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Several researchers have used crude glycerol as a source of substrate for methane production and power generation, which is a way of adding value to this residue that has a high chemical oxygen demand (COD) and is rich in impurities. This review article summarizes recent data and discussions on the use of crude glycerol as substrate and co-substrate for anaerobic digestion. In general, the dilution of glycerol has been used to avoid problems of inhibition due to the presence of inorganic salts of chloride and sulphates, and due to accumulation of metabolites. However, other methods have been proposed, such as the use of halo-tolerant biomass. It can be concluded that the anaerobic digestion of crude glycerol is technically viable, and an anaerobic reactor treating 25 m^3 per day of crude glycerol can produce 4.4 MW of thermal energy, which can be converted to 4.4 GW of heat or 1.2 GW of electricity.

Keywords: biogas; biodiesel by-product; glycerine; methane; renewable energy

Introduction

Future strategies for securing energy resources must include technologies for production of biofuels such as bioethanol, biodiesel and biogas [1]. Several countries use biodiesel in the energy matrix in order to reduce the dependency on fossil fuels as well as to promote sustainable development. In Brazil, a resolution from the beginning of July 2008 established the mandatory minimum percentage of biodiesel to be added to diesel as 4%, increasing this percentage to 5% from early 2010 [2]. In Argentina, the diesel is supplemented with 7% of biodiesel [3], while in the 27 European Union member countries this value is 5.75% [4], and in the United States of America the preferred diesel blend consists of 20% of biodiesel (B20) [5].

Considering that, for each kilogram of biodiesel produced, approximately 100 g of glycerol is generated as a by-product of the transesterification reaction [6], the worldwide production of glycerol was approximately 3,000,000 tons in 2011, and can increase to 4,600,000 tons in 2020 [7] with future scaling up of biodiesel production. Therefore, the supply of this substance is already exceeding demand [8]. Moreover, this residue contains around 20% of impurities, a rather high amount, affecting the industrial process and increasing the costs of purification. In order to maintain economic and environmental sustainability of biodiesel production, it is therefore essential to find alternative uses for this crude glycerol.

In recent years, the use of crude glycerol as an organic substrate for biological synthesis of other materials has increased, the main products being 1,3-propanediol [9], formate and ethanol [10], propionic acid [11], butyric acid and acetic acid [12], butanol [13], dihydroxyacetone [14], succinic acid [15], hydrogen [16], and methane [8].

This paper presents a literature review on the use of crude glycerol as a substrate for methane production aimed at generating energy, including the main bottlenecks during the anaerobic digestion of this waste. In the articles cited, it was observed that anaerobic digestion of glycerol is technically feasible, provided some operational strategy is implemented to increase the tolerance of the anaerobic consortia towards the high concentration of organic matter and toxic compounds present in the crude glycerol. First, the chemistry of crude glycerol and the biochemistry of the anaerobic digestion of this waste are explained. In the second step, the toxic effects of glycerol and the impurities present in crude glycerol waste on the anaerobic sludge is discussed. Next, the article shows some results that have been found for the methane production potential (MPP) using crude glycerol as a substrate and co-substrate for anaerobic reactors. Finally, there is a discussion on the current situation and future prospects of the use of such waste in anaerobic biotechnology, and an example of its application for energy production on an industrial scale.

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Glycerol

Glycerol (or glycerine or 1,2,3-propanetriol) is a colourless, odourless, viscous and non toxic alcohol, which liquefies at 17.8°C [17]. The chemical formula of glycerol is $C_3H_5(OH)_3$. Glycerol can be obtained from biological fermentation [18], by chemical synthesis from petrochemicals, by hydrogenation of sucrose in the presence of a catalyst under high pressure and temperature [19], during the production of bioethanol [20], or as a by-product of the production of soap [21] and of the reaction of transesterification of vegetable and animal oils for biodiesel production [22].

Pure glycerol (over 95% purity) can be used by the chemical industry [21]. However, glycerol derived from biodiesel production has impurities that affect and increase the operational costs of these industrial processes. The main impurities in crude glycerol are methanol, salts of potassium and sodium, heavy metals and soap [23]; and water, fatty acids and other organic impurities [8]. The concentration of these impurities in the crude glycerol, as well as some physicochemical parameters such as pH, density, colour, and concentration of organic matter, varies depending on the nature of the animal or vegetable oil used and on the industrial process used for the biodiesel production [22,24].

Among the impurities in the crude glycerol derived from biodiesel, there are three that can significantly affect microbial metabolism, as they are considered toxic or recalcitrant compounds: long-chain fatty acids (LCFAs) [25], chloride [26], and sulphates [27], which will addressed in subsections 'Long-chain fatty acids' and 'Inorganic salts' of this review. The LCFAs come from the triglycerides in the transesterification reaction and are dissolved in glycerol [24]. Chloride and sulphates are formed during the acidification phase of the glycerol–biodiesel mixture, when hydrochloric acid or sulphuric acid is used, respectively, in order to prevent soap formation [28].

Metabolism of glycerol by anaerobic microorganisms

A large number of microorganisms can grow on a medium containing glycerol and use it as a source of carbon and energy. The glycerol uptake by microorganisms can occur by passive transport [29] or active transport [30], both under aerobic conditions [23] and under anaerobic conditions [1,6].

The metabolic pathways of anaerobic fermentation of glycerol are well established and, according to Biebl *et al.* [31], occur by means of a reductive or an oxidative pathway. Via a reductive pathway, glycerol undergoes dehydration, mediated by the co-enzyme glycerol dehydratase, producing 3-hydroxypropionaldehyde, which in turn is reduced to 1,3-propanediol (1,3-PDO), mediated by the enzyme 1,3-propanediol dehydrogenase (Figure 1).

The oxidative route consists of dehydrogenating glycerol by the enzyme glycerol dehydrogenase, forming dihydroxyacetone, which after phosphorylation is mediated by the enzyme dihydroxyacetone kinase, and can be converted to succinate, which is subsequently converted to propionate or to pyruvate. The reactions that lead to the formation of compounds from pyruvate vary with the environmental conditions and with the enzymes that mediate the reaction, i.e. from organism to organism, and can lead to simpler compounds such as 2,3-butanediol, lactate, butyrate, ethanol, formate, acetate, hydrogen and carbon dioxide [8,31].

When the anaerobic process is aimed at power generation, the presence of microorganisms capable of forming formate, acetate, hydrogen and carbon dioxide (as bicarbonate) is necessary, since these are the only compounds that can be converted directly to methane (CH_4). Nevertheless, not all reactions involved in the acetogenesis occur spontaneously under standard environmental conditions (neutral pH, 25°C and 1 atm), such as the reactions producing propionate, butyrate and ethanol, which need a mechanism for the removal of H_2 from the medium to make those reactions thermodynamically feasible. The main mechanism for removing H_2 from the medium is its consumption by the *hydrogenotrophic methanogenic archaea*. Only when the consumption is fast enough to maintain the H_2 pressure below or around 10^{-4} to 10^{-6} atm, does the biodegradation of propionate, butyrate or ethanol become exergonic, releasing energy for the acetogenic bacteria, making these reactions thermodynamically favourable [32]. Subsequently, the metabolites of the acetogenesis are assimilated by *acetoclastic methanogenic archaea* and converted to CH_4 and CO_2.

Toxic and inhibitory effects of the anaerobic digestion of crude glycerol

Metabolic pathways of the anaerobic digestion of glycerol may be inhibited if some external factor interferes with the biodegradation process. In addition to environmental and operational factors (such as pH, temperature and alkalinity), the accumulation of intermediates [33] and the presence of toxic substances may inhibit the anaerobic digestion of crude glycerol [34–36].

Accumulation of intermediaries

The limiting step of digestion of waste is defined by Lawrence [37] as 'that step which will cause process failure to occur under imposed conditions of kinetic stress'. Considering that glycerol is a compound that is readily available for use by acidogenic bacteria, the limiting step will then be either the acetogenic or the methanogenic step. During the anaerobic digestion of glycerol, some organic acids (acetic, propionic, butyric, valeric and others) formed by the fermentative acidogenic bacteria, cannot be consumed by the acetogenic or methanogenic *archaea* at the same rate at which they are produced. This is because the cellular yield coefficient ($Y_{x/s}$) of the acidogenic bacteria (0.15–0.17 g of

Figure 1. Metabolic pathways of the fermentation of glycerol, forming more simple compounds (adapted from Biebl *et al.* [31]).

volatile solids [VS] per gram of chemical oxygen demand [COD]) is much higher than that of the acetogenic bacteria (0.025–0.051 g VS/g COD) or methanogenic *archaea* (0.020–0.054 g VS/g COD) [38]. The production of these acids must be counterbalanced by the alkalinity present in the bulk liquid, otherwise inhibition of methanogenic activity may occur by accumulation of these metabolites, leading to a collapse of the system, regardless of the pH value [39].

In an anaerobic system, this accumulation of organic acids was the result of an organic overload, as shown in experiments by Fountoulakis *et al.* [40], where crude glycerol was used as co-substrate in the digestion of sewage sludge. To avoid such problems, tests of specific methanogenic activity (SMA) may be performed to determine the maximum load tolerated by a given amount of sludge [41].

The COD of crude glycerol is in the range between 925 and 1600 g/L [8,34,42], which may hinder its use as a substrate. To avoid organic overloading, Viana [43] and Siles López *et al.* [8] diluted glycerol in water during their tests, while Siles *et al.* [44] proposed dilution with pre-treated wastewater originating from washing operations at the biodiesel plant.

The accumulation of metabolites during anaerobic digestion of crude glycerol can also be used for production of volatile fatty acids (VFA), which are then digested in a second reactor. This two-stage system, an acidogenic reactor (operated at high organic load and low hydraulic retention time [HRT]) followed by a methanogenic reactor not only facilitates the control of the operational parameters of both reactors separately but also can increase the efficiency of conversion of methane and generate hydrogen [45]. Luo *et al.* [45] used two continuous stirred tank reactors (CSTR) in series; the first operated at an organic loading rate (OLR) of up to 97 kg COD/(m^3·d) and an HRT of 1 day, while the second operated at an OLR of 7 kg COD/(m^3·d) and an HRT of 14 days. They found that the two-stage system was able to recover 11% more energy, as methane or hydrogen, than a single-stage system.

Long-chain fatty acids

The LCFAs are a part of the constitution of the triglycerides used in the transesterification reaction, and part of them remains dissolved in the crude glycerol [34]. These LCFAs, although biodegradable, are detrimental to anaerobic digestion [46]. Studies have shown the inhibitory potential of

these fatty acids for acetogenic bacteria [47], acetoclastic methanogenic *archaea* [46,48] and hydrogenotrophic methanogenic *archaea* [46,49]. The inhibition of anaerobic organisms is caused mainly because LCFAs adhere to the cell wall of the microorganisms, causing two deleterious effects: (i) the LCFA layer formed on the cell wall prevents the passage of nutrients through the membrane, causing the death of microorganisms; and (ii) the LCFAs have relatively low density and, when attached to the bacterial cell wall, can cause flotation of biomass and washout from the reactor [50]. The presence of these compounds in the composition of crude glycerol is shown by Thompson and He [24], Hazimah *et al.* [35] and Yong *et al.* [51]. On the other hand, Hanaki *et al.* [49] studied the toxicity potential of several LCFAs to the anaerobic sludge, and concluded that the acidogenic bacteria were tolerant to LCFAs. This is an indication that a two-stage system can cope with such compounds.

The main anaerobic route of LCFA biodegradation is via a β-oxidation pathway, performed by acetogenic bacteria, in which each degraded molecule of the LCFA forms a carboxylic acid with two fewer carbon atoms, until complete conversion into acetic acid and hydrogen [52]. Assuming that the microorganisms involved in the anaerobic digestion of LCFAs are not inhibited, the biodegradation process is complemented by acetoclastic and hydrogenotrophic methanogenic *archaea*, responsible for consuming the molecules of acetic acid and hydrogen, respectively, to form methane and carbon dioxide [46,47].

LCFAs are found in high concentrations in the floating sludge that is formed on the top of the settler used for separation of the glycerol–biodiesel blend. This system does not completely eliminate the LCFAs from the glycerol, although its concentration considerably decreases. Table 1 shows the concentration of several LCFAs found in crude glycerol derived from the transesterification of palm oil, considering its average density of glycerol to be equal to 1.25 kg/L [53].

Several researchers have investigated the toxicity of LCFAs, which is assessed by the index IC_{50}, or 'Inhibitory Concentration 50', which is the concentration that causes a 50% inhibition of the bacterial metabolism [25,54,55]. Table 2 shows the values of IC_{50} for several LCFAs.

High concentrations of LCFAs can cause complete deterioration of the anaerobic system [57]. To solve this problem, Rinzema *et al.* [58] operated a system fed with synthetic wastewater composed of sodium caproate (C10:0) and sodium laurate (C12:0), at increasing loading rates, whilst gradually adapting the microorganisms to the biodegradation of LCFAs. The use of inoculum already adapted to wastewater rich in lipids and/or LCFAs may contribute to a decrease in the adaptation period [57].

Considering that one of the main problems of high concentrations of LCFAs is the flotation and washout of biomass, increasing the settleability of the sludge would enhance its resistance to flotation and improve system performance for anaerobic digestion of this material [59]. Nevertheless, the results presented by Pereira *et al.* [55] showed that flocculent sludge had better performance when degrading this type of compounds than did granular sludge. This contradicts the publication of Show *et al.* [60], which demonstrated that methanogenic granular sludge had an activity superior to that of flocculent sludge.

Hutňan *et al.* [42] observed sludge flotation in an upflow anaerobic sludge blanket (UASB) reactor degrading crude glycerol using flocculent sludge as an inoculum. The problem could be resolved by re-inoculating the reactor with granular sludge. With this measure, it was possible to operate the reactor with an OLR of up to 6.5 kg COD/($m^3 \cdot d$), which is almost double the maximum OLR when flocculent sludge was used. On the other hand, no significant increase in specific methane production was observed by Siles López *et al.* [8] when comparing flocculent sludge and granular sludge for the anaerobic digestion of the same kind of pretreated crude glycerol (pre-acidified to remove K^+ from the catalyst). Viana [43] also observed flotation of sludge from a UASB reactor during anaerobic digestion of crude glycerol.

Table 2. Concentration that causes a 50% inhibition of the bacterial metabolism for different LCFAs.

LCFA	IC_{50} (g/L)	LCFA	IC_{50} (g/L)
$C_{8:0}$	1.44[48]	$C_{16:0}$	4.15[25]
$C_{10:0}$	1.02[48]–1.41[47]	$C_{18:0}$	0.50[56]–4.14[25]
$C_{12:0}$	0.86[48]	$C_{18:1}$	0.08[57]–2.78[25]
$C_{14:0}$	1.20[48]	$C_{18:2}$	0.35[54]–0.59[25]

Note: $C_{x:y}$: 'x' is the number of carbon atoms in the LCFA, 'y' is the number of double bonds. Superscript numbers are literature references.

Table 1. Concentration of LCFAs found in residual glycerol derived from the transesterification of palm oil.

Concentration of LCFA (g/L)								
$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	Ref.
0.6–33.1	0.6–1.5	0.6–2.5	1.5–1.9	1.5–2.5	0.6–15.0	25.0–30.0	n.i.	[35]
37.9	11.8	51.0	7.9	3.0	0.9	6.1	0.8	[51]

Note: $C_{x:y}$: 'x' is the number of carbon in LCFA, 'y' is the number of double bonds; n.i.: no information provided by the author.

However, this researcher did not attribute this phenomenon to the presence of LCFA in the influent; rather, the low concentration of nutrients in the system was the most likely cause of flotation of sludge since the shortage of essential chemicals can cause cell death and subsequent flotation of the biomass [61].

The addition of magnesium salts [62], bentonite and calcium salts [63] (except for calcium carbonate because of its low solubility in water [49]) to the crude glycerol can reduce the toxic effect of LCFAs. These minerals induce the formation of insoluble salts of LCFAs [49,62], eliminating the free forms of the acids and making them unavailable to microorganisms. Another alternative is the removal of LCFA by means of centrifugation after acidification [64], which can also enhance the anaerobic biodegradability of crude glycerol. However, this alternative must include a further neutralization step, as the low pH would deteriorate the process, which increases the salts' concentration in the system.

Inorganic salts

Chloride (Cl^-) or sulphate (SO_4^{2-}) in the crude glycerol comes from the hydrochloric acid or sulphuric acid that is used in the neutralization step of the transesterification process. The chemical reaction responsible for the formation of chloride is basically the ion Cl^- (from the hydrochloric acid) binding to the catalyst cation (usually Na^+ or K^+), giving the highly saline characteristics of crude glycerol [65].

Considering that the concentration of chloride necessary to cause strong inhibition of methanogenesis is typically in the range of 4 to $9\,g\,Cl^-/L$ [26,66,67] and that the glycerol derived from biodiesel can contain between 34 and $46\,g\,Cl^-/L$ [36,68], most probably the crude glycerol will be toxic to the anaerobic consortia if used as an undiluted substrate. The toxic effect of chloride on microorganisms occurs through cell plasmolysis, i.e. shrinkage of cell volume by water loss or loss of cellular activity due to high osmotic pressure [67].

To mitigate the problem of inhibition due to high chloride concentration in the effluent of anaerobic reactors, the biomass may be acclimated to this extreme environment, as shown by Ma *et al.* [34]. These authors operated a UASB reactor, replacing gradually pure glycerol by glycerol with high salinity ($29.0\,mS/cm$) as co-substrate during the biological treatment of effluents from the potato industry. With this operational strategy, they achieved a COD removal efficiency of 75%. Viana [43] operated a laboratory-scale UASB reactor ($14.85\,L$) fed with crude glycerol and nutrients, for 400 days, decreasing gradually the dilution of crude glycerol in the influent from 1:1500 to 1:5 (w/w). This author found that the reactor converted 97.5% of the organic matter in the effluent without problems of instability, even with chloride concentrations of up to $14\,g\,Cl^-/L$ in the bulk of the liquid. The importance of acclimation of the biomass

to a saline environment has been well established by Vallero *et al.* [66].

The dilution of crude glycerol was also used by Suehara *et al.* [69] to avoid microbial inhibition by high salinity. Dilution can be expensive at an industrial scale, and an alternative is the use of other wastewater streams, such as those from washing processes or sewage, which can also be a source of macro- and micronutrients [70]. Tests conducted by Siles *et al.* [44] showed that dilution of glycerol with effluent generated during the purification step of biodiesel can be environmentally and economically viable, provided that the concentration of toxic and recalcitrant substances present in this wastewater does not exceed the limits tolerated by the anaerobic microorganisms [65].

The addition of external compatible solutes (non-toxic substances that protect cellular components against high osmotic pressure) to the high saline influent of an anaerobic reactor can increase the tolerance of the high osmotic pressure of this environment. The main compatible solutes are β-glutamine, α-glutamate, N-acetyl-β-lysine and glycine-betaine [71]. Vyrides *et al.* [71] found a more than four times increase (compared with the control) in the CH_4 production by adding $0.1\,mM$ of betaine in a laboratory-scale submerged anaerobic membrane bioreactor (SAMBR) treating synthetic wastewater composed of glucose and NaCl ($34\,g/L$).

In addition to the strategies of reduction of osmotic stress described above, there is the physical–chemical pretreatment for precipitation of the salts present in the crude glycerol, as demonstrated by Siles *et al.* [44] and Siles López *et al.* [8]. According to the latter authors, the cation of the catalyst (KOH) is recovered by acidifying glycerol with phosphoric acid to form insoluble potassium phosphate, which is separated by centrifugation. The latter authors also suggested that, apart from reducing the salt concentration in the glycerol prior to the anaerobic digestion, the recovered potassium phosphate can be used in agriculture as a fertilizer.

Besides the toxic effect of the chloride anion, it should be noted that the cations (Na^+ or K^+) may also be strongly inhibitory to microorganisms at high concentrations (from $8\,g\,Na^+/L$ and $12\,g\,K^+/L$) [72], which can also cause bacterial cell plasmolysis [73,74].

Similar to chloride formed during the neutralization step with hydrochloric acid, when sulphuric acid is used as the catalyst, sulphates are generated and found in large quantities in the crude glycerol. Sulphates can inhibit the activity of anaerobic bacteria by competition for electron donors between methanogenic *archaea* and sulphate-reducing bacteria (SRB). However, the crude glycerol contains up to $255\,mg\,SO_4^{2-}/L$ [34], which probably will not cause problems when using it as substrate in an anaerobic system for methane production. According to Rinzema and Lettinga [75], in an environment where the ratio of COD/SO_4^{2-} is below 10, there is a predominance of SRB compared

with methanogenic *archaea*. Assuming an average COD concentration for the crude glycerol equal to 1260 g/L, this ratio would reach 4941, disfavouring the competition for SRB.

The presence of sulphur compounds causes economic losses when the goal of an anaerobic digestion is to produce energy via methane. This is because the Gibbs free energy ($\Delta G°$) for the reaction of anaerobic sulphate reduction is -48 kJ/mol, whereas the $\Delta G°$ for the reaction of acetoclastic methanogenesis is -31 kJ/mol. This implies that methanogenesis takes place only after virtually all the sulphate has been reduced by SRB, consuming part of the available acetic acid [27]. Thus, each gram of SO_4^{2-} is responsible for consuming 0.625 g of acetic acid, which would be sufficient to produce about 0.233 L of CH_4 under standard temperature and pressure conditions.

Under anaerobic conditions, this SO_4^{2-} is reduced by SRB, forming hydrogen sulphide (H_2S), a corrosive and toxic gas [76] gas that can cause problems of malodours and deterioration of the methanogenic step during anaerobic digestion of waste [27]. Also, H_2S can cause precipitation of essential nutrients for bacterial growth in the form of insoluble metal sulphide, e.g. FeS_2, MnS, CuS and ZnS [27]. According to the literature review done by Chen *et al.* [77], the amount of sulphide should not exceed 125 mg H_2S/L at a pH between 7 and 8, in order to avoid inhibition of the anaerobic microorganisms, and should not exceed 250 mg H_2S/L at a pH between 6.4 and 7.2. Moreover, H_2S in the biogas composition reduces the concentration of CH_4, reducing the lower heating value of the biogas and increasing the costs for biogas purification [78]. Moreover, H_2S reacts with water vapour and forms sulphuric acid (H_2SO_4), which causes corrosion in equipment [79]. According to Ryckebosch *et al.* [76], there are several techniques that can be used to remove H_2S from biogas before using as fuel: (i) adsorption using iron oxide or hydroxide; (ii) absorption with water or an organic solvent; (iii) semi-permeable membrane separation; (iv) biological filtration; (v) adsorption on activated carbon.
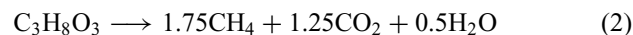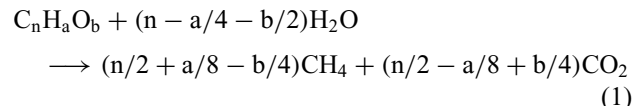
Anaerobic biodegradability and MPP

Anaerobic biodegradability represents the fraction of organic matter that can be converted into biogas (methane and carbon dioxide) under controlled conditions. Tests to determine the anaerobic biodegradability of crude glycerol were carried out by Siles López *et al.* [8] in batch reactors of 1 L under mesophilic conditions. The authors found that glycerol was 100% biodegraded using a sludge loading rate (SLR) of between 0.21 and 0.38 kg COD/(kg VS · d). Similar results were obtained by Siles *et al.* [44] studying the anaerobic digestion of crude glycerol at a maximum SLR of 0.36 kg COD/(kg VS · d). The high biodegradability of glycerol found in these studies may have been obtained because, prior to the tests, glycerol was pretreated in order

to remove the organic and inorganic impurities that would cause inhibition of bacterial activity.

In fact, it is expected that the anaerobic biodegradability of crude glycerol does not reach 100% since some substances that are present in it can inhibit the methanogenic activity and/or are recalcitrant. This was shown by Viana *et al.* [68], who assessed the anaerobic biodegradability of several types of crude glycerol without any pretreatment and found biodegradability efficiencies of between 65.9% and 85.6%. Their results showed that the nature of the vegetable oil from which the crude glycerol originated, as well as the transesterification process used, affected the anaerobic biodegradability.

The theoretical mass of methane produced per mole of glycerol can be calculated by the equation developed by Buswell and Muellepi (1952) [80] (Equation (1)), from which Equation (2) was deduced:

$$C_nH_aO_b + (n - a/4 - b/2)H_2O$$
$$\longrightarrow (n/2 + a/8 - b/4)CH_4 + (n/2 - a/8 + b/4)CO_2 \tag{1}$$

$$C_3H_8O_3 \longrightarrow 1.75CH_4 + 1.25CO_2 + 0.5H_2O \tag{2}$$

Considering the stoichiometry of the reaction expressed by Equation (2), the theoretical MPP is 0.426 m^3 CH_4/kg glycerol at STP. However, in practice, cell growth and environmental and operational conditions, as well as the presence of toxic compounds, interfere with this result. From the results of anaerobic biodegradability, it is possible to calculate the MPP (MPP), i.e. the maximum volume of methane that can be produced by a certain amount of substrate. In laboratory-scale assays, the MPP is the ratio between the cumulative production of methane after 30 days and the mass of substrate used in the test [81].

Table 3 shows values of the MPP obtained from several experiments using crude glycerol as the sole source of organic matter for anaerobic digestion. Based on this table, it is possible to note that when granular sludge is used as inoculum, the values of MPP are higher than when flocculent sludge is used. According to Hwu *et al.* [59], this can be due to the increase in the performance of granular sludge for degrading LCFAs present in the crude glycerol. However, the most likely cause of the MPP increase is the pretreatment of the glycerol, which eliminates toxic and recalcitrant compounds. Experiments carried out by Siles López *et al.* [8] showed that improving the pretreatment for removing impurities caused an increase in the MPP.

Stoichiometrically, anaerobic digestion of methanol will produce less methane than digestion of glycerol; therefore, the removal of methanol can also raise the MPP of the crude glycerol.

Viana *et al.* [68] suggest that the physical and chemical characteristics of the crude glycerol, as well as the nature of the vegetable oil that the biodiesel originated from, interfere directly in the biodegradability of the respective crude

Table 3. MPP of different types of residual glycerol samples.

MPP (m^3 CH_4/kg glycerol)	Experimental conditions	Glycerol characteristics	Reference
0.291[a]	Flocculent sludge	Pretreated w/H_3PO_4	[8]
0.295[a]	Granular sludge	Pretreated w/H_3PO_4	
0.411[a]	Granular sludge	Pretreated w/H_3PO_4 + distillation	
0.221	Granular + flocculent sludge	GSC with no pretreatment	[68]
0.265	Granular + flocculent sludge	GSY with no pretreatment	
0.322	Granular + flocculent sludge	GCT with no pretreatment	
0.243	Granular + flocculent sludge	GCN with no pretreatment	

Note: GSC: glycerol from soybean oil and cottonseed (3:2); GSY: glycerol from soybean oil; GCT: glycerol from castor oil; GCN: glycerol from canola oil.
[a]MPP at STP.

glycerol and consequently in the MPP (Table 3). In addition, other substances present in the crude glycerol may increase the MPP due to their molecular structure, such as palmitic acid (C16:1) that is present in the crude glycerol at concentrations between 1.5 and 3.0 g/L [35,51]. The carbon chain of this LCFA suffices to produce seven times more CH_4 than does the pure glycerol (see Equation (3), obtained from Equation (1).

$$C_{16}H_{32}O_2 + 7.0H_2O \longrightarrow 11.5CH_4 + 4.5CO_2 \quad (3)$$
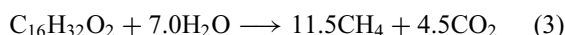
Anaerobic reactors fed with crude glycerol

Viana [43] used a laboratory-scale UASB reactor (14.85 L) for biogas production from glycerol generated by the biodiesel industry. The glycerol was obtained from the transesterification of a mixture of soybean and cottonseed oils (2:3, v/v). The system was operated at a temperature of approximately 30°C. The UASB reactor was started up by gradually increasing the OLR from 2 to 10 kg COD/(m^3·d) and by decreasing the dilution of the crude glycerol in the influent from 1:1500 to 1:5, w/w. With this operational strategy, the reactor achieved a COD removal efficiency of 97.5% and a methane production of 0.380 m^3 CH_4/kg glycerol, despite the high salinity of the bulk liquid (approximately 14 g Cl^-/L).

Kolesárová et al. [82] found specific methane production reaching up to 0415 m^3 CH_4/kg crude glycerol, which is close to the theoretical MPP (0.426 m^3 CH_4/kg glycerol). The researchers operated a continuous stirred tank reactor (CSTR) of 1.2 m^3, for 513 days, at a maximum OLR of 2.2 kg COD/(m^3·d), and removed about 99% of dissolved organic matter. The results also showed that the anaerobic sludge was able to adapt to a high-salinity environment (up to 30 g/L of dissolved inorganic salts).

Hutňan et al. [42] evaluated two types of reactors (UASB and an anaerobic sequential batch reactor, ASBR) for anaerobic digestion of crude glycerol with high salinity (21.3 g/L of dissolved inorganic salts). The laboratory-scale UASB reactor (3.7 L) was first inoculated with flocculent sludge, and was able to convert 65% of the organic matter to methane without accumulation of VFA.

However, when the researchers increased the OLR from 3.45 to 4.32 kg COD/(m^3·d), the reactor showed signs of instability, and the efficiency of the system decreased to 32%. During operation with the highest OLR, the authors noted the occurrence of severe sludge flotation, probably caused by the presence of LCFAs. To solve this problem, a mixture of granular sludges from different anaerobic reactors was used for re-inoculation of the UASB reactor. With this new strategy of operation, the system was able to convert up to 61% of organic matter to methane, with an OLR of 6.5 kg COD/(m^3·d). Considering that the concentration of CH_4 in the biogas was 61.1%, the average specific methane production (SMP) was 0.513 m^3 CH_4/kg glycerol during the approximately 75 days of experiment, which is slightly higher than when the system was operated with flocculent sludge (0.420 m^3 CH_4/kg glycerol). The laboratory-scale ASBR (4 L) was operated with an OLR of up to 5.6 kg COD/(m^3·d). The methanation reached 90%, with an SMP of 0.526 m^3 CH_4/kg of glycerol. When the researchers increased the OLR to 8.0 kg COD/(m^3·d), the methanation decreased to 31% and VFA accumulated. A critical evaluation of the results obtained by Hutňan et al. [42] shows that the causes of the system failure were probably (i) the short adaptation of the sludge before increasing the OLR and/or (ii) the high concentrations of salts and LCFAs present in the crude glycerol.

Yang et al. [83] evaluated the methane production by anaerobic fixed-bed reactors operated under thermophilic conditions and fed with synthetic wastewater containing glycerol as the sole source of substrate in a semi-continuous mode. With this operational strategy, the system converted about 87% of dissolved organic matter into methane and was able to produce 0.450 m^3 CH_4/kg glycerol at an OLR of 0.7 kg COD/(m^3·d). Despite this high efficiency of methanation, the researchers did not assess the behaviour of the reactor at OLRs above 1 kg COD/(m^3·d). This increase probably would not affect system performance, as several other researchers operated reactors fed with glycerol at higher loading rates without major problems [8,43].

Siles López et al. [8] evaluated the performance and stability of the anaerobic digestion process used to produce

biogas from glycerol that was chemically pretreated to remove impurities. Six laboratory-scale reactors (working volume of 1 L) were fed in batch mode, each with a different type of inoculum (non-granular and granular sludge). The best results were obtained when the researchers used granular sludge and fed the reactors with distilled glycerol. With these operational conditions, the SMP achieved 0.411 m^3 CH_4/kg glycerol.

The use of glycerol as a co-substrate

Crude glycerol was extensively tested as a co-substrate for anaerobic digestion of different organic wastes. Fountoulakis and Manios [53] tested crude glycerol as a co-substrate in anaerobic digestion of a mixture of two wastewaters: one from an olive processing plant and another from a slaughterhouse (at a ratio of 1:4). The authors found that by adding 1% (v/v) of crude glycerol the methane production increased from 0.479 to 1.210 L CH_4/d. However, using the results presented by these researchers, it is possible to conclude that this increase is only due to the anaerobic digestion of glycerol added and not a result of improved digestion of the wastewater. This analysis was performed assuming a COD of 1250 g/L for glycerol [8,34,42] and that glycerol is almost 100% anaerobically biodegradable. With these conditions, the amount of glycerol used by the authors can produce about 0.751 L CH_4/d, which is close to the difference obtained between operations with and without supplemental crude glycerol.

More recently, Fountoulakis *et al.* [40] investigated the effect of the addition of crude glycerol during the co-digestion of excess sludge from an activated sludge plant used for sewage treatment. The results of batch tests showed that the supplementation with 3% (v/v) of glycerol to the reactor (1 L of working volume), equivalent to an OLR of approximately 38 kg COD/(m^3·d), led to inhibition of bacterial activity, probably caused by organic overloading. After 20 days of operation under these conditions, VFA accumulated, and the pH decreased from 6.5 to 5.3, decreasing the efficiency in removing organic matter to a mere 58%. Moreover, the concentration of toxic compounds present in crude glycerol may have influenced the deterioration of the system. However, when the authors added only 1% (v/v) of glycerol to the reactors (OLR of 12.5 kg COD/(m^3·d)), the system remained stable, and the COD removal efficiency was approximately 90%.

Fountoulakis *et al.* [40] used the data of the batch reactors to operate an anaerobic CSTR of 3 L for the digestion of excess sludge of an activated sludge plant, supplemented with 1% (v/v) of crude glycerol. At the beginning of the experiment, the daily volume of methane produced (1.857 L CH_4/d) was equal to the sum of the methane production from the digestion of the excess sludge (1.106 L CH_4/d) and of the digestion of the glycerol (0.751 L CH_4/d). After 80 days, however, the daily production of methane was equal to 2.353 L CH_4/d. Thus, an increase in the

methane production of 42% occurred due to the action of crude glycerol as co-substrate. This may be due to an increase in active biomass caused by the digestion of an easy-to-degrade substrate.

Kolesárová *et al.* [82] operated a pilot-scale CSTR (1.2 m^3), fed with crude glycerol as sole substrate, for 513 days. Then the researchers substituted the influent with a mixture comprising crude glycerol and rapeseed meal (1:1 v/w). Using the data obtained by the authors, it was possible to calculate the specific methane production of the rapeseed meal, 0.385 m^3 CH_4/kg. This value is lower than that found by Antonopoulou *et al.* [84] (0.450 m^3 CH_4/kg). Therefore, the crude glycerol seems to be not effective as a co-substrate for anaerobic digestion of rapeseed.

The use of glycerol as co-substrate was successful in a study conducted by Ma *et al.* [34], comparing the MPP of two UASB reactors (each with a working volume of 2.3 L). One reactor was fed with potato processing wastewater (control), whilst the other was fed with the same wastewater, supplemented with three different types of glycerol as co-substrate. After the starting period and acclimation, the reactor supplemented with glycerol was first fed with 2 mL of pure glycerol per litre of wastewater (equivalent to an OLR of 2.2 kg COD/(m^3·d), due to the glycerol). After 32 days, pure glycerol was substituted by sulphate-rich (255 mg SO_4^{2-}/L) crude glycerol. Finally, maintaining the same loading rate, glycerol with a high conductivity (about 29 mS/cm) was used. The best results occurred when pure or crude glycerol was added to the wastewater, reaching an average organic matter removal of 85%. When the high-conductivity glycerol was used, the efficiency decreased to 75%, which is still typical for anaerobic processes, even when dealing with saline wastewater. Although glycerol was responsible for only 18% of the organic load, the production of CH_4 increased by 24%.

Amon *et al.* [85] were able to increase the methane production by adding crude glycerol as co-substrate for anaerobic digestion of a mixture of corn silage (31%), corn (15%) and pig manure (54%). Based on the amount of methane produced by a reactor fed with 100% of the mixture and another fed with 100% of crude glycerol, it is possible to conclude that the anaerobic biodegradability of the mixture increased by 17% and 22% when the crude glycerol was added at concentrations of 3% and 6% (v/v), respectively.

The use of crude glycerol as a co-substrate was also evaluated for the anaerobic digestion of corn silage by Hutňan *et al.* [42]. The researchers used an anaerobic reactor of 2.450 m^3 and concluded that the specific methane production due to glycerol was approximately 0.541 m^3/kg glycerol. This value is similar to that obtained in laboratory tests performed by the same authors when using glycerol as the sole source of organic matter. From this, it is concluded that glycerol had no noticeable influence on the digestion of corn silage. These results are in agreement with those of Fountoulakis and Manios [53], who added 1% (v/v) of

glycerol to an anaerobic reactor fed with organic solid waste and found that the increase in CH_4 production occurred only because of digestion of glycerol.

Špalková *et al.* [86] operated two laboratory-scale reactors (6 L) for evaluating the MPP of maize silage and crude glycerol. During the last stage of the operational period, both reactors were operated at an OLR of 3.2 kg $COD/(m^3 \cdot d)$, one with sole maize silage and the other with a mixture of 4830 g of maize and 805 mL of crude glycerol. Under this condition, the total volume of biogas produced by both reactors, with and without supplementation with crude glycerol, was approximately the same (1240 L). The researchers affirmed that both substrates have approximately the same specific methane production (based on COD). However, the mass balance shows that the crude glycerol did not contribute to increased anaerobic biodegradability of maize silage, and has higher MPP (based on either mass or COD).

Daun *et al.* [87] also compared the production of biogas in two batch reactors, one fed with a mixture of water and cow manure (1:1) and another using the same mixture but with supplementation of 5% (v/v) glycerol, which is equivalent to 0.221 kg of glycerol (considering a glycerol density of 1.26 kg/L). The authors reported that, after 14 days of reaction time, the reactor containing glycerol was able to increase methane production by approximately 4 L. According to the data shown in this work, the use of glycerol for co-digestion of manure was not effective, as the anaerobic digestion of 0.221 kg of glycerol can generate up to 70 L of methane after 14 days of retention time (considering specific methane production of 0.426 m^3 CH_4/kg glycerol and an efficiency of 75% of substrate conversion), which is far beyond the values found by these authors.

The digestion of pig manure with and without glycerol as co-substrate was evaluated by Alvarez *et al.* [88]. The authors used batch reactors with 385 mL of working volume and added 9% of crude glycerol for improving methane production, resulting in 0.377 L of methane. However, considering that the biodegradability of the crude glycerol is approximately 75% [68,86], and that its COD concentration is 1390 g/L, the amount of methane can be estimated as 0.389 L (0.060 L + 0.328 L due to digestion of pig manure and crude glycerol, respectively). Therefore, the crude glycerol did not improve the degradation of pig manure.

Final considerations

In the last five years, data related to anaerobic digestion of crude glycerol showed clear operational limitations, both when the biomass is exposed to high concentrations of this residue as the sole source of substrate and when it is co-digested with other organic materials. Limitations associated with accumulation of metabolites, due to the high COD of glycerol and associated with the presence of toxic compounds – the main ones being LCFAs and inorganic salts of chloride and sulphates, make its use in anaerobic

digestion a challenge. No definitive solution was found to overcome the difficulties mentioned above, but dilution and gradual adaptation of the sludge to this material appear to be the most frequently used strategies. Adding steps like chemical precipitation, centrifugation, distillation; addition of compatible solutes; 'gas stripping' or the use of two-step biological reactors makes the process more complex and expensive. However, research has advanced in order to find viable solutions for the anaerobic digestion of this waste, such as the one developed by Forrest *et al.* [12], who used halophilic cultures as inoculum for the production of organic acids from the anaerobic digestion of crude glycerol. Thus, these researchers were able to increase tolerance to high salinity of the medium.

The research related to the use of glycerol for improving anaerobic digestion of complex waste indicates that this operational procedure is not viable because only very rarely is the increase in methane production a result of improved conversion of the main carbon source, rather than being the effect of glycerol conversion only. On the other hand, this strategy can be useful to increase the active biomass concentration, which will then use the main substrate when all easily biodegradable co-substrate has been consumed. However, in this case, the glycerol ought to be removed from the influent, and the problem of the accumulation of huge amounts of glycerol, as produced by the biodiesel industry, will not be solved.

As reported in the section 'Accumulation of intermediaries' of this review, during the anaerobic digestion of crude glycerol, there may be accumulation of intermediates, depending on the glycerol concentration in the influent. To avoid this problem and to add value to these metabolites, a two-phase anaerobic system can be an alternative. This system consists of applying an organic overload in the first reactor in order to produce organic acids and/or hydrogen. Hydrogen can be used as a source for power generation, whereas the organic acids can be methanized in the second reactor [45]. According to Luo *et al.* [45], the overall net energy produced in such a system was found to be 11% higher than that produced by a single-step methanogenic reactor.

A preliminary analysis of the anaerobic digestion of crude glycerol for energy production can be made based on the analysis of a small biodiesel plant that produces 250 m^3 of biodiesel per day. This plant also produces about 25 m^3 of crude glycerol per day, since each kilogram of produced biodiesel results in the production of 100 g of crude glycerol [6]. According to Viana [43], it is necessary to dilute the glycerol five times before feeding the reactors, which results in a COD solution of approximately 252 kg COD/m^3 [8,34,42]. Considering that an upflow anaerobic reactor can be steadily operated at an OLR of 10 kg $COD/(m^3 \cdot d)$, with a COD removal efficiency of 90% [43], the volume of such a reactor should be around 3145 m^3. In this case, the reactor is expected to produce 16,128 m^3/d of biogas, with 60% methane in its composition. Theoretically, this

is sufficient to generate 6.25 GW of thermal energy, which can be converted to 5.5 GW of heat at an efficiency of 85% or 3.1 GW of electricity at an efficiency of up to 48%.

Acknowledgements

References

[1] Y. Dharmadi, A. Murarka, and R. Gonzalez, *Anaerobic fermentation of glycerol by* Escherichia coli*: A new platform for metabolic engineering*, Biotechnol. Bioeng. 94 (2006), pp. 821–829.

[2] Ministério de minas e energia (Ministry of Mines and Energy), Conselho nacional de política energética (National Energy Policy Council), *Resolução nº 6, de 16 de setembro de 2009 (Brazilian Resolution nº. 6, from September 16, 2009)*, Diário oficial da União (Official journal of the Union), 10/16/2009, n.204, s.1 (in Portuguese).

[3] Secretaría de Energía (Secretary of Energy), *Resolución nº 554, de 5 de julio de 2010 (Argentine Resolution nº 554, from July 5, 2010)*, Boletin oficial de la Republica Argentina (Official journal of the Republic of Argentina), 07/12/2010, n. 31.941, s.1, pp. 12–14 (in Spanish).

[4] *Directive 2003/30/EC of the European Parliament and of the Council of 8 May 2003 on the promotion of the use of biofuels or other renewable fuels for transport*, O.J. L 123, 17 May 2003, pp. 42–46.

[5] J. Yanowitz and R.L. McCormick, *Effect of biodiesel blends on North American heavy-duty diesel engine emissions*, Eur. J. Lipid Sci. Technol. 111 (2009), pp. 763–772.

[6] S.S. Yazdani and R. Gonzalez, *Anaerobic fermentation of glycerol: A path to economic viability for the biofuels industry*, Curr. Opin. Biotechnol. 18 (2007), pp. 213–219.

[7] Organisation for Economic Co-operation and Development (OECD), Food and Agriculture Organization of the United Nations (FAO), *Biofuels*, in *OECD-FAO Agricultural Outlook 2011–2020*, OECD, Paris, 2011, pp. 77–94.

[8] J.A. Siles López, M.D.L.A. Martín Santos, A.F. Chica Pérez, and A. Martín Martín, *Anaerobic digestion of glycerol derived from biodiesel manufacturing*, Bioresour. Technol. 100 (2009), pp. 5609–5615.

[9] S. Papanikolaou, S. Fakas, M. Fick, I. Chevalot, M. Galiotoupanayotou, M. Komaitis, I. Marc, and G. Aggelis, *Biotechnological valorization of raw glycerol discharged after bio-diesel (fatty acid methyl esters) manufacturing process: Production of 1,3-propanediol, citric acid and single cell oil*, Biomass Bioenergy 32 (2008), pp. 60–71.

[10] G.N. Jarvis, E.R. Moore, and J.H. Thiele, *Formate and ethanol are the major products of glycerol fermentation produced by a* Klebsiella planticola *strain isolated from red deer*, J. Appl. Microbiol. 83 (1997), pp. 166–174.

[11] A. Zhang and S.-T. Yang, *Propionic acid production from glycerol by metabolicallly engineered* Propionibacterium acidipropionici, Process Biochem. 44 (2009), pp. 1346–1351.

[12] A.K. Forrest, R. Sierra, and M.T. Holtzapple, *Effect of biodiesel glycerol type and fermentor configuration on mixed-acid fermentations*, Bioresour. Technol. 101 (2010), pp. 9185–9189.

[13] H. Biebl, *Fermentation of glycerol by* Clostridium pasteurianum – *batch and continuous culture studies*, J. Ind. Microbiol. Biotechnol. 27 (2001), pp. 18–26.

[14] C. Gätgens, U. Degner, S. Bringer-Meyer, and U. Herrmann, *Biotransformation of glycerol to dihydroxyacetone by recombinant* Gluconobacter oxydans *DSM 2343*, Appl. Microbiol. Biotechnol. 76 (2007), pp. 553–539.

[15] P.C. Lee, W.G. Lee, S.Y. Lee, and H.N. Chang, *Succinic acid production with reduced by-product formation in the fermentation of* Anaerobiospirillum succiniciproducens *using glycerol as a carbon source*, Biotechnol. Bioeng. 72 (2001), pp. 41–48.

[16] D.M. Rossi, J.B. Costa, E.A. Souza, M.C.R. Peralba, D. Samios, and M.A.Z. Ayub, *Comparison of different pretreatment methods for hydrogen production using environmental microbial consortia on residual glycerol from biodiesel*, Int. J. Hydrogen Energy 36 (2011), pp. 4814–4819.

[17] N. Pachauri and B. He, *Value-added utilization of crude glycerol from biodiesel production: A survey of current research activities*, Proceedings of the American Society of Agricultural and Biological Engineers (ASABE), Portland, Oregon, 2006.

[18] M.J. Taherzadeh, L. Adler, and G. Lidén, *Strategies for enhancing fermentative production of glycerol—a review*, Enzyme Microb. Technol. 31 (2002), pp. 53–66.

[19] F.D. Lópes, J.L.G. Revilla, and M.H. Munilla, *Glicerol (Glycerol)*, in *Manual de derivados da cana-de-açúcar: Diversificação, matérias-primas, derivados do bagaço do melaço, outros derivados, resíduos, energia (Guide of the sugar cane by-products: diversification, raw-material, bagasse and molasses by-products, other by-products, residues, energy)*, Cuban Research Institute of Derivatives Sugar Cane (ICIDCA), ed., Brazilian Association of the Technological Research Institutes (ABIPTI), Brasilia, 1999, pp. 393–397.

[20] A.S. Aldiguier, S. Alfenore, X. Cameleyre, G. Goma, J.L. Uribelarrea, S.E. Guillouet, and C. Molina-Jouve, *Synergistic temperature and ethanol effect on* Saccharomyces cerevisiae *dynamic behaviour in ethanol bio-fuel production*, Bioprocess Biosyst. Eng. 26 (2004), pp. 217–222.

[21] Z.X. Wang, J. Zhuge, H. Fang, and B.A. Prior, *Glycerol production by microbial fermentation: A review*, Biotechnol. Adv. 19 (2001), pp. 201–223.

[22] F. Ma and M.A. Hanna, *Biodiesel production: A review*, Bioresour. Technol. 70 (1999), pp. 1–15.

[23] A. Rywińska, W. Rymowicz, B. Zarowska, and M. Wojtatowicz, *Biosynthesis of citric acid from glycerol by acetate mutants of* Yarrowia lipolytica *in fed-batch fermentation*, Food Technol. Biotechnol. 47 (2009), pp. 1–6.

[24] J.C. Thompson and B.B. He, *Characterization of crude glycerol from biodiesel production from multiple feedstocks*, Appl. Eng. Agric. 22 (2006), pp. 261–265.

[25] H. Shin, S.H. Kim, C.Y. Le, and S.Y. Nam, *Inhibitory effects of long-chain fatty acids on VFA degradation and beta-oxidation*, Water Sci. Technol. 47 (10) (2003), pp. 139–146.

[26] R. Riffat and K. Krongthamchat, *Specific methanogenic activity of halophilic and mixed cultures in saline wastewater*, Int. J. Environ. Sci. Technol. 2 (2006), pp. 291–299.

[27] L.W. Hulshoff Pol, P.N. Lens, A.J.M. Stams, and G. Lettinga, *Anaerobic treatment of sulphate-rich wastewaters*, Biodegradation 9 (1998), pp. 213–224.

[28] C.W. Chiu, M.A. Dasari, W.R. Sutterlin, and G.J.A. Suppes, *Removal of residual catalyst from simulated biodiesel's crude glycerol for glycerol hydrogenolysis to propylene glycol*, Ind. Eng. Chem. Res. 45 (2006), pp. 791–795.

[29] R.T. Voegele, G.D. Sweet, and W. Boos, *Glycerol kinase of* Escherichia coli *is activated by interaction with the glycerol facilitator*, J. Bacteriol. 175 (1993), pp. 1087–1094.

[30] B. Holst, C. Lunde, F. Lages, R. Oliveira, and C. Lucas, *GUP1 and its close homologue GUP2, encoding multi-membrane-spanning proteins involved in active glycerol uptake in* Saccharomyces cerevisiae, Mol. Microbiol. 37 (2000), pp. 108–124.

[31] H. Biebl, K. Menzel, A.P. Zeng, and W.D. Deckwer, *Microbial production of 1,3-propanediol*, Appl. Microbiol. Biotechnol. 52 (1999), pp. 289–297.

[32] S.R. Harper and F.G. Pohland, *Recent developments in hydrogen management during anaerobic biological wastewater treatment*, Biotechnol. Bioeng. 28 (1986), pp. 585–602.

[33] G. Lyberatos and I.V. Skiadas, *Modelling of anaerobic digestion—a review*, Global Nest: Int. J. 1 (1999), pp. 63–76.

[34] J. Ma, M. van Wambeke, M. Carballa, and W. Verstraete, *Improvement of the anaerobic treatment of potato processing wastewater in a UASB reactor by co-digestion with glycerol*, Biotechnol. Lett. 30 (2008), pp. 861–867.

[35] A.H. Hazimah, T.L. Ooi, and A. Salmiah, *Recovery of glycerol and diglycerol from glycerol pitch*, J. Oil Palm Res. 15 (2003), pp. 1–5.

[36] M. Carmona, J.L. Valverde, A. Pérez, J. Warchol, and J.F. Rodriguez, *Purification of glycerol/water solutions from biodiesel synthesis by ion exchange: Sodium removal Part I*, J. Chem. Technol. Biotechnol. 84 (2009), pp. 738–744.

[37] A.W. Lawrence, *Application of process kinetics to design of anaerobic processes*, in *Advances in Chemistry*, R.F. Gould, ed., American Chemical Society, Washington, DC, 1971, pp. 163–190.

[38] S.G. Pavlostathis and E. Giraldo-Gomez, *Kinetics of anaerobic treatment*, Water Sci. Technol. 24 (8) (1991), pp. 35–61.

[39] H.N. Gavala, I. Angelidaki, and B.K. Ahring, *Kinetics and modeling of anaerobic digestion process*, Adv. Biochem. Eng. Biotechnol. 81 (2003), pp. 57–93.

[40] M.S. Fountoulakis, I. Petousi, and T. Manios, *Co-digestion of sewage sludge with glycerol to boost biogas production*, Waste Manage. 30 (2010), pp. 1849–1853.

[41] M. Jawed and V. Tare, *Microbial composition assessment of anaerobic biomass through methanogenic activity tests*, Water SA 25 (1999), pp. 345–350.

[42] M. Hutňan, N. Kolesárová, I. Bodík, V. Špalková, and M. Lazor, *Possibilities of anaerobic treatment of crude glycerol from biodiesel production*, 36th International Conference of Slovak Society of Chemical Engineering, Tatranské Matliare, Slovakia, 2009.

[43] M.B. Viana, *Produção de biogás a partir do glicerol oriundo de biodiesel (Biogas production from glycerol generated on biodiesel industry)*, M.Sc. diss., University of São Paulo, 2011.

[44] J.A. Siles, M.A. Martín, A.F. Chica, and A. Martín, *Anaerobic co-digestion of glycerol and wastewater derived from biodiesel manufacturing*, Bioresour. Technol. 101 (2010), pp. 6315–6321.

[45] G. Luo, L. Xie, Q. Zhou, and I. Angelidaki, *Enhancement of bioenergy production from organic wastes by two-stage anaerobic hydrogen and methane production process*, Bioresour. Technol. 102 (2011), pp. 8700–8706.

[46] J. Lalman and D.M. Bagley, *Anaerobic degradation and inhibitory effects of linoleic acid*, Water. Res. 34 (2000), pp. 4220–4228.

[47] A Rinzema, M. Boone, K.V. Knippenberg, and G. Lettinga, *Bactericidal effect of long chain fatty acids in anaerobic digestion*, Environ. Res. 66 (1994), pp. 40–49.

[48] I.W. Koster and A. Cramer, *Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids*, Appl. Environ. Microbiol. 53 (1987), pp. 403–409.

[49] K. Hanaki, T. Matsuo, and M. Nagase, *Mechanism of inhibition caused by long-chain fatty acids in anaerobic digestion process*, Biotechnol. Bioeng. 23 (1981), pp. 1591–1610.

[50] C.-S. Hwu, S.-K. Tseng, C.-Y. Yuan, Z. Kulik, and G. Lettinga, *Biosorption of long-chain fatty acids in UASB treatment process*, Water Res. 32 (1998), pp. 1571–1579.

[51] K.C. Yong, T.L. Ooi, K. Dzulkefly, W.M.Z. Wanyunus, and A.H. Hazimah, *Characterization of glycerol residue from a palm kernel oil methyl ester plant*, J. Oil Palm Res. 13 (2001), pp. 1–6.

[52] C. Weng and J. Jeris, *Biochemical mechanisms in the methane fermentation of glutamic and oleic acids*, Water Res. 10 (1976), pp. 9–18.

[53] M.S. Fountoulakis and T. Manios, *Enhanced methane and hydrogen production from municipal solid waste and agro-industrial by-products co-digested with crude glycerol*, Bioresour. Technol. 100 (2009), pp. 3043–3047.

[54] J.A. Lalman and I. Komjarova, *Impact of long chain fatty acids on glucose fermentation under mesophilic conditions*, Environ. Technol. (2004), pp. 391–401.

[55] M.A. Pereira, O.C. Pires, M. Mota, and M.M. Alves, *Anaerobic degradation of oleic acid by suspended and granular sludge: Identification of palmitic acid as a key intermediate*, Water Sci. Technol. 45 (10) (2004), pp. 139–144.

[56] I. Angelidaki and B.K. Ahring, *Effects of free long-chain fatty acids on thermophilic anaerobic digestion*, Appl. Microb. Biotechnol. 37 (1992), pp. 808–812.

[57] M.M. Alves, J.A. Vieira, R.M. Pereira, M.A. Pereira, and M. Mota, *Effects of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. Part II: Oleic acid toxicity and biodegradability*, Water Res. 35 (2001), pp. 264–270.

[58] A. Rinzema, P.A. Alphenaar, and G. Lettinga, *Anaerobic digestion of long chain fatty acids in UASB-reactors and expanded granular sludge bed reactors*, Process Biochem. 28 (1993), pp. 527–537.

[59] C.-S. Hwu, B. Donlon, and G. Lettinga, *Comparative toxicity of long-chain fatty acid to anaerobic sludges from various origins*, Water Sci. Technol. 34 (5–6) (1996), pp. 351–358.

[60] K.-Y. Show, Y. Wang, S.-F. Foong, and J.-H. Tay, *Accelerated start-up and enhanced granulation in upflow anaerobic sludge blanket reactors*, Water Res. 38 (2004), pp. 2292–2304.

[61] K.V. Rajeshwari, M. Balakrishnan, A. Kansal, K. Lata, and V.V.N. Kishored, *State-of-the-art of anaerobic digestion technology for industrial wastewater treatment*, Renew. Sustainable Energy Rev. 4 (2000), pp. 135–156.

[62] H. Galbraith, T.B. Miller, A.M. Paton, and J.K. Thompson, *Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol*, J. Appl. Bacteriol. 34 (1971), pp. 803–813.

[63] I. Angelidaki, S.P. Petersen, and B.K. Ahring, *Effects of lipids on thermophilic anaerobic digestion and reduction of lipid inhibition upon addition of bentonite*, Appl. Microbiol. Biotechnol. 33 (1990), pp. 469–472.

[64] D. Pyle and Z. Wen, *Production of omega-3 fatty acid-rich microalgae from biodiesel derived crude glycerol: Effects of glycerol impurities on algal growth and DHA production*, Annual International Meeting of the American Society of Agricultural and Biological Engineers, Providence, Rhode Island, 2008.

[65] N. Kolesárová, M. Hutňan, V. Špalková, and M. Lazor, *Biodiesel by-products as potential substrates for biogas production*, 37th International Conference of the Slovak Society

of Chemical Engineering, Tatranské Matliare, Slovakia, 2010.

[66] M.V.G. Vallero, G. Lettinga, and P.N.L. Lens, *Long-term adaptation of methanol-fed thermophilic (55 °C) sulfate-reducing reactors to NaCl*, J. Ind. Microbiol. Biotechnol. 30 (2003), pp. 375–382.

[67] K. Vijayaraghavan and T.K. Ramanujam, *Effect of chloride and condensable tannin in anaerobic degradation of tannery wastewaters*, Bioprocess Eng. 20 (1999), pp. 499–503.

[68] M.B. Viana, A.V. Freitas, R.C. Leitão, and S.T. Santaella, *Anaerobic biodegradability, methane production potential and toxicity of the glycerol generated on biodiesel industry*, 10th Latin American Workshop and Symposium on Anaerobic Digestion, Ouro Preto, Brazil, 2011.

[69] K.-I. Suehara, Y. Kawamoto, E. Fujii, J. Kohda, Y. Nakano, and T. Yano, *Biological treatment of wastewater discharged from biodiesel fuel production plant with alkali-catalyzed transesterification*, J. Biosci. Bioeng. 100 (2005), pp. 437–442.

[70] E. Foresti, M. Zaiat, and M. Vallero, *Anaerobic processes as the core technology for sustainable domestic wastewater treatment: Consolidated applications, new trends, perspectives, and challenges*, Rev. Environ. Sci. Biotechnol. 5 (2006), pp. 3–19.

[71] I. Vyrides, H. Santos, A. Mingote, M.J. Ray, and D.C. Stuckey, *Are compatible solutes compatible with biological treatment of saline wastewater? Batch and continuous studies using submerged anaerobic membrane bioreactors (SAMBRs)*, Environ. Sci. Technol. 44 (2010), pp. 7437–7442.

[72] P.L. McCarty, *Anaerobic waste treatment fundamentals – part three: Toxic materials and their control*, Public Works 95 (1964), pp. 91–94.

[73] L.A. de Baere, M. Devocht, P. van Assche, and W. Verstraete, *Influence of high NaCl and NH₄Cl salt levels on methanogenic associations*, Water Res. 18 (1984), pp. 543–548.

[74] D.W. Yerkes, S. Boonyakitombut, and R.E. Speece, *Antagonism of sodium toxicity by the compatible solute betaine in anaerobic methanogenic systems*, Water Sci. Technol. 37 (6–7) (1997), pp. 15–24.

[75] A. Rinzema and G. Lettinga, *Anaerobic treatment of sulfate-containing waste water*, in *Biotreatment Systems*, D.L. Wise, ed., CRC Press, Boca Raton, 1988, pp. 65–109.

[76] E. Ryckebosch, M. Drouillon, and H. Vervaeren, *Techniques for transformation of biogas to biomethane*, Biomass Bioenergy 35 (2011), pp. 1633–1645.

[77] Y. Chen, J.J. Cheng, and K.S. Creamer, *Inhibition of anaerobic digestion process: A review*, Bioresour. Technol. 99 (2008), pp. 4044–4064.

[78] A.B. Jensen and C. Webb, *Treatment of H₂S-containing gases: A review of microbiological alternatives*, Enzyme Microb. Technol. 17 (1995), pp. 2–10.

[79] S.V. Mikhalovsky and Y.P. Zaitsev, *Catalytic properties of activated carbons I. Gas-phase oxidation of hydrogen sulphide*, Carbon 35 (1997), pp. 1367–1374.

[80] A.M. Buswell and H.F. Muellepi, *Mechanism of methane fermentation*, Ind. Eng. Chem. 44 (1952), pp. 550–552.

[81] C.P. Pabón Pereira, *Anaerobic digestion in sustainable biomass chains*, Ph.D. diss., Wageningen University, 2009.

[82] N. Kolesárová, M. Hutňan, V. Špalková, R. Kuffa, and I. Bodík, *Anaerobic treatment of biodiesel by-products in a pilot scale reactor*, Chem. Pap. 65 (2011), pp. 447–453.

[83] Y. Yang, K. Tsukahara, and S. Sawayama, *Biodegradation and methane production from glycerol-containing synthetic wastes with fixed-bed bioreactor under mesophilic and thermophilic anaerobic conditions*, Process Biochem. 43 (2008), pp. 362–367.

[84] G. Antonopoulou, K. Stamatelatou, and G. Lyberatos, *Exploitation of rapeseed and sunflower residues for methane generation through anaerobic digestion: The effect of pretreatment*, Chem. Eng. Trans. 20 (2010), pp. 253–258.

[85] T. Amon, B. Amon, V. Kryvoruchko, V. Bodiroza, E. Pötsch, and W. Zollitsch, *Optimising methane yield from anaerobic digestion of manure: Effects of dairy systems and of glycerine supplementation*, Int. Congr. Ser. 1293 (2006), pp. 217–220.

[86] V. Špalková, M. Hutňan, M. Lazor, and N. Kolesárová, *Selected problems of anaerobic treatment of maize silage*, 36th International Conference of Slovak Society of Chemical Engineering, Tatranské Matliare, Slovakia, 2009.

[87] L.G. Daun, R.A.C. Mesquita, R.K. Kimura, A.C. Gonçalves, and R.A.V. Ramos, *Alternativa para o uso do glicerol obtido da produção de biodiesel em biodigestores anaeróbios como otimizador da produção de biogas (Alternative for the use of glycerol from biodiesel production as an improver for biogas production)*, 8th Latin-American Congress on Electricity Generation and Transmission, Ubatuba, 2009.

[88] J.A. Álvarez, L. Otero, and J.M. Lema, *A methodology for optimising feed composition for anaerobic co-digestion of agro-industrial wastes*, Bioresour. Technol. 101 (2011), pp. 1153–1158.