Methane production via anaerobic digestion of glycerol: a comparison of conventional (CSTR) and high-rate (PABR) digesters

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Abstract

BACKGROUND: Biodiesel is an alternative to fossil fuels and can be used directly in internal combustion engines when mixed with diesel. The economic feasibility of biodiesel production necessitates the valorisation of glycerol, which is produced in large quantities (equal to 10% of the biodiesel produced). Anaerobic digestion is applicable to a variety of organic residues yielding biogas rich in methane. In order to estimate the net potential of glycerol to yield methane, pure glycerol was selected to avoid any effect from the impurities in crude glycerol.

RESULTS: The anaerobic digestion of pure glycerol was studied in two types of bioreactors: a continuous stirred tank reactor (CSTR) and a baffled reactor (periodic anaerobic baffled reactor, PABR). Both reactors were operated in mesophilic conditions (35 °C) at various organic loading rates. The maximum glycerol loading achieved in a CSTR was 0.25 g COD L–1 d–1, yielding 0.074 ± 0.009 L CH4 L–1 d–1. On the other hand, PABR allowed glycerol degradation at a loading of 3 g COD L–1 d–1 yielding 0.993 ± 0.102 L CH4 L–1 d–1.

CONCLUSION: PABR was proved to be more efficient since it was subjected to a 10-fold higher organic loading rate than CSTR. Moreover, its performance was much higher in terms of COD removal and methane productivity.

INTRODUCTION

Global warming and environmental pollution caused by the use of fossil fuels has shifted attention to renewable fuels such as biodiesel, as an answer to the energy problem and the increasing consequences of the greenhouse effect.1 According to the Kyoto protocol, industrialized countries must reduce their greenhouse gas emissions 5% below the 1990 levels in the period from 2008 to 2012. The use of biodiesel reduces emissions of atmospheric CO2, since it replaces the use of fossil fuels. In Europe, the production of biodiesel has increased significantly in recent years; 3 184 000 tons were produced in 2005, 5 713 000 tons in 2007 and 9 046 000 tons in 2009.2 Biofuels consumed for transport corresponded to 4% of the road transport fuel consumption3 in 2009. Biodiesel representing 75% of the total biofuel production in the European Union,4 is a biodegradable fuel,5 which is produced via the reaction of transesterification of triglycerides.6–8 Triglycerides are components of bio-oils such as rapeseed, sunflower and cotton.9 During this reaction, molecules of triglycerides react with an alcohol (preferably with methanol due to its low cost) in the presence of a catalyst (KOH or NaOH) and produce a two-phase mixture.10 This mixture consists of esters (upper phase) and glycerol (lower phase).

The glycerol phase constitutes 10–20% of the mass of the corresponding biodiesel generated. Consequently, biodiesel production is accompanied by the production of glycerol in huge amounts. The surplus glycerol has had a negative impact on crude glycerol prices,1 causing a financial crisis to many industries associated with glycerol production.

Glycerol may be utilized as an ingredient in food, cosmetics, pharmaceuticals and tobacco industries.11,12 Purification of the glycerol originating from biodiesel production is required in these cases, resulting in raising the cost. An alternative way of glycerol valorization is to use it as an ‘energy’ source. This may be achieved through biotechnological processes such as fermentation and anaerobic digestion.13,14 Several studies have focused on the anaerobic degradation of glycerol using pure cultures, for the production of metabolites, such as fermentation and anaerobic digestion.13,14 Microorganisms, such as Klebsiella pneumoniae, Citrobacter freundii, Enterobacter ∗ Correspondence to: K. Stamatelatou, Department of Environmental Engineering, Democritus University of Thrace, Vas. Sofias 12, 67100 Xanthi, Greece. Email: astamat@env.duth.gr

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agglomerans and Clostridium butyricum have been used to produce 1–3 propanediol,17,18 which is a very important high value added product. In addition, hydrogen may be derived during fermentation of glycerol.19–22 In general, most studies have focused on pure cultures and the production of metabolites, instead of mixed cultures and methane production, respectively. From an ‘energy’ point of view, it is worthwhile to develop processes that transform glycerol directly to biofuels with low energy and financial cost, since product separation remains the main disadvantage of fermentation processes aiming to produce high value added products.

Biogas (a mixture of methane and carbon dioxide) is another important biofuel, which may be produced during the biodegradation of organic matter. Methane may be used as a fuel in internal combustion engines. In addition to the methane generation, other significant advantages of anaerobic digestion are the low sludge production and the waste stabilization.24 A few studies have focused on biogas production from glycerol,25 particularly the glycerol generated during biodiesel production. Crude glycerol has also been added as a supplement in co-digestion with other wastes (such as municipal solid wastes, agro-industrial by-products and cattle slurry) to enhance methane production.27,28

Crude glycerol contains free fatty acids, methanol, biodiesel and other organic compounds. The maximum productivity of biogas from glycerol may be influenced by the inhibitory effect of glycerol itself in high concentrations and the other impurities as well. In order to determine the methane productivity of glycerol alone without the effect of the other compounds in crude glycerol, pure glycerol was used in this study for operating continuous mode bioreactors such as an anaerobic CSTR (continuous stirred tank reactor) and PABR (periodic anaerobic baffled reactor). The first one is a conventional type of reactor, which has been used for the anaerobic treatment of many wastes. The PABR is a high-rate reactor, similar to the ABR (anaerobic baffled reactor), but it has the option of switching the feeding point (and consequently, the effluent point) from compartment to compartment periodically. In this way, the PABR flexibly adapts its dynamic behavior from full compartmentalization (zero switching frequency; the feed enters the bioreactor at a single compartment and the PABR behaves as a single-compartment bioreactor) to full homogeneity (infinite switching frequency; the feed enters the bioreactor at all compartments almost simultaneously and the PABR behaves as a single-compartment bioreactor). This flexibility in adapting the level of compartmentalization of the bioreactor allows the biomass to withstand the fluctuations of the feed concentration and therefore culture adaptation is easier under varying conditions.

MATERIALS AND METHODS

Analytical methods

Measurements of soluble chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS) and alkalinity were carried out according to Standard Methods.31 The measurement of pH was done using a pH-meter (HANNA HI 8224). For the quantification of VFAs (acetate, propionate, isobutyrate, butyrate, iso-valerate and valerate), 1 mL of sample acidified with 30 µL of 20% H2SO4 was injected into a gas chromatograph (Varian CP-30) equipped with a flame ionization detector and a capillary column (30 m, 0.53 mm ID, Nitroterephthalic acid modified polyethylene glycol film 1.0 µm). The oven temperature was raised from 105 to 160 °C, at a rate of 15 °C min−1, and subsequently, to 235 °C at a rate of 20 °C min−1 and was held for 3 min. Helium was used as the carrier gas at 15 mL min−1, the injector temperature was set at 175 °C and the detector at 225 °C. Biogas production was measured at STP using an oil-displacement technique in a U-shaped tube. The content of methane and CO2 in the biogas were determined using a gas chromatograph (SRI 8610c, with two columns in series: molecular sieve column, 6 ft, OD 1/8 inch, ID 2.1 mm and silica gel column, 6 ft, OD 1/8 inch) equipped with a thermal conductivity detector (TCD). The column oven temperature was set at 80 °C, the injector valve at 90 °C and the TCD oven was programmed at 100 °C. Helium was used as carrier gas at 20 mL min−1. Glycerol concentration was determined using an enzymatic reagent kit (K-GCROL, Megazyme).

Inoculum and medium

Anaerobic mesophilic sludge from the Patras wastewater treatment plant was used as inoculum for the experiments. The main characteristics of the sludge were typical for this type of process: TSS 27.77 ± 3.99 g L−1, VSS 13.91 ± 1.92 g L−1, pH 7.59 ± 0.35 and soluble (after filtration) COD 0.35 ± 0.02 g COD L−1. The feed medium was glycerol (99.5 %) diluted in an aqueous solution containing nutrients and trace metals. The solutions of nutrients and trace metals used to supplement the feeding media were (in g L−1): sol.A: (NH4)2HPO4 (7.21), sol.B: FeSO4·7H2O (0.7), sol.C: CaCl2·2H2O (22.5), NH4CL (35.9), MgCl2·6H2O (16.2), KCl (117), MnCl2·4H2O (1.8), CoCl2·6H2O (2.7), H3BO3 (0.51), CuCl2·6H2O (0.24), Na2MoO4·2H2O (0.23), ZnCl2 (0.19), NiCl2·6H2O (0.2), H2WO4 (0.01).

Operation of CSTR

A cylindrical stainless steel (5 L total volume) reactor, with double wall, was used for the experiments. Warm water (35 °C) flowed within the double wall space to maintain mesophilic conditions in the interior of the reactor. Sludge suspension was achieved through continuous stirring. During start-up, the reactor was filled with 3 L of anaerobic sludge and remained for 24 h in batch mode in order to enhance microorganisms acclimation at these new conditions. Afterwards, the reactor was switched to continuous mode. The feed solution was kept in a refrigerator at 4C. HRT was maintained at 20 d with the use of a peristaltic pump which was turned on every 8 h. The outflow tube was fixed on the top of the reactor. This configuration allowed the reactor to maintain a constant volume by overflow. The feeding medium consisted of 1.18 g L−1 (NH4)2HPO4, 5.5 g L−1 NaHCO3, 10 mL L−1 of the trace metal solution C and 0.3 g L−1 yeast extract, together with glycerol at various concentrations 5, 7.5 and 10 g COD L−1 corresponding to an organic loading rate (OLR) of 0.25, 0.375 and 0.5 g COD L−1 d−1 respectively. The bioreactor was operated at the same OLR until a steady state was reached whenever possible. At steady state, the data exhibiting a standard deviation lower than 15% of the mean value was selected to calculate the mean biogas and methane productivity as well as other average values.

Operation of PABR

15 L PABR (periodic anaerobic baffled reactor) consisting of four compartments was used (Fig. 1).29,30 The reactor was placed in a water bath to keep the temperature constant at 35 °C. Every compartment was separated into two sections (downflow and upflow respectively) through a vertical baffle.29,30 Outside the reactor, four electro-valves (controlled by a PC) changed the
feeding compartment periodically, at a certain frequency set by the operator (for example, every 2 d). For start-up, the bioreactor was filled with 2 L of anaerobic sludge distributed evenly to all four compartments. After 1 day of batch operation, the reactor was switched to continuous operation. The feeding solution was kept in a refrigerator at 4 °C and was added by peristaltic pump every 3 h. The HRT was set at 10 d and the switching period at 2 d (the feeding compartment was switched every 12 h). The feeding medium consisted of 10 mL L–1 of solutions A, B and C, 0.6 g L–1 yeast extract and 5.5 g L–1 NaHCO3, together with glycerol at various concentrations (10, 15, 20, 25, 30, 37.5 g COD L–1 ). Since PABR operates at higher OLR than a conventional bioreactor, it was decided to increase the yeast supply slightly to avoid any limitation of microorganism growth. The PABR was operated at various OLRs and calculation of the average biogas and methane productivity as well as the other parameter values was done as described above.

RESULTS
Anaerobic digestion of glycerol in CSTR
During the reactor operation, five phases can be distinguished. In the first one (phase a), the OLR was set at 0.25 g COD L–1 d–1 (Fig. 2). At this OLR, the COD removal was 95%. Then, the OLR was doubled to 0.5 g COD L–1 d–1 (phase b). This caused VFA accumulation (around 3 g L–1 ) and, after a short increase, the biogas production decreased significantly. It is well known that anaerobic digestion is inhibited at high VFA concentration. Propionate and acetate concentrations are indicators for process imbalance.32 As a consequence, OLR was decreased by one fourth to 0.375 g COD L–1 d–1 (phase c). The reactor did not seem to recover and the OLR was returned to the initial OLR at 0.25 g COD L–1 d–1 (phase d). The accumulation of VFAs (consisting of mainly acetate and propionate) was up to 2.8 g COD L–1 (Fig. 3(b)) and lasted approximately 90 d until the microorganisms achieved to degrade them. In this phase, pH reduced below 6.5 due to VFAs accumulation (Fig. 3(b)). The pH drop indicates imbalance in the process since the methanogenic microorganisms are strongly inhibited at low pH values.33 When the reactor recovered at 0.25 g COD L–1 d–1, the productivity of biogas and methane was 0.109 ± 0.018 L L–1 d–1 and 0.074 ± 0.009 L L–1 d–1 respectively. The COD removal was 94% on the average. The glycerol concentration in the effluent, at steady state, was less than 0.03 g L–1. The alkalinity and pH varied between usual limits for this type of process (3.98 ± 0.50 g CaCO3 L–1 and 7.22 ± 0.04 respectively), and the solids were stabilized to 2.45 ± 0.26 g TSS L–1 and 1.42 ± 0.17 g VSS L–1. The operation of the CSTR during phase d was stable with an average methane yield of 0.30 L g–1 COD added. In the last phase (phase e), the OLR was set again to 0.375 g COD L–1 d–1 in the hope that the well-acclimated culture during phase d could now withstand a higher OLR. However, this increase in the OLR was accompanied by an increase in the COD and VFA concentrations (>2 g COD L–1) as shown in Fig. 3(a), (b), indicating that the process was kinetically limited at this OLR.

Anaerobic digestion of glycerol in a PABR
The PABR was operated at a constant HRT of 10 d, while increasing the glycerol concentration in the feed. The evolution of biogas and methane production during the operation phases of the PABR is shown in Fig. 4. Each phase corresponds to a different organic loading rate. During the first 100 d, it was necessary to change the HRT often to avoid acid build-up. After that, the OLR was set to 1 g COD L–1 d–1 and the biogas and methane productivity were stabilized at 0.510 ± 0.027 L L–1 d–1 and 0.322 ± 0.023 L L–1 d–1 respectively (phase a). After stabilization, the OLR was increased to 1.5 (phase b), 2 (phase c) and 2.5 g COD L–1 d–1 (phase d). During phases c and d, the PABR was allowed to reach steady state;
Acetate
COD L–1 (individually in g L–1, acetic acid: 2.03, propionic acid: 1.62, butyric acid: 1.39, valeric acid: 0.72, isomers of butyric and valeric acids in traces) which resulted in a rapid drop of the pH to 5.2. Under these conditions, the PABR was considered to be in an acidified state. To overcome the acidification, continuous operation was ceased and the bioreactor was switched to a batch mode (phase f) after removing the acidified supernatant and adjusting the pH to 6.5–7 with the addition of NaHCO₃. Moreover, a small quantity (1.2 L) of fresh methanogenic sludge, containing approximately 15 gVSS L⁻¹, was added to the reactor to enhance COD consumption and equilibrate the loss of methanogenic biomass due to the removal of supernatant. To secure recovery, the OLR was increased progressively (phases f to g) from 0 to 3 g COD L⁻¹ d⁻¹, while the removal of COD and the low VFA levels indicated stability. The PABR presented the highest productivity of biogas and methane (1.673 ± 0.039 and 0.993 ± 0.051 L L⁻¹ d⁻¹, respectively) while operated at an OLR of 3 g COD L⁻¹ d⁻¹ (phase g). In this phase, the COD removal decreased to 95%. The pH and alkalinity varied around typical values for this type of process in all phases, when the PABR was stable (7.12–7.29 and 3–4.9 g CaCO₃ L⁻¹). The concentration of glycerol in the effluent, at steady state, was below 0.03 g L⁻¹.

While the PABR operated at steady state, at OLRs of 2, 2.5 and 3 g COD L⁻¹ d⁻¹, the dynamic behavior of all compartments was monitored for 2 d (equal to the switching period), by taking samples every 4 h and measuring the COD and VFAs (Fig. 5). The concentration of the COD and VFAs changed periodically within 2 d as expected, since the input of each compartment changes with time; the input consisted of fresh glycerol feeding medium for one fourth of the period (0.5 d) or the effluent of the adjacent compartment for the rest three fourths of the period (1.5 d). The amplitude of the wave-like curve increased with the organic loading rate, while the lower part of the curves (corresponding to the effluent phase of the compartment) was maintained at low levels. This indicates the capacity of each compartment to accept a high load in a certain time interval and withstand the accumulation of VFAs, achieving a decrease of the organic loading until the time when the effluent of the PABR comes out of the specific compartment. Comparing the response of COD and VFAs (expressed in g COD L⁻¹ so that this comparison is allowed), one can see that there is a small difference between the two parameters during an OLR of 2 and 2.5 g COD L⁻¹ d⁻¹, while this difference becomes larger during an OLR of 3 g COD L⁻¹ d⁻¹. This difference between the overall COD and total VFAs, expressed in COD units, indicated that part of the organic matter in the effluent should be attributed to unconverted glycerol or compounds other than VFAs. The concentration of these compounds seems to be negligible in the first two phases, while it becomes evident in the last phase, meaning that the operation started to be kinetically limiting above an OLR of 2.5 g COD L⁻¹ d⁻¹. Note that in the case of the CSTR, the organic loading limit was ten times less (0.25 g COD L⁻¹ d⁻¹). As a result, the achieved methane productivity of the PABR was ten times higher than that of the CSTR.

**DISCUSSION**

Anaerobic digestion of pure glycerol has not been studied extensively. Yang et al. studied anaerobic digestion of a glycerol based synthetic medium at mesophilic and thermophilic conditions using a packed bed reactor (which allows higher
conversion rates due to biomass retention and can be compared to PABR). They reported a dissolved organic carbon (DOC) conversion of 80% at an organic loading rate of 0.25 g L⁻¹ d⁻¹ at mesophilic conditions but when the OLR was increased above 0.25 g L⁻¹ d⁻¹ to 0.5, 0.7 and finally to 1 g L⁻¹ d⁻¹, the DOC conversion decreased to 60% and 50% and finally dropped to zero. When the temperature was turned to thermophilic levels, the bioreactor could be operated at higher organic loading rates (0.25, 0.5, 0.7 and 1 g L⁻¹ d⁻¹) with higher DOC conversion (80–90%). Assuming that the equivalence of COD to DOC is 2.667 (based on the stoichiometry of the carbon oxidation reaction), the OLR of 0.25 g DOC L⁻¹ d⁻¹ is equivalent to 0.67 g COD L⁻¹ d⁻¹ which is much lower than the OLR (1–3 g COD L⁻¹ d⁻¹) imposed on PABR. Moreover, the COD conversion in PABR was higher. The performance of the packed bed reactor in thermophilic conditions is similar to PABR at mesophilic conditions.

An interesting approach on the OLR effect on glycerol anaerobic digestion has been followed by Siles et al. They used various types of glycerol (acidified and distilled from biodiesel manufacturing) to feed batch reactors inoculated with granular and non-granular sludge. The highest methane production rates were obtained in the case of granular sludge-acidified glycerol (OLR: 0.21–0.38 g COD g⁻¹ VSS d⁻¹). When acidified glycerol was used on non-granular sludge, the OLR ranged from 0.12 to 0.26 g COD g⁻¹ VSS d⁻¹ and when distilled glycerol was used on granular sludge, the OLR was 0.075 to 0.080 g COD g⁻¹ VSS d⁻¹. The COD removed per COD added was almost 1, 0.74 and 0.85 respectively. In the present study, the loading on CSTR in terms of g COD g⁻¹ VSS d⁻¹ was 0.17 (at an ORL of 0.25 g COD L⁻¹ d⁻¹ and 1.42 g VSS L⁻¹), almost double than the one Siles et al. observed for the distilled glycerol (which is the most comparable case) and the COD removed per COD added was 0.94. This indicates that glycerol derived from biodiesel manufacturing (after acidification to recover the KOH and distillation to remove the impurities) may be more toxic to anaerobic mixed culture consortia than pure commercial glycerol used in the present study. Perhaps, the biomass inoculum used in the present study was better acclimatized than the one used in Siles et al. Operation practices and reactor design favoring acclimation and biomass accumulation result in high methane production rates. Viana et al. reported a high COD removal (97%) during the anaerobic digestion of crude glycerol in a UASB (upflow anaerobic sludge blanket) while increasing the OLR gradually from 2 to 10 g COD L⁻¹ d⁻¹, despite the high salinity of the bulk wastewater. However, this was reported in a review paper and no more details could be extracted regarding the procedure followed so that comparisons with other works can be made. Lower performance of UASB was observed by Nuchdang and Phakkornule, who also used crude glycerol as feedstock. They reported a high consumption of glycerol at OLR between 1.3–2.6 g COD L⁻¹ d⁻¹ with a maximum methane production rate at OLR of 1.6 g COD L⁻¹ d⁻¹. Under the optimal OLR, the COD reduction was 86%. The further increase of the OLR to 5.6 g COD L⁻¹ d⁻¹ resulted in propionate accumulation. Phukingngam et al. operated a five-compartment ABR system in a rectangular configuration, at 25°C. They examined the effect of the OLR of biodiesel waste on methane productivity. Glycerol concentration in this mixture was 2.2–2.4 g L⁻¹ while methanol was present at significant concentrations. Glycerol removal decreased from 98% to 80% approximately, as the OLR increased from 0.5 to 1.5 to 2.1–3 g COD L⁻¹ d⁻¹.

A number of papers are focused on the crude glycerol co-treatment with other wastes. According to Kolesarova et al., crude glycerol digestion in a CSTR, with recirculation, provided stable biogas production of 760 L per L of crude glycerol. They also used crude glycerol with rapeseed meal and found that feeding a mixture consisting of 500 mL of crude glycerol and 500 g of rapeseed meal (loading rate: 1.33 g L⁻¹ d⁻¹), the average biogas production reached 0.58 L L⁻¹ d⁻¹. The use of different units in expressing the loading rate (mass loading instead of COD loading) does not permit comparisons with the results of the present study. Moreover, crude glycerol has been mixed with cattle slurry in order to enhance biogas production. It was found that the optimal concentration of crude glycerol in the feed was between 5 and 10%. In this range crude glycerol addition contributed to the increase of biogas production. Nevertheless, when crude glycerol supplementation increased to 15%, the process failed.

The limitations of anaerobic digestion of pure glycerol seem to stem from the accumulation of metabolites, since other
constituents of crude glycerol that may be toxic (such as long chain fatty acids, salts of chloride and sulphate) were not present in this work. Proper adaptation of the microbial mass and use of efficient reactor designs such as UASB, ABR and PABR seem to be decisive parameters for converting glycerol to methane.

The utilization of glycerol in co-digestion with complex waste such as sludge, manure and lignocellulosic residues, slightly improved the process performance as concluded by Vaina et al. According to Vaina et al., the presence of inhibitory metabolites is concomitant with low efficiency of the process. In the present work, metabolic compounds other than VFA seem to be produced at an organic loading rate of 3 g COD L⁻¹ d⁻¹ when the efficiency of the PABR declined. Propionate was dominant among the VFA was 0.33 L CH₄ g⁻¹ COD added in the PABR. Moreover, the PABR, at an organic loading rate of 3 g COD L⁻¹ d⁻¹ when the efficiency of the process failure. Metabolic compounds other than VFA seem to be produced at an organic loading rate of 3 g COD L⁻¹ d⁻¹ when the efficiency of the PABR declined. Propionate was dominant among the VFA was 0.33 L CH₄ g⁻¹ COD added in the PABR. Moreover, the PABR, at an organic loading rate of 3 g COD L⁻¹ d⁻¹ when the efficiency of the process. As in the case of other feedstocks, PABR was proved to be more stable in comparison with the CSTR at higher OLR when converting pure glycerol. In comparison with other high-rate systems, PABR seems to be more efficient with the exception of the study of Vaina et al. It should be stressed, however, that these comparisons should be made skeptically since most of the studies cited herein concern the anaerobic digestion of crude glycerol, while the present work permits conclusions to be drawn directly about the anaerobic biodegradability of glycerol itself without the possible interferences of the other constituents of crude glycerol.

CONCLUSIONS

Glycerol is a highly biodegradable compound under anaerobic conditions. The efficiency of a conventional anaerobic digester, such as a CSTR was compared with that of a high-rate reactor such as the PABR. In fact, the maximum OLR applied to the PABR was 10 times that in the CSTR, at an OLR of 3 g COD L⁻¹ d⁻¹ the methane productivity was 0.993 ± 0.102 L L⁻¹ d⁻¹ and the yield was 0.33 L CH₄ g⁻¹ COD added in the PABR. Moreover, the PABR, compared with other high-rate systems for glycerol digestion presented in the literature, seems to be more efficient.

REFERENCES


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