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Biogas Production and Cleanup by Biofiltration for a Potential Use as an Alternative Energy Source

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1. Introduction

As many countries have taken advantage of the richness of crude oil, fossil fuels have become the main energy source, and human activities have become entirely dependent on petroleum products. However, this is not sustainable because of the huge environmental cost of harvesting and utilizing vast amounts of fossil fuels (Fairley, 2011). Therefore, the need for alternative fuels has become critical, especially for a new generation of advanced biofuels that can maximize petroleum (crude oil) displacement and minimize the side effects of burning fossil fuels. The primary objective is then to produce biofuels from corn stalks or other 'cellulosic plants' (or even from municipal garbage) and jet fuels from dedicated energy crops such as the fast-growing Camelina sativa (Fairley, 2011). The challenges are then to develop the agriculture for these plants and improve their utilization at an industrial scale. In this way, net reductions in petroleum use and greenhouse-gas emissions will be long-lasting and ethical. Bridging this gap will require continued investment, research, government regulations and development of technology. The International Energy Agency (IEA) has recommend the maximized use of farm, forestry and municipal wastes as well as increased cultivation of dedicated energy crops away from lands that provide carbon sequestration and other critical environmental services. One way to develop biofuels along an environmental friendly path is to draft a set of standards and practices that biofuels producers must comply with, either voluntarily or by mandate (Fairley, 2011).

In large cities, such as Mexico City with a population of more than 20 million, concerns about waste disposal and the use of alternative energy sources has steadily increased. This population produces a tremendous amount of solid waste, more than 12,000 tons per day. On the other hand, to provide sufficient food for this population, many markets are distributed throughout the city. The central market for food distribution in Mexico City, Central de Abasto (CEDA), is the second largest market in the world, receiving 25,000 tons of food products and producing 895 tons of organic solid waste each day (84% of the total solid waste produced is organic waste, 50% of that is from fruits and vegetables).

Fruit and vegetable waste (FVW) is produced in large quantities in markets in many large cities (Mata-Alvarez et al., 1992; Misi and Forster, 2002; Bouallagui et al., 2003; Bouallagui et al., 2005). The application of an anaerobic digestion process for simultaneous waste treatment and renewable energy production from the organic fraction of these residues could therefore be of great interest (Bouallagui et al., 2005). The high biodegradability of FVW promotes the rapid production of volatile fatty acids (VFAs), resulting in a rapid decrease in pH, which in turn could inhibit methanogenic activity (Bouallagui et al., 2003; Bouallagui et al., 2009). A strategy to avoid the acidification of the system is the addition of cosubstrates. Data obtained during the codigestion of FVW and other substrates resulted in the design of an efficient digestion process, improving methane yields through the positive synergistic effects of the mixed materials exhibiting complementary characteristics and the supply of missing nutrients from the cosubstrate (Agdag and Sponza, 2005, Habiba et al. 2009, Bouallagui et al. 2009). In a recently published study (Garcia-Peña et al., 2011), a 30-Liter anaerobic digestion reactor operated with a mixture of FVW:MR (meat residues) (75:25) had a stable CH₄ production percentage of 53 \pm 2 % and a sustained pH of 6.9 \pm 0.5 (naturally regulated) in a co-digestion process. The adequate and sustained performance and stable CH₄ production were a result of an appropriate buffering capacity and highly stable operation of the experimental system. However, the biogas produced during this anaerobic process needs to be cleaned before use by eliminating a relatively high content of other compounds as CO_2 and H_2S .

Biogas consists of approximately 60–70% (v/v) methane (CH₄), 30–40% (v/v) carbon dioxide (CO₂), 1–2% (v/v) nitrogen (N₂), 1000–3000 ppmv H₂S, 20-30 ppmv of VFAs and 10–30 ppmv of ammonia (NH₃), depending on the organic substrate used during the anaerobic process (Angelidaki et al., 2003). Hydrogen sulfide (H₂S) is one of the most commonly reported reduced sulfur compounds, and represents up to 2% (v/v). However, this H₂S concentration can be higher when a rich protein feedstock is used. H₂S elimination is thus required because it reduces the life span of combustion engines by corrosion, forms SO₂ upon combustion and is a malodorous and toxic compound (Angelidaki et al., 2003, Pride, 2002). Malodorous gases include mainly H₂S (around 3000 ppmv) and some volatile fatty acids (VFAs).

Reducing CO_2 and H_2S content will significantly improve the quality of biogas. There have been many technologies developed for the separation of CO_2 from gas streams, including absorption by chemical solvents, physical absorption, cryogenic separation, membrane separation and CO_2 fixation by biological or chemical methods (Abatzoglou and Boivin , 2009, Granite and O'Brien, 2005). These techniques are of significant industrial importance and are generally applied during natural gas sweetening and in the removal of CO_2 from flue gases of power plants.

H₂S is currently removed using chemical, physical or biological methods. The most commonly used method is chemical absorption by selective amines, such as diglycolamine, monoethanolamine and methyldiethanolamine, but also by absorption into aqueous solutions, physical absorption on solid adsorbents or conversion to low-solubility metal sulfides (Horikawa et al., 2004, Osorio and Torres, 2009). Water scrubbing systems are also frequently used because of their simplicity and low cost (Kapdi et al., 2005, Rasi et al., 2008). Their use allows the production of high quality CH₄ enriched gas from biogas by chemical absorption where a packed bed column and a bubble column are normally used to provide

liquid/gas contact (Krumdieck et al., 2008). However, the main drawbacks of these chemical technologies are the high energy requirement, the stability and selectivity of the chemicals used, the high cost of the chemicals and their regeneration, the negative environmental impacts from liquid wastes, the large equipment size requirements and the high equipment corrosion rate (Tippayawong and Thanompngchart, 2010, Fortuny et al., 2008).

Biological treatments are cost effective and environmentally friendly processes (Shareenfdeen et al., 2003, Ng et al., 2004, Maestre et al., 2010). Biofiltration is one of the most promising clean technologies for reducing emissions of malodorous gases and other pollutants into the atmosphere (van Groenestijn and Hesselink, 1993, van Groenestijn and Kraakman, 2005). This technology has been proven to effectively control reduced sulfur compounds in diluted gas streams (Yang et al., 1994, Smet et al., 1998, Ergas et al., 1995, Chung et al., 1996, Devinny et al., 1999; Gabriel and Deshusses, 2003, Kim and Deshusses, 2005). However, the elimination of H₂S from fuel gases requires systems that can handle high loads of pollutants for extended periods of time (Maestre et al., 2010). Surprisingly, there is still a limited number of reports on the removal of high concentrations of H_2S (>1000 ppmv) using biofilters, biotrickling filters and bioscrubbers. On the other hand, two processes have been effectively applied for the removal of high concentrations of H₂S from biogas or fuel gas in industrial processes: the Thiopaq process (Paques, The Netherlands) and the Biopuric process (Biothane, USA). The first one is a chemical process that uses a conventional caustic scrubber and an expanded bed bioreactor for the recovery of spent caustic and elemental sulfur generation. The Biopuric process combines a chemical scrubber with a subsequent biological treatment.

Although H₂S treatment for industrial processes has already been applied through the above-mentioned commercial systems, there is a need for the development of alternative and sustainable biological processes. Regarding the development of biofiltration and/or biotrickling filter systems to eliminate high H₂S concentrations, Rattanapan et al., 2009, compared the elimination of 200 to 4000 ppmv of H₂S in two biofiltration systems. One of the biofilters was a sulfide oxidizing bacterium immobilized on Granular Activated Carbon (GAC) (biofilter A) and the other was GAC without cell immobilization (biofilter B). The results showed that in the GAC system, the H₂S was autocatalytically oxidized when it absorbed into the CAG, reaching a removal percentage of 85%. The removal was enhanced to over 98% (even at a concentration as high as 4000 ppmv) through the biological activity in biofilter A. In this last system, the maximum elimination capacity was approximately 125 gH₂S/m³GAC h. In addition, Fortuny et al., 2008, reported the performance of a biotrickling filter system for treating high concentrations of H₂S in simulated biogas using a single reactor. Two laboratory-scale biotrickling filters filled with different packing materials were evaluated, the inlet H₂S concentration ranged from 900 to 12000 ppmv. During long-term operation, a removal percentage of 90% was determined with an extremely high H₂S concentration (6000 ppmv). Maximum elimination capacities of 280 and 250 g H_2S/m^3 h were obtained at empty-bed residence times of 167 and 180 s, respectively. During this study, the main end products of the biological oxidation of H₂S were sulfate and elemental sulfur; the final percentage of these products varied as a function of the ratio of O_2/H_2S supplied (v/v). At a value of 5.3, corresponding to an inlet H_2S concentration of 3000, the main product was sulfate (60-70%), whereas at the higher H₂S concentration of 6000 ppmv, the sulfate recovery decreased to 20-30%. Elemental sulfur production varied inversely with the O_2/H_2S supplied (v/v), it was low at a ratio of 5.3 and increased up to 68-78% as the ratio decreased.

In a biofiltration system, a gas stream is passed through a packed bed on which pollutantdegrading organisms are immobilized as biofilms. Biotrickling filters use the same principle, but an additional liquid phase will flow through the reactor. In both systems, the microorganisms in the biofilms transform the absorbed H₂S by metabolic activity into elemental sulfur or sulfate depending on the amount of available oxygen. Oxygen is thus the key parameter that controls the level of oxidation. Sulfur production (Eq. 1) results from the partial oxidation of sulfide instead of complete oxidation to sulfate (Eq. 2) when oxygen is limited, as is shown in Equations 1 and 2 (Kennes and Veiga, 2001).

$$H_2S + 0.5O_2 \rightarrow S_0 + H_2O$$
 (1)

$$H_2S + 2O_2 \cdot SO_4^{-2} + 2H +$$
 (2)

As the performance of a biofiltration system depends on the microbial community present in the reactor, the determination of the microorganism and the microbial activity responsible for the behavior of the process is very important. However, there is still a lack of understanding of the structure and dynamics of microbial communities and the physiological role of the main microbial population as well as the correlation between the global performance of the system with the metabolic activities of the microorganisms involved in the process. This knowledge could allow control of the reactor behavior and the design of enhanced processes to eliminate high concentrations of H_2S in the gas phase because the performance of the process depends on the robustness of the microbial communities (Maestre et al., 2010).

Some authors have characterized microbial population diversity present in different gas phase reactors by analysis of biomarkers such as phospholipid fatty acids (Webster et al., 1997), molecular techniques such as fluorescent in situ hybridization (FISH) (Moller et al., 1996), cloning and sequencing of ribosomal RNA genes (Roy et al., 2003), terminal restriction fragment length polymorphism (Maestre et al., 2009) and denaturing gradient gel electrophoresis (Borin et al., 2006). There are only a few studies in the literature that focused on determining the microbial diversity of microorganisms capable of removing reduced sulfur compounds in biofilters or gas phase bioreactors using molecular biological approaches. Ding et al., 2006, reported the changes in the microbial diversity of a biofiltertreating methanol and H₂S. In this study, the biofilter's initial microbial community had a high diversity, but after the biofiltration system was fed with H₂S, the microbial diversity decreased to adapt to the low pH and use H₂S as an energy source. Maestre et al., 2010 studied and described the bacterial composition of a lab-scale biotrickling filter (BTF) treating high loads of H₂S using 16S rRNA gene clone libraries. The authors reported the diversity, the community structure and the changes in the microbial population on days 42 and 189 of reactor operation. The main changes in microbial diversity were observed at the beginning of the process and again when steady state operation was reached (i.e., neutral pH and at an inlet H₂S concentration of 2000 ppmv). At steady state, the major sequences associated with SOB included Thiothrix spp., Thiobacillus spp., and Sulfurimonas denitrificans. Additionally, FISH analysis was used to determine the spatial distribution of sulfuroxidizing bacteria (SOB) along the length of the reactor under pseudo-steady state operation. The aerobic species were found to be predominantly along the system, but some

facultative anaerobes were also found. The anaerobic microorganisms were associated with higher H₂S concentrations (inlet) with lower oxygen availability. The distribution of a microbial community was associated with changes in the dissolved oxygen (DO) concentration, and the accumulation of elemental sulfur and the pH (Maestre et al., 2010). Recently, Omri et al., 2011 studied the microbial community structure of the three layers (bottom, middle and top) of a biofilter using the polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis. The results obtained showed a high microbial diversity for bacteria, with the relative diversity of the bacterial community represented by the number of peaks in the profiles. Significant differences were observed between the microbial communities of the three layers of the biofilter. The Simpsons diversity index was used to determine the microbial diversity in the system, and the results indicated that the bottom and middle layers exhibited high diversity (1/D of 13.6 and 10.8,respectively). However, the microbial distribution in the top layer (1/D=8.75) was associated with the vertical gradient of the substrate, as higher H₂S concentrations near the inlet allowed the growth of sulfur-oxidizing bacteria and low pH provided a favorable environment for the oxidation of H₂S. The predominant bacteria in samples of the operation were found to be *Pseudomonas* sp, *Moraxellacea*, *Acinetobacter* and *Exiguobacterium* belonging to the phyla Pseudomonadaceae, gamma-Proteobacteria and Firmicutes.

In the present chapter, the data obtained for the potential use of FVW and meat residues for methane production will be presented. The results demonstrating how a codigestion process of FVW and MR enhanced methane production by increasing the C/N ratio and controlling the natural pH in a 30L reactor will also be analyzed and discussed. At different stages of the start up of the anaerobic digestion system, methane production increased from 14 to 50% as a result of the use of a protein rich feedstock (MR). However, the H₂S concentration also increased in the biogas stream under these conditions. Due to the increased H₂S content, and considering that this compound does not allow for the efficient use of methane as fuel, a biofiltration system was evaluated in the elimination of H₂S. The results obtained for the elimination of H₂S and VFAs (average concentrations of 1500 ppmv and less than 10 ppmv, respectively) in the gas stream from an anaerobic process by a biofiltration system will then be presented. The microbial population in the biofilter when operating at steady state conditions is also presented and discussed.

2. Materials and methods

2.1 Microbial culture

Microbial culture in the biofiltration system was adapted to 10% (v/v) acetic acid and 40 mM Na₂SO₃.5H₂O in mineral media and used for VFA and H₂S degradation experiments, respectively. In situ immobilization in the biofilter was facilitated by recirculating the solution containing the microbial culture previously adapted (Ramirez-Saenz et al., 2009).

2.2 Experimental setup

2.2.1 Anaerobic digestion system

The anaerobic digestion process was evaluated in an anaerobic digestion reactor (ADR) consisting of a stainless steel tubular reactor with a total volume of 30 L into which 20 L of a (50:50) mixture of FVW and meat residues (MR) was initially packed. After the initial period

of operation (20 days), the ADR was inoculated with cow manure (10% v/v) to enrich the methanogenic population. The reactor was stirred by recirculating the FVW twice a day. The pH was initially set at 7 by the addition of a 0.8 N NaOH solution. Later in the process, the pH was naturally regulated by the metabolic intermediates produced during digestion. The bioreactor was kept at room temperature and operated in a fed-batch mode. To avoid inhibition due to metabolic products and to ensure a sufficient supply of organic matter, 2.5Kg of different compositions of fresh feedstock mixtures were fed periodically (approximately every 12-15 days), and an equal volume of exhausted sludge was removed.

2.2.2 Biofiltration system

A lava rock biofilter was used to evaluate the degradation of H₂S from the AD gas stream. The experimental setup for the biofilter used in this study was previously described (Ramirez-Saenz et al 2009). The gas stream was humidified and fed in the top of the biofilter using a mass flow controller. Sample ports were located in the output and input of the gas stream. For H₂S degradation experiments, the biofiltration system was fed at the top with an air-diluted gas stream originated from the ADR, as previously reported (Ramirez-Saenz et al., 2009). Periodic water additions (once a week) were used to control moisture loss and to avoid SO₄-² accumulation. Recirculation was provided at a flux of 0.5 L/min over 1 h. All experiments were conducted at room temperature (20-25°C).

2.2.3 H₂S elimination tests in the biofilter

Different dilutions of the biogas stream produced in the AD at an initial concentration of 3000 ppmv of H_2S were prepared by mixing the biogas with humidified air. Two empty-bed residence times (EBRT), 31 and 85 s, were chosen for the performance of the reactor during the H_2S and VFA biodegradation tests. Increasing mass loading rates from 99 g/m³h to 400 g/m³h (corresponding to 850 and 3000 ppmv H_2S) were used for evaluating H_2S removal at both EBRT. Gas samples of the inlet and outlet ports of the biofilter were periodically collected and diluted in 10 L Tedlar bags before taking measurements to determine the H_2S and VFA consumption in the biofiltration system. Details of the analysis conditions were previously reported in Ramirez-Saenz et al., 2009.

2.3 Analytical methods

The fruit and vegetable waste samples were analyzed for total solids (TS) and volatile solids (VS) according to the standard methods of the American Public Health Association (APHA, 2005).

Biogas production in the anaerobic digester was periodically measured using a water displacement setup in which the biogas was passed through a 5% NaOH solution (Anaerobic Lab Work, 1992). Biogas samples were taken periodically from the gas collection lines prior to the water displacement setup, and the gas composition was analyzed using a gas chromatograph (GowMac Series 550, Bethlehem, PA) equipped with a thermal conductivity detector. A CTR1-packed column (Alltech Co., Deerfield, IL) was used for the analysis. The analysis conditions were the same as those reported previously (Garcia-Peña et al., 2009). VFA samples were analyzed in a gas chromatograph (Buck Scientific, East Norwalk, CT) as previously reported (Garcia-Peña et al., 2009).

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Biofilter samples were analyzed for H₂S consumption by measuring H2S concentrations of the inlet and outlet of the biofilter using a gas analyzer (Testo 350XL, Clean Air Engineering, Inc., Pittsburgh, PA).

2.4 Microbial characterization and identification in the biofiltration system

DNA samples of the microbial consortium along different lengths of the biofilter were extracted using an Easy-DNATM Kit (Invitrogen, USA) following the manufacturer's instructions. The 16S rRNA gene was amplified using universal bacterial primers. Polymerase chain reaction (PCR) was conducted as previously reported (Garcia-Peña et al., 2011). The PCR amplification products were purified using a QIAquick PCR Purification Kit (Qiagen, UK). The PCR-amplified DNA products were separated by DGGE on 8% polyacrylamide gels with a linear gradient of 5–30% denaturant (100% denaturant was 40% [v/v] formamide plus 42% [w/v] urea) using the DCodeTM System (Bio-Rad, Hercules, CA, USA). After purification, the PCR products were sent to a sequencing service (Macrogen Inc., Korea). The nucleotide sequences of each 16S rRNA gene were aligned using the Clustal X software (Higgins et al., 1996). Identification of the sequences was made after performing BLAST searches of the NCBI database.

2.5 Data analyses

The data obtained for the microbial population by DGGE were further analyzed by Jaccard's (3) and Sorensen-Dice's (4) indexes. Similarity indices are frequently used to study the coexistence of species or the similarity of sampling sites. A matrix of similarity coefficients, between either species or locations, may be used to analyze changes in microbial populations over time or at different locations (Real and Vargas, 1996). Jaccard's index is one of the most useful and widely used indices to determine similarity between binary samples. Jaccard's index may be expressed as follows:

$$J = \frac{n_{AB}}{n_A + n_B - n_{AB}} \tag{3}$$

where n_{AB} is the number of bands in both samples A and B, n_A is the number of bands present in sample A, and n_B is the number of bands present in sample B.

Sorensen-Dice's index, also known as Sørensen's similarity coefficient, is also used to compare the similarity of two samples. It can also be applied to the presence/absence of data. The index is described by the expression, the terms of the equations are the same as describe above:

$$S_D = \frac{2n_{AB}}{n_A + n_B} \tag{4}$$

3. Results

3.1 Methane production from FVW

Evaluation of the feasibility of producing methane from fruit and vegetable waste (FVW) was performed in two different stages: an initial stage with different conditions for high biogas and methane yields and VS removal evaluated in batch experiments. The evaluation

of the anaerobic digestion process in a fed-batch mode and the feasibility of the long operation time under stable conditions were determined in a second stage of the project.

The design and efficient operation of an anaerobic digestion system can be determined by establishing the physical and chemical characteristics of the organic waste and the culture conditions, which have an effect on biogas production and process stability of the system. The residue mixture used as feedstock was composed of products most frequently and consistently sold in the market (i.e., excluding seasonal products). The FVW mixture (equal proportions of each residue, w/w) showed a total solid (TS) content of approximately 73-100 g/Kg waste (approximately 10%), a pH of 4, and a moisture content of 90% (Garcia-Peña et al., 2011). The FVW had a higher soluble carbohydrate content (only contained fruits and vegetables and did not include a source of protein), a high moisture content, and could be considered a highly degradable substrate. These properties make it an ideal candidate for CH_4 production.

Since the FVW is highly degradable, large amounts of volatile fatty acids (VFA) are produced and a rapid acidification of the system could inhibit the biological activity of the methanogens. Some reports have demonstrated that pH control is one of the most important parameters in achieving high biogas production (Mata-Alvarez 1992, Bouallagui et al., 2005). Additionally, as mentioned above, the FVW contains no nitrogen source. Some experiments were also performed to evaluate the effect of adding a nitrogen source, considering that an adequate C/N ratio is necessary to enhance the anaerobic digestion process. Different conditions were thus tested to optimize biogas and methane production using FVW, including buffered and nitrogen supplemented systems with and without inoculation.

Figure 1 shows the data obtained for TS removal, biogas production (m³/kgVS) and final pH under the evaluated conditions. As an overall result, the inoculation, pH control and nitrogen source addition all had positive effects on VS reduction and biogas yield. Higher degradation percentages of approximately 86 % of the initial total volatile solid (tVS) were obtained in the inoculated and pH-regulated system (IpH) as well as in the inoculated, pH-regulated and nitrogen-added system (IpHN) in a 28-day culture. Higher VS removal was correlated with higher biogas production. The highest biogas production (0.42 m³/kgVS) was obtained in the inoculated system with pH control and nitrogen addition (IpHN), reaching a VS removal percentage of 86%.

Lower biogas productions of 0.15 m³/kgVS and 0.08 m³/kgVS were measured in the systems without inoculum (WIpH and WIpHN) and had correspondingly low VS removals of 42 and 34%, respectively.

The results suggested a correlation of pH with biogas productivity, with higher productivity occurring at pH values close to the optimum pH of 7. Methane production (approximately 45-53+0.5% v/v in the biogas mixture) only occurred in the inoculated systems, with the highest methane percentage (53 %, v/v) observed for the IpHN system.

Biogas and CH₄ production were in the range (0.16-0.47 m³/kgVS) of those reported by other authors for anaerobic digestion processes using FVW as a feedstock (Rajeshwari et al., 1998, Alvarez 2004, Mata-Alvarez et al., 1992; Boullagui et al., 2003). In the inoculated systems, both nitrogen addition and pH control had positive influences on biogas production. Therefore, these conditions should be used to produce the maximum amount of methane from FVW.

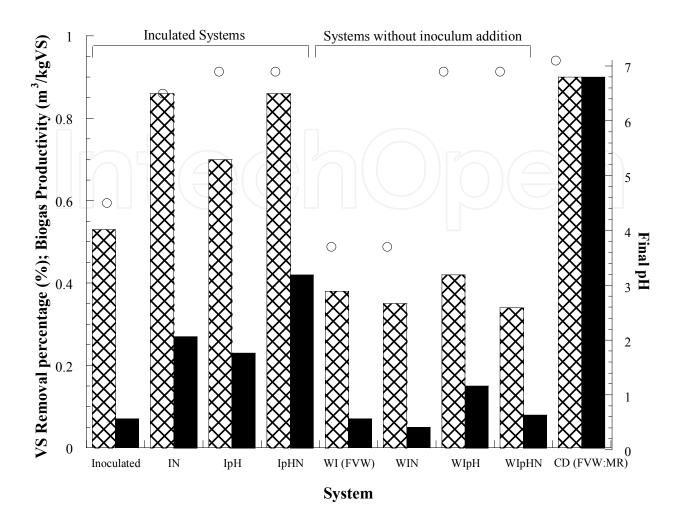


Fig. 1. VS removal percentage (%/100); Biogas productivity in m³/kgVS; (\bigcirc) Final pH obtained for different conditions evaluated in the batch systems. I, FVW inoculated with cow manure (10%); IN, FVW inoculated and supplemented with NH₄Cl as a nitrogen source; IpH FVW inoculated and salts added (buffer) to control pH; IpHN, FVW inoculated, buffering salts, and NH₄Cl added; WI, FVW without inoculation (Control); WIN, FVW and NH₄Cl; WIpH, FVW and buffering salts; and WIpHN, FVW buffering salts and NH₄. Modified from Garcia-Peña et al., 2011.

Similar conditions were obtained by the codigestion of a mixture of FVW and meat residues (obtained from meat packaging operations at the same market). The meat residues (MR) provide a high nitrogen concentration, and protein hydrolysis could result in natural pH control due to NH₄ production. For the codigestion system of FVW and MR, the highest biogas yield of 0.9 m³/kgVS (methane yield of 0.45 m³/KgVS) was observed, reaching an organic matter degradation of 93% (Figure 1).

The feasibility of an anaerobic digestion process using FVW and MR was then evaluated in a 30 L ADR system. To determine the effect of MR addition on biogas and methane production, experiments were carried out using different MR proportions. The biogas productivity and methane percentages obtained under different conditions after 130 days of operation are presented in Table 1.

	Time of operation (days)	Biogas production (m³/kgVS)	VS removal (%)	Methane (%) ^b	Methane production (m³/kgTS)
Start-up	0-19	1.03	89	0	0
Inoculation	20-43	0.64	81	16	0.10
75:25	43-55	0.5	70	28	0.14
50:50	55-64	0.4	73	30	0.12
100:0	64-76	0.24	80	14	0.033
50:50	76-83	0.2	75	30	0.06
75:25	83-130	0.25ª	78	53	0.135
Current operation		0.4	65	63	0.252

Table 1. Biogas production, VS removal and methane production during the start-up of the ADS. a average, b biogas was composed mainly of CO_2 and CH_4 , with the remaining biogas percentage (v/v) accounted for by CO_2 .

In the first stage, no methane was observed in the biogas effluent, thus biogas production resulted from the hydrolysis of the easily degradable components of the feedstock. After 20 days of operation, the anaerobic digestion reactor (ADR) was inoculated with (13 %, v/v cow manure) and a new, strong biological activity was observed. In this period, the CH₄ content in the biogas was 16 % (v/v) due to the initial activity of the methanogenic population introduced into the ADR with the inoculum. The methane percentage increased from 16 to 30% (v/v) as the proportion of MR (50:50) increased. When steady state was reached (after 80 days of operation) with a 75:25 mixture of FVW and MR, the CH₄ percentage was stable at 53 ± 2 %, and the pH was stable at 6.9 ± 0.5 (naturally regulated during this last stage of the process).

For the next stage (on the 70th day of operation), a 50:50 mixture of FVW:MR was added and the CH₄ percentage recovered to 30%. Once stable operation was achieved after 83 days, the biogas production showed a constant value of approximately 0.25 m³/kgVS and a methane percentage of 53%, corresponding to a methane production of 0.135 m³/kgVS and a VS removal of 78%. The reactor was regularly fed with a 75:25 mixture of FVW:MR. Under these conditions, the CH₄ percentage was stable at 53 ± 2 %, and the pH was stable at 6.9 ± 0.5.

An appropriate buffering capacity and a highly stable experimental system were observed with Organic Loading Rates (OLRs) in the range of 2.4 to 2.7 g COD/L day (Hydraulic Retention Times (HRT) in the range of 15–20 days). The natural pH regulation during the stable operation of the ADS was a result of NH₃ release from protein hydrolysis. The results could also be explained as an effect of the alkalinity in the system. At normal percentages of CO_2 in the digester gas, between 25 and 45%, a total alkalinity of at least 500 to 900 mg/L is required to keep the pH above 6.5. Higher CO_2 partial pressure makes alkalinity requirements larger (Rittmann and McCarty, 2001). When the meat residues were introduced into the ADS, the alkalinity started to increase considering that the moles of

bicarbonate alkalinity was equal to the moles of NH₄ according to the stoichiometric equation for methanogenesis of an organic mixture (carbohydrates and proteins) (Garcia-Peña et al 2011). Additionally, at stable and at long operation times the CO₂ percentage was lower than those obtained during the start-up of the ADS. The required alkalinity was 3445 mg/L, while an alkalinity as CaCO₃ of 4804.6 mg/L was calculated under the experimental conditions (i.e., 70% removal efficiency and an initial substrate concentration of 50 gCOD) using the stoichiometric equation for an organic mixture of carbohydrates and protein (50:50) (Garcia-Peña et al, 2011). This total alkalinity value was high enough to avoid a possible acidification of the Anaerobic Digestion System (ADS), and the high buffering capacity allowed stable operation without external control. The increase in methane production after the 80th day of operation could have resulted from an increase in the methanogenic population and its adaptation to the operating conditions of the ADS. The high VS removal, the increased methane yield, and the natural pH control during the stable period of the ADS was due to an adequate ratio of nutrients and the availability of proteins for new cell synthesis (Garcia-Peña et al 2011).

3.2 H₂S elimination from the biogas stream using a biofiltration system

A characterization of the three potential biofilter packing materials was performed. The highest water retention capacity (WRC) was found in vermiculite (65%), while the WRC for lava rock was 15%. Although vermiculite showed a higher WRC, lava rock favored water irrigation, which ensured that the desired moisture level was maintained and avoided sulfate accumulation at the same time.

The acetic, propionic, butyric and valeric acid could also be present in the biogas stream, their assimilation was determined in batch experiments. The biodegradation of different loadings of acetic and propionic acids as individual substrates were also evaluated in the lava rock biofilter as it as previously described elsewhere (Ramirez-Saenz et al., 2009).

As the MR proportion increased in the ADS, the H₂S concentration in the biogas stream also increased. The biodegradation of H₂S was determined in the lava rock biofilter under two different empty-bed residence times (EBRT). Results for H₂S elimination capacity as a function of H₂S inlet loading in the lava rock biofilter, operated at 85 sec and 31 sec EBRT, are depicted in Figure 2. As shown in Figure 2A, at an EBRT of 85 sec, the relationship between the inlet loading and the elimination capacity was linear, and the critical H₂S elimination capacity defined by Devinny et al., 1999 (i.e., deviation from the 100% removal capacity) was not yet reached at an inlet loading of 144 g/m³h. Under these operation conditions, the removal efficiency of H₂S for loadings between 36 and 144 g/m³h was always above 98 %. Furthermore, the EC reached a maximum of 142 g/m³h when the H₂S loading was 144 g/m³h.

For an EBRT of 31 sec (Figure 2B), the H₂S elimination capacity was found to be linear with respect to H₂S inlet loading up to 200 g/m³h (100% removal efficiency). A higher inlet loading of 300 g/m³h reduced the removal efficiency in the system to 85 %. An inlet loading of 400 g/m³h (corresponding to 3000 ppmv) caused the removal efficiency to drop to 75%, which suggested inhibition of biological activity and/or insufficient mass transfer. In this case, the critical H₂S EC was 200 g/m³h, whereas a maximum H₂S EC value of 232 g/m³h was achieved in the biofilter. At the same time, however, the removal of VFAs present in the gaseous stream (approximately 10 ppmv) reached 99%.



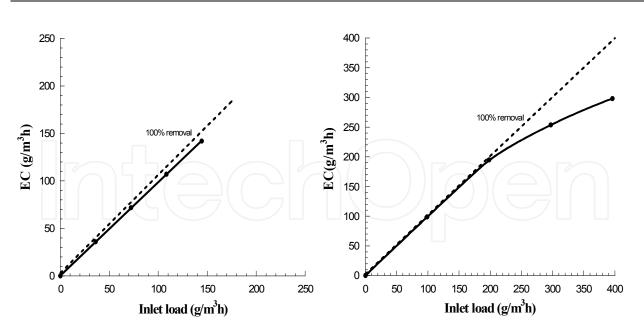


Fig. 2. H2S elimination capacities as a function of H2S inlet loadings in a biofilter operated at A) 85 sec EBRT and B) 31 sec EBRT. The points and solid lines represent the experimental data, and the dashed line (--) is the 100% removal line measured at both EBRTs.

For long operation times, the biofilter nearly eliminated all the H₂S from the biogas stream. The H₂S concentrations of the AD gas stream were previously diluted to maintain an inlet concentration of 1500 ppmv and to allow complete elimination (99% removal efficiency), but the high removal efficiency was maintained over 90 days and complete biodegradation of VFAs was also observed. Fifty days after the start-up period, a technical failure in the AD system blocked the feeding of the biofilter, no data was obtained during that time. After operation conditions were restored, an inlet H₂S concentration was maintained at approximately 1500 ppmv from day 103 to 194 at an EBRT of 31 sec. Under these conditions, a removal efficiency of 95% was maintained for 90 days. Higher concentrations, around 3000 ppmv, caused a drop in the biofilter efficiency to 50% (Ramirez-Saenz et al., 2009). The biofilter was fed with the ADS gas stream every two weeks, which corresponded to the HRT of the AD system.

According to the stoichiometry of aerobic biological H_2S oxidation (Eq. 1 and 2) and the sulfate determinations obtained between days 103 and 194 of the operational period of the biofilter, 51 to 60% of the H_2S was completely oxidized to sulfate. These data are correlated with those reported by Fortuny et al., 2008 with respect to the H_2S conversion to sulfate. The elimination of these compounds allowed the potential use of the biogas while maintaining the methane (CH₄) content throughout the process.

3.3 Microbial community characterization

Samples from three different positions of the biofiltration system were taken to evaluate the spatial distribution of the microbial population. Figure 3 shows a picture of the biofiltration system and the positions where the samples were collected. The samples at the top of the reactor "a" correspond to the inlet of the biogas stream mixed with different air fluxes, samples "b" and "c" correspond to the middle part of the reactor and sample "d" was located at the bottom of the biofilter (outlet of the biogas stream).



Fig. 3. Biofiltration system coupled to an anaerobic digestion system. Arrows indicate the position of sampling used for DGGE analysis.

Additionally, the changes in the bacterial community were also determined by taking samples during long-term operation of the biofiltration system (Samples from 1a to 9a). The analysis was performed by a DDGE system using 16S rRNA as a bacteria-specific target for PCR amplification. Figure 4 shows an example of denaturing gradient gel DDGE (15% to 60%) from samples of different times of cultivation compared with the initial bacterial community. In summary, around 13 bands for the total bacterial community were systematically detected over long-term operation of the biofiltration system. (Samples 1a to 3a correspond to days 5, 10 and 20th of operation. Sample 4a was obtained at day 45th of operation, when the inlet load was increased to 3000 ppmv. Samples from 5a to 8a were obtained in days 90, 110 130 and 150 of operation, respectively.) In view of the total bacterial community, the bands remained constant until variations in intensity appeared. In lane 4a, lower intensity bands revealed a weakening pattern, which suggest a decrease in certain types of bacteria, when the H₂S concentration increased from 1500 ppm to 3000 ppm. Both the decrease in removal efficiency and the decrease in the microbial population could be explained by the toxicity of the extremely high H₂S concentration. This factor was assumed to be responsible for the disappearance of some of the microbial species. Increased intensity

in some bands in the gel (boxes A and B) demonstrated intensifications of specific band patterns. These data suggested the eventual dominance of H₂S-oxidizing bacteria (SOB) and bacteria able to consume VFA.

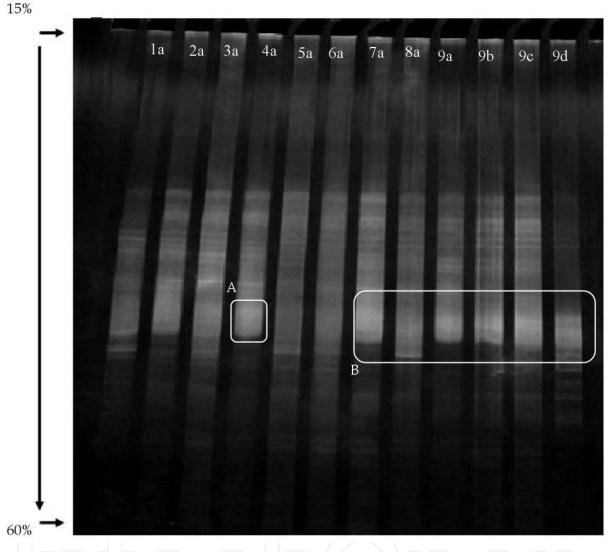


Fig. 4. Polyacrylamide denaturing gradient gel (15–60%) with DGGE profiles of 16 S rRNA gene fragments of the samples taken from different operation times (Lanes from 1a to 8a) and locations of the biofilter (Lanes from 9a, inlet to 9d, outlet), (6-h run, 200 V, 60 °C).

To compare bacterial community between different samples and to determine possible changes in composition, the presence or absence of a band in a DDGE gel was analyzed using a binary system. A 0 value was assigned when the band was absent (i.e., different band is considered a different microorganism) and 1 when the band in two or more samples was present (i.e., same microorganism) at similar positions in the gel. Jaccard's index and the Sorensen-Dice index could then be calculated. Table 2 shows the matrix constructed using the DDGE gel containing different band patterns obtained at different times of operation (lanes 1a to 9a) and at different lengths along the biofilter (9a, 9b, 9c and 9d). Nineteen different bands (arbitrarily named A to S) were found in the samples analyzed by gradient DDGE.

Once the number of bands that were similar or different between the two samples was determined, the similarity of the different samples was determined by calculating the Jaccard and Sorensen-Dice indexes. Two different aspects were analyzed: the similarity of the samples during the time of cultivation (lanes 1a to 9a) and the similarity at a different position in the reactor (lane 9a compared to 9b, 9c and 9d).

Band/Lane	1a	2a	3a	4a	5a	6a	7a	8a	9a	2b	2c	2d
A	1	1	1	1	1	1	1	1	1	1	1	1
В	1	4	1	1	1	1	1	1	1	1	1	1
С	0	0	1	0	1	1	1	0	0	0	0	0
D	0	1	1	1	1	1	1	1	1	1	1	0
Е	0	1	1	1	0	1	1	1	1	1	1	0
F	1	1	1	1	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1	1	1	1
Н	1	1	1	1	1	1	1	1	1	1	1	0
Ι	1	1	1	1	1	1	1	1	1	0	1	0
J	1	1	1	0	1	1	1	1	1	1	1	0
К	1	1	1	1	0	0	1	1	1	1	1	0
L	1	1	0	1	0	0	0	1	0	0	0	0
М	1	0	1	0	0	0	0	0	0	0	0	0
Ν	1	0	1	0	1	1	0	1	0	0	0	1
0	1	0	1	0	1	1	1	0	0	0	0	1
Р	0	1	1	0	1	1	1	1	0	0	0	1
Q	0	0	0	0	0	1	1	0	0	0	1	1
R	1	1	1	0	1	1	1	0	0	1	1	1
S	0	0	0	0	0	0	0	0	0	0	0	1
Total	13	13	16	10	13	15	15	13	10	10	12	10

Table 2. Matrix constructed using the DDGE gel containing different band patterns. Lanes 1a-8a correspond to samples at 8 different times of cultivation. Lanes 9a to 9d were samples at different lengths of the biofiltration system.

Regarding time of cultivation, the bands A, B and E to J constantly appeared in the microbial community, suggesting little change in the microbial populations during the operation of the biofilter. The most similar microbial communities were found in lanes 6a and 7a, with a Jaccard index of 0.875 and a Sorensen-Dice index of 0.933, which corresponded to the steady state of the biofiltration system at an average H₂S inlet concentration of 1500 ppmv and a removal efficiency of 95%. In contrast, the least similarity was found between lanes 4a and 5a, 6a and 7a (Jaccar's indexes of 0.438, 0.47 and 0.56, respectively). In lane 4a, the microbial community sample was exposed to an increased H₂S concentration of 3000 ppmv. These data differed from those found by Maestre et al., 2010. These authors reported a wide phylogenetic diversity and showed that the initial populations became more specific, being the SOB the dominant community.

The similarity between the microbial communities along the biofilter was also calculated. For this purpose, the bands in lane 9a were compared with the bands in lanes 9b, 9c and 9d. Significant differences in the microbial population were observed at different lengths along the biofilter. The highest divergence was found between lanes 9d and 9b, with a Jaccard index of 0.333 and a Sorensen-Dice index of 0.5. These data could be partially explained by the H₂S concentration gradient: a higher concentration at the inlet of the biofilter and a lower concentration at the outlet (sample 9d). The accumulation of metabolic products could also explain the divergence. The highest similarity was found in samples of lanes 9b and 9c, which corresponded to the middle of the reactor, where apparently the environmental conditions were more homogeneous (Jaccard index of 0.833 and Sorensen-Dice index of 0.909). These data are in agreement with the results obtained by Maestre et al., 2010 and Omri et al., 2011 about the divergence in microbial populations along the reactor.

Sequence analysis of DNA extracted from single bands representing specific species were then used as an approach for further community characterization. Sequence analyses of bands (Table 3) revealed the predominant bacteria in the biofiltration system. The structure of the bacterial community sequenced was associated with microbial activity in the system as a function of the pollutant eliminated in the biofiltration system.

↑ 1ª 4ª 5ª 9ª 9 ^b 9 ^c	Band No.	Closest relative	Identity (%)
40% 1	1	Agromyces mediolanus	100
	2	Arcobacter butzleri	99%
3	3	Bacillus cereus	98%
5	4	Bosea thiooxidans	94%
2	5	Butirivibrio fibrisolvens	99%
6	6	Thiobacillus sp.	100%
60%	7	Uncultured bacteria	98%

Table 3. Sequence analysis and species identification of the major (7) DGGE bands for the biofilter samples.

Band sequencing results showed that the dominant members of SOB consisted of *Bosea thiooxidans* and *Thiobacillus* sp (Table 3). Das et al., 1996 reported that *Bosea thiooxidans* was a new gram-negative bacterium isolated from agricultural soil and capable of oxidizing reduced inorganic sulfur compounds. Data showed that this microorganism was strictly aerobic. Experiments conducted to evaluate thiosulfate oxidation showed that the growth yield varied with the concentration of this compound; the greatest growth was observed at a

concentration of 5 g/L. Under these conditions, conversion of thiosulfate to sulfate was stoichiometric, and the pH of the medium decreased from 8.0 to 6.6. The distance matrix phylogenetic tree based on the level of difference between *Bosea thiooxidans* and 19 reference strains of the alpha subclass of the Proteobacteria indicated that strain BI-42 belonged to a new lineage located between the methylotrophs, the genus *Beijerinckia*, and the *Rhodopseudomonas palustris* group. No close relationship was found between the strain and other sulfur-oxidizing bacteria, such as *Thiobacillus acidophilus* and *Acidiphilium* species (Das et al., 1996). Microorganisms utilize sulfur compounds for the biosynthesis of cellular material or transform these compounds as part of a respiratory energy-generating process. Most of the known sulfur-oxidizing bacteria belong to the genus *Thiobacillus*, *Thiothrix, Beggiatoa, Thiomicrospira, Achromatium, Desulfovibrio, Desulfomonas, Desulfococcus,* and *Desulfuromonas*. Furthermore, members of the genus *Thiobacillus* have been studied extensively to increase understanding of the coupling of oxidation of reduced inorganic sulfur compounds to energy biosynthesis and assimilation of carbon dioxide.

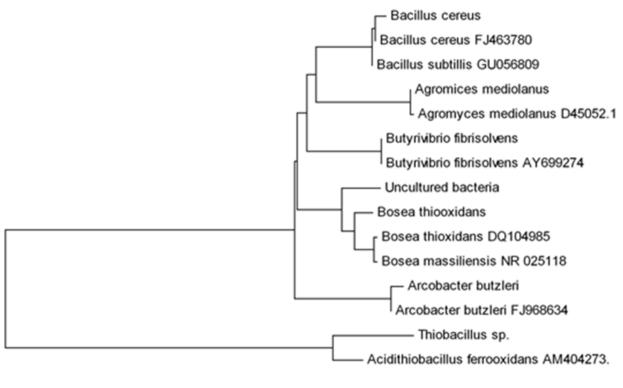




Fig. 5. Neighbor-joining tree of partial 16S rRNA sequences (approximately 750 bp) recovered by denaturing gradient gel electrophoresis (DGGE) bands in the biofilter. The bar indicates 1% sequence variation.

The presence of *Arcobacter butzleri*, belonging to the Phylum Proteobacteria (e-Proteobacteria), could be associated with VFA degradation (these compounds were introduced into the biofilter through the biogas stream). This microorganism is able to grow under both aerobic and anaerobic conditions over a wide temperature range (15-42 °C). However, optimal growth occurs under microaerobic conditions (3–10% O₂). *Arcobacter*

butzleri was also recently found as a member of the microbial population in a microbial fuel cell (MFC) used to produce electricity from synthetic domestic wastewater that contained a mixture of VFAs as electron donors (Freguia et al., 2010). Similar results were obtained by Nien et al., 2011, where an *Arcobacter butzleri* strain, ED-1, was also determined to be part of the microbial community of a MFC fed with acetate. Although aerobic species were predominant because the metabolic activity determined (sulfate as the main product), the DGGE showed that profile some facultative anaerobes were as part of the microbial population, which could be related to the trophic properties of the community, and the different substrates in the biogas stream (H₂S and VFAs).

Some of the species found in the present study agreed with those previously reported in the literature for biofiltration systems used in the removal of reduced sulfur compounds. For example, Ding et al., 2006 studied a packed compost biofilter for the treatment of a mixture of H₂S and methanol using 16S rRNA sequencing analysis. The authors established that the microbial community was composed of strains of *Thiobacillus, Sulfobacillus,* and *Alicyclobacillus hesperidensis*. In a biofilter packed with compost, activated carbon and sludge used for the removal of H₂S, Chung, 2007 determined a microbial population composed of *Pseudomonas citronellolis, P. fluorescens, P. putida, S. capitis, Bacillus subtilis* and *Paracoccus denitrificans*. In a recently published work (Omri et al., 2011), it was reported that most bacteria in the operation samples were of the genera *Pseudomonas* sp., *Moraxellacea, Acinetobacter* and *Exiguobacterium*, which belong to the phyla Pseudomonadaceae, gamma-Proteobacteria and Firmicutes.

A neighbor-joining tree (Fig 5.) of partial 16S rRNA sequences (approximately 750 bp) was constructed in MEGA4 (Tamura et al., 2007) by considering sequences obtained and comparing them with others in the data bank.

4. Conclusion

The feasibility of CH₄ was demonstrated. High VS removal, the increased methane yield, and the natural pH control during the stable period of the ADS was obtained by codigestion of VFW and MR, due to an adequate ratio of nutrients and the availability of proteins for new cell synthesis. However, the increasing MR concentration in the ADS increased the H₂S concentration in the gas stream. The elimination of H₂S and VFAs by a biofiltration system was successfully determined, reaching high removal efficiencies of both compounds (95% and 99%, respectively). This approach could allow the potential use of the biogas maintaining the methane (CH₄) content throughout the process. The microbial population characterization of the bioifltration system showed that dominant members of SOB were *Bosea thiooxidans* and *Thiobacillus* sp. Some facultative anaerobes were also determined in the system, which could be explained by the composition of the biogas stream and the conditions at different length of the reactor.

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6. References

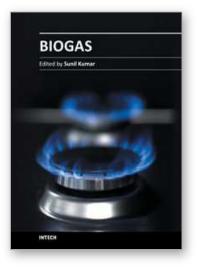
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This book contains research on the chemistry of each step of biogas generation, along with engineering principles and practices, feasibility of biogas production in processing technologies, especially anaerobic digestion of waste and gas production system, its modeling, kinetics along with other associated aspects, utilization and purification of biogas, economy and energy issues, pipe design for biogas energy, microbiological aspects, phyto-fermentation, biogas plant constructions, assessment of ecological potential, biogas generation from sludge, rheological characterization, etc.

How to reference

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