The obtained results demonstrated that it was possible to partially prevent LCFA inhibition by the addition of adsorbents like clay mineral bentonite. Those additives can compete with biomass for LCFA adsorption sites, and consequently, influence the kinetics of the LCFA adsorption and inhibition process.

Keywords

Anaerobic digestion; LCFA inhibition; adsorption; suspended and granular biomass.

INTRODUCTION

Lipids are interesting substrates for the anaerobic digestion process, according to their potential methane yields. Lipids are initially hydrolyzed to glycerol and long chain fatty acids (LCFA), which are further metabolized to hydrogen (H₂) and acetate (Ac) by syntrophic acetogenic bacteria. The degradation pathway of LCFA, known as β -oxidation has been reported to be the rate-limiting step of the anaerobic process (Lalman and Bagley, 2002). Initially, LCFA inhibitory-toxic effects have been related to cell damages, permanent toxic effect (Angelidaki *et al.*, 1990), bactericidal effect (Rinzema *et al.*, 1994) and cell lysis (Hwu and Lettinga, 1997) and are known to affect both syntrophic acetogens and methanogens (Lalman and Bagley, 2002). Further studies reported that LCFA exert a reversible inhibitory effect and that, after a lag phase, microorganisms are able to efficiently degrade the accumulated LCFA (Hwu *et al.*, 1998; Shin *et al.*, 2003). Physical adsorption of LCFA onto the active biomass has been proposed as the main inhibition mechanism, creating a physical barrier that hinders the transfer of substrates and metabolites (Pereira *et al.*, 2005).

Therefore, methods to prevent or overcome inhibition would have a significant advantage for the safe and stable operation of digestion plants. Although LCFA are inhibitory for the anaerobic biogas process at low concentrations, several improving strategies has been reported. Pulse exposure to LCFA or discontinuous feeding (Cavaleiro *et al.*, 2008), co-digestion with other substrates (Fernandez *et al.*, 2005; Kuang *et al.*, 2002), addition of easily-degradable co-substrates, like glucose and cysteine (Kuang *et al.*, 2006), addition of biofibers as adsorbents (Nielsen and Ahring, 2006) or an increase of the ratio biomass/substrate (Palatsi *et al.*, 2009) have been used to overcome LCFA inhibition.

The aim of the present work is to study the adsorption process of lipids (LCFA) onto anaerobic biomass, the effect of adsorption over LCFA inhibition process, and the possibility to introduce competitive additives as LCFA inhibition prevention or recovery strategy, using batch tests and semi-continuous anaerobic reactors.

MATERIALS AND METHODS

Analytical Methods

Total solids (TS), volatile solids (VS), volatile suspended solids (VSS), chemical oxygen demand (COD) and pH were determined according to Standard Methods (APHA, AWA, WEF, 1995). Methane content (%CH₄) and volatile fatty acids concentration (VFA; Acetate (Ac), Propionate (Pr), iso-Butyrate (iso-But), n-Butyrate (n-But), iso-Valerate (iso-Val), n-Valerate (n-Val) and Hexanoate (Hex)) were measured with GC-TCD and FID, as described elsewhere (Campos *et al.*, 2008; Angelidaki *et al.*, 2009). LCFA – laurate (C12:0), myristate (C14:0), palmitate (C16:0), palmitoleate (C16:1), stearate (C18:0) and oleate (C18:1) - were determined as fatty acids methyl esters (FAME) with a CP-3800 gas chromatograph (Varian, USA), fitted with CP7489:CP-Sil 88 FAME capillary column (50m0.25mm0.2µm, Varian, USA) and FID detection, according to Palatsi *et al.* (2009).

Experimental set-up

Adsorbents as recovery agents

To test the addition of adsorbents, as strategies to recover LCFA inhibition, semi-continuous anaerobic manure based reactors were used. Digested thermophilic effluent from a biogas pilot-scale plant (PP), digesting cow manure located at DTU (Kongens Lyngby, Denmark), with an average concentration of 3.0% TS and 2.2% VS, was used as inoculum for experiments. To impose LCFA inhibition to the biogas process, a synthetic LCFA mixture (LCFAM), consisting of sodium oleate (C18:1), sodium stearate (C18:0) and sodium palmitate (C16:0) in a ratio of 40:10:50 (w/w/w) respectively (analytical grade, BDH Chemicals Ltd), was used. This LCFAM simulated the 3 major constituents in slaughterhouse wastewater sludge (Hwu *et al.*, 1998), which is considered to be an interesting co-substrate, often used in manure based biogas plants. Commercial powder bentonite (Al₂O₃ 4SiO₂ H₂O, Sigma-Aldrich) was used as adsorbent for testing the adsorption competition as recovery strategy.

Semi-continuous reactors consisted on glass vials (2.2 L total volume; 1.0 L working volume) closed with a rubber stopper and with attached maprene tubes, inserted for feeding and sampling (liquid/gas). Semi-continuous feeding was applied with diluted manure (2.5% TS and 2.0% VS) during all experimental time (organic loading rate (OLR) of 1.0 g_{VS} L⁻¹ day⁻¹ and a hydraulic retention time (HRT) of 20 days). The manure was diluted with distilled water in order to decrease the ammonia level and ensure that LCFA was the only inhibitor in the experiments. The inhibitory LCFAM pulse was introduced as sodium oleate powder (4 g L⁻¹). The tested recovery actions consisted on the addition of bentonite powder (R_{bentonite}), in the quantity of 5 g_{VS} L⁻¹ (ratio 1:1 LCFA/adsorbent), 48h after LCFA inhibitory pulse. That experimental set-up (inhibition + recovery action) was reproduced twice (2 LCFAM pulses). A control reactor (R_{control}), not inhibited, and fed daily with diluted fresh manure, was also run during the whole experimental period. More information about the experimental set-up, biomass characterization and LCFAM inhibitory effect, can be found in Palatsi *et al.* (2009 and 2010). Experimental set-up is summarized in Table 1.

Adsorbents as prevention agents

To test the addition of adsorbents as strategies to prevent LCFA inhibition (not to overcome), discontinuous batch assays experiments were designed. Mesophilic granular sludge was sampled from industrial fruit juice processing industry UASB reactor in Lleida (Spain). Granular sludge was selected to better monitor adsorption process (including image analysis). Sodium oleate powder salt (Riedel-de Haën/Sigma-Aldrich; 82% C18:1/LCFA) was selected as LCFA substrate model due to its high solubility, while bentonite was introduced again as analytical grade reagent (4SiO H₂O, Sigma-Aldrich). Toxicity assays of increasing LCFA concentrations, a complete characterization of granule biomass (in terms of methanogenic activity, surface area and microbial community structure), and the

estimation of the LCFA adsorption isotherms over inactivated biomass and bentonite, were previously performed to better design experimental set-up. Complete information can be found in Palatsi *et al.* (2010).

The tested prevention strategy consisted in the addition of bentonite ($5 \text{ g}_{\text{bentonite}} \text{ L}^{-1}$), previously incubated with C18:1 ($0.5 \text{ g}_{\text{C18:1}} \text{ L}^{-1}$), in buffered media vials (T) during four days, to force bentonite adsorption prior to the inoculation with active granular sludge (120 mL with a media working volume of 50 mL). Control vials (C), with oleate and biomass but without bentonite, and blank vials (BL), with only biomass, were also run. All vials were maintained at 35°C under continuous shaking (150 rpm) and anaerobic conditions. Each treatment was performed in triplicate for CH_4 analysis, and 12 vials per treatment were withdrawn periodically to determinate LCFA_L , LCFAs and VFA profile. More information about the experimental set-up can be found in Palatsi *et al.* (2010). A summary of experimental set-up is reported in Table 1.

Table 1. Summary of experimental set-up.

| Experiment | Reactor configuration | Temp ($^\circ\text{C}$) | Biomass | LCFA pulse (g/L) | Strategy |
|------------|-----------------------|---------------------------|-----------|-----------------------------|-----------------------------------------------------------------------------------------------------------|
| Recovery | Semi-continuous | 55 | Suspended | 4.0 | application of $5 \text{ g}_{\text{bentonite}}/\text{L}$ after 48h |
| Prevention | Batch | 35 | Granular | 0.5 | LCFA incubation with $5 \text{ g}_{\text{bentonite}}/\text{L}$ during 4 days, prior to sludge inoculation |

RESULTS AND DISCUSSION

Adsorbents as recovery agents

The time course of the methane production ($\text{L}_{\text{CH}_4} \text{ L}^{-1} \text{ d}^{-1}$), VFA concentration (mM), and (C18:1) oleate - (C16:0) palmitate evolution (mg L^{-1}) during recovery strategy experiments (Table 1) is reported in Figure 1. Table 2 summarized the main process parameters.

The application of LCFA_M pulse (4 g L^{-1} , Table 1) clearly inhibited the anaerobic process, reducing the methane production till values close to zero and detecting a clear accumulation of VFA in the both reactors liquid media (R_{feed} and $R_{\text{bentonite}}$ in Figure 1), although the system was able to recover the degradation activity. Addition of adsorbents, ($R_{\text{bentonite}}$) as recovery strategy improved the recovery time from 4-5 days to 2-3 days, did not increase the maximum accumulated VFA concentration and, these vials showed a higher utilization of LCFA_M potential, compared to control reactor without adsorbent addition (R_{feed}), according to Figure 1 and Table 2. The recovery time was calculated as the time between the beginning of the recovery action and the time when the methane production rate ($\text{L}_{\text{CH}_4} \text{ L}^{-1} \text{ d}^{-1}$) exceeded the mean for control reactor (R_{control}), only feed with manure.

Table 2. Process parameters during experiments with bentonite as recovery agent.

| | Max. Prod.Rate ($\text{L CH}_4/\text{Lday}$) | | Max VFA (mM) | | Recovery time (days) | |
|------------------------|---------------------------------------------------|-----------|-----------------|-----------|-------------------------|-----------|
| | 1st pulse | 2nd pulse | 1st pulse | 2nd pulse | 1st pulse | 2nd pulse |
| R_{control} | 0.37 | 0.39 | 4.8 | 4.3 | - | - |
| R_{feed} | 1.14 | 1.64 | 56.3 | 65.3 | 4 | 5 |
| $R_{\text{bentonite}}$ | 1.47 | 1.88 | 51.2 | 41.0 | 2 | 3 |

Beccari *et al.* (1999) also observed positive effect of bentonite addition during anaerobic degradation of olive oil mill wastewaters, while Nielsen and Ahring (2006) reduced oleate inhibition by adding bio-fibers (anaerobically digested fibers) to continuously fed reactors digesting manure. Those reports

proposed that adsorbents were able to bind the lipids or LCFA on their surface, lowering the adsorption to the microbial cells, and thus stimulating methane production. Adsorption is considered as a rapid physico-chemical mediated phenomenon, while desorption is biologically mediated (Hwu *et al.*, 1998; Ning *et al.*, 1996). Bentonite was added to the reactors 2 days after the LCFA_M pulse, and consequently a significant part of LCFA_M may have already been adsorbed to the biomass. This previous absorption onto biomass might have been the reason for the absence of an initial clear effect in Figure 1, where the concentration of total LCFA (C18:1 or C16:0), just after the application of recovery strategy in R_{bentonite}, was quite similar to R_{feed}. By measuring the soluble fraction of LCFA (LCFA_L) in the second pulse, i.e. the fraction non-associated to particles, a lower concentration of LCFA_L was found in R_{bentonite} (81.4 mg_{C18:1} L⁻¹ or 110.7 mg_{C16:0} L⁻¹), compared to R_{feed} (179.4 mg_{C18:1} L⁻¹ or 270.7 mg_{C16:0} L⁻¹), 24 hours after the introduction of the recovery strategy (data not shown). Furthermore, the LCFA degradation rates (mg_{LCFA} L⁻¹ d⁻¹) were higher for R_{bentonite} reactor. This was consistent with the assumption that remaining non-particle-associated LCFA was adsorbed on the bentonite, producing a faster system recovery.

The effect of the adsorbents competition (Figure 1) reinforced the hypothesis of the surface- inhibition related process. Other possible processes like flocculation, aggregation or complex structures formation, like adsorbent-cell-LCFA (Hulshoff *et al.*, 2004; Kuang *et al.*, 2002 and 2006) needs to be considered in further research.

Adsorbents as prevention agents

Figure 2 shows the time course evolution of main detected LCFA, oleate and palmitate, in the solid and liquid phase (LCFA_S and LCFA_L), VFA accumulation and CH₄ production for the prevention experiments, in bentonite addition (T), controls (C) and blank (BL) vials. All monitored parameters were expressed in equivalent chemical oxygen demand units (COD) to facilitate comparison and enclose mass balance.

Since LCFA (0.5 g_{C18:1} L⁻¹) was incubated during 4 days in the buffered media and, in the presence or absence of bentonite (Table 1), it was considered (from adsorption isotherms, data not shown) that at time 0 all the LCFA were completely solubilised in the C vials, and completely adsorbed on bentonite in T vials (Figure 2). Initial palmitate (C16:0) concentration (day 1 in Figure 2) was due to the synthesis grade of sodium oleate salt reagent (see Materials and Methods part) and not to the beginning of a C18:1 degradation process.

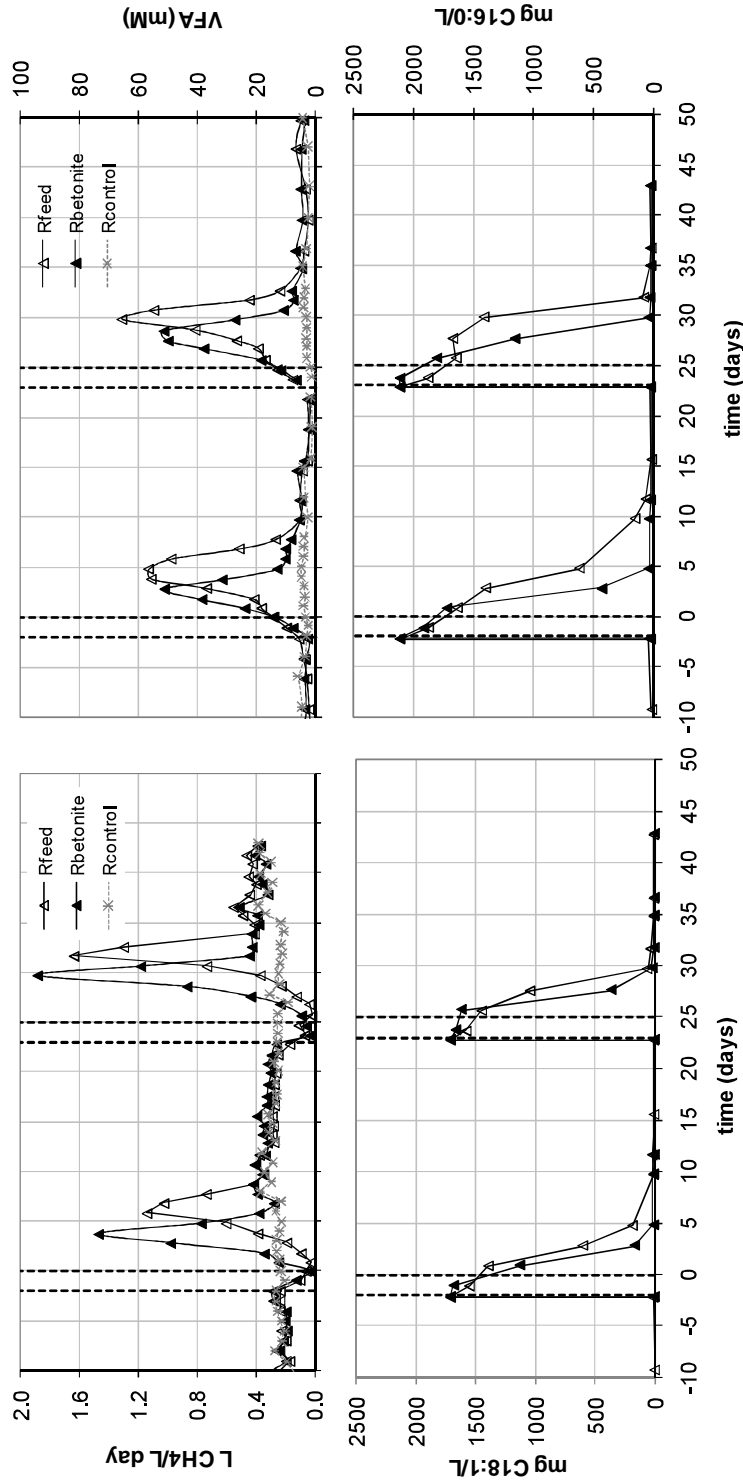


Figure 1. Time course of the methane production ($L_{CH_4} L^{-1} d^{-1}$), VFA concentration (mM) and (C18:1) oleate - (C16:0) palmitate evolution ($mg L^{-1}$) during recovery strategy experiments. Vertical discontinuous lines indicate the LCFA_M pulses ($4 g L^{-1}$) and the time of recovery actions application.

The disappearance of C18:1 from the liquid media in control vials (C), where no competitive adsorbent (bentonite) was added was very fast, in less than 5 days (Figure 2), in accordance with previously estimated isotherms and with the definition of adsorption as a fast process compared with LCFA degradation (Hwu *et al.*, 1998). In the present experiment, and from the evolution of C18:1 degradation (Figure 2), it was possible to observe differences between treatments, being the LCFA degradation rate higher for the treatment where bentonite was added (T).

No intermediates of oleate β -oxidation process were detected (data not shown), regards acetate. The reported differences between T and C vials, in terms of LCFA consumption, were also clearly detected by the acetate evolution profile, with a clear Ac accumulation only in C vials (Figure 2).

The inhibition caused by the LCFA pulse, also resulted in slower methane production in C vials, compared to the bentonite vials (T), where no delay in methane formation was detected (Figure 2). Methanogenesis has been reported to be more susceptible to LCFA inhibition compared to acetogenesis (Lalman and Bagley, 2002; Mykhaylovin *et al.*, 2005). Nevertheless, as in previous experiments, LCFA inhibition was a reversible process as methane formation was able to recover.

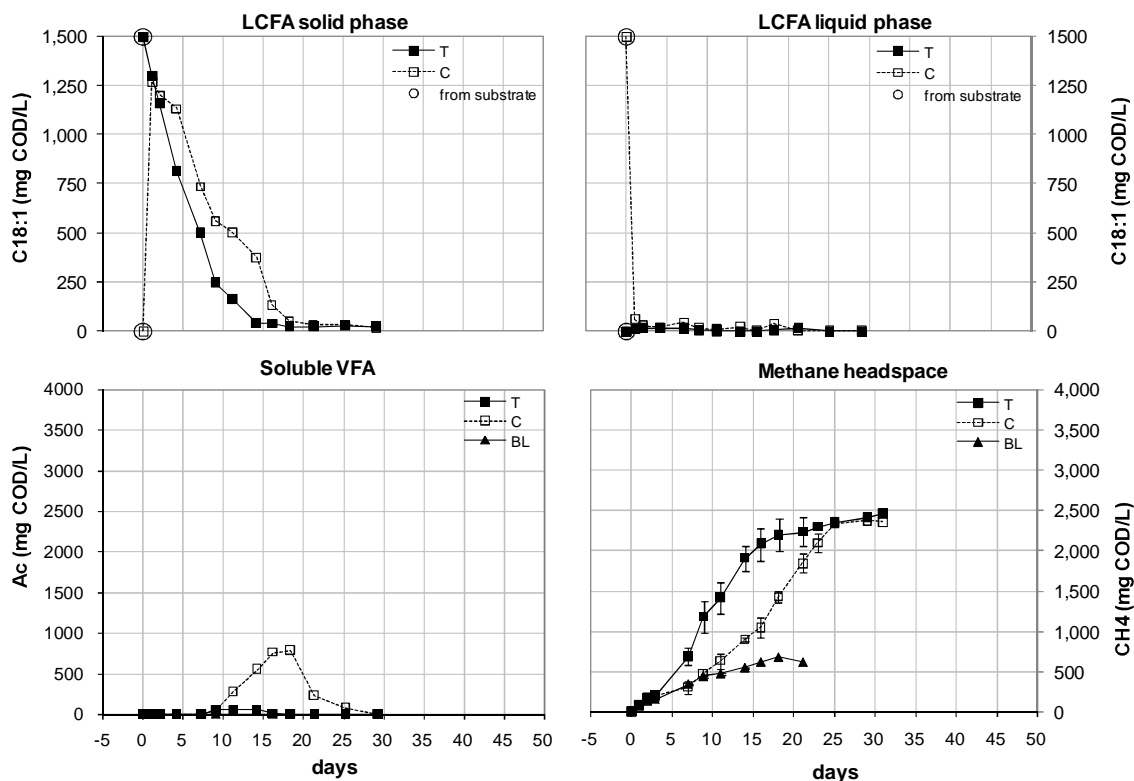


Figure 2. Time course of the oleate and palmitate (C18:1, C16:0), acetate concentration (Ac) and methane production (CH₄), during recovery strategy experiments. All parameters are expressed in COD units.

CONCLUSIONS

The results of the present batch tests demonstrated that it is possible to partially prevent LCFA inhibition by the addition of competitive adsorbents, like clay mineral bentonite. Those additives can compete with biomass for LCFA adsorption sites and, consequently, influence the kinetics of the LCFA adsorption and inhibition process.

Also, the addition of inorganic materials, as cheap clay minerals like bentonite, appeared to be a promising strategy for industrial scale implementation as remediation solution for lipid inhibited processes.

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Modelling anaerobic digestion of complex particulate/lipid-protein rich substrates: Balancing complexity and model utility using ADM1

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Abstract

Mathematical models have an important role to play in anaerobic digestion (AD) development and application in order to fully exploit the capacity of this technology. There is significant accumulated developmental experience in anaerobic digestion from which to draw lessons that illustrate the utility of modelling in developing and understanding a process as well as for control and optimisation. Anaerobic digestion of lipid /protein-rich substrates offers not only the prospect of sustainable elimination of a solid organic waste but also of its valorisation through the recovery of valuable biogas. AD of lipid/ protein-rich substrates is generally limited by hydrolysis and the key parameters of interest are the extent of the biodegradability (the *how-much*) and the hydrolysis rate (the *how-fast*). An adequate characterisation of the substrate in terms of biodegradable (and therefore biomethanisable) COD is key for an accurate prediction of methane productivity. On the other hand an adequate estimation of the coefficient for the first order apparent hydrolysis kinetics is the another crucial element necessary for an accurate prediction of the process rate, key for process design, economics and control purposes. Solids hydrolysis is characterised by a high degree of diversity and uncertainty in the mechanisms of how and in the rates at which solids become bioavailable and hydrolysed. There is a need to manage that uncertainty efficiently. Existing AD mathematical models are capable of describing these two elements of the process and can therefore be used as tools for design and control. Models need however adequate information processed from standard characterisation measurements into model variables to provide accurate predictions. The IWA Anaerobic Digestion Model No.1 is a widely used highly standardised model for anaerobic digestion processes that can be easily adapted to describe AD of high lipid/protein solid substrates. Both good substrate characterisation from typical measurements into ADM1 variables and good interpretation of data from biodegradability tests are needed to inform ADM1 for good prediction of process behaviour, rates and yields.

Keywords

Hydrolysis; solid substrates; ADM1; biodegradability; substrate characterisation.

MODELS IN ANAEROBIC DIGESTION

Models, in a broad sense, are representations of real (or envisaged) systems which make conceptual ideas of the system and, as necessary, its components and their functional relationships apparent. These ideas can be represented in many ways and also mathematically. The mathematical formulation of conceptual ideas related to a real system produces a mathematical model of that system, which can be used for e.g. a quantitative description of its response to external inputs.

In essence a mathematical model can be conceived as a machine transforming inputs (u) into outputs (y) through established relationships. The characteristics of these relationships between inputs and outputs define the basic structure of a model. The outputs are variables of interest to the model user (e.g. biogas production, COD concentration, efficiency) while the inputs are manipulated variables and/or perturbations that affect the system (e.g. flow rates, influent concentrations, temperature).

Some models consist simply of transfer functions which transform inputs directly to outputs. State-space mathematical models however use the so-called state variables (x) as intermediates between inputs and outputs. These state variables define the state of the system at a certain instant in time (e.g. species concentrations in a reaction compartment) determined from their recent past values and the inputs (u) to the system (e.g. a reactor influent composition and flow) through a function known as the state-transition equation (e.g. a mass balance). The defined outputs (y) (e.g. gas flow rate, concentration itself, removal efficiency) are generated from the state variables (x) through the so-called observation equations (Dochain and Vanrolleghem, 2001).

All the constituents of the expressions in a mathematical model are either variables, constants or parameters. Variables include inputs, outputs and states; constants are those constituents that never change their value for any model application (e.g. physical or geometric constants) and parameters are those constituents for which values can change depending on circumstances and application. Parameter values (e.g. kinetic, stoichiometric or operational) must therefore be determined for each particular model application.

Model characteristics affect the model user

Some characteristics in a model structure like level of mechanistic vs. empiricism or level of aggregation vs. segregation, are very related to model properties that are relevant to the final model user such as accuracy, interpretability of results, range of applicability of the model, its complexity and number of parameters or time required for model simulation.

As a general rule, though not strictly, mechanistic and highly segregated models are usually more accurate, their results more interpretable and they have a wider range of applicability. On the other hand they will in general be more complex and include a larger number of parameters that will need identification. They may require long simulation times particularly in the cases of dynamic, highly segregated and/or multidimensional space dependent models.

Highly empirical and very aggregated models are in general less accurate, their results less interpretable and have a narrower range of applicability. They are normally formulated with less complexity and fewer parameters. They tend to require shorter simulation times, particularly lumped parameter models.

Consideration of the model characteristics is important for selecting a modelling approach and structure which is best suited to a specific application and the rules indicated above can be of great help. It is the objective pursued or the application who defines the type of modelling we need.

Typical objectives for models describing anaerobic degradation of solid lipid/protein-rich substrates are normally the prediction of methane yield and productivity and of effluent composition.

In addition to these and considering the number of existing pretreatment options available for enhancement of biodegradability and/or hydrolysis in solids AD, the capability to incorporate the effect of these pretreatments into its structure would be also a big plus for a good mathematical model.

THE IWA ADM1

The IWA task group of mathematical modelling of anaerobic digestion developed a general purpose model for anaerobic digestion processes. The motivations for developing such a generic model were to seek for: (i) an increased model application for design, operation and optimisation; (ii) a common basis

for further model developments and validation with comparable results; (iii) an improvement in the technology transfer to industry (Batstone *et al.*, 2002). The resulting ADM1 is widely used today as a reference model for research and design purposes. Many applications and developments based on the ADM1 have been recently reported (Batstone *et al.*, 2005).

The ADM1 considers three types of processes: (i) the (bio)chemical conversions degrading stepwise solid and soluble substrates to final products mainly methane and carbon dioxide. Biomass decay and hydrolysis of solids and particulates are included among these biochemical conversions; (ii) the physicochemical processes consisting of the acid-base equilibrium (ion dissociations); (iii) the mass transfer processes between liquid and gas phase.

Figure 1 presents schematically the conversions and species considered in the ADM1. The ADM1 model is rigorous in COD balance, this feature is key for its applicability for prediction of biogas productivity from a well characterised substrates. If complete information about biodegradable COD is provided to the model, the stoichiometric prediction of methane production will be accurate as well. The ADM1 describes solid disintegration and hydrolysis with a first order kinetics since it has been experimentally observed as a good approximation.

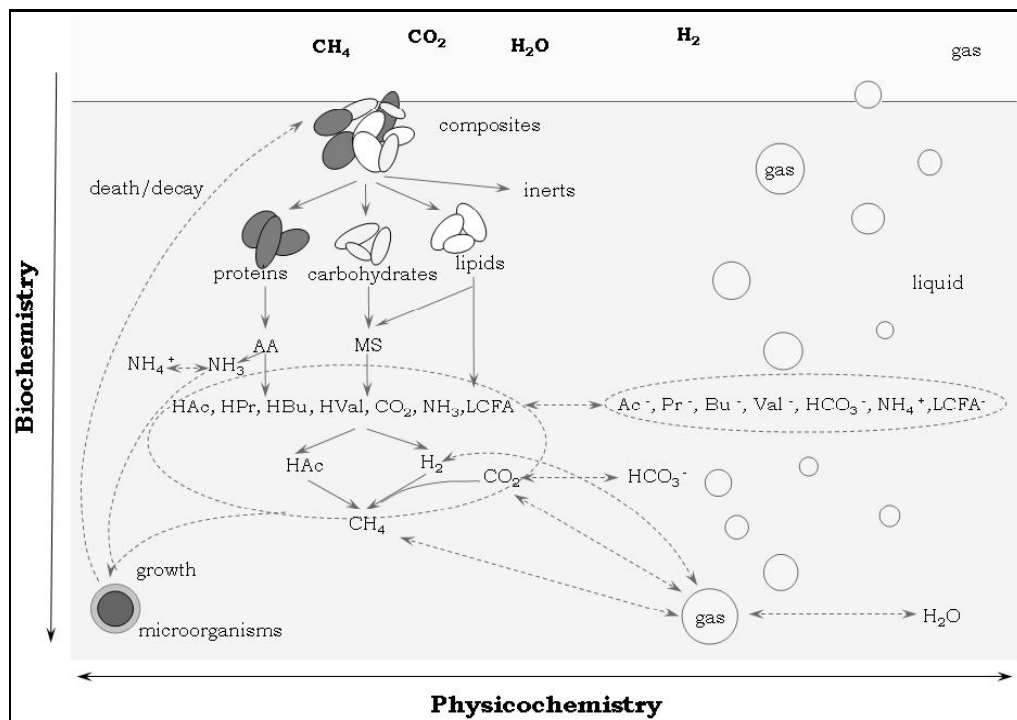


Figure 1. Overview of the processes involved in anaerobic digestion as described in the IWA Anaerobic Digestion Model No.1 (adapted from Batstone *et al.* 2002).

If hydrolysis is the limiting kinetic step, good estimations of the first order kinetic coefficients for hydrolysis, from batch BMP tests or similar, could allow the model to accurately predict process dynamics. This is however not sufficient if it is not accompanied by a good substrate characterisation that can define in particular how much of the substrate COD is biodegradable and will therefore end as methane. Failing to properly characterise the substrate degradability and composition in terms of the model variables is an often encountered cause for inaccurate predictions of biogas production. The biodegradability and composition of the substrate in ADM1 is defined by the stoichiometry of hydrolysis and disintegration distributed into lipids, proteins and carbohydrates (LPC) and the inert

fraction, which is that COD considered not to react biologically and therefore not producing any methane.

SUBSTRATE CHARACTERISATION FOR ADM1: EXAMPLE FOR MANURE

A major purpose of the substrate characterization is to achieve an accurate model prediction of the extent to which the substrate will be converted to methane.

Typically the information available from experimental measurements and essays might include (depending on the level of detail of the characterisation study): Total Solids (TS) and Volatile Solids (VS), Chemical Oxygen Demand (COD), Soluble Chemical Oxygen Demand (Soluble COD), Kjeldhal Nitrogen (TKN-N), Ammonium Nitrogen (NH_4^+ -N), Total Alkalinity, VFAs content, Proteins content, Lipids content, Carbohydrates content, pH, Density, Other measures: Liquid fraction, soluble fraction conductivity. Table 1 presents a characterisation sheet for a sample of manure.

Table 1. Composition of a manure sample in terms of standard measurements.

| MANURE RESIDUE | |
|-------------------------------------------|---------|
| CHARACTERIZATION | INITIAL |
| Liquid fraction (%) | 98.3 |
| pH | 6.9 |
| Soluble fr. conductivity (mS/cm) | 29.5 |
| Density (kgww/L) | 1.00 |
| TS (g TS/kgww) | 17.30 |
| VS (g VS/kgww) | 11.70 |
| COD (g O ₂ /kgww) | 28.90 |
| Soluble COD (g O ₂ /kgww) | 15.30 |
| TKN-N (g N/kg ww) | 3.30 |
| NH ₄ -N (g N/kg ww) | 3.10 |
| Total Alkalinity (g CaCO ₃ /L) | 7.70 |
| Chloride (g/kg ww) | 0.50 |
| Sulphate (g/kg ww) | 0.04 |
| COD/N ratio | 8.90 |
| VFA-COD (g VFA-COD/kg ww) | 12.20 |
| Acetic Acid (g COD/kg ww) | 12.20 |
| Propionic Acid (g COD/kg ww) | 0.00 |
| Butyric Acid (g COD/kg ww) | 0.00 |
| Valeric Acid (g COD/kg ww) | 0.00 |
| Proteins (g/kg ww) | 1.10 |
| Lipids (g/kg ww) | 1.50 |
| Carbohydrates (g/kg ww) | 9.20 |

The challenge to enable model application for describing the anaerobic digestion of a LPC rich solid substrate is to transform these experimental measurements into model variables as defined in the ADM1 structure with special attention on maximising the accuracy on the estimation of biodegradable COD.

Using the batch BMP assay information

The experimental methane production in a BMP batch test from an initial substrate sample provides direct information of the percentage of biodegradable COD and rate assuming that the process was controlled by hydrolysis and not inhibited in between.

This value by difference with the total substrate COD provides the estimation of the fraction of so-called inert COD to be used in the ADM1 characterisation. This fraction of inerts can be evenly distributed into lipids, proteins and carbohydrates in absence of detailed characterisation of these

fractions at the beginning and end of the essay. This information on degradability and characterisation accompanied with that on the first order rate coefficient also estimated from the methane slope of a hydrolysis limited BMP essay might be sufficient to obtain a good prediction of methane production using ADM1.

Lumped disintegration and hydrolysis

Although ADM1 assumes an initial disintegration step from a so-called complex substrate, experimentally, disintegration and hydrolysis appear often as a single step first order process.

The definition of a complex substrate which first disintegrates into the solid components (protein, lipids, carbohydrates (LPC) and inerts) obtained from the characterization might be inadequate given the uncertainty in these process and variability between substrates considering the little contribution to describing the system dynamics if the whole process is observed as a first order in most cases anyway.

DISINTEGRATION PROCESS



It is for this reason that it appears as adequate to characterise substrates as a function of hydrolysable lipids, proteins, carbohydrates (LPC) and inerts directly. For each complex substrate e.g. manure, a spreadsheet can be set up such that all the characterisation information is gathered taking into account standards analyses and, very importantly, the results of the BMP tests in order to estimate the inert fraction and lipids, proteins and carbohydrates as defined in ADM1.

The growing interest of the inert fraction

Inerts are defined as those components which are not biodegraded in a significantly long time in an essay and therefore considered that will not be either in the anaerobic digestion plant. These components however include substances that may have very interesting characteristics as soil fertilisers. It is for this reason that more detailed study of their composition based on elementary analyses and rigorous mass balances before and after the anaerobic treatment can provide interesting results about a potentially valuable by product of AD.

The formula of the inerts can be estimated from the characterisation data, depending on the level detail available, but it appears as promising to also conduct specific studies on their long term biodegradability, nitrogen content, etc.

MODELLING PRETREATMENT EFFECTS

Existing pretreatment technologies are oriented at increasing either the biodegradability of a given substrate, its hydrolysis rate or both. Very interesting recent work illustrates how the effect of pretreatments can well be described by the ADM1 and other models in an approach considering biodegradability and hydrolysis rate kinetic coefficients alone (Batstone *et al.*, 2010; Ge *et al.*, 2010). By conducting BMP essays after different pretreatments, some appears as causing an increase in final total methane productivity (impact on biodegradability) while others cause an increase in the hydrolysis rate but show no change in final total methane productivity (impact on hydrolysis kinetic coefficient only) and other on both. Figure 2 tries to illustrate this idea.

Under this approach the impact of pretreatments on biodegradability should be described by modifying the inerts fraction in the characterisation of the substrate fed into the ADM1 model, no model modification is therefore required, only at substrate characterisation level. On the other hand those pretreatments showing an impact on the hydrolysis rate but not on the final methane production can be

modelled by modifying the first order hydrolysis rate coefficient accordingly. These pretreatments therefore imply a model modification at kinetic parameter level.

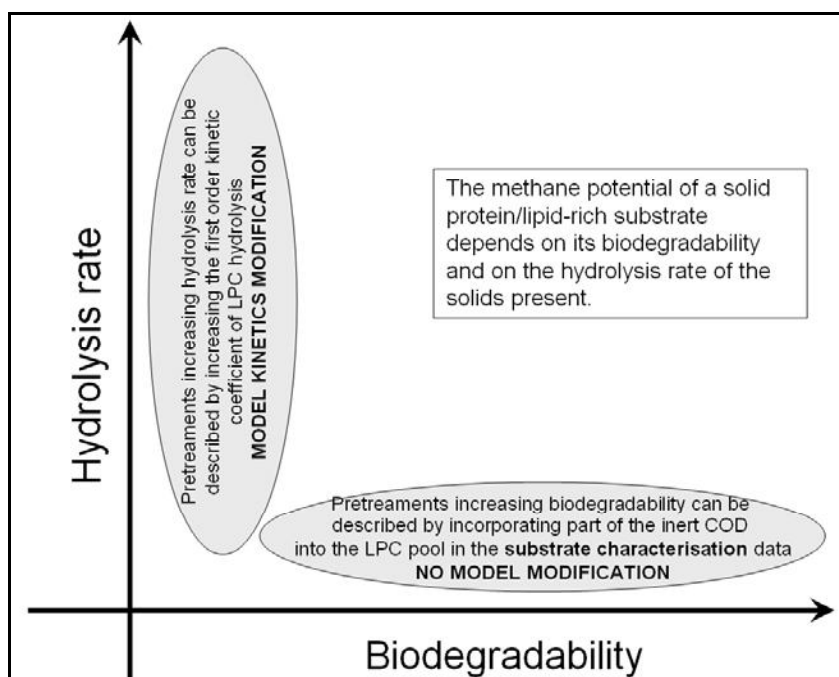


Figure 2. The impact of pretreatments can be graphically represented as a modification on biodegradability, in hydrolysis rate kinetic coefficient (grey areas) or both.

CONCLUSIONS

Mathematical models can be used to predict methane production yield and rate from solid lipids/protein-rich substrates only if they incorporate rigorous COD balance and if adequate information in terms of biodegradability and hydrolysis rate is available.

The IWA ADM1 can be easily applicable to these substrates if accompanied by comprehensive substrate characterisation in terms of COD and determination of the inert fraction. The model first order hydrolysis kinetic coefficients can be estimated from well designed BMP essays for each specific substrate type.

Accurate determination of the biodegradability fraction of a solid substrate is critical to achieve a good prediction of methane productivity, irrespective of the model used and of the accuracy on kinetic parameters estimation.

Detailed substrate specific characterisation in terms of hydrolysable lipids, protein and carbohydrates is preferred to the use of a complex substrate component given the level on uncertainty and specificity to each type of residue that this would involve confronted with the potential perceived improvement in model accuracy.

Investigation of the inert fraction composition and long term degradability deserves more attention and can prove highly useful for AD digestate valorisation as soil fertiliser.

Impact of pretreatment technologies can be easily incorporated into the ADM1 via increased biodegradable fraction, hydrolysis rate kinetic coefficient or both.

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A new reactor configuration design for the slaughterhouse waste treatment

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Abstract

Existing high rate anaerobic technology such as UASB, EGSB and IC reactors based on granular sludge is not robust when applied to industrial effluents with high lipids content. After discovering the potential of converting fat and long chain fatty acids to methane, the development of a compact high rate anaerobic reactor technology for the treatment of effluents of high lipid content was pursued. In this paper the basic principles of the IASB technology (patented) are described and results of a pilot plant treating a slaughterhouse effluent are presented.

Keywords

Long Chain Fatty Acids; Slaughterhouse wastewater, anaerobic reactor technology

INTRODUCTION

High-rate anaerobic technology is an accepted technology for industrial wastewater treatment. More than 2,000 full-scale installations are running worldwide (Van Lier, 2007) and mainly treat wastewaters containing readily degradable organic pollutants such as volatile fatty acids and carbohydrates. Lipids do not belong to this group, since their hydrolysis results in the production of long chain fatty acids (LCFA). Until recently these were considered toxic to anaerobic bacteria and a nuisance because they induce floatation of biomass (Hwu, 1997). Since the success of conventional anaerobic treatment systems is based on optimisation of biomass sedimentation, floatation leads to washout and subsequent process disruption. Therefore, lipids are normally removed from wastewater prior to anaerobic treatment using e.g. dissolved air floatation.

Pereira *et al.* (2002) showed that lipids are not toxic and can be converted to biogas. As to prevent washout induced by LCFA adsorption, a sequential process including at least a feeding and reaction phase was proposed as the preferred technology for anaerobic LCFA removal from wastewater (Pereira *et al.*, 2005). It was further postulated that the specific contact area between bacteria and LCFA should be maximised as to maximise LCFA adsorption and minimise mass transfer limitations. The sequential process was applied at lab scale by Cavaleiro *et al.* (2009). Volumetric loading rates up to 20 kg COD/m³/day were achieved on lab scale with 80% conversion to methane, with a synthetic effluent made by 50% COD as oleic acid. Furthermore, the feeding phase could be prolonged with every cycle, showing that a continuous process for LCFA treatment should be possible. From the current problems encountered at industrial scale with LCFA and the research results from Pereira *et al.* (2002-2005) two main principles may be postulated for the design of a reactor capable of high-rate anaerobic treatment of LCFA containing wastewater. These form the base of the proposed reactor concept:

- i. Maximise the contact area between biomass and LCFA as to optimize LCFA adsorption, since LCFA adsorption forms the first step in effective LCFA conversion to biogas.
- ii. Use floatation as the primary biomass retention technique, since LCFA induced floatation is currently the main reason why LCFA are removed prior to anaerobic treatment.

These two principles imply that conventional primary biomass retention techniques such as granulation or biomass fixation cannot be applied. However, a settling step is still needed, because sludge settles well again after effective LCFA conversion. This settled sludge can subsequently be intimately contacted with LCFA containing wastewater as to maximise adsorption. Thus, a sludge recycle loop should be present over the reactor. This loop could further provide the mild shear stress needed to maximise the sludge surface area. Additionally, it provides the means to control mixing intensity inside the reactor and reduce possible mass transfer limitations even further. A critical feature of this reactor is to limit the amount of LCFA accumulated onto the cells, since this causes problems of mass transfer (Pereira *et al.*, 2005). According to the kinetics determined by Pereira *et al.* (2004), the optimal amount of COD-LCFA that should be accumulated onto the cells in order to maximize the LCFA-to-methane conversion rate was about 1 g COD-LCFA/g TS. This value was however observed in batch assays, in specific and more defined conditions than the ones prevailing in a continuous reactor fed with a real wastewater. Furthermore Pereira *et al.* (2003) also determined that 1 g COD-LCFA/g TS had already a negative effect in the measured specific methanogenic activity.

Summarising, an effective reactor would need to provide the following:

1. Primary biomass retention through floatation.
2. Secondary biomass retention through settling.
3. Contact area maximisation using mild shear stress.
4. Mass transfer maximisation by adequately controlling mixing intensity.
5. LCFA adsorption induction through intimate contact between influent and recycled settled sludge.

This resulted in a novel patented reactor concept (Alves *et al.*, 2007). The invention is an apparatus specifically designed for the high rate anaerobic treatment of (waste)waters with relatively high concentrations of lipidic compounds, referred to as the Inverted Anaerobic Sludge Blanket (IASB) reactor. Contrary to conventional anaerobic reactors, it avoids the need of sludge with good settling properties and exploits the problem of sludge flotation due to long chain fatty acid (LCFA) or biogas adsorption onto the sludge and/or biogas encapsulation by the sludge. Furthermore, it provides an increased specific sludge surface area for better LCFA degradation. It is fed from the top and is equipped with a separation step at the bottom. Reactor contents are thoroughly mixed by the novel combined action of a gas lift loop and a liquid recycle over the reactor. The reactor can be operated in continuous and sequential mode. Although it is specifically designed for lipid degradation, its application is not limited to this. Figure 1 shows a schematic representation of the reactor concept.

The main results obtained in the proof of concept of the IASB reactor are presented in this paper.

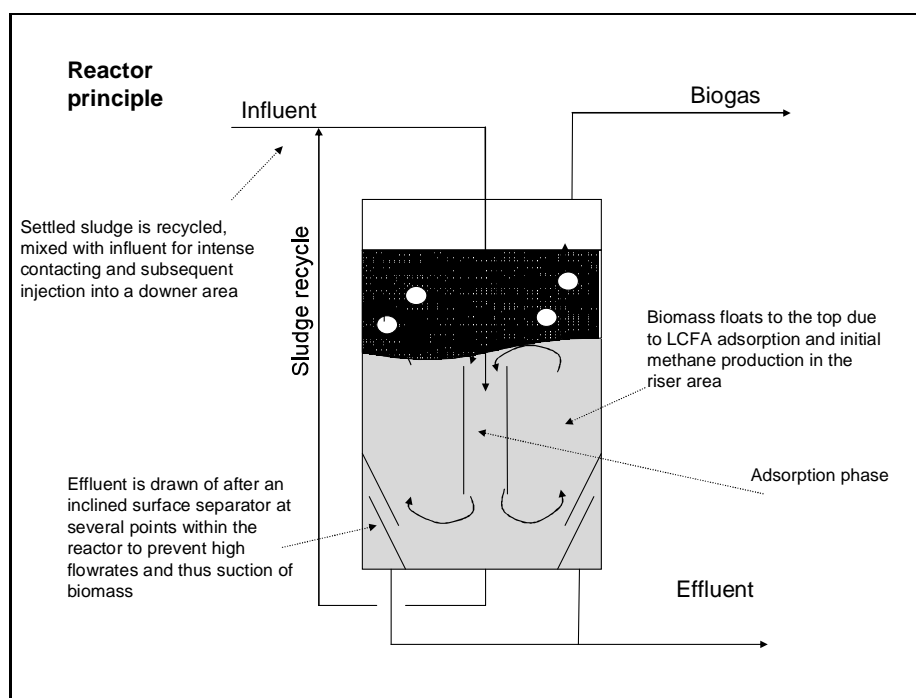


Figure 1. IASB Reactor concept.

MATERIALS AND METHODS

Experimental set-up and routine analysis

The pilot scale reactor had a total volume of 1032 L, was constructed in stainless steel and was composed of three parts: (i) reaction, (ii) separation and (iii) effluent discharge. The operating temperature was set at 37 C. Figure 2 presents the pilot scale reactor “on site”, located on the “Matadouro do Barroso e Alto Tâmega”, Montalegre, north of Portugal. The raw effluent composition was highly variable (Table 1).

Routine analysis was performed according to Standard Methods (VSS, TSS) and according to adapted Hach Lange methods (COD, P, N, S). Long chain fatty acids were quantified by the method described by Neves *et al.* (2009).

Table 1. Chemical characterization of the slaughterhouse effluent.

| Parameters | Average \pm standard deviation | Range |
|-------------------------------------------------------|----------------------------------|------------|
| Total COD, g/L | 10.7 \pm 5.6 (n=110) | [4.1-33.1] |
| Soluble COD, g/L | 4.1 \pm 2.1 (n=110) | [1.1-12.9] |
| Total Nitrogen, mgN-NH ₄ ⁺ /L | 694 \pm 429 (n=41) | [111-2554] |
| Soluble Nitrogen, mgN-NH ₄ ⁺ /L | 131 \pm 110 (n=41) | [23-457] |
| Sulphate, mg S-SO ₄ ²⁻ /L | 130 \pm 105 (n=25) | [11-554] |
| pH | 6.6 \pm 0.4 (n= 165) | [5.9-8.6] |



Figure 2. Pilot scale reactor (Montealegre, Portugal)

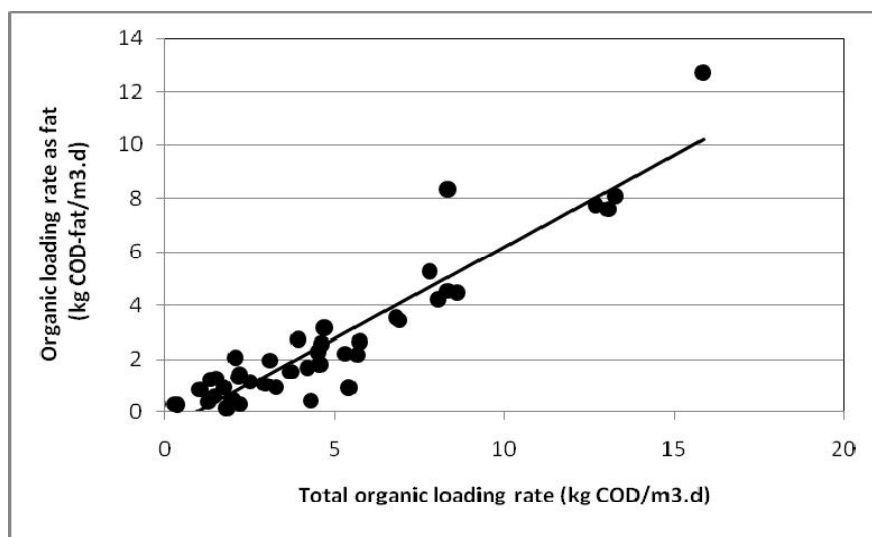


Figure 3. Relationship between the total and organic loading rate (as fat) fed to the reactor, during the operation period II.

Reactor operation was divided in two periods. In the first period, the raw wastewater (composition in Table 1) was fed for a period of about 100 days. The ratio between fat loading rate and total organic loading rate was very variable between 0.6 and 45% in this first operation period. In the operation period II that lasted 80 days, besides feeding the wastewater, animal fat was periodically introduced in the reactor, in order to increase the fat loading rate. Figure 3 represents the relationship between the total organic load and the organic load as fat fed to the reactor, which accounted, on average, to 63% of the total organic loading rate.

RESULTS AND DISCUSSION

The COD removal efficiency of the IASB reactor during the period I, was variable with an average of $83 \pm 7\%$ for total COD and 92.3 ± 3 for soluble and colloidal COD. The organic loading rate was relatively low at 1.5 ± 1.0 kgCOD/m³ d. Figure 4 represents the influent and effluent COD concentration during Period I.

During the period II, the organic loading rate achieved a value of 16 kgCOD/m³·d and the fat removal efficiency was consistently above 85% for loading rate above 10 kg COD/m³·day (Figure 5).

Passeggi *et al.*, (2009) demonstrated the effectiveness of an anaerobic reactor fed with a dairy industrial wastewater that was constituted by two UASB reactors (in parallel), an external sludge flotation tank and an external settler. The advantage of the present reactor concept as compared to the one described by this author is its compactness.

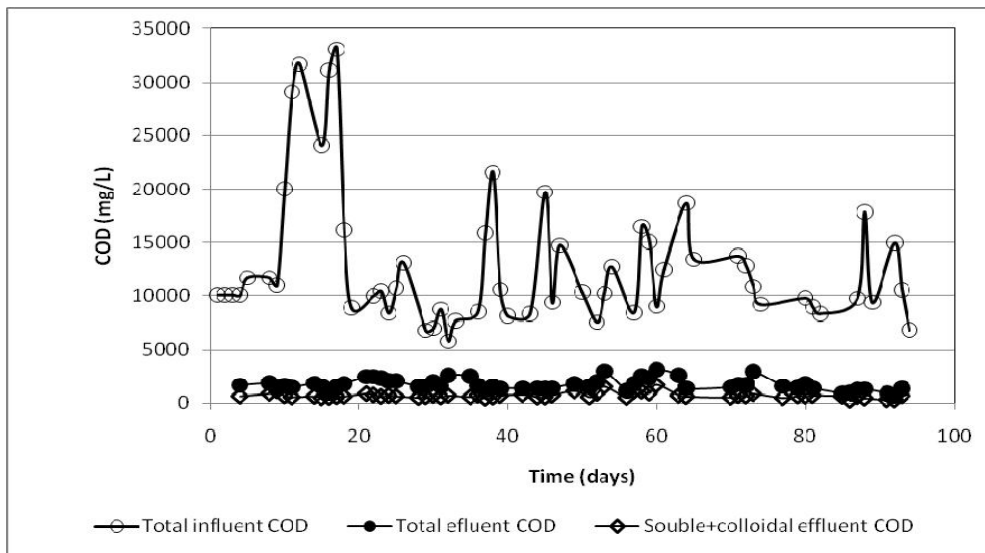


Figure 4. Influent, total and soluble and colloidal effluent COD during operation Period I.

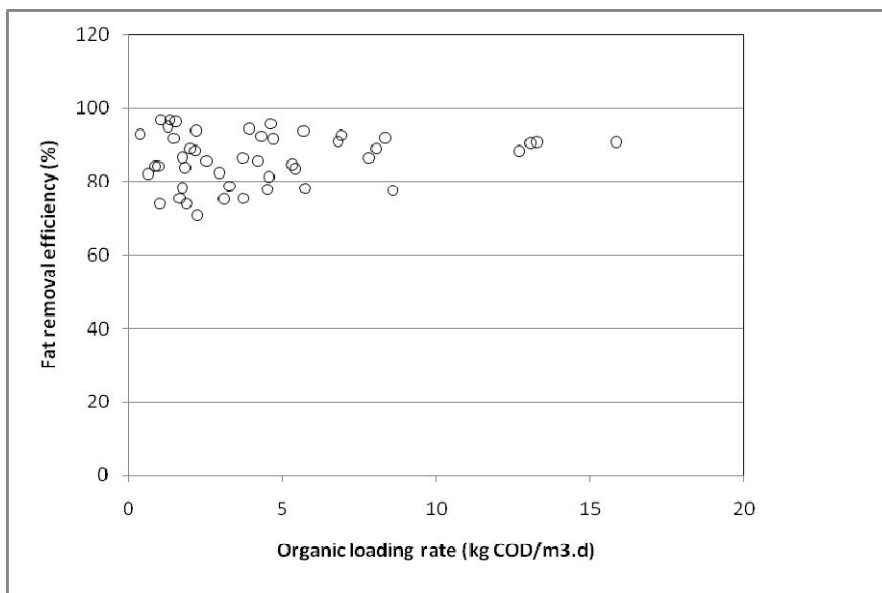


Figure 5. Influence of the organic loading rate on the fat removal efficiency, during period II.

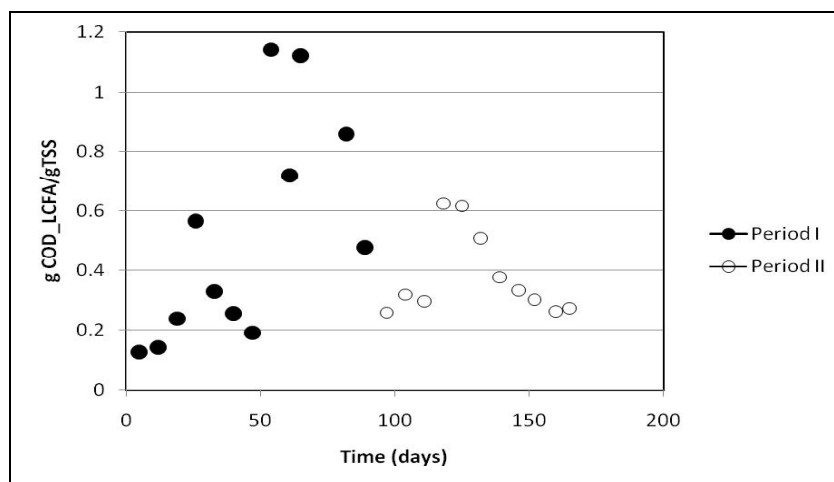


Figure 6. Accumulation of total LCFA in the floating sludge (reaction section).

The accumulation of LCFA in the reaction-flotation section was determined (Figure 6). Interestingly in the Operation Period II the amount of LCFA accumulated decreased considerably, even when the fat loading rate increased up to 12 kgCOD-Fat/m³.d. The critical value previously determined of 1 gCOD-LCFA/gTS was exceeded only sporadically in the Period I. In Period II a maximum of 0.6 gCOD-LCFA/gTS was not exceeded.

The average effluent VSS concentration in the first and second operation period respectively were 526±148 and 347±123 mg/L.

CONCLUSIONS

- The IASB reactor has proven to remove efficiently fat from an effluent with high lipids content up to 63% of the total organic loading rate.
- Fat removal efficiencies higher than 85% were achieved for organic loading rate between 10 and 16 kg COD/m³.d, (63% as animal fat).
- The effluent VSS concentration did not change significantly when the fat loading rate increased in the Operation Period II.
- Although sludge flotation was promoted in the top of the reactor the mild shear conditions applied in the reaction section, promoted an efficient LCFA degradation and no excessive LCFA accumulation was observed.

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Experiences on anaerobic digestion of poultry slaughterhouse waste

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Abstract

Anaerobic digestion can be a suitable option when considering management and financial valorization of slaughterhouse wastes, fundamentally due to the use of biogas produced in the process and even more if high methane production is expected thanks to their high lipid content. Although, this high lipid and protein content may redound in difficulties during the control of the process because of volatile fatty acids and long chain fatty acids accumulation, as well as inhibitor concentrations of free ammonia. The digestion with other substrates can reduce inhibition and organic overload problems due to dilution of nitrogen content and improvement of biodegradability. This is the reason why the organic fraction of municipal solid wastes, agricultural wastes (maize) and activated carbon were used as co-substrates in the anaerobic digestion of different slaughterhouse wastes. Specifically, continuous and mesophilic experiments of intestinal content and entrails of poultry slaughterhouse (solid slaughterhouse wastes) were performed, without a cosubstrate addition and in co-digestion with the organic fraction of municipal solid wastes. Infrared spectroscopy and thermogravimetry analysis were used to get an insight in the degree of stabilization reached by the organic matter once the anaerobic process ended.

Keywords

Anaerobic digestion; methane; slaughterhouse; thermogravimetry.

INTRODUCTION

Anaerobic digestion of organic matter has been reported as a widely used technology in the efficient treatment of organic waste and the simultaneous production of a renewable energy source through the use of biogas.

Slaughterhouses generate meat and products marketed for human consumption, pollutant solid waste and other by-products (skins, fats, bones...), as well as substantial volumes of wastewater as a result of cleaning operations. Animal by-products are all bodies or parts of animals and products of animal origin not intended for human consumption, either because they are not fit for human consumption or there is no market for them as foodstuff. Consumer demand for meats with a low unsaturated fat content in order to reduce cholesterol has led to the expansion of the poultry industry and hence to an increase in lipid and protein waste. This waste causes important environmental problems as a result of organic pollution and microbial loads, and the increasing problems of its removal must be addressed as a result of legislative constraints and the cost of treatment and disposal.

Slaughterhouse waste is an ideal substrate for anaerobic digestion and elimination more than 90% chemical oxygen demand (COD) can be attained. Lipids represent an important fraction of the organic charge in slaughterhouse waste. They consist mainly of triglycerides and long chain fatty acids (LCFA). Triglycerides may be hydrolyzed to LCFA and glycerol. Accumulation of LCFA may inhibit anaerobic digestion, because they are toxic for acetogens and methanogens, the two main groups involved in LCFA degradation (Salminen *et al.*, 2002).

Analyzing anaerobic process stability of the end product and assessment of organic matter of waste during the process is necessary for obtaining the best information about the stabilization process carried out. In this way, Fourier Transform infrared spectroscopy (FTIR) and thermal analysis are among the most promising tools for characterizing the heterogeneous organic matter, providing important information on the chemical characteristics of the samples.

In the present study we present some experiences carried out about the biomethanization (anaerobic digestion) of both slaughterhouse waste (SHW) and mixtures of solid slaughterhouse waste with the organic fraction of municipal solid waste (OFMSW) at semi-pilot scale in mesophilic semi-continuously fed digesters (Cuetos, 2008) and some results of the transformations undergone by the organic fraction of slaughterhouse waste during anaerobic digestion through FTIR and thermogravimetry combined with mass spectrometry (TG-MS), with the main objective of determining the stability level of the end product (Cuetos, 2009).

MATERIALS & METHODS

The inoculum used for starting up the digesters was obtained from the sludge digester of an urban wastewater treatment plant of the North West of Spain, with total solids (TS) and volatile solids (VSS) concentration of 11.3 and 7.0 g L⁻¹, respectively. The digester treated a mixture of primary sludge and waste activated sludge. The temperature of the digestion process was 32°C and the average hydraulic retention time (HRT) was 26 days. The bacterial consortia were adapted for 60 days to an ammonia-rich environment, since the adaptability of anaerobic microorganisms, by pre-exposing the cultures to non-inhibitory fat and high concentrations of free ammonia, is fundamental to carry out a successful anaerobic process.

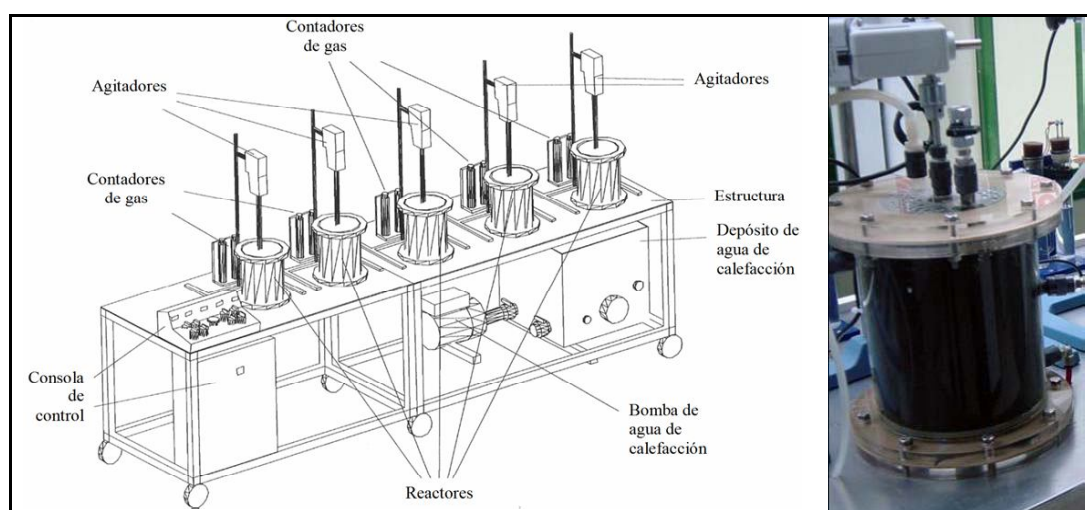
Two feeds were prepared for the study being periodically analyzed to always ensure the same solids content. A SHW feed was prepared by diluting the sample with distilled water to the desired feed load; the SHW:H₂O ratio being 1:5 in weight. The percentages of total solids (TS) and volatile solids (VS) of this feed were 4.7 and 4.3%, respectively. A combined mixture with slaughterhouse waste was prepared with an SHW:OFMSW co-digestion ratio of 1:5 in weight. The mix as prepared had a TS and VS percentages of 10 and 9.3%, respectively.

The digestion of SHW and its co-digestion with OFMSW was carried out in two completely mixed stirred digesters, with a working volume of 3 litres (Figure 1) and thermostated at 34±1°C. Both digesters had a side inlet via which the systems were fed daily. Digestion of the slaughterhouse waste was carried out in Digester A. Co-digestion of the mixtures of SHW together with OFMSW was carried out in Digester B.

The initial HRT was 50 days and it was successively reduced and the organic loading was gradually increased in accordance with the parameters shown in Table 1. For each experimental condition, the reactors were continuously operated for two consecutive HRTs to ensure a situation of steady state before changing their operational conditions.

Table 1. Digester operational parameters in the second reactor set-up carried out.

| Digester | Description | Days | HRT (d) | Loading rate ($\text{kgVS}_{\text{feed}} \text{m}^{-3} \text{d}^{-1}$) |
|----------|--------------------------|---------|---------|-----------------------------------------------------------------------------|
| A | SHW Digestion | 0-100 | 50 | 0.90 |
| A | SHW Digestion | 101-175 | 36 | 1.16 |
| A | SHW Digestion | 176-225 | 25 | 1.70 |
| B | SHW + OFMSW Co-digestion | 0-100 | 50 | 1.85 |
| B | SHW + OFMSW Co-digestion | 101-175 | 36 | 2.56 |
| B | SHW + OFMSW Co-digestion | 176-225 | 25 | 3.70 |

**Figure 1.** Semi-pilot plant.

The following parameters were monitored periodically during the digestion process: pH, total solids (TS), volatile solids (VS), alkalinity, chemical oxygen demand (COD), ammonia, yield and composition of the biogas produced and concentration of volatile fatty acids (VFA). All these variables were measured twice a week, except for ammonia, which was monitored once a week, and gas production, which was daily measured.

The analyses of pH, total and volatile solids (TS, VS), alkalinity and ammonia were carried out according to Standard Methods. The chemical oxygen demand (COD) was determined using a Hanna Instruments Series C99 multi-parameter photometer. The homogenized sample was digested in the presence of dichromate at 150°C for 2 hours in a Hanna C9800 reactor.

Daily gas production was measured using a reversible device with liquid displacement with a wet-tip counter. Biogas composition was analyzed using a Varian CP-3800 GC gas chromatograph equipped with a thermal conductivity detector (TCD). A 4-m long column packed with Haysep 80/100 Mesh followed by a 1-m long Molecular Sieve column 13 x 80/100 Mesh (1.0 m x 1/8" x 2.0 m) were used to separate methane (CH_4), carbon dioxide (CO_2), nitrogen (N_2), hydrogen (H_2) and oxygen (O_2). The carrier gas was helium and the columns operated at 331kPa at a temperature of 50°C. Standard 234 from Supelco was used to calibrate the apparatus.

Volatile fatty acids (VFA) were determined on the same gas chromatograph, using a flame ionization detector (FID) equipped with a Nukol capillary column (30 m x 0.25 mm x 0.25 μm) from Supelco. The carrier gas was helium. Injector and detector temperatures were 220 and 250°C, respectively. The oven temperature was set at 150°C for 3 min and thereafter increased to 180°C. The detection limit for VFA analysis was 5.0 mg L⁻¹. The system was calibrated with a mixture of standard volatile acids from Supelco (for the analysis of fatty acids C2-C7). Samples were previously centrifuged (10 min, 3500 x g) and the supernatant filtrated through 0.45 μm cellulose filters.

The original wastes (SHW and OFMSW) and the final effluents of reactors were dried at 105°C in a furnace for 48 h and then ground in laboratory ball mill (Retsch mill model MM200) prior to FTIR and TG analysis. Total nitrogen, total organic carbon and C/N ratios were determined as described in Cuetos *et al.* (2008) for the effluents obtained from the reactors studied.

Two milligram of separate dried milled samples were ground up with 200 mg KBr (FTIR grade) and homogenized in an agate mortar. KBr pellets were compressed under vacuum in a standard device underpressure of 6,000 kg/cm for 10 min. Infrared spectra were recorded as described Cuetos *et al.* (2009). The original wastes and the digestates were analyzed and mean values of three replicates were estimated for each sample.

TG experiments were carried out with a thermobalance coupled to a quadrupole mass spectrometer as described Gómez *et al.* (2007a). The heating rate applied to the dried and milled samples was 15°C/min from 20 to 650°C with a flow rate of 100 mL/min of synthetic air. Three replicates of each sample were analyzed and the mean values calculated.

RESULTS

The main results corresponding to the steady state periods, for different HRT and loading, are presented in Table 2. To avoid problems of system instability, a set of reactors was assembled employing an HRT of 50 days. The reactors were kept under the operational conditions reported in the Materials section during two consecutive hydraulic retention times. Under these conditions, operation of the reactors was highly stable, a fact confirmed by the presence of stable pH, alkalinity and COD.

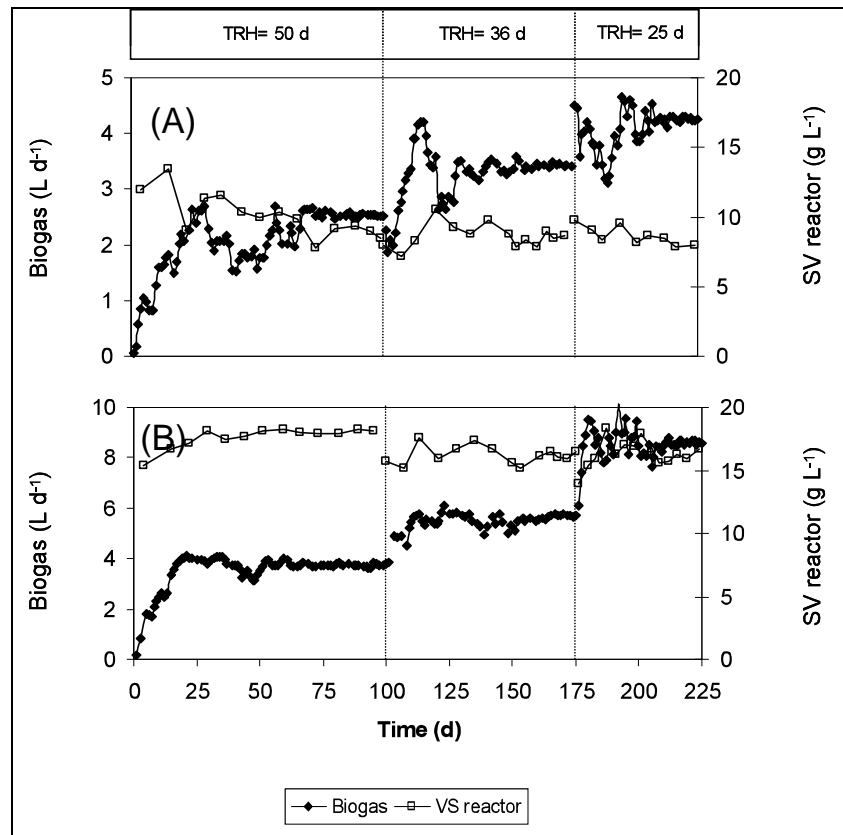
Levels of VFA of below 2.5 and 1.0 g L⁻¹, respectively, were detected at the commencement of digestion of the SHW and co-digestion with the OFMSW. A short time after start-up, and without having completed one operational hydraulic retention time, no values of VFA were detected in either of the two reactors (*results not showed*).

The average values of total ammonia during the steady state in the digesters with an HRT of 50 days (Table 3) were below the toxic limits reported in the literature (Henze, 1983). With the change of HRT and loading the system needed a period of time to adapt to the newly imposed conditions until reaching steady profiles (Figure 2).

Table 2. Steady-state operational parameters of Digesters A and B.

| Parameters | Feed | | | | | |
|---------------------------------------------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
| | SHW | | | SHW+OFMSW | | |
| HRT (d) | 50 d | 36 d | 25 d | 50d | 36d | 25d |
| Biogas (L d ⁻¹) | 2.5±0.5 | 3.4±0.7 | 4.3±0.9 | 3.7±0.6 | 5.6±0.8 | 8.6±0.9 |
| CH ₄ (%) | 64.3±2.2 | 65.6±0.3 | 66.2±0.5 | 59.4±4.1 | 61.9±1.4 | 64.5±2.6 |
| TS (g L ⁻¹) | 16.1±2.3 | 12.7±1.9 | 13.5±1.8 | 27.2±0.7 | 21.8±1.4 | 23.1±2.2 |
| VS (g L ⁻¹) | 10.1±0.5 | 8.7±0.8 | 8.7±0.7 | 18.1±0.1 | 16.1±0.5 | 16.2±0.5 |
| pH | 7.6±0.2 | 7.7±0.2 | 7.8±0.1 | 7.7±0.1 | 7.8±0.1 | 7.9±0.1 |
| Total ammonia (mg L ⁻¹) | 2143.3±88.4 | 3022.2±149.4 | 3210.8±103.9 | 2106.8±59.2 | 3830.2±73.8 | 4099.7±53.9 |
| Free ammonia (mg L ⁻¹) | 115.0±12.7 | 203.3±48.8 | 270.2±24.6 | 121.9±4.8 | 237.9±26.5 | 337.4±24.9 |
| VFA (mg L ⁻¹) | ND | ND | ND | ND | ND | ND |
| COD (mg L ⁻¹) | 18798.6±998.7 | 17116.0±966.6 | 17156.3±758.6 | 36139.2±153.4 | 26395.8±502.9 | 27603.0±472.6 |
| Fat removal (%) | 89.5±1.3 | 81.8±1.0 | 81.6±0.5 | 91.7±0.4 | 91.9±0.7 | 92.2±0.5 |
| Methane yield (m ³ kg ⁻¹ VS _{feed}) | 0.6±0.1 | 0.7±0.2 | 0.6±0.1 | 0.4±0.1 | 0.5±0.1 | 0.5±0.1 |

ND: not detected


Figure 2. Daily biogas and VS concentration in Digesters A and B.

However, the release of ammonia from the proteins provoked an increase in alkalinity concentration of the system, leading to an average value for the period higher than 5000 mg L^{-1} , the recommended value for standard rate sewage sludge digesters. Due to the high protein concentrations in the waste, pH values in the reactors were expected to be slightly higher than those reported as suitable for the development of methanogenic microorganisms with another type organic substrate; pH values remained between 7.5 and 8.0. Therefore, as a result of the increase in pH, the free ammonia concentration is high, but not inhibitory for the development of microorganisms.

The changes in FTIR spectra were evaluated by calculation of the ratio between the intensity of major peaks (Table 3) (Lguirati *et al.* 2005; Ouatmane *et al.* 2000). The peaks at 2,930, 2,851, 1,630, 1,530 and 1,034 cm^{-1} were chosen for these calculations. The 1630/2930, 1630/2851 and 1530/2930 ratios show the relationship between aromatic C/aliphatic C and aromatic C/carboxylic C, while the 2930/1034 ratio explain the correlation between aliphatic C and polysaccharide. Easily degradable organic matter constituents, such as aliphatic components, polysaccharides and alcohols are oxidized and the volatile content of wastes is reduced, increasing the aromaticity degree (Gomez *et al.* 2007).

In this sense, these ratios could be used as stability indexes since the changes in these ratios indicate changes in organic matter composition. Ratio analysis allowed distinguishing between anaerobic systems at different HRT, a behaviour that could not be easily appreciated from FTIR spectra. The 1630/2930, 1630/2851 and 1530/2930 ratios decreased as HRT was decreased while 2930/1034 ratio increased with the decrease of HRT. This may be rationalized by an increase in lipid content as consequence of a lower efficiency in the anaerobic process, originated from the decrease in fat degradation. An increase in the value of these ratios was expected with the increment in the HRT as consequence of the stabilization process.

Table 3. Ratios of selected FTIR peaks from digestate samples (Cuetos, 2009).

| Digester | 1630/2851 ^a | 1630/2930 ^b | 1530/2930 ^c | 2930/1034 ^d |
|-----------------------|------------------------|------------------------|------------------------|------------------------|
| SHW failed-25 | 0.43 ± 0.03 | 0.41 ± 0.03 | 0.26 ± 0.02 | 2.26 ± 0.20 |
| SHW + OFMSW failed-25 | 0.57 ± 0.04 | 0.46 ± 0.03 | 0.36 ± 0.02 | 2.27 ± 0.20 |
| SHW-50 | 1.70 ± 0.15 | 1.02 ± 0.09 | 0.70 ± 0.06 | 0.77 ± 0.06 |
| SHW-36 | 1.70 ± 0.15 | 1.03 ± 0.09 | 0.71 ± 0.06 | 0.79 ± 0.07 |
| SHW-25 | 1.06 ± 0.09 | 0.72 ± 0.06 | 0.44 ± 0.03 | 1.57 ± 0.13 |
| SHW + OFMSW-50 | 3.05 ± 0.25 | 1.19 ± 0.09 | 0.72 ± 0.06 | 0.51 ± 0.04 |
| SHW + OFMSW-36 | 2.04 ± 0.20 | 1.08 ± 0.09 | 0.77 ± 0.06 | 0.85 ± 0.07 |
| SHW + OFMSW-25 | 1.50 ± 0.10 | 0.88 ± 0.07 | 0.63 ± 0.05 | 1.62 ± 0.14 |

^a Relationship between aromatic C/aliphatic C

^b Relationship between aromatic C/carboxylic C

^c Relationship between aromatic C/aliphatic C

^d Relationship between aliphatic C/polysaccharide

Digestate SHW-50 presented thermal oxidation at a temperature close to 200°C , while digestates SHW-36 and SHW-25 initiated this oxidation at a lower temperature. The SHW failed-25 sample presented a peak with a comparable intensity to that of the SHW waste. In this case, it could be attributable to easily biodegradable materials that have not yet been consumed by microbial populations as consequence of the overloading and subsequent collapse of anaerobic digestion suffered by this system.

The degradation of organic matter by means of anaerobic digestion leads to an enrichment in aliphatic components (Gomez *et al.*, 2007; Francioso *et al.*, 2009) in the study of the digestion of sludge in a two phase process reported the formation exclusively in the methanogenesis phase of alkanes (Cn-12 and Cn-13) by means of HS–SPME–GC–MS. These compounds usually derive from oils or fuels, but their presence in methanogenesis suggests a biological origin.

The continuous mass loss experienced by the digestate sample SHW-50 and SHW-36 may be explained by the higher content of the aliphatic components. In the high-temperature range, corresponding to the weight loss suffered by structurally more complex materials, the intensity of the peaks and the temperature at which they take place changed with the decrease in HRT.

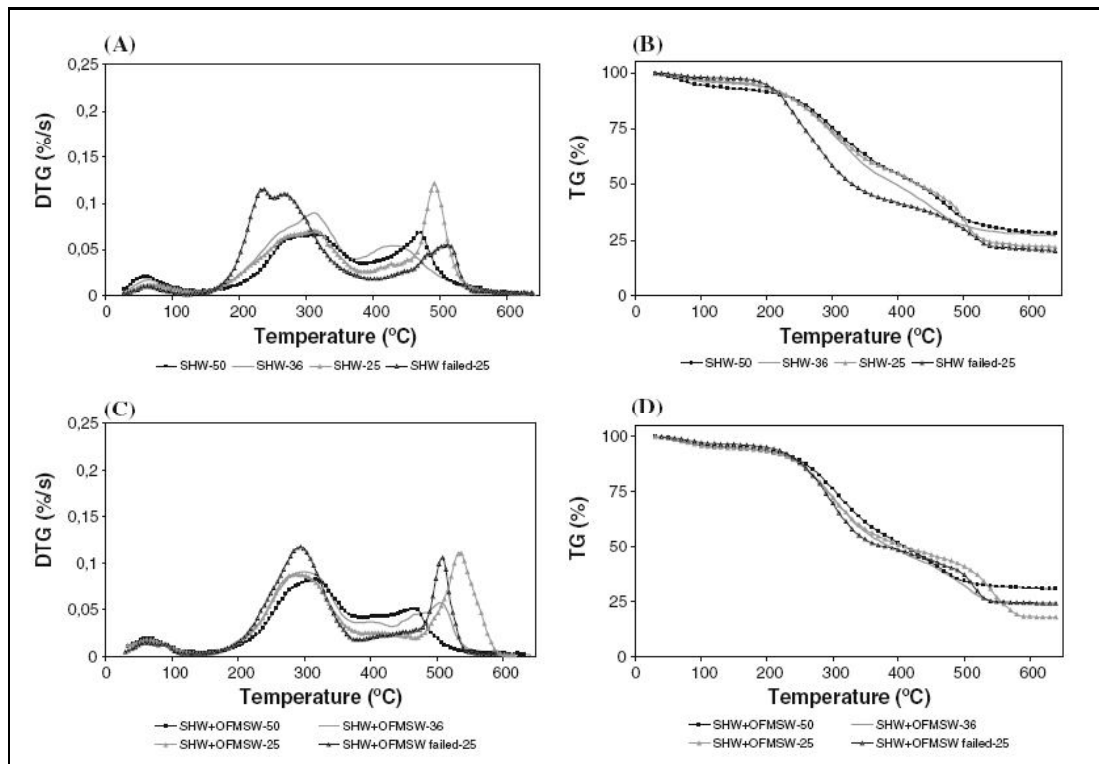


Figure 4. Evolution of the weight-loss profiles (TG–DTG) of the obtained digestates from co-digestion process under the different operational conditions considered.

The intensity of this peak for reactors with higher HRT (SHW-50 and SHW-36) was lower, indicating that complex materials generated during the process are consumed subsequently, showing a preferential degradation during stabilization of the organic matter (in accordance with the increase in the aromatic structures in final effluents showed by FTIR). On the other hand, the DTG from sample of digestate SHW-25 presented a narrow peak with a greater intensity displaced to the right on the temperature scale compared with the other digestates from successful anaerobic process. This fact could be related to the formation of complex compounds during metabolic activity that have not yet been consumed by the microorganisms due to the smaller HRT applied to the digester.

CONCLUSIONS

It was possible to carry out anaerobic digestion of SHW and co-digestion of mixtures of SHW with OFMSW by progressively decreasing the HRT from 50 days to 25 days while increasing the organic loading from 0.9 and 1.85 kgVS m⁻³d⁻¹ to 1.70 and 3.70 kgVS m⁻³d⁻¹, respectively. In consequence, the

adaptability of anaerobic microorganisms to a fat-and free ammonia-rich medium was observed by pre-exposing the cultures to non-inhibitory concentrations.

A successful stabilization process of organic matter led to an increase in aromaticity degree and a reduction of volatile compounds as stabilization process was carried out. FTIR spectra obtained from successful anaerobic systems presented a high amount of unsaturated bands of digestates. These reactors also showed a reduction in the low-temperature range (~300°C) of derivate thermogravimetry (DTG) profiles, related to easily oxidized materials, while the weight loss in the high-temperature range (450–550°C) was variable for the different systems, so the intensity of the weight loss peak in the high-temperature range decreased in the reactors with higher hydraulic retention times, whereas its intensity increased and the peak was displaced to higher temperatures for the digesters with lower HRT.

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Full scale application of anaerobic digestion of slaughterhouse wastes – long term experiences, problems and resulting strategies

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Abstract

In this contribution a four year-period of operation of a full scale plant operated with blood, colon, gut and fat scrubber content is described. Through the production of methane from diverse slaughterhouse waste products almost half of the energy demand of the slaughterhouse is covered. The semi-continuous operation of the biogas plant results in a characteristic pattern of methane concentration and productivity through the week. High values of Ammonia-Nitrogen result from the high protein content of the substrate and represent approx. 82 to 86% of the TKN. The concentrations of free ammonia are calculated based on simple dissociation-constants and through equilibrium-simulation taking mayor acid components into account. The results suggest that high concentrations of volatile fatty acids are necessary to keep values of unionized Ammonia (NH₃) low.

Keywords

Anaerobic digestion; slaughterhouse wastes; ammonia; toxicity; volatile fatty acids; TSE; prion protein.

INTRODUCTION

Rendering of animal proteins has been an accepted pathway for treatment of slaughterhouse wastes for a long time. Due to the appearance of BSE in Europe, the European Commission banned rendered animal protein from the food given to farmed animals in 2000 (European Commission, 2000). The result was an increase of gate fees for the treatment of slaughterhouse wastes on the one hand and a new regulation from the European Commission and the European Parliament laying down health rules regarding animal by-products not intended for human consumption (European Commission, 2002) on the other hand.

Only specific parts of animals in which concentrations of presumable BSE or TSE-infectious tissues are not present may be used in biogas plants. Experiments have shown that the concentrations of those infectious proteins (“Prions”, Prusiner, 1982) may not be reduced substantially under anaerobic conditions (Kirchmayr *et al.*, 2006).

Every year, more than 16 million tons of materials of animal origin not intended for human consumption, the bulk of which derive from healthy animals, are produced in the EU (European Commission, 2010). In Austria 582,000 tons of ABP’s per year (including catering waste) need to be treated. From there 347,000 t is slaughterhouse waste (derived directly from slaughtering and from the meat processing industry) which equals around 40 kg per capita and year (Lebensministerium, 2010).

In the Regulation (EC) No 1774/2002 and the Regulation replacing the latter Reg. (EC) No 1069/2009 the treatment of slaughterhouse wastes (animal by-products, ABP's) from animals slaughtered for human consumption in biogas plants is explicitly pointed out and parameters for the operation of those biogas plants are defined. These treatment parameters consist principally of general hygienic rules and a pasteurisation or hygienisation unit is defined, which may not be bypassed (treatment at minimum 70°C during 60 minutes with maximum particle size of 12mm or a corresponding validated process).

Based on this clear European legislation, several abattoirs deliver ABP's to biogas plants which treat slaughterhouse waste in codigestion with other wastes. Only sparse information is available on slaughterhouses constructing their own biogas plant to treat their wastes and to gain energy for slaughterhouses.

Rudolf Grossfurtner GmbH, Austria's biggest pig slaughtering facility runs a biogas plant operated only on ABP's of pigs such as blood, colon, fat scrubber content (after dissolved air flotation) and rumen content from the nearby bovine slaughtering facility. In the actual plant setup the biogas formed allows a production of approx. 4.7 MWh/d of electricity and 7 MWh/d of heat. The electricity produced from biogas is fed into the national power grid and the heat is delivered to the slaughtering facility. The specific heat-load-profile of the slaughterhouse showed that only during 35% of the time the heat produced by the CHP could directly be consumed in the slaughterhouse. Therefore a hot water storage tank serves as a buffer to match the steady heat production with the heat demand of the slaughterhouse. Thus nearly all of the heat produced in the biogas plant is used in the slaughterhouse, covering 45% of its thermal energy demand (Kirchmayr *et al.*, 2009).

The handling of a biogas plant operated with ABP's requires sensitivity not only in the field of hygiene and toxicity of nitrogen, but also regarding odour emissions (Kirchmayr *et al.*, 2007). The manipulation hall, the buffer tank and eventually open storage lagoons have to be connected to a sufficient exhaust air treatment or in the latter two tanks connected to the gas collection system due to the emission of hydrophobic substances. Even the digestate used as agricultural fertilizer may cause nuisances, which have to be overcome by low-emission spreading.

In this pig slaughtering facility around 5 L of blood, 6 L of hind gut (and their content) and 10 L of fat scrubber content (after dissolved air flotation) per slaughtered animal are produced and treated in the biogas plant. These figures include a little bit of process water and therefore are slightly higher than those reported by Freudenreich and Bach (1993).

Pilot scale applications and experiences of anaerobic digestion of slaughterhouse wastes (Edstöm *et al.*, 2006; Hejnfeldt and Angelidaki, 2009; Lopez *et al.*, 2006; Siegrist *et al.*, 2006; Wu *et al.*, 2009) have already been reported, but no full-scale application has been described so far.

High concentrations of free ammonia cause microbial inhibition. Between the different strains of microorganisms involved in anaerobic digestion, the methanogens are the least tolerant and therefore most likely to be inhibited (Koster, 1986; Koster 1988; Angelidaki and Ahring, 1993; Braun *et al.*, 2010; Schnurer and Nordberg, 2008). The inhibition results in bad degradation rates which go along with poor biogas yields and an accumulation of volatile fatty acids. Practical experiences with full-scale bio-waste and slaughterhouse waste digesters show that ammonium concentrations exceeding 5 g/L NH_4^+ -N still allow stable operation (Kirchmayr *et al.*, 2007; Resch *et al.*, 2006). In this biogas plant the monitored concentration of ammonia nitrogen (NH_4^+ -N) in the fermenters ranges (depending on the substrate throughput and composition) from 5.3 to 8.5 g/L. Such high levels cause microbial inhibition and drops in the methane potential (Angelidaki and Ahring, 1993; Hansen *et al.*, 1998).

Inhibition of the biogas process is related to total ammonia concentration (Kayhanian, 1999; Sprott and Patel, 1986; Wiegant and Zeeman, 1986) and free or unionized Ammonia (NH_3 , UAN – unionized ammonia nitrogen), which is considered to be the toxic form (McCarty and McKinney, 1961).

Free Ammonia is a function of Ammonium (NH_4^+) concentration, pH, CO_2 , the concentration of volatile fatty acids and the digester temperature. Thus thermophilic fermenter temperature increases the part of NH_3 , increasing gas pressure and higher concentrations of volatile fatty acids will decrease the inhibiting effect of high ammonia concentrations.

ANAEROBIC DIGESTION OF PRION-PROTEIN CONTAINING MATERIAL

The behaviour of the TSE causing infectious agent denominated prion protein (Prusiner, 1982) through the application of different deactivation protocols (for example: high temperature, pressure and chemicals) has already been described (Taylor *et al.*, 1995, 1998; Taylor, 1999, 2001; Oberthür, 2001), however, the behavior of PrPSC under anaerobic degradation conditions has not been reported. Under mesophilic conditions (35–38°C) no or very little reduction of the PrPSC titer could be observed. Degradation experiments of TSE positive brain homogenates (22A/SV mouse-scrapie, 301V/VM mouse BSE and bovine BSE) under thermophilic anaerobic conditions (55°C, CO_2 atmosphere) showed a reduction of the luminescence on the Western blot. PrPSC deriving from bovine BSE shows the highest stability, followed by 301V/VM mouse BSE and 22A/SV mouse scrapie.

The luminescence declined within the incubation time of 302 hours to 20–40% of the initial values which equals a reduction of the PrPSC titer of about one order of magnitude.

Under the experimental conditions PrPSC of both prion protein sources (bovine BSE and 301V/VM) showed a clear matrix dependent behavior. The relative luminescence reduction graph of TSE positive brain homogenate in water and active anaerobic sludge is similar.

In sterilized (120–130°C/20 min) and poisoned anaerobic sludge (NaN_3) the PrPSC titer reduces much faster. Both PrPSC sources show the fastest reduction of the luminescence in sterilized sludge, although the preliminary experiments showed an improved detection of PrPSC in steam pressure sterilized sludge than in gamma irradiated (26 kGray) anaerobic sludge.

The prion protein in its physiological and not TSE associated form (PrPC) is a cell-surface protein (Hornemann and Glockshuber, 1996; Harris, 2001). Although the pathway of PrPSC formation is not identified yet, parts of membranes could play an essential role (Lehmann and Harris, 1995; Vey *et al.*, 1996; Naslavsky *et al.*, 1997; Caughey *et al.*, 2001; Harris, 2001).

The current knowledge regarding PrPC and PrPSC available and the higher amounts of cell wall fragments present in deactivated anaerobic sludge substantiates the hypothesis of a major involvement of membranes in the enhanced reduction of the PrPSC titer observed.

No PrPSC could be detected without a previous detergent treatment. Consequently PrPSC will attach to the solid fraction in wastewater or the effluent of waste water treatment plants. These estimations of Cohen *et al.* (2001), Gale and Stanfield (2001), and Gale (2002) in their TSE-risk assessment of wastewater were confirmed by our experimental results.

Infectivity

In the experiments described here, the reduction of the prion protein titer under anaerobic degradation conditions was examined. After an incubation time of more than 380 hours under mesophilic anaerobic conditions (35 °C) and 302 hours under thermophilic anaerobic conditions (55 °C) respectively, PrPSC could still be detected.

Beyond doubt, PrPSC plays an essential role in the TSE infectious agent (Büeler *et al.*, 1993; Blättler *et al.*, 2003). There is serious concern about PrPSC as to whether it is the only fraction of the TSE infectious agent (Wille *et al.*, 1996; Manuelidis, 1997; Farquhar *et al.*, 1998; Shaked *et al.*, 1999, 2001; Manousis *et al.*, 2000; Somerville, 2000; Dormont, 2002; Manuelidis, 2002). This leads to the conclusion that the reduction of infectivity may only be confirmed by means of bio-assays. Further research is necessary to correlate the results described here with the supposed reduction of infectivity under the same conditions.

Technical relevance

The anaerobic degradation process has been described as a reaction of the first order (Pavlostathis and Giraldo-Gomez, 1991a, b). The deactivation of TSE infectivity by means of heat has also been characterized as a reaction of the first order (Oberthür, 2001). In the rendering process a reduction of TSE infectivity by a factor of 10²–10³ has been reported by Taylor *et al.* (1995, 1997) and Oberthür (2001). Furthermore, through the reduction by anaerobic degradation investigated here, it is obvious that the decrease of PrPSC would require an elevated solids retention time in the biogas reactor. Practice shows that the average solid retention time in biogas reactors should not fall below 30 days. Therefore a considerable degradation of PrPSC by a factor 10⁴–10⁶ within 10 days would give the anaerobic digestion of rendered animal material an opportunity of being considered as an alternative MBM treatment possibility, as in the end an overall PrPSC reduction factor of 10⁶ could be summarized. The experiments described here do not show a PrPSC reduction within an incubation time short enough to bring the anaerobic digestion of TSE infectious tissue into technical considerations. In order to confirm those experiments the reduction of TSE infectivity by incubation with anaerobic sludge has to be assessed.

DESCRIPTION AND OPERATION OF THE BIOGAS PLANT

The plant design corresponds to the classical concept of an agricultural biogas plant: two main fermenters of 600 m³ and 1,000 m³, respectively, a secondary fermentation tank (without heating, 1,000 m³) and a storage tank (3,200 m³) were constructed. The biogas plant is operated with selected fractions of the pig slaughtering process such as pig blood, minced hind gut including content and fat scrubber content (after dissolved air flotation, DAF).

According to the European Regulation (EC) No. 1774/2002, the fat scrubber content has a maximum particle size of 6 mm and the other substrate fractions are crushed to a maximum particle size of 12 mm. The substrate is pumped from the slaughterhouse to a buffer tank and subsequently pasteurised (70°C/60 min). Before feeding, the substrate is cooled to 55°C in order to minimise a possible damage of the bacterial biomass in the biogas fermenter. In average approx. 20 m³ of slaughterhouse wastes per day are fed in parallel to the two digesters.

Rumen content from the neighbouring cattle slaughterhouse is delivered to the biogas plant and fed through a feeding screw directly into the smaller digester. Both digesters are operated at mesophilic temperatures (35°C). Depending on the substrate composition ammonia-nitrogen levels vary between

5.3 and 6.2 g/L in fermenter 1 and between 6.0 and 7.4 g/L in fermenter 3 respectively, corresponding to a TKN concentration between 7.1 and 8.5 g/L.

Loading rates may be calculated based on the real-case-scenario of feeding 5 days per week or as weekly mean values. The real daily organic loading rate (5 days/week feeding) of the biogas plant is 5.07 kg/m³.d VS (10.8 kg/m³.d COD) in fermenter 1 and 2.95 kg/m³.d VS (6.28 kg/m³.d COD) in fermenter 2 respectively.

Due to reduced blood addition and high water intake there are two periods with lowered NH₄⁺-N values. Based on those different Ammonia (and TKN)-concentrations a probable correlation between milieu conditions and gas production may be calculated. Comparing maximum methane yield and real produced as recovery percentage) over the monitoring period of 4 years a fair correlation can be identified between recovery percentage and milieu conditions such as TKN, NH₄⁺-N, free ammonia NH₃-N or volatile fatty acids. Although, regression analysis show a tendency of decreasing daily gas yield and increasing volatile fatty acids on increasing nitrogen (TKN; NH₃-N or NH₄⁺-N) concentrations.

Only values in fermenter 3 show a reasonable correlation between the concentration of NH₄⁺-N and volatile fatty acids. Linear regression analysis show a coefficient of determination for fermenter 3 of r²=0.4712 (F2: r²=0.057; F1: r²=0.1608).

The analysis of correlation between Nitrogen content (TKN, NH₄⁺-N or free NH₃-N) and Gas or Methane production or gas quality shows values of the coefficient of determination below 0.1.

The influence of ammonia-nitrogen (NH₄⁺) or free ammonia (NH₃) has been described under batch conditions using increasing concentrations of volatile fatty acids as an indicator for process instabilities (Angelidaki and Ahring, 1993; Edström *et al.*, 2003). The concentration of free ammonia has been calculated based on two methods. Based on chemical dissociation constants, pH-values and ammonia-concentrations quite high values for free ammonia concentrations are the result. These values will not represent the real concentrations present in the liquid because they do not consider the acid components and gas bubbles also present in the liquid. Under continuous fermentation conditions, carbamates and other acid components such as volatile fatty acids will reduce the concentration of free ammonia due to their counterion-character to NH₄⁺. In a steady state fermentation situation, there will be equilibrium of volatile components such as volatile fatty acids, ammonia and CO₂. Using a process-simulation software (ASPEN PLUS), the equilibrium concentrations of free ammonia were calculated. In these values the equilibrium between liquid and gaseous phase (based on the law of Henry) and acid components (all forms of CO₂, Acetic and Propionic Acid) are considered. These real concentrations of free ammonia (having acid components into account) are in a very constant range between 100 and 200 mg/L. In comparison the simple calculation results in values ranging from 500 to 2000 mg/L. The latter values would far exceed values of NH₃-toxicity reported. The resulting values of the simulation are close to those inhibition values described to unadapted cultures (Braun *et al.*, 1981; de Baere *et al.*, 1984).

Due to the bad correlation none of the resulting values can be used to describe the behaviour of the methane recovery rate.

In fact this result suggests that the high concentrations of volatile fatty acids observed were necessary to keep the concentrations of free ammonia on a very low level. This further suggests that under those conditions volatile fatty acids are not suitable to be used as an indicator for process-stability.

The handling of a biogas plant operated with slaughterhouse wastes (ABP's) requires high sensitivity in regards to hygiene, odour emissions and the influence of ammonia-nitrogen to the activity of micro-organisms. This example shows that the operation of a biogas plant only with slaughterhouse wastes is possible, even covering half of the energy demand of the slaughterhouse.

The operation of this biogas plant shows that anaerobic digestion of high protein containing wastes under sub-optimal (inhibited steady state), but stable conditions is feasible. The micro-organisms will adapt to ammonia-nitrogen (NH_4^+ -N) concentrations up to approx. 7.5 g/L transforming more than 85% of the total nitrogen into ammonia-nitrogen. High concentrations of volatile fatty acids in the range of 5 to 25 g/L remain in the fermentation liquid to keep values of free ammonia (NH_3) ranging from 100 to 200 mg/L, causing probable additional odour nuisances.

REMOVAL OF NITROGEN

The influence of high nitrogen concentrations on the micro-organisms involved in the anaerobic degradation cascade has been described for a long time (Kroeker *et al.*, 1979; van Velsen, 1979).

To overcome nitrogen influence two strategies were assessed in laboratory-scale: widening of the tight C:N ratio by adding a further carbon-source on the one hand or reducing ammonia-nitrogen by simple stripping on the other. Continuous (in a 2L lab-scale fermenter) and discontinuous (batch, 2L) experiments in adding further C to sludge of fermenter 3 from previous described biogas plant by means of glycerine, starch and/or fat did not show a higher biogas yield nor a significant reduction in volatile fatty acids (Resch *et al.*, 2007). Using a laboratory-scale steam-stripping column the ammonia-nitrogen concentration of the sludge in fermenter 2 was reduced stepwise to 4g/L. Using this as a substrate to feed a lab-scale fermenter, varying the organic loading rate and hydraulic retention time (using sludge of fermenter 2 as inoculum), a substantial reduction of volatile fatty acids (90%), enhanced degradation of COD (46 % or 100 % of degradable fraction, based on batch-test assays) and a significant increase in the methane yield up to 41% (at TKN-values of 4g/L) compared to the reference could be observed (Resch *et al.*, 2010, in preparation).

Based on this experiments a full-scale nitrogen-removal plant based (Patent pending) was designed for the described Biogas plant at the Slaughterhouse Rudolf Großfurtner GmbH.

Adding a 20 fold overdosage of a trace element solution (based on Scherer *et al.*, 1983) to sludge of fermenter 2 of the previous described biogas plant using this as a substrate for a continuous operated lab scale fermenter (6L), inoculated with sludge from fermenter 3, could reduce the concentration of volatile fatty acids and showed a significant higher performance. The reference scenario without trace element addition showed remained at levels of VFA corresponding to those in the full scale fermenter.

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Slaughterhouse waste co-digestion – 15 years of full-scale operation

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Abstract

At Tekniska Verken in Linköping, Sweden, there is a long time experience of handling and, through co-digestion, producing biogas from large volumes of slaughterhouse waste. This experience has led to a profound understanding about the process of anaerobic digestion of slaughterhouse waste, both on a macroscopic as well as on a microscopic scale. Throughout the years, experiences from R & D and plant operations have led to the implementation of several process improving technical/biological solutions. Hence, we can in this paper show that the improvements have had several positive effects on the process, including energy savings, better odour control, better gas quality, higher organic loading rates and higher biogas production from less material. Furthermore, all the above have been accomplished with maintained or increased process stability. In addition to the above we can show that much of the mechanism behind process stability in anaerobic digestion of slaughterhouse waste relates to the plant operation, which allow the microbiological consortia to adapt to the substrate. Because digestion of proteinaceous substrates like slaughterhouse waste leads to high ammonia loads, special requirements in ammonia tolerance are placed on the microbiota of the anaerobic digestion. Biochemical assays revealed that the main route for methane production proceed through syntrophic acetate oxidation, which require longer retention times than methane production by acetoclastic methanogens. Thus, we can show that the long retention time of the plant, accomplished by a low dilution of the substrate, is a vital component of the maintained process stability when treating high protein substrates like slaughterhouse waste.

Keywords

Anaerobic digestion; co-digestion; full-scale; slaughterhouse waste; syntrophic acetate oxidation.

INTRODUCTION

Slaughterhouse waste is the very energy-rich waste stream of meat industry (Edström, 2003). As such, it is an attractive material to treat through anaerobic digestion for the production of biogas. However, there are many potential technical and microbiological problems associated with anaerobic digestion of slaughterhouse waste. These include the practical handling, European Union Animal By-Products (ABP) Regulation (EC 1774/2002), protein content (Hejnfelt, 2009), high degradation and volatile fatty acid (VFA) formation rates, etc. (e.g. Salminen *et al.*, 2001; Salminen and Rintala, 2002). The present work reports on the experiences, production results and R & D activities at a full scale co-digestion biogas plant treating slaughterhouse waste in Linköping (Sweden) during the period 1997-2010.

BACKGROUND

Anaerobic digestion

Anaerobic digestion of organic material is a complex microbiological process requiring the combined activity of several groups of microorganisms with different metabolic capacities (Zinder, 1984), which

need to work in a synchronized manner in order to obtain a stable biogas process. One of the key organisms are the methanogens, producing methane mainly from acetate or hydrogen.

Protein-rich substrate, such as slaughterhouse waste, is a well-known source of sulphide-formation during anaerobic degradation. The increased concentration of sulphides in the digester lead to higher concentrations of corrosive H_2S in the biogas and can further lead to sulphide inhibition of the methanogens (Ochieng' Otieno, 1996; Chen, 2008). When the proteins in slaughterhouse waste are degraded, not only sulphides are formed but also ammonia (Hejnfelt, 2009). The released ammonia increases the pH in the digester and with a large ratio of slaughterhouse waste in the substrate mixture, the pH tends to reach over 8.0, which can be growth limiting for some methanogens (Jiunn-Jyi, 1997). The above-optimal pH, together with a high production of VFA from protein and fat in the slaughterhouse waste can lead to VFA accumulation, and subsequent process overload and pH drop. If the organic load to the digester is not decreased at that point, the process overload will lead to a permanent pH drop and, finally, to a total inhibition of the methanogenesis and process collapse will follow.

Although much of the released ammonia (NH_3) from protein degradation is in equilibrium with the less harmful ionized ammonium species (NH_4^+), the non-ionized form is itself also a source of inhibition of microorganisms. This is so because the neutral NH_3 can easily transfer over cell membranes of bacteria and archaea, and upon entering the cell disrupt e.g. intra-cellular pH and concentrations of other ions (Chen, 2008). At increased pH and temperature the equilibrium of ammonia and ammonium is shifted towards the toxic ammonia species, resulting in a positive correlation between toxicity effects with increasing pH and temperature (Siegrist, 2002).

Among the methanogens, the acetate-utilizing methanogens have been suggested to be responsible for 70-80 % of the methane produced (Zinder, 1984). However, recent results suggest that an alternative methane producing pathway is activated at elevated levels of ammonia (Nordberg and Schnürer, 2008). In this pathway, acetate is converted to hydrogen and carbon dioxide by syntrophic acetate oxidizers (SAO), followed by the subsequent reduction of carbon dioxide to methane by a hydrogen utilizing methanogens, i.e. methane is produced only by hydrogen utilizing methanogens. Development of SAO has been shown to occur due to a selective inhibition of acetate-utilizing methanogens by ammonia, released during the degradation of proteins (Schnürer and Nordberg, 2008).

Establishment of co-digestion plant in Linköping

In the city of Linköping, located in the south-eastern part of Sweden, a plant for co-digestion of slaughterhouse waste was built in 1995. The plant, called Linköping Biogas (LB), which started operation in 1996, was a joint-venture project involving the city of Linköping, through its municipal company Tekniska Verken i Linköping AB (TVAB) and a local slaughterhouse, run by Swedish Meats co-op, and the Federation of Swedish Farmers (LRF). The main incentive for construction of the plant was a desire to improve the local air quality in the city, by switching from buses running on diesel, to buses run on biomethane. At the same time, the slaughterhouse was in need of an improved disposal method for its waste products. Currently, the plant is operated by the regional production company Svensk Biogas AB (SvB), a subsidiary of TVAB, and has since start-up continuously supplied vehicle-fuel quality biomethane, upgraded in water scrubbers, to all the city buses. TVAB has an in-house Biogas R & D department, which continuously support and develop their plants and several achievements have also resulted in patents (Holm *et al.*, 2004; Ejlertsson, 2005; Holm *et al.*, 2005). Extensive biogas process research has also been performed in collaboration with both Linköping University and the Swedish University of Agricultural Sciences.

Co-digestion plant design and operation

The co-digestion plant consists of three basic parts: 1) reception and storage, 2) pasteurization equipment, and 3) anaerobic digesters (Figure 1), and the process is a temperature phased anaerobic digestion (TPAD) of 65-70 °C and 38 °C. Yearly capacity of the plant is 55 000 metric tons, and the proportion of slaughterhouse waste in the total substrate mixture has varied between 35 and 75 % (w/w, yearly average). During 2009/2010, the capacity of the plant is being expanded to 100 000 tons/year.

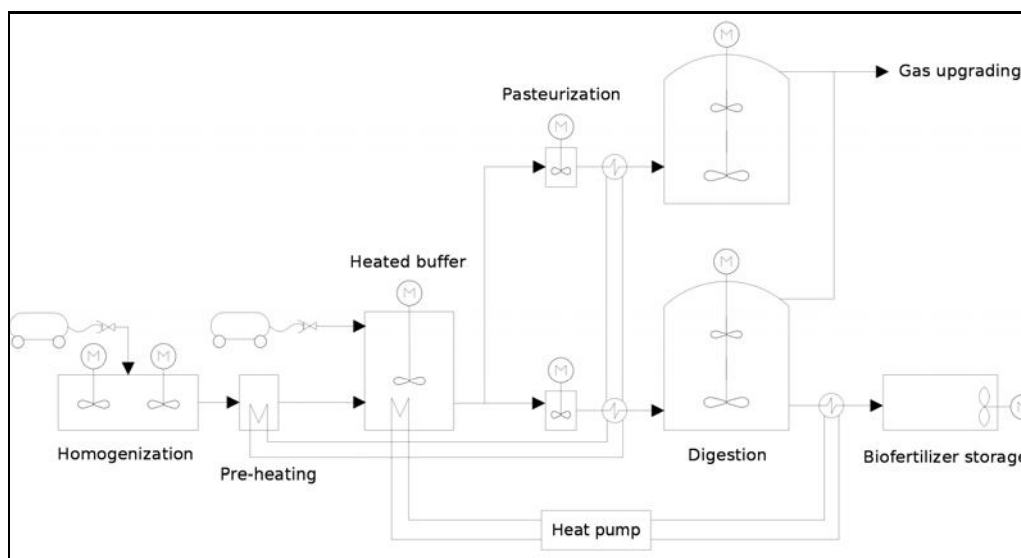


Figure 5. Schematic diagram of the process at Linköping Biogas plant.

The waste is delivered to the plant by closed trucks in a grinded (≤ 12 mm) pumpable form and is either transferred into a combined homogenization and buffer tank or directly into a second, heated buffer tank. A reception pit for solid material exists, but is seldom used due to odor and inconvenience of handling. After homogenization the substrate mixture is pumped to the heated buffer tank. The temperature of the second buffer tank is kept above 65 °C, to avoid foaming, and to provide a stable thermal disintegration of the substrate. Substrate which is delivered warm is pumped directly to the heated buffer tank, to save energy on substrate heating. Before loading of the digesters, the substrate is pasteurized in a batch process for one hour at 70 °C, to fully comply with the EU ABP regulation (EC 1774/2002) for category three materials. The anaerobic digestion takes place in two mesophilic (38 °C) continuously stirred tank reactors (CSTR) run in parallel, with a total volume of 7400 m³. The hydraulic retention time (HRT) is 45-55 days. Process heat was previously supplied from a steam boiler, operating on internal biogas or external fuel. However, in 2007 the steam boiler was taken out of operation and the process heat was supplied through the city's waterborne district heating system. The gas composition is, on average, 68 % CH₄, 31 % CO₂ and <100 ppm H₂S. No significant modification to the plant has been necessary, as a consequence of ABP regulation implementation, since the plant was already equipped with the required pasteurization function. However, the precise categorization of different substrates of animal origin has changed over the years, as legislation and its interpretation have changed.

MATERIALS & METHODS

Operating and analysis data

Operating data on biogas production, biogas composition and the amount and type of incoming substrates were collected from the plant's SCADA-system. pH was analyzed with a WTW 526 pH

meter (WTW Inolab, USA), according to Swedish Standard SS 028122:2. Partial (bicarbonate) alkalinity was analyzed by titration to pH 5.4, with simultaneous removal of CO₂, in accordance with Swedish standard SS-EN ISO 9963 Part 2. Total solids (TS) and volatile solids (VS) were analyzed according to Swedish Standard SS 028113 issue 1 of 1981-05-20. VFAs was analyzed with a modified spectroscopic HACH method (HACH no. 8196), accredited by SWEDAC (Swedish Board for Accreditation and Conformity Assessment). Ammonium nitrogen was analyzed according to FOSS Tecator's Kjeltex method (sub note 3502), on a Kjeltex 2200 (Foss Tecator, Denmark). The method gives the total ammonium nitrogen concentration (NH₄⁺-N (aq) + NH₃-N (aq)).

Labeling experiments

Inoculation of digester samples with isotopically labeled acetate was performed in order to distinguish between methane formation by acetate utilizing methanogens or via syntrophic acetate oxidation. Aliquots of digester content (20 mL), were transferred during flushing (N₂/CO₂; 80/20) to sterile serum vials (118 mL). The bottles were closed with butyl rubber stoppers and aluminium caps and the labeling studies were started by the addition of (2-¹⁴C)-acetate (Amersham, England) to a final concentration of 10 kBq/ml. The culture was incubated at 37 °C and the degradation of (2-¹⁴C)-acetate and the concomitant formation of ¹⁴CH₄ and ¹⁴CO₂ were determined by scintillation counting according to Schnürer *et al.* (1994). The labeling pattern was analyzed when approximately 90 % of the labeled acetate had been converted. Finally, the ratio of ¹⁴CO₂/¹⁴CH₄ was determined and values above 1 were considered as evidence for SAO.

Practical experiences

The general experiences of plant operation were conferred by Peter Johansson (former Plant Manager, Linköping Biogas AB) and Jonas I Ahlbert (former Production Manager, Svensk Biogas AB) (Personal communication, 2010).

RESULTS

Operational strategy development

During the two first year of operation about 50 % (w/w) of the substrate consisted of cattle manure. This is a common way to avoid possible problems with process overloading, nitrogen/ammonia inhibition and micronutrient deficiency (Tafdrup, 1994; Edström, 2003; Alvarez, 2008). However, diluting the substrate mixture with manure has the negative effect of decreasing the amount of methane produced per reactor volume, since the methane yield of manure is far lower than that of slaughterhouse waste (Deublein and Steinhauser, 2008). To increase the profitability of the plant, and to meet the increased local demand for biomethane as a vehicle fuel, a gradual replacement of manure, with more slaughterhouse waste and other organic wastes with higher methane yields has been implemented.

Organic load of digesters and biogas production

At start-up, the plant was designed for a substrate mixture with a TS maximum of 8 %. However, as a result of the constant endeavour to increase the organic load and thereby methane production, the TS of the incoming substrate mixture, sampled in the heated buffer tank, has during 2009/2010 reached a TS of 17 % as a yearly average (Figure 2), with individual samples during 2009/2010 sometimes reaching 20 %. Exchanging steam injection with district heating for pasteurization in 2007, also led to a thicker substrate mixture, since added water no longer enter the system through the steam.

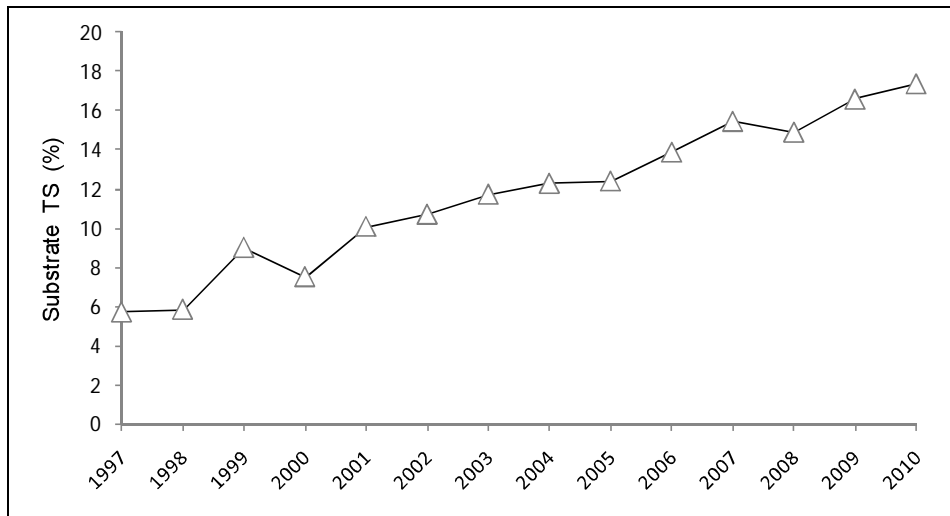


Figure 6. Total solids (TS) of incoming substrate (yearly average) during 1997-2010 (data for 2010 up until 2010-05-06), sampled in the heated buffer tank.

As can be seen in Figure 3, the plant has experienced an almost unbroken increase of yearly biogas production. In 2009 the average volumetric biogas production reached an average of $3.6 \text{ Nm}^3/(\text{m}^3 \text{ R-d})$ and a yearly total production of 9.6 million Nm^3 .

Main achievements in process stabilization and optimization

The cut down in manure use put a focus on process development, which was facilitated by three main appendages to the operational strategy of the plant; 1) addition of ferrous chloride, 2) addition of hydrochloric acid and 3) addition of the process additive KMB1 (Figure 3).

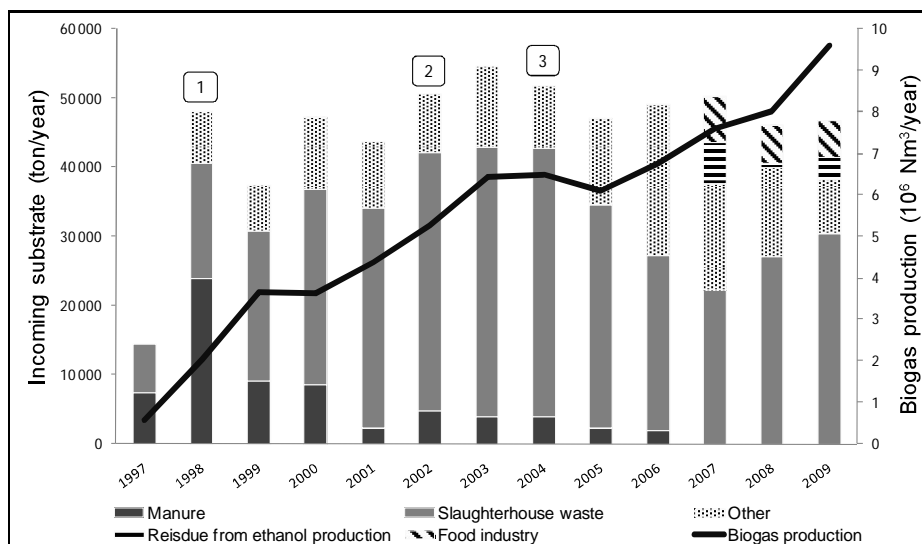


Figure 7. Annual substrate amount, substrate composition and biogas production during 1997 - 2009. Number captions denote implementation of process additives: [1] FeCl_2 ; [2] hydrochloric acid; [3] KMB1.

Addition of ferrous chloride

Sulphide-associated problems, such as corrosive H_2S in the biogas and sulphide-inhibition of the methanogenesis, are both reduced by precipitation of sulphides with Fe(II) . At LB, the addition of ferrous chloride to the homogenization and pasteurization tanks commenced in May 1998 and as a

result, the sulphide concentration in the digesters and the concentration of H₂S in the biogas were reduced, as well as the sulphur load on the water scrubbers (Vallin, 2007). Since the addition of the precipitant is made already in the homogenization and pasteurization tanks, the H₂S-induced odors from the buffer and pasteurization tanks are also reduced.

Addition of hydrochloric acid

It was hypothesized, that the two negative effects from degradation of proteins and release of ammonia, i.e. increased pH above optimum for methanogenesis, and the direct toxic effects of ammonia at high pH, could be counteracted by addition of acid to the digester in a controlled way. The acid would bring the pH closer to the optimum of the methanogens, and following the pH decrease, would shift the ammonia-ammonium equilibrium towards the non-toxic form. Laboratory tests, with addition of hydrochloric acid to co-digestion of slaughterhouse waste, manure, fat and hydrolyzed yeast in reactors operated under mesophilic conditions were performed in 1999-2000 (Ejlertsson, 2005). Positive effects on volumetric gas production and VFA levels were seen in the digesters where pH was lowered with hydrochloric acid, compared to control digesters without any acid addition. The acid used should be a non-oxidizing inorganic acid which is not consumed by the microorganisms in the digester.

Full-scale acid addition was started at LB in March 2002. On comparison of the operation performance of the plant in 2000-2001 with 2002-2003, the following direct and indirect effects were observed (Vallin, 2007): digester loading rate could be increased with 70 %, gas production increased (results are adjusted, to take into account time when one of the digesters were taken out of service for maintenance), acetate concentration decreased by 43 % and partial alkalinity concentration increased from 11 000 mg/L to 17 000 mg/L. The gas production increase was a result of the increased loading rate, because the specific methane yield per kg VS was unchanged. Also, the partial alkalinity increase was partly due to longer HRT and increased amount of slaughterhouse waste in the substrate mixture. There was also no visible effect on digester pH. However, the operation was in general more stable.

Addition of process additive KMB1

To further enhance process stability, and to increase the efficiency of the plant, a process additive known as KMB1 was developed at TVAB. The main effects of the additive were: 1) higher methane yield; 2) more stable production and; 3) higher organic loading rate without process disturbances and heavy foaming (Vallin, 2007). Also, the additive enabled the decrease and final removal of manure in the substrate mixture, and has been added to the LB plant since November 2003.

VFA and pH after process improvements

After implementation of the three process improving additives mentioned above, a closer study of the process reveal the positive effects (data from 2004-2005). In the heated buffer tank the VFA levels fluctuate to a great extent and can occasionally get very high (up to 16 000 mg/L) while the pH is low (Figure 4). The differences in the properties of the buffer tank substrate are most likely due to variable composition and age of the incoming substrates. However, even though the buffer tank substrate display a low pH and high, fluctuating VFA concentrations (average 8400 mg/L, pH 5.5), the concentration of VFA in the digester is low and stable (average 1600 mg/L, max 2800 mg/L) and the digester fluid has a stable pH of 8.0 (7.9-8.1).

Ammonium concentration and methane production pathway

Since the plant's early years of operation, the total NH₄-N concentration has been high. The average for both digesters during 1999-2010 has been 5060 mg/L, with a maximum yearly average of 5880 mg/L (digester 1, 2006) and a minimum yearly average of 4120 (digester 2, 2002) (Figure 5). Labeling

experiments were performed in 2008 to establish what type of methane formation pathway is prevalent – methane formation by acetate utilizing methanogens or via syntrophic acetate oxidation?

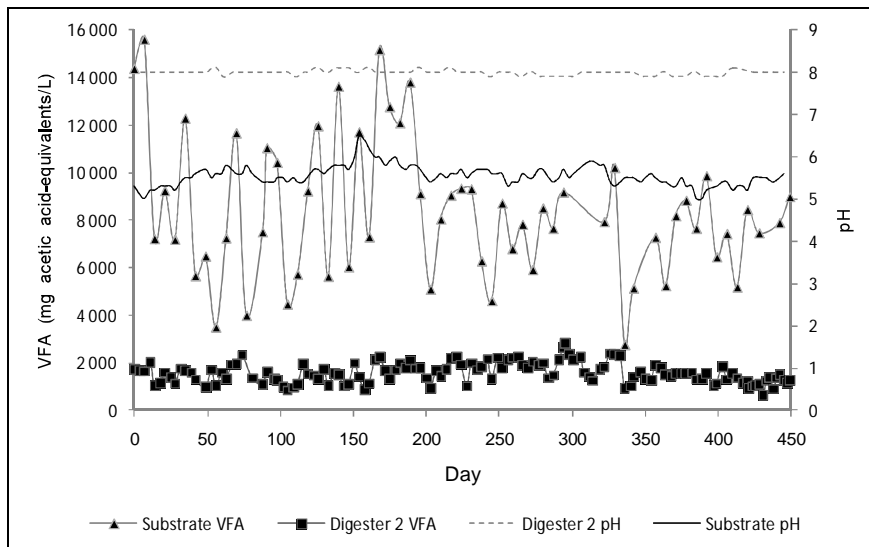


Figure 8. Volatile fatty acid (VFA) concentrations (mg acetic acid-equivalents/L) and pH levels in the heated buffer tank (denoted: substrate) and in the biogas digester (data from 2004-2005). Even though VFA levels are high and fluctuating and the pH is low in the heated buffer tank, the VFA concentration in the digester is low and stable, and the digester fluid has a stable pH.

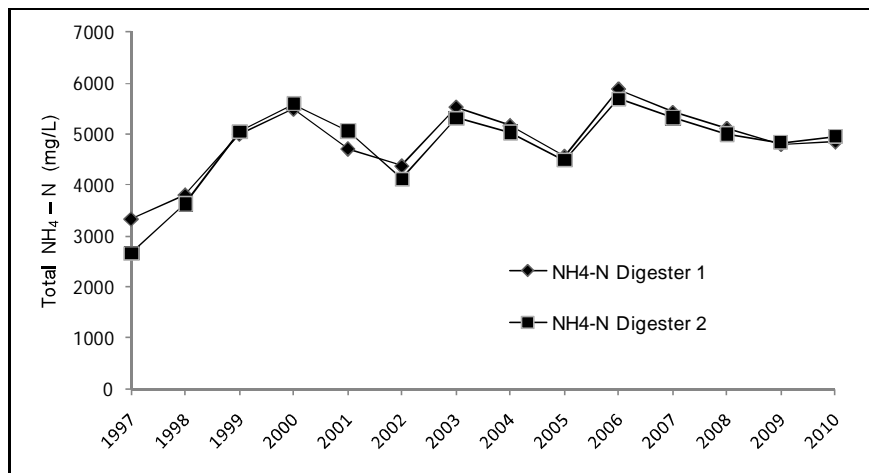


Figure 9. Total ammonium ($\text{NH}_4^+\text{-N (aq)} + \text{NH}_3\text{-N (aq)}$) concentrations (mg/L) in the digesters during 1997-2010.

The labeling analysis showed production of high levels of labeled carbon dioxide in relation to labeled methane. The $^{14}\text{CO}_2/^{14}\text{CH}_4$ quota was determined to be 16; clearly showing that methane production in the digester occurred mainly through syntrophic acetate oxidation and hydrogenotrophic methanogenesis. Since the digester is operated at high ammonium levels (5300 mg $\text{NH}_4^+\text{-N/L}$ at the time of sampling), this is a result that was expected and in accordance with the previous studies that have shown development of SAO in response to increasing ammonia levels (Schnürer and Nordberg, 2008). The development of this prevailing metabolic pathway is likely the explanation to the stable operation of the process even at high ammonia levels. Given that methanogenesis via syntrophic acetate oxidation involves a hydrogenotrophic methanogens, that tolerates higher levels of ammonia

than acetoclastic methanogens, methane production from acetate can still proceed even though the acetoclastic methanogens are inhibited. Furthermore, isolation and characterization of several ammonia tolerant hydrogen utilizing methanogens, as well as ammonia tolerant syntrophic acetate oxidizing bacteria, support this suggested mechanism for ammonia adaptation in biogas processes (Schnürer *et al.*, 1996, Schnürer *et al.*, 1999, Karlsson *et al.*, 2010).

Practical experiences

The general plant operation experiences of anaerobic digestion of slaughterhouse waste concern two main themes: 1) logistics and transportation and 2) process and technology.

An open and good relationship with the local slaughterhouse has been essential for the successful handling of the substrate. At the slaughterhouse, the waste is grinded to ≤ 12 mm and treated with formic acid. The grinding at the slaughterhouse allows for transportation of the substrate in slurry form, and a closed-system handling at the biogas plant. The fact that the substrate can be pumped in a closed system decreases odor issues significantly, especially compared to open handling of slaughterhouse waste transported in containers. Transportation in containers, when tried in the early days of the plant, also caused problems with icing of substrate during cold winter months. Slaughterhouse waste is in general difficult to store; without acid treatment or heated storage, it is very prone to foaming. Without formic acid treatment, the slaughterhouse waste would already start foaming at the slaughterhouse, thus causing significant problems during storage, transport and subsequent storage at the biogas plant. The thermal disintegration of the substrate in the heated buffer tank, and the fact that the substrate temperature is over 65 °C in large parts of the system, reduces potential problems with clogging and eases pumping of the substrate, due to reduced viscosity. To achieve a stable process, the type of material co-digested with the slaughterhouse waste is important, and the complimentary substrates should work well in the plant, both from a practical and process point of view, and with regards to logistics. If waste reception logistics are well-planned, this leads to an even substrate mixture over time, and thus an even organic loading rate and a stable biogas process.

CONCLUSIONS

From 15 years of full-scale co-digestion of slaughterhouse waste, the following conclusions are drawn:

- It is possible to operate CSTR co-digestion of slaughterhouse waste, at substrate TS levels significantly over the original design level (up to 20 % TS, compared to design level of 8 % TS.)
- Stable storage of the slaughterhouse waste, without foaming, is possible due to formic acid treatment at the slaughterhouse and heated storage at the biogas plant.
- The plant is operating well at high levels of ammonium, and the long (45-55 day) HRT enables establishment of a mesophilic syntrophic acetate oxidizing culture, which has comparably low growth rates. The generation time of a SAO culture was calculated to be approx. 28 day (Schnürer *et al.*, 1994) which can be compared with the times of around 2-12 day for acetate utilizing methanogens (Jetten *et al.*, 1992).
- With optimization of process parameters, substrate composition and through the addition of process additives, it has been possible to achieve a continued increase of the biogas production, with basically the original plant capacity.

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