STRATEGIES TO OPTIMIZE METHANE PORDUCTION FORM NON CONVENTIONAL WASTE

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ABSTRACT

The greatest technological challenge for human society today is the replacement of fossil fuels by energy sources that are renewable and carbon neutral. The most likely way that this challenge will be met is by using microorganisms, which have the potential to generate large flows of renewable energy. Wastewaters, sludges, residues, and other ‘wastes of today’ must be viewed as resources, within the context “waste-to-energy”. Converting the energy value in these wastes to useful energy provides two simultaneous benefits: the generation of renewable energy and the minimization of environmental pollution. Anaerobic technology has been traditionally applied for the treatment of carbon rich wastewater and organic residues. One of the main advantages of anaerobic digestion is the possibility of recovering renewable energy in the form of biogas, which is a versatile energy carrier that can be used for electricity production, heating purposes, vehicle and jet fuel and replacement of natural gas by injection of upgraded biogas in the natural gas grid. In addition, biogas may be considered as starting compound for biotechnological production of chemicals.

The wide range of feedstocks suitable for biogas production includes animal waste, municipal sludge, industrial wastewater and organic fractions of municipal solid waste, as well energy crops. Nonetheless, other substrates should be considered because of their high methanisation potential. Examples of non-conventional and/or recalcitrant wastes already attempted to produce biogas include fish oil, slaughterhouse waste, fish waste, poultry waste, animal by-products (cat 2), herbaceous species and other lignocellulosic materials, such as gorse, and macroalgae (seaweeds). However, these substrates present some common problems, which can be grouped in 3 categories: lipid rich wastes, which by hydrolysis form Long Chain Fatty Acids (LCFA); protein rich wastes, which by hydrolysis produce ammonia (NH_3); and cellulose rich wastes, particularly those that form complex structures of cellulose and lignin.

The economy of the anaerobic digestion plants, especially when treating non-conventional wastes, depends on the availability and biodegradability of the co-substrates. Two opportunities to improve the economy of these plants should be considered. First, pre- and co-treatments can increase the wastes solubilisation. In this category are included the pre-treatment with high temperature and/or pressure, addition of enzyme preparations, and bioaugmentation with microorganisms with known lipolytic, proteolytic, and cellulolytic activity. However, to increase the methane production special measures are needed to prevent the subsequent methanogenesis inhibition. Second, the high energy value of lipids makes them an ideal co-substrate to increase the economical feasibility of co-digestion plants. However, without a proper feeding strategy addition of waste lipids to an anaerobic digestion plant is risky, if accumulation of LCFA is not prevented and a given threshold is exceeded. Understanding anaerobic degradation of lipids has therefore an immediate economical impact on anaerobic digestion plants.

This paper reviews the anaerobic digestion of non-conventional/recalcitrant wastes and the use pre-treatments, bioaugmentation and co-digestion techniques to simultaneously achieve their treatment and increase their energetic valorisation.

Keywords: anaerobic digestion, bioaugmentation, biogas, pre-treatments, macroalgae, waste.

ANAEROBIC DIGESTION OF NON-CONVENTIONAL WASTES

Increasing demand for sustainable development has stimulated political interest in measures to decrease pollution and greenhouse gas production by human activities. In response, several national governments have prepared objectives for the next decades, bringing anaerobic waste treatment with biogas production into the forefront. According to data from the Barometer Biogas between 2000 and 2009 the biogas produced in Europe increased 4,8x. However, there are large asymmetries of biogas technology implementation in Europe. In Germany, 51500 ton oil equivalent (TOE) were produced per 1000 inhabitants as biogas primary energy in 2009, whereas in Portugal only 2200 TOE/1000 inhabitants were produced in the same period (EurObserv'ER, 2010).

Anaerobic digestion has a strong potential for treating biodegradable solid waste. It is considered as an organic recycling technology, as it produces renewable energy from combustion of the biogas obtained. This could replace fossil fuel-derived energy and reduce environmental impacts including global warming and acid rain. On the other hand, the anaerobic digestion of organic waste produces a digestate, which can be used in agriculture after stabilization or composting step. Anaerobic digestion of organic solid waste is an established technology in Europe, with more than 200 full-scale plants treating almost 6 million tons per year (De Baere and Mattheeuws, 2010). Anaerobic digestion of complex organic substrates proceeds through a series of parallel and sequential steps, with several groups of microorganisms involved (Costa et al., 2013d). The biochemical methane potential (BMP) is a key parameter for assessing design, economic and managing issues for the full-scale implementation of the anaerobic digestion process. This parameter depends on the solid waste composition. A wide variety of substances, present in high concentrations in waste, can cause inhibition or failure of anaerobic digestion. The hydrolysis is usually the limiting step during the biodegradation of recalcitrant wastes. Improved efficiency of the anaerobic processes can lead to higher biogas yield meaning that new substrates, usually regarded as difficult to degrade, need to be addressed. Difficult wastes can be grouped according to the type of problem they cause in anaerobic digestion plants. Lipid, protein and lignocellulosic rich wastes should be addressed due to the wide abundance and to the specific drawbacks associated to the wastes when digested. Anaerobic digestion of energy crops (aquatic and/or terrestrial), rich in lignocellulosic material and/or proteins, may also follow in the same category.

When protein rich wastes (such as chicken feathers) are hydrolyzed, ammonia (and eventually hydrogen sulphide) is produced. Ammonia (in the non-ionised form NH_3) can inhibit the anaerobic digestion process and 100 mg L^{-1} should not be exceeded if the sludge was not previously acclimated. Digestion of lignocellulosic substrates is limited by the low hydrolysis rate. Some inhibition effects due to accumulation of hydrolysis products such as cellobiose, glucose, volatile fatty acids (VFA), and ammonia may occur (Costa et al., 2012a). Lipid rich wastes can cause problems of biomass flotation and washout due to lipids and LCFA adsorption. Self-granulation of biomass and/or granules maintenance is related to these problems as well. At neutral pH, LCFA act as surfactants lowering the surface tension; consequently, granules disintegrate. Furthermore, lipids and LCFA can impose mass transfer limitations (Pereira et al., 2005). These problems have resulted in process failure (Hwu and Lettinga, 1997) and lipids addition as co-substrate can cause a persistent problem of inhibition, if the adequate dose is exceeded, and LCFA accumulation prevails.

Recent works proved that lipids can be degraded either in continuous high-rate anaerobic reactors treating a synthetic and a real lipid-rich wastewater (Cavaleiro et al., 2009; Gonçalves et al., 2012). Moreover, successful biodegradation of recalcitrant and/or non-conventional wastes is possible (Table 1). However, many times less than 50% of the energetic potential of these wastes is used, either because the anaerobic consortium is unable to properly perform the hydrolysis/solubilisation, either because no proper measures are considered to prevent the process inhibition.

Table 1 Biochemical Methane Potential (BMP) of non-conventional wastes

Waste/Biomass	BMP [L CH4 kg-1 VS]	Reference
Animal by-products (cat.2)	317 ± 7	Pozdniakova et al. 2012
Chicken Feathers	123 ± 3	Costa et al. 2012b
Enteromorpha sp.	154 ± 7	Costa et al. 2012c
Gracilaria sp.	182 ± 23	Costa et al. 2012c
Gracilaria vermiculophylla	295 ± 26	Costa et al. 2013b
Gorse	160	Eiroa et al. 2012
Greaves	707 ± 46	Cavaleiro et al. 2013
Mackerel	350	Eiroa et al. 2012
Mixed Sludge	335 ± 27	Costa et al. 2012c
Needle	260	Eiroa et al. 2012
Poultry Litter	145 ± 14	Costa et al. 2012a
Rinds	756 ± 56	Cavaleiro et al. 2013
Sardine	250	Eiroa et al. 2012
Spent Grain	191 ± 3	Costa et al. 2013c
Trub	251 ± 2	Costa et al. 2013b
Tuna	280	Eiroa et al. 2012
Ulex Europaeus	308 ± 1	Costa et al. 2013a (submitted)
Ulva sp.	196 ± 9	Costa et al. 2012c

OPTIMIZATION STRATEGIES

PRE-TREATMENTS

The conversion of complex particulate materials, rich in lipids, proteins and lignocellulose, to methane in anaerobic digesters is frequently limited by the hydrolysis step (Vavilin et al., 1996). An efficient hydrolysis is crucial to make complex substrates accessible for the anaerobic bacteria and ultimately optimize methane production from the waste to be treated. Several pre-treatment techniques have been applied to enhance the hydrolysis and anaerobic biodegradability of organic wastes. Physical treatments destroy particles aggregation, decrease particles size and disrupt cell structure through mechanical methods (e.g. grinding or maceration), high temperatures, microwaves or ultrasounds; chemical processes can be achieved through the addition of acids or bases, that disrupt the molecular structure of the materials due to the high pH change; biological methods involve the action of viable enzyme-producing microorganisms (bioaugmentation) or enzyme preparations.

Several physical, thermo and thermochemical pre-treatments have been performed to increase the hydrolysis/solubilisation of non-conventional wastes. Poultry litter (Costa et al., 2012a) and chicken feathers (Costa et al., 2012b) wastes were pre-treated with lime ($Ca(OH)_2$) and sodium hydroxide (NaOH). The variables tested were the temperature (20 and 90 ºC), contact time (30, 60 and 120 min), pressure (1.01, 1.27 and 4 bar), and concentration (0.05, 0.1 e 0.2 g alkali g^{-1} TS waste). The wastes solubilisation increased with temperature, contact time and alkali concentration. The optimal pressure was 1.27 bar because the efficiency decrease at higher pressures. Although the soluble chemical oxygen demand (COD) resulting from thermochemical pre-treatment was higher when using NaOH, subsequent substrate conversion to methane was less effective than in the tests pre-treated with lime, probably due to the accumulation of high concentrations of intermediates, such as VFA, ammonia and furfural. Pre-treatment during 120 minutes with $Ca(OH)_2$ at 90 ºC and 1.27 bar give the best BMP, i.e. 137 L CH_4 kg^{-1} VS and 105 L CH_4 kg^{-1} VS, respectively for poultry litter and chicken feathers wastes. Overall, BMP obtained in assays with pre-treated feather waste were lower than in the test with raw waste, i.e. 123 L CH_4 kg^{-1} VS. The pre-treatments caused the accumulation of high concentrations of ammonia, up to 2.7 g

NH_4^+-N L^{-1}, due to the high content of proteins in the chicken feathers waste, which caused the methanogenesis inhibition.

Six pre-treatments were tested to increase the meat processing wastes (greaves and rinds) solubilisation and subsequent methane production (Cavaleiro et al., 2013), using NaOH (0.3 g g^{-1} TS), NaOH at 55 °C, autoclaving with NaOH, high temperature (70 °C), enzyme (lipase from *Candida rugosa* at 10 U g^{-1} fat) addition, and enzyme addition plus autoclaving. A maximum value of 1220 g COD kg^{-1} raw waste was obtained for greaves with the combined action of base and temperature, although high concentrations, around 1000 g COD kg^{-1} raw waste, were also accomplished with enzymatic pre-treatments. For rinds, maximum values of 900–1000 g COD kg^{-1} were obtained with base and autoclaving + enzyme addition. The highest percentage of fat hydrolysis (52–54%) occurred for the pre-treatment of greaves with base + temperature and for the pre-treatment of rinds with base alone. Pre-treating the greaves did not improve BMP values and imposing temperatures between 55–121 °C even decreased the methane recovered by 36–57%. A 25% higher BPM value (919 L CH_4 kg^{-1} VS) was obtained in assays amended with rinds pre-treated with heat. In the other situations, BMP was not significantly different or was lower than that of untreated waste. Anaerobic biodegradability of the samples pre-treated with NaOH and NaOH at 55 °C was severely reduced.

The effect of several physical (washing, drying and maceration) and thermochemical (NaOH, temperature and pressure) pre-treatments were also tested to increase the solubilisation and methanisation of the macroalgae *Gracilaria vermicullophylla* (Costa et al., 2013b). The washing and macerated sample achieved the higher specific methane production (481 L CH_4 kg^{-1} VS). The maceration increased the surface area making the substrate more accessible to the inoculum, facilitating a fast hydrolyses and providing higher biodegradation rates. The washing process removed some inhibitory compounds in the raw samples, for instance, excess salt. One of the disadvantages of using seaweeds as energy crops for biogas production, compared with terrestrial energy crops is the high water content. Although the specific methane production was smaller after drying the *G. vermiculophylla*, it was possible to verify a methane production boost in terms of methane yield per mass of seaweed. Indeed, the washed, dried and macerated sample achieved 240 L CH_4 kg^{-1} macroalgae, while the washed and macerated sample produced only 45 ± 1 L CH_4 kg^{-1} macroalgae. Therefore, drying the sample may be the only way to digest seaweeds in a continuous reactor, to increase the organic loading rate. Regarding the thermochemical pre-treated samples, no significant effects were observed in the biodegradability results, with BMP ranging from 353–380 L CH_4 kg^{-1} VS and the final solubilisation percentage (pre-treatment + anaerobic digestion) from 82–87%. However it should be noted the highest biodegradability rate of the assays with higher initial soluble COD. Therefore, the pre-treatment of the samples caused changes in the structure of the organic matter, which facilitated the initial production of methane.

BIOAUGMENTATION

The physical and thermochemical pre-treatments are considered very expensive methods, and many times they are economically unfeasible. Therefore, addition of hydrolytic microorganisms can be an alternative to enhance the hydrolysis–fermentation process. There are several described microorganisms with cellulolytic and proteolytic activity that could be used to biodegrade the lignocellulosic portion and proteins in lignocellulosic- and protein-rich wastes. Low energy and no chemical requirements are the main advantages of biological pre- or co-treatment.

Poultry litter was bioaugmented with mesophilic (*Clostridium cellulolyticum*) and thermophilic (*Clostridium thermocellum* and *Caldicellulosiruptor saccharolyticus*) microorganisms to increase the solubilisation of the lignocellulosic material (Costa et al., 2012a). Addition of *C. cellulolyticum* had a positive effect in the specific methane production (102 L CH_4 kg^{-1} VS), compared with BMP obtained in the raw waste non-bioaugmented tests (90 L CH_4 kg^{-1} VS). However, in the trials with *C. thermocellum* and *C. saccharolyticus* as bioaugmented species no significant differences on methane production were observed, when compared to the non-bioaugmented assays. These results are explained by the use of a non-acclimated mesophilic inoculum, which had low methanogenic activity at the optimal growth, i.e. 55 °C and 65 °C, respectively for *C. thermocellum* and *C. saccharolyticus*. This assumption is confirmed by the high solubilisation of the waste in both assays, 62% and 74% *vs.* 52% in non-bioaugmented assay, and by the accumulation of by-products, confirming an efficient hydrolysis of the cellulosic material in the poultry litter and that methanogenesis was the rate-limiting step in the conversion of cellulosic material.

Bioaugmentation of chicken feathers waste (rich in protein) with *Fervidobacterium pennivorans* resulted in a BMP of 45 L CH_4 kg^{-1} VS and a solubilisation yield of 64% (Costa et al. 2012b). This BMP value corresponds to less than one third of the result obtained in the non-bioaugmented test (123 L CH_4 kg^{-1} VS). Nevertheless, the solubilisation values in the bioaugmented assays had an increase of approximately 20% suggesting that the microorganism acted on the substrate, promoting their biodegradation. Afterwards, the methanogenesis was inhibited by intermediates generated, namely ammonia (2.8 g NH_4^+-N L^{-1}) and VFA (18.0 g L^{-1}). Also, the use of a mesophilic inoculum at 65 °C may have introduced a delay in the methane production due to the adaptation span of the microorganism to a higher temperature.

CO-DIGESTION

There is a long tradition of anaerobic sewage sludge and animal manure treatment. Presently agricultural applications are mainly based in co-digestion of manure with available co-substrates such as harvest residues, top and leaves of sugar beets, organic wastes from agriculture related activities, food waste, collected municipal biowaste from households and aquatic and terrestrial energy crops (Weiland 2010). The advantages of co-digestion include: dilution of the potential toxicity of any of the involved co-substrates, nutrients balance, synergistic effects on microorganisms, increasing the load of biodegradable organic matter, higher methane yields per unit of digester volume; and the economic advantages can also be significant, derived from the fact of sharing equipment (Mata-Alvarez et al. 2000).

Lipids have high potential for methane production. However, LCFA are produced during their degradation, which can be toxic for the anaerobic consortium, inhibiting the methanogenic activity. In the study of Neves et al. (2009), four 5 L mesophilic (37 °C) continuously stirred tank reactors were used to co-digest cow manure and food waste (1:1 TS:TS). After a stable operation for 148 days, oily waste effluent from a canned fish processing industry was fed in the form of pulses, to determine the safe threshold that should not be exceeded. The oil concentration rose up to 9, 12, 15 and 18 g COD_{oil} $L^{-1}_{reactor}$, after the pulse feeding in the reactor. The highest fat concentration of 18 g COD_{oil} $L^{-1}_{reactor}$ promoted a persistent inhibition in the process of the continuous reactor, although in batch assays, the reactor content evidenced a capacity to degrade more oil and to degrade the accumulated organic matter. All the other pulses had a positive effect in the methane production. The threshold input of oily waste to enhance the methane production in the co-digestion of cow manure and food waste was 12 g COD_{oil} $L^{-1}_{reactor}$. This corresponds to a continuous feeding of 100/10 ($V_{manure}/V_{food\ waste}$) with intermittent oil pulses of 5% (V_{oil}/V_{manure}).

Marine biomass, such as macroalgae (seaweeds), has the potential of becoming a viable energy crop. However, the production of energy from macroalgae is still committed for reasons of economic viability (Jones and Mayfield, 2011). One possibility is the cultivation of macroalgae using nutrients from wastewater treatment plants (WWTP) and subsequent production of biogas from the cultivated biomass alone or in co-digestion with sewage sludge in existing digesters. Costa et al. (2012c) performed several batch co-digestion assays using macroalgae (*Ulva* sp. (U) and *Gracilaria vermiculophylla* (GV)) and sewage sludge (primary (PS), secondary (SS) and mixed sludge (MS)) as substrates. The methane yield was similar for all the assays, in the range 42–45%, indicating a similar degree of biodegradation of all substrates. In the assays with *Ulva* sp. and mixed sludge the methane production rate increased significantly (2–3x), compared to the digestion of 100% U. These results indicate that the co-digestion of macroalgae with sewage sludge seems an attractive choice, with a synergetic effect. The methane production rate of the co-digestion process, compared with the sewage sludge digestion alone was increased 26% and the overall biodegradability was not negatively affected. The ratio 15% U + 85% MS had the highest BMP (296 L CH_4 kg^{-1} VS). *Ulva* sp. seems economically more attractive than *G.*

vermiculophylla because the methane production rate was 1.7x higher, while the BMP was very similar. This difference is very important because it may represent, for instance, the difference between an anaerobic digester operating with a hydraulic retention time of 10 or 20 d, with all the increasing investment and operational costs that such difference involves. Regarding the different sludge tested, the highest BMP was obtained with the 15% U + 85% PS (358 L CH_4 kg^{-1} VS).

Glycerol is a by-product of biodiesel manufacturing industry with more than two thousand applications, especially if refined to pure state. However, its refining is very expensive and its increasing production exceeds the commercial demand (Siles et al. 2010). Options for the biological conversion of glycerol into added value products are becoming increasingly important. Currently, several approaches are being studied to improve the productivity of mixed-culture methods. Co-digestion with nitrogen-rich wastes is a promising option since it increases the energy yield of the co-substrate, whereas the anaerobic digestion process approaches the optimum humidity and C/N ratio. Costa et al. (2013c) demonstrated that the co-digestion of brewery waste (10% trub + 90% spent grain) with 10% glycerol caused an increase of 65% in the BMP (328 L CH_4 kg^{-1}COD), relatively to the assay without glycerol (BMP = 199 L CH_4 kg^{-1} COD). It seems that the addition of glycerol up to 10% had a synergetic effect on the wastes biodegradability since the methane yield increased to 91-94%. However, higher concentrations (20 and 33% of glycerol) inhibited the methanogenic activity. Coupled to the decrease in the BMP and methane yields, an increase in the soluble COD, VFA and LCFA was observed.

Addition of 2, 5, 10 and 20% of glycerol to the macroalgae *G. vermicullophyla* and to the mixture *G. vermicullophyla* (15% TS) and sewage sludge (85% TS) was also studied (Costa et al., 2013b). Regarding the glycerol and macroalgae co-digestion, the highest BMP was obtained in the assay with 2% glycerol (599 L CH_4 kg^{-1} VS). This value represents an increase of 18% in the specific methane production compared with the value obtained in the sole digestion of *G. vermiculophylla*. In this assay almost all available substrate was converted to methane as indicated by the PM of 96%. Inhibition of the methanogenic activity started with concentrations higher than 5% of glycerol, as observed by the accumulation of metabolites and toxic compounds, namely VFA, LCFA and ammonia. A significant increase in the specific methane production was observed with the co-digestion of macroalgae and sewage sludge (605 L CH_4 kg^{-1} VS) compared with the initial assays with only *G. vermiculophylla*. However, the subsequent addition of glycerol (2 and 5%) had no significant effect on the methane production. In the assay with 10% glycerol, a severe inhibition occurred probably due to the high concentration of VFA, LCFA and ammonia.

The co-digestion of two non-conventional wastes, namely fish waste with gorse, was studied in order to improve methane production from fish waste (Eiroa et al., 2012). However, the methane production was similar in all assays, around 200 L CH_4 kg^{-1} VS. Therefore, the biochemical methane potential of fish waste was not improved by adding gorse. The high lignocellulosic content of gorse suggests the need of a pre-treatment to increase its biodegradability.

CONCLUSIONS

Non-conventional/recalcitrant wastes should be seen as a promising source of bioenergy by their anaerobic biodegradation. However, their recalcitrant nature makes them difficult to completely biodegrade without the use of special strategies that can improve the efficiency of the anaerobic digestion process, either by improving their hydrolysis/solubilisation or their methanisation. Physical and thermochemical pre-treatments can significantly enhance the hydrolysis and solubilisation of recalcitrant wastes, however a proper dilution should be used to prevent the subsequent methanogenesis inhibition. Bioaugmentation treatments should be performed in two steps, a previous inoculation of the waste with the microorganism, with subsequent anaerobic degradation at mesophilic temperatures assays. Another possibility consists in the previous acclimation of the anaerobic inoculum to the optimal growth of the microorganism.

Controlled intermittent inputs of oil can enhance the methane production in co-digestion assays. However, special attention should be considered when adding lipids (e.g. glycerol) as co-substrate because, although it can be a powerful way to improve the methane production, it can easily inhibit the anaerobic digestion process if secure thresholds are exceeded.

From the point of view of a WWTP, it is possible to envisage macroalgae cultivation as an important post treatment method, where carbon dioxide and nutrients are used to the macroalgae growth, and macroalgae are subsequently used for energy production..

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of the Portuguese Science Foundation (FCT) and European Social Fund (ESF, POPH-QREN) through the grant given to José Carlos Costa (SFRH/BDP/48962/2008), and through the project FCOMP-01-0124-FEDER-027914 (PTDC/AAG-TEC/3048/2012), financed by FEDER through COMPETE – Programa Operacional Factores de Competitividade.

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