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# Biogas production from glycerol in a multistage anaerobic digestor

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## ABSTRACT

The possibility of utilizing crude glycerol, a residue from biodiesel production, for biogas production is tested. For this purpose a multistage anaerobic digestor with semi-continuous feed of substrate is used. It was observed that the microbial pre-treatment and methanogenesis could be distributed in a series of compartments maintaining pH values suitable for methanogenic activity. Very high methane content (95% vol.) is detected in the biogas. When the digester is overloaded by substrate, pH drops because of acid accumulation. In this case the methane production ceases. This problem could be overcome by selective extraction of the accumulated carboxylic acids. The anaerobic digestion of glycerol may serve both for energy production by methane and for formation of value-added products like propionic acid and 2,3-butanediol.

**KEYWORDS:** waste glycerol utilization, biogas production, multistage digestor

## 1. INTRODUCTION

Crude glycerol is the main waste product from biodiesel manufacturing. Its amount is equivalent to the methanol used for the trans-estherification and exceeds the traditional market demands. On the other hand this waste product contains about 20% water and it is contaminated by the catalyst and some methanol. Since the market is interested only in pure glycerol and its price is not high, the purification of this waste product is not economically feasible. That is why different new applications of waste glycerol for production of value-added chemicals are sought in the recent years [1-3]. Many of them study the production of various chemicals by chemical conversions, like propylene glycol, 1,3-propanediol, epichlorohydrin, some of its derivatives suitable as additives to gasoline and diesel, e.g., glycerol tertiary butyl ether (GTBE) [3], etc.

There are many chemical compounds produced by glycerol by microbial methods. It is well-known that 1,3-dihydroxy-acetone [4], 1,3-propanediol [5], succinic acid [6], propionic acid [7], some polyesthers, like poly(hydroxyalkanoates) are produced due to the microbial activity [2]. The latter, i.e., the poly-(hydroxyalkanoates) are interesting due to their biodegradability and therefore they are applicable in packaging of various goods with little impact on the environment [8]. Furthermore, 2,3-butanediol is also produced by fermentation of glycerol [9] being a precursor for butadiene, methyl-ethyl-ketone and some other practical applications. Different bacteria (from the genera *Klebsiella, Clostridium, Enterobacter* etc.) are capable of metabolizing glycerol, leading to main basic products with some differences in the side-products of metabolism [2]. The metabolic scheme for glycerol conversion by the bacteria *Klebsiella* sp. is shown in Scheme 1 given and discussed by Saxena *et al.* [10] and Zhang *et al.* [11]. There is a work, claiming that formation of

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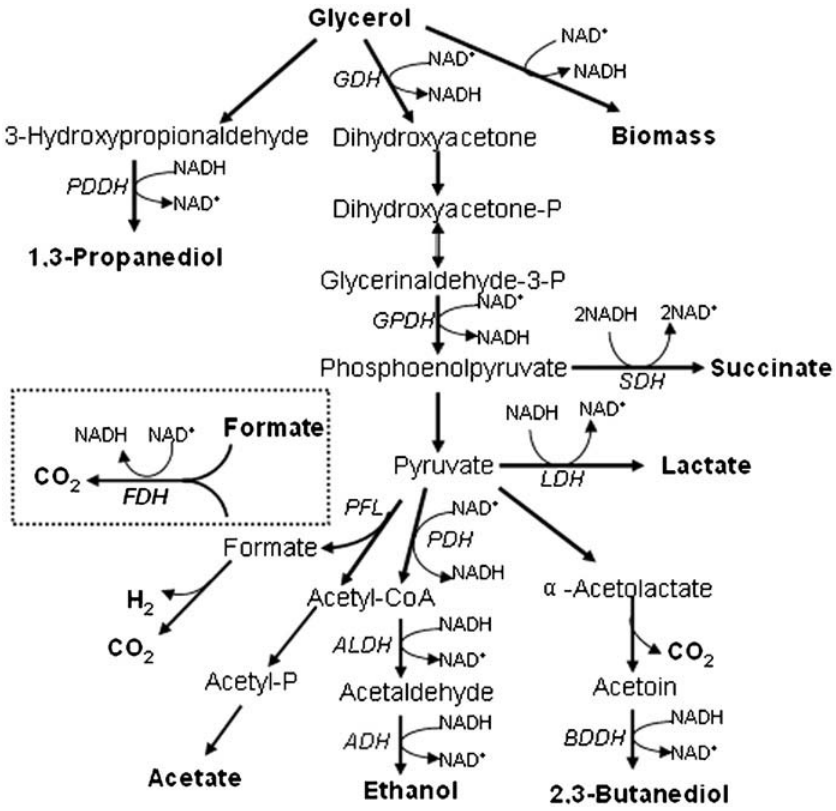


**Scheme 1**

propionic acid as an intermediate product is possible during anaerobic digestion [12].

Another way for waste glycerol utilization is to produce energy in the form of biogas [13, 14]. This biogas could be used for partial energy supply of the main biodiesel plant. Methanogenic bacteria can produce methane from the products of metabolism of the other bacteria, listed above. It can be produced either after acetic acid decarboxylation or carbon dioxide reduction by hydrogen:

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 \quad (1)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (2)$$

The method of carbon isotopes was extensively used to establish the pathway of methane production, as summarized by Conrad [15].

Hydrogen and carbon dioxide are produced intermittently by the degradation of the formic acid, as it is shown in Scheme 1 (according to Saxena *et al.* [10]). An important hindrance for this application is the rapid accumulation of carboxylic acids leading to strong inhibition of the methanogenesis and shift to production of gas with very low methane content [13, 14]. Compared to the traditional complex substrates for biomethanation glycerol has a very simple molecule and therefore it quickly yields intermediates and final products as organic acids and alcohols (cf. Scheme 1). The acids lower rapidly the pH to inhibit the methanogenic bacteria. It was reported however, that small amounts of glycerol can boost the biogas production based on traditional substrates (animal waste, activated sludge, different wastewaters, etc.), see Wohlgemut [16] and Fountoulakis & Manios [17].

The purpose of the present study is to demonstrate how to overcome, at least partially, the effect of this strong acidification on the biogas production from crude glycerol. For this purpose a baffled bioreactor separated into 8 compartments is used. It was reported earlier by Grobicki & Stuckey [18], that this type of digesters are stable towards

various disturbances in the feed, pH, temperature variations, etc.

## 2. MATERIALS AND METHODS

### 2.1. Materials and equipment

Crude glycerol leftover from biodiesel production containing 20% wt. of water with pH ~ 5 was used. The acidity of this substrate corresponded to pH 5.5. No pH-adjustment was carried out.

A principal sketch of the used bioreactor is shown in Figure 1. It consists of eight rectangular sections with equal volumes of 33 liters separated by stationary baffles with static mixers. The total bioreactor volume was 270 liters. The reactor was initially inoculated by activated sludge using residues from ethanol distillation as a carbon source. The inoculum and the initial feed were equal for each section. The gas space above the liquid was common for the whole reactor. The cultivation was carried out under naturally attained anaerobic conditions, without deliberate scavenging of the bioreactor with inert gas. After full development of the biomass, indicated by the release of combustible biogas, the feeding of the reactor started in the first section with crude glycerol. The excess liquid from each section enters the next one through overflows and below the separating baffles and leaves the apparatus from the end section. The process was carried out in a fed-batch mode, with a feeding of four times daily. The temperature was maintained at 32$^{o}$C by thermostat with a sensor dipped in the fermentor. Different amounts of crude glycerol (from 0.1 to 1 l/day) were added. The production rate of the obtained gas was periodically measured after collection in a gas-holder under water. Samples from each compartment were taken regularly. They were analyzed for substrate and intermediates and for pH too. For this purpose the samples were filtrated by filters with 0.22 micrometers pore diameter and have been analyzed by HPLC for different intermediate components.

### 2.2. Analyses

The analyses were carried out by HPLC system Perkin Elmer Series 10, with Bio-Rad column for organic acid analysis (Aminex HPX-87H). The organic acids were determined by Knauer UV-detector at 210 nm, whereas for analyses of alcohols an RI detector was used. Solution of 0.01 N sulfuric acid was used as a mobile phase at an elution flow rate of 0.6 l/min at 65$^{o}$C. The analyzed intermediates were identified by their retention times compared to added standards. The pH-values were measured off-line by glass probe coupled to standard pH-meter.

The qualitative biogas content was determined by passing gas samples through solutions of copper sulfate (for detection of hydrogen sulfide and mercaptanes) and of calcium chloride (for carbon dioxide). The methane content was evaluated volumetrically after absorption of carbon dioxide by a solution of potassium hydroxide.

### 2.3. Bacterial strain determinations

After development of the microbial consortium the microbial genera in each compartment were determined using different cultivation media for screening procedure. The viable cell counts of aerobes and anaerobes were carried out using

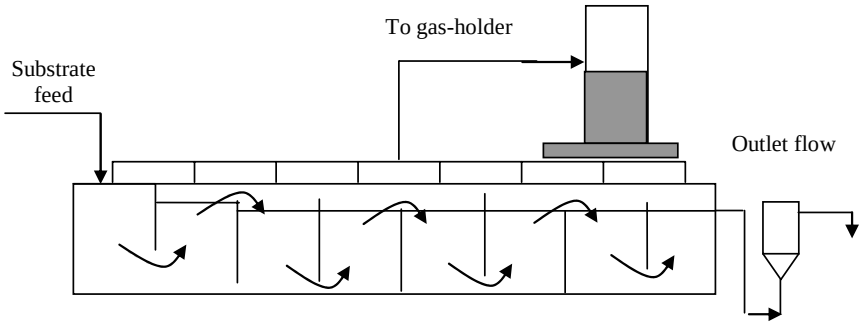


**Figure 1.** A sketch of the experimental set-up.

decimal dilutions and plating on different media and cultivation conditions. Terrific broth agar, Luria-Bertani agar, Mc Conkey agar, Kligler iron agar and XLD agar were used for growth of aerobes. They were purchased from Sigma-Aldrich (USA). Thyoglycolate medium (AppliChem GmbH) was used for cultivation of anaerobes under anaerobic conditions. It was supplied by AnaeroPack™ system (Remel, Germany). The tests were done at 37°C and 45°C.

The initial identification of the strains was done by examination of their phenotypic and biochemical characteristics. The strains were observed for their cell morphology by immersed microscopy and Gram reaction. Catalase activity was tested using 20% $H_2O_2$. Biochemical tests of the strains were done using API® 20E and API® 50 CH identification kits for carbohydrate metabolism (bioMerieux, France).

In particular, the glycerol-degrading microorganisms were tested in batch cultures, grown in media with a sole carbon source glycerol, varying between 10 and 50 g/l.

## 3. RESULTS AND DISCUSSION

### 3.1. Biogas production

The qualitative analysis of the biogas content showed some carbon dioxide and lack of sulfur compounds (i.e., hydrogen sulfide and mercaptanes). The volumetric analysis showed very low content of carbon dioxide (less than 20% vol.). The lack of sulfur compounds is expected because of the use of glycerol as a substrate. The low percentage of carbon dioxide in the gas leads us to the conclusion, that the route of $CO_2$ reduction by hydrogen is predominant to the acetate decarboxylation but both routes take place. This conclusion is supported by the microbial profile shown in Table 1.

### 3.2. Microbial profile

The inoculating activated sludge contains a broad variety of microbes, but different species of them can grow and develop, depending on the substrates and the experimental conditions. In our particular case glycerol as a carbon source was used. The steady state microbial profile along the

**Table 1.** Microbial profile in the bioreactor with glycerol as a substrate.

| Compartment No. | Anaerobes | Aerobes | Genus | Methane production | Source |
|---|---|---|---|---|---|
| 1 | ~1 x $10^1$ | 1 x $10^2$ | Moulds, *Bacillus* | - | |
| 2 | ~1 x $10^5$ | 1 x $10^3$ | *Klebsiella*, *Methanosarcina* | Acetate; $CO_2$+$H_2$ | Krzycki *et al.* [19] Fuchs *et al.* [20] Zyakun et al. [21] Gelwicks *et al.* [22] |
| 3 | ~1 x $10^5$ | 1 x $10^3$ | *Klebsiella*, *Methanobacterium* | $CO_2$+$H_2$ | Games *et al.* [23] Fuchs *et al.* [20] Belyaev *et al.* [24] Balabane *et al.* [25] |
| 4 | ~1 x $10^6$ | 2 x $10^3$ | *Klebsiella*, *Methanobacterium* | $CO_2$+$H_2$ | ” |
| 5 | ~4-5 x $10^6$ | 6-8 x $10^2$ | *Klebsiella*, *Methanobacterium* | $CO_2$+$H_2$ | ” |
| 6 | ~2 x $10^6$ | 4 x $10^2$ | *Klebsiella*, *Methanobrevibacter* | $CO_2$+$H_2$ | ” |
| 7 | ~1 x $10^6$ | 1-2 x $10^3$ | *Klebsiella*, *Methanobrevibacter* | $CO_2$+$H_2$ | ” |
| 8 | ~1 x $10^5$ | 1 x $10^2$ | *Methanobrevibacter*, *Klebsiella* | $CO_2$+$H_2$ | ” |

reactor is shown in Table 1. One can see that the prevailing microbes are from the genus *Klebsiella*, therefore we can speculate with the metabolic routes shown in Scheme 1. Except the first compartment, where moulds and *Bacillus* bacteria were observed, in all other cells, the methanogenes were predominant. In the second compartment acetate decarboxylation by *Methanosarcina* was considerable. *Methanosarcina* grow during the catabolism of acetate to $CO_2$ and $CH_4$ [26]. This is the acetate cleavage or aceticlastic reaction, where methane is formed without oxidation of the methyl group of acetate. In all other compartments methanogenes utilizing carbon dioxide and hydrogen are in consortium with the *Klebsiella* bacteria probably following the metabolic routes shown in Scheme 1. Propionic acid was found in the reactor, which is not typical for the Scheme 1.

The microbial profile and the cell count for the methanogenes follow qualitatively the pH profile shown in Figure 4; i.e., the methanogene concentrations in compartment 2 and 3, where the pH values are the lowest are one order of magnitude lower than in the next compartments. The studies of the methanogenic pathways by these two and other mechanisms are summarized by Conrad [15] in a review article. However it was stressed there, that a general conclusion for a single pathway of methane production in each separate case cannot be drawn without careful experimental work.

### 3.3. Metabolite concentration profiles

Some of the experimental results for the intermediate concentrations and pH time profiles for three bioreactor compartments are shown in Figure 2a-d. One can see that along the bioreactor the intermediate piruvate vanishes. In the middle of the reactor (compartment 4) a considerable pH drop is observed, due to the accumulation of volatile fatty acids (acetic and propionic ones). The low pH values inhibit the methanogenesis. Fortunately the situation is improved in the next compartments because of the acid decarboxylation and their concentration becomes negligible and the pH is within the optimum range for the methanogenes.

Different profiles along the bioreactor compartments for different periods of time are shown in Figure 3a-c. It can be seen that the pH values in the first and intermediate compartments drop to values between 5 and 5.5. This acidity does not allow the methanogenes to produce methane, but in the two last compartments the pH values are between 6.5 and 7.5. Additional experiments show that overloading with glycerol (i.e., with a feeding rate higher than 0.8 l/day) leads to gradual pH drop in six of the compartments resulting in the cessation of methanogenesis. In such cases the gas becomes rich in carbon dioxide and cannot burn. For a reference, before glycerol addition (t = 0) the pH values in the compartments 5 to 8 are higher than 6; i.e., conditions are favorable for methanogenesis. After seven days the pH is suitable for methane production in the compartments 7 and 8 only. However, this situation is improved and on the $12^{th}$ day the pH profile is better than in the beginning.

The profiles of some intermediate and final products at the $12^{th}$ day after glycerol addition are shown in Figure 4. Glycerol could be found in compartments 1, 2 and 3. Practically no acetic acid was detected. However, higher concentrations of propionic acid were observed in the fermentor, besides the last three compartments, where the pH values were high enough for methane formation. That is why the problem with the accumulation of propionic acid along the reactor is a crucial one. One can suggest addition of some alkaline agent to correct the pH value where it is necessary. However, after decarboxylation and carbon dioxide release the alkalinity will increase to high levels being toxic for the bacteria. That is why two possible alternative ways to maintain the optimum pH values may have to be considered: remove selectively the excessive acids, e.g. by ion-exchange, or feed the reactor very slowly to avoid the fatty acid accumulation. The first one is associated with the simultaneous recovery of value-added products, like the organic acids in Scheme 1. The second one is not suitable for treatment of waste glycerol, but for enhancing the methanization based on another substrate.

Considerable amounts of 2,3-butanediol were detected in the broth. It is a competitive process for production of a value-added product besides the biogas.

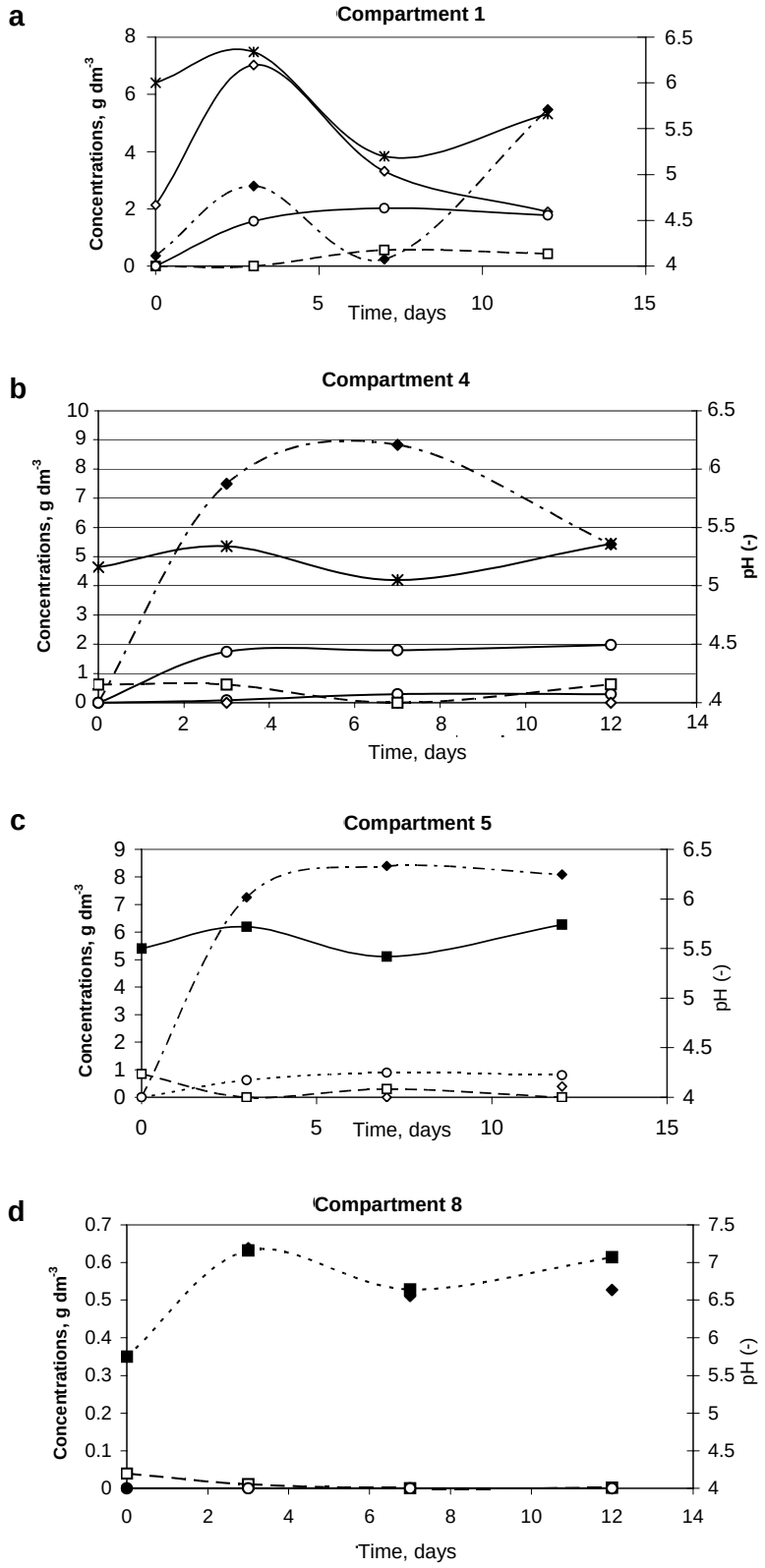


**Figure 2a-d.** Time development of some intermediates and pH in different reactor compartments (a-d). (◊) - piruvic acid; (□) - acetic acid; (○) - propionic acid; (♦) - 2,3-butanediol; (■) - pH. Feed 1 kg crude glycerol.

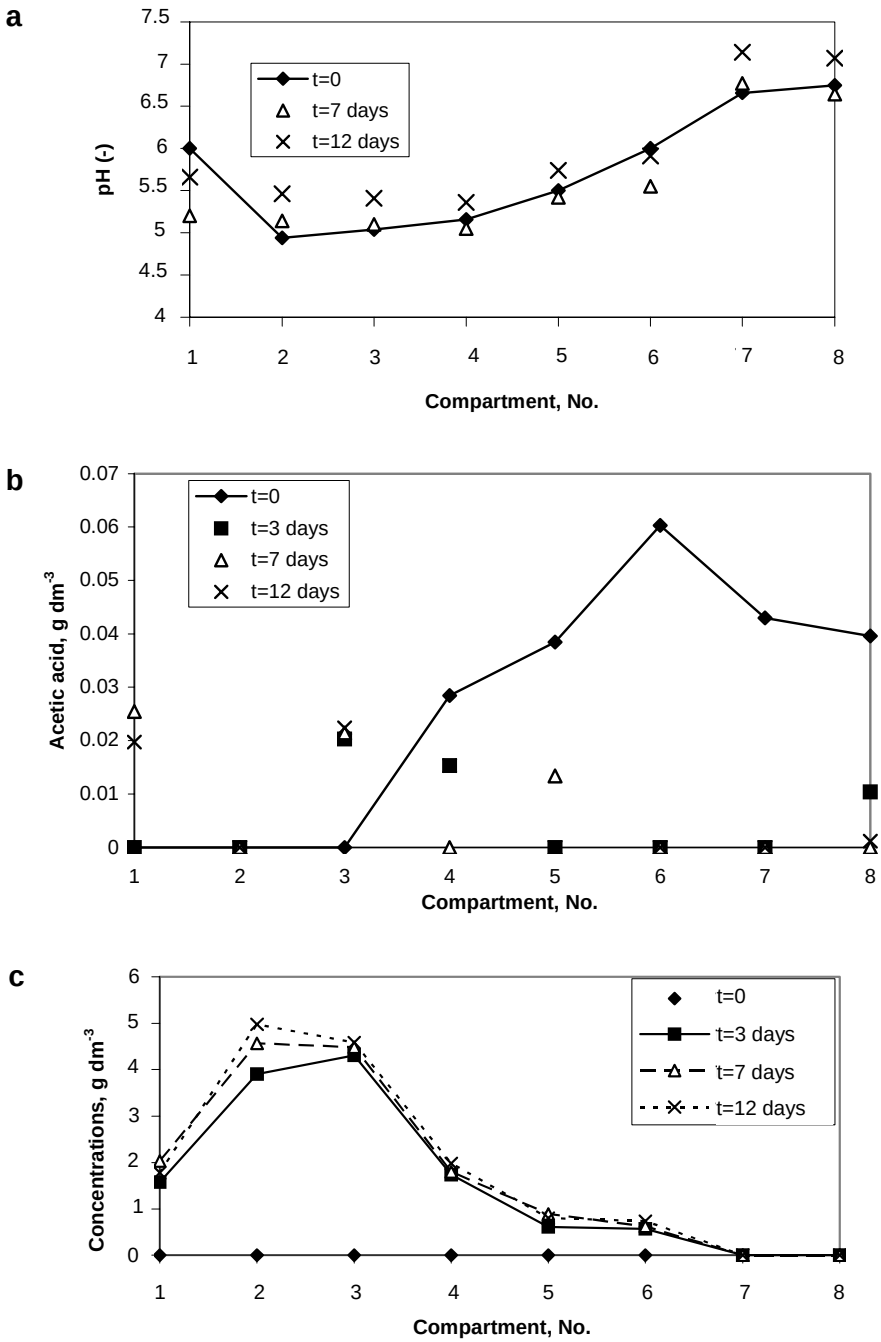


**Figure 3a-c.** Profiles for some intermediates and pH at different times after substrate feed. (a) - pH; (b) - acetic acid; (c) - propionic acid. Feed 1 kg crude glycerol.

The amounts of accumulated biogas and the biogas formation rate during the same period are shown in Figure 5. One can see that the daily amount for the 6$^{th}$ day is the highest one, whereas the one released before and after this day are lower. Afterwards the daily biogas release oscillates and drops to an average value of about 4 m$^3$/day. These results show that the chosen reactor configuration enables a very stable process, because within appropriate substrate feeding limits there are always compartments with suitable pH conditions for methanogenesis.

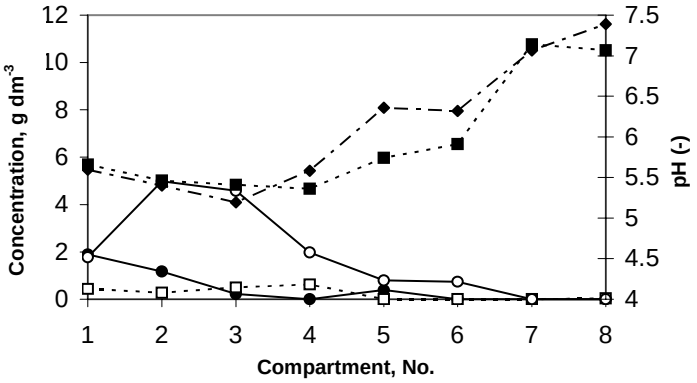


**Figure 4.** Profiles of substrate, intermediate products and pH on the 12th day after feed with glycerol (●) - glycerol; (□) - acetic acid; (○) - propionic acid; (♦) - 2,3-butanediol; (■) - pH. Feed 1 kg crude glycerol.

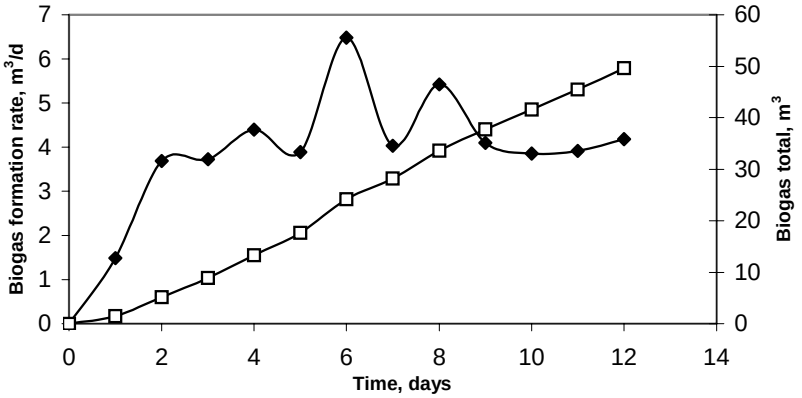


**Figure 5.** Time profile of biogas formation and accumulation. (♦) - biogas formation rate; (□) - total amount of accumulated gas. Feed 1 kg crude glycerol.

## CONCLUSION

1. The multistage anaerobic digester is suitable for glycerol conversion into biogas. It is because the microbial pre-treatment and methanogenesis are distributed in series of compartments, some of them with pH values suitable for methanogenic activity. Very high methane content (95% vol.) is detected in the biogas.
2. When the digester is overloaded, pH drops because of acid accumulation and the methane production ceases. This problem could be overcome by selective extraction of the accumulated acids.
3. The anaerobic digestion of glycerol may serve both for energy production by methane and for formation of propionic acid and 2,3-butanediol.

## ACKNOWLEDGMENTS

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