Valorization of the liquid fraction of a mixture of livestock waste and cheese whey for biogas production through high-rate anaerobic co-digestion and for electricity production in a Microbial Fuel Cell (MFC)

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Abstract

The objective of this work is the evaluation of two alternative ways of valorizing the liquid fraction of a mixture of different kinds of livestock waste and cheese whey, namely (a) biogas production through the anaerobic co-digestion of in a Periodic Anaerobic Baffled Reactor (PABR) and (b) electrical energy generation in a Microbial Fuel Cell (MFC).

The different kinds of livestock manure (pig manure, cow manure, poultry manure, sheep manure and cheese whey) were collected from the region of Metsovo, Greece. The mixture was passed through a pretreatment process producing a liquid fraction that was treated (a) in a pilot-scale Periodic Anaerobic Baffled Reactor (PABR) for methane production and (b) in a lab-scale Microbial Fuel Cell for electricity production.

In the present study, the experimental data obtained from a previous study [1] were used for the evaluation of a simple mathematical model, based on Monod kinetics, for the PABR using Aquasim 2.1 [2]. The simple model was able to satisfactorily describe the behavior of the PABR in terms of dissolved COD consumption.

In addition, the same liquid waste, filtered (0.70 μm) and diluted at different initial concentrations, was used as feedstock for electricity production, using a two-chamber microbial fuel cell (MFC). The experiments showed that the MFC performance was not limited by the wastewater strength since the substrate removal efficiency and maximum power density were not affected by the increase of the initial concentration. The required time showed a linear relationship with the initial concentration of the substrate.

1. INTRODUCTION

Livestock manure obtained from farms constitute mainly organic waste, which contains a high concentration of nitrogen (N) and phosphorus (P), also in addition to residues of some harmful substances such as growth hormones, antibiotics and heavy metals which could pollute the environment, and also cause human diseases [1]. The efficient utilization of livestock waste is of significant importance due to agriculture's high social and economic impact especially on rural and mountainous regions.

The Periodic Anaerobic Baffled Reactor (PABR) is a novel bioreactor, designed to operate at high organic loading rates. The PABR is 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 in a single compartment and the PABR behaves as an ABR) 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 fluctuations of the feed concentration and therefore culture adaptation is easier under varying conditions [2, 3].

Anaerobic digestion –especially when using a PABR- is characterized by high complexity and non-linearity and by a difficulty to collect adequate amounts of experimental data required for modeling purposes [4]. In order to describe the performance and kinetics of the anaerobic digestion process for methane generation, several models have been proposed over the last decades. In contrast with the, universally acceptable, thus extremely complicated –due to its involvement of a large number of kinetic parameters- Anaerobic Digestion Model No1 (ADM1), the current study examines the use of a very simple model, consisting of a minimal number of state variables and kinetic and stoichiometric parameters to describe the behavior of a high-rated pilot-scale Periodic Anaerobic Baffled Reactor. This model, assumes that the organic matter (in terms of dissolved COD) is consumed through Monod kinetics towards methane [5].

Two-chamber MFC

The MFC is a bioreactor that converts the chemical energy of the bonds of organic compounds to electrical energy, through catalytic reactions of microorganisms under anaerobic conditions, offering the advantage of simultaneously treating waste while generating electricity [6-9].

In the present study, a dual chamber MFC (H-type) was constructed and operated. An H-type MFC consists of two chambers (anode and cathode) where the two electrodes are immersed. The bacteria grow on dissolved organic matter in the anode chamber under anaerobic conditions. These bacteria transfer electrons to the anode electrode. The electrons then pass through an external circuit to the cathode electrode producing electrical current. Protons migrate through the solution and a proton exchange membrane to the cathode, where they combine with oxygen and electrons transferred through the circuit to form water [7, 10]. In this work, after the systems' acclimatization using anaerobic sludge and glucose as fuel, the feedstock used in the anodic compartment was the liquid fraction of the mixture of livestock waste and cheese whey at varying concentrations. In these experiments, the substrate removal efficiency was determined and the duration of electricity supply was correlated with the initial substrate concentration.

2. MATERIALS AND METHODS

The measurements of dissolved and total chemical oxygen demand (COD), total (TS) and volatile (VS) solids, total (TSS) and volatile (VSS) suspended solids, total nitrogen (Kjeldahl) and Total Organic Carbon (TOC), alkalinity and temperature were carried out according to Standard Methods [11]. The total carbohydrates were measured according to the analysis of Josefsson [12]. The pH and conductivity were measured using a digital pH-meter (WTW INOLAB PH720) and conductivity meter (WTW INOLAB), respectively. The methane content of biogas was quantified indirectly using an experimental set-up in which CO_2 was absorbed by NaOH. The biogas production rate was measured using an oil displacement technique [13].

Anaerobic co-digestion

The livestock waste and cheese whey used in this study were collected from local farms from the region of Metsovo, Greece. The basic characteristics of each component are given in Table 1:

Table 1 Feedstock characteristics [1]

	Moisture (%)	TS (g/g fresh)	VS (g/g fresh)	pH (20^{o}C)	tCOD (g/g TS)
cattle manure	74.74	0.26	0.12	8.47	0.75
poultry manure	61.94	0.38	0.34	7.45	0.7
sheep manure	74.15	0.26	0.23	7.44	0.83
cow manure	87.8	0.12	0.097	8.68	1.18
pig manure	86.3	0.14	0.09	7.30	1.02
whey	93.02	0.07	0.05	6.03	2.18

The feed mixture percentages used are given in Table 2. The feed was made representative of the relative quantities of production in the region of Metsovo for Phase#1 (see experimental procedure).

Table 2 Feedstock composition [1]

	Annual production (tn/year)	Ratio (%)
cattle manure	1792	3.93
poultry manure	15832	34.71
sheep manure	2812	6.17
cow manure	3663	8.03
pig manure	20640	45.25
whey	873	1.91
Total	45612	100

A solids/liquid separation step was used as pretreatment, because of the inability of the PABR to treat feedstock with high solids levels. Initially, the mixture was diluted in water at 60^{O}C and was vigorously stirred for 30 minutes. Then the slurry was filtered under pressure using a cloth filter. The liquid phase (filtrate) retained ~63% of the organic content of the waste. The characteristics of the liquid phase were suitable for feeding the PABR (Table 3).

Table 3 Feedstock Characteristics after pretreatment

pH	7.46
TSS (g/L)	5.72
Conductivity (S/cm)	3.93
VSS(g/L)	4.60
dCOD (g/L)	3.46
tCOD(g/L)	11.02
TS(g/L)	9.4
VS(g/L)	7.14
Total Carbohydrates (g/L)	1.19
Dissolved Carbohydrates (mg/L)	0.28
Total Kjeldahl Nitrogen (mg/L)	631
Ammonium Nitrogen (mg/L)	378.8
Organic Nitrogen (mg/L)	252.2
Total Phosphorus (mg/L)	120.4
Orthophosphates PO_4^{3-}P (mg/L)	41.5
Organic phosphorus (mg/L)	78.9

The liquid phase was further processed in order to be used as feedstock for the MFC, as described in the experimental procedure section.

The efficiency of this novel type of bioreactor to treat livestock waste at various organic loading rates and Hydraulic Retention Times was evaluated, using a pilot-scale PABR digester. The operating volume of the pilot scale four-compartment PABR was 77L. It was equipped with sampling valves in every compartment placed in the middle-height of the compartment. There were also four biogas vents on the top of the reactor. The PABR consists of two concentric cylinders of which the interior is full of water maintained at 35^{o}C through temperature control. The reactor was operated anaerobically under mesophilic conditions. Feeding was performed via a peristaltic pump controlled by an on/off time controller. More information about the characteristics of the PABR and the experimental procedure –as a whole- can be found in [1].

In all cases the performance of the PABR in terms of COD removal and biogas production was recorded and evaluated. The hydraulic retention time (HRT) was 6 d, the switching period T was 2 d and the organic loading rate was 0.6 $g_{COD}/L_{reactor}$/d.

The PABR was initially fed at an HRT of 22.3 d and a switching period (T) of 2 d, with an influent COD of 13.36 g/L for an operation period of 148 d (phase #1). The experimental procedure was continued to further phases with different operating parameters as shown in [1]. The feed characteristics varied from phase to phase – sometimes even during a single phase- due to the nature of the livestock waste and the seasonal features of each kind of waste. The COD value presented at Table 4 is the mean value of the influent total COD concentration measured. During the experiment, gas and liquid samples were taken at regular intervals and biogas production, biogas content of CH_4, Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), pH, alkalinity, soluble and total COD were determined.

Table 4 Operating parameters

HRT (d)	22.3
Switching period (d)	2
Influent tCOD (g/L)	13.36
Organic Loading Rate ($g_{COD}/L_{reactor}/d$)	0.6

A model was developed in Aquasim 2.1 [2]. The basic assumption of the model is that the organic matter is consumed with simple Monod kinetics, as shown in eq. (1):

$$r = \mu max \cdot \frac{S}{Ks + S} \cdot X \qquad (1)$$

Where S is the concentration of COD, μ_{max} is the maximum specific growth rate on each COD fraction [=] day^{-1}, K_s is the saturation constant [=] g/L, X is the biomass concentration [=] g/L and r is the consumption rate of COD [=] g/(L*d).

A stoichiometric coefficient (yield of methane on the substrate) Y_{meth}= 6.6901l_{meth}*$l_{reactor}$/gCOD obtained by Lyberatos et al. [3] was assumed for methane production and similarly a stoichiometric coefficient Yx/s = 0.05 g_{CODx}/g_{CODs} for the production of biomass from COD was assumed as in [3]. Experimental data (dCOD values) describing the operation of the PABR were used for the estimation of all the kinetic parameters. The initial dCOD of the four compartments was: $dCOD_{ini}$= 1.46 g/L.

Moreover, for the prediction of the behavior of the PABR it was necessary to take into consideration the high levels of biomass retention in the four compartments of the reactor (described by the high solids retention time in comparison to the HRT) by assuming a biomass retention factor R_b.

The retention factor R_b, the saturation constant K_s, the maximum specific growth rate on each COD fraction μ_{max} and the initial biomass concentration XB_{ini} were estimated through fitting of the COD experimental data for the specific operational phase.

Two-chamber MFC

The MFC used in this work consists of two (anode and cathode) 310 ml Plexiglas cylindrical chambers (operating volume of 250 ml each) connected via a glass tube. The solutions in the two bottles, where the two electrodes of the MFC were immersed, were kept in electrolytic contact via a polymer electrolyte membrane (PEM, Nafion 117), with a 3.77 cm^2 surface area. The PEM was sequentially boiled (80°C) in H_2O_2 (0.1M) for one hour, washed in deionized water, immersed in 0.1M H_2SO_4 for 30 min, and washed again in deionized water (stored in it prior to use). It was then placed in the middle of the chamber-connecting tube by a clamp.

Carbon fiber paper (3 cm x 2.3 cm, Toray, TGP-H-060, 10wt%, wet proofing) was used as the anode electrode and carbon cloth coated with a Pt catalyst (3 cm x 2.3 cm, ETEK, 0.5 mg/cm^2) as the cathode electrode. Electrodes were soaked in deionized water for 1 day before tests. The electrodes were connected with a copper wire coming out of the rubber stopple to provide the connection points for the external circuit which contained were connected via copper wires with a 1 kΩ external resistor, forming a closed electrical circuit. Both chambers were mixed with a magnetic stir bar. Experiments were conducted in a 35°C temperature-controlled box. The current, the MFC voltage, and the temperature were continuously monitored, while the dCOD was measured at selected time intervals after the addition of wastewater. The fuel cell voltage U_{cell} was monitored and recorded at 50 seconds intervals, using a data acquisition system (Advantech ADAM-4019+), connected to a personal computer, while the current I passing through the cell was measured using a precision multimeter (Mastech MY 61).

The produced power density was calculated from the eq. 2:

$$\mathbf{P_{Dual}} = \frac{\mathbf{I \cdot U_{cell}}}{\mathbf{A}} \qquad (\mathbf{2})$$

where I denotes the current, Ucell the cell voltage, and A the geometric surface area of the anode ($A=13.8\ cm^2$).

Polarization curves were obtained after adding the fresh substrate and establishing a constant power output, by varying the external resistance (external load) in the range of 0.1-1000 kΩ and recording the corresponding steady-state (quasi – steady – state) MFC voltage and current values.

The Coulombic Efficiency (CE) was calculated using the eq.3:

$$\mathbf{CE} = \frac{\mathbf{M \cdot \int_0^{t_b} Idt}}{\mathbf{FbV_{an}\Delta COD}} \qquad (\mathbf{3})$$

Where M is the molecular weight of oxygen (=32), F is Faraday's constant (=96,485 C/mol), b is the number of electrons exchanged per mol of oxygen (=4), V_{an} is the volume of liquid in the anode compartment and ΔCOD is the change in dissolved COD over time

For ensuring anaerobic conditions in the anodic compartment, the anodic chamber was sealed with a rubber stopple and the anolyte was sparged with a gaseous mixture of N_2/CO_2 for 5 minutes to remove dissolved oxygen.

All experiments were conducted in batch mode. The MFC was inoculated using anaerobic sludge obtained from the Athens (Greece) Wastewater Treatment Plant. For the inoculation, a mixed solution of 25ml anaerobic sludge with 225 ml fresh nutrient medium was injected into the anode chamber. The main characteristics of the sludge were pH = 7.055 ± 0.02, dissolved chemical oxygen demand (dCOD) = 0.2 ± 0.01 g/L**,** total chemical oxygen demand = 18.7 g/L, TSS (total suspended solids) = 25.04 ± 0.48 g/L, VSS (volatile suspended solids) = 10.97 ± 0.18 g/L. Nutrient medium containing the following (per liter): 5.288 g $NaH_2PO_4 \cdot 2H_2O$, 3.447 g $Na_2HPO_4 \cdot 2H_2O$, 0.16g KCl, 5 g $NaHCO_3$, 10 mL of a trace elements solution [13] and glucose at a concentration of 0.5 g dCOD/L. $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 2H_2O$ were added in medium forming Phosphate Buffer Solution (PBS, pH 7), while KCl was added to increase the solution conductivity.

After the inoculation stage, electricity generation during the filtered livestock waste and whey wastewater treatment was studied. Prior to use the liquid phase of the pretreatment step was filtered (0.70 μm), in order to remove the solids present and the final filtrate was diluted from initial dCOD value of 3.46 g dCOD/L in the range of 0.4 to 3.1 g dCOD/L.

Following the dilution, the above filtrate was used as fuel in the anodic chamber at different initial concentrations (0.4-3.1 g dCOD/L). PBS, $NaHCO_3$, salt and trace element solution were added in each experimental cycle at same quantities as used for the preparation of nutrient solution during inoculation stage. The cathode compartment was continuously aerated and filled with the PBS, where KCl (0.16 g/L) was added.

3. RESULTS AND DISCUSSION

Anaerobic co-digestion with PABR

The PABR exhibited great stability during the process. The effluent tCOD concentration varied in the range 2 - 2.2 g/L while the influent tCOD concentration varied around 13.36 g/L (tCOD removal 79.9%). The Volatile Solids concentration remained below 1 g/L in all four compartments of the reactor. The mean biogas production rate was approximately 0.13 L/L/d, the pH and alkalinity levels were normal and the system was not under kinetic limitation. The mean methane composition of the biogas produced was 70.3%. The experimental data were used for the development of a simple mathematical model for the prediction of the behaviour of the PABR. More details about the data of the whole experimental procedure can be found in [1].

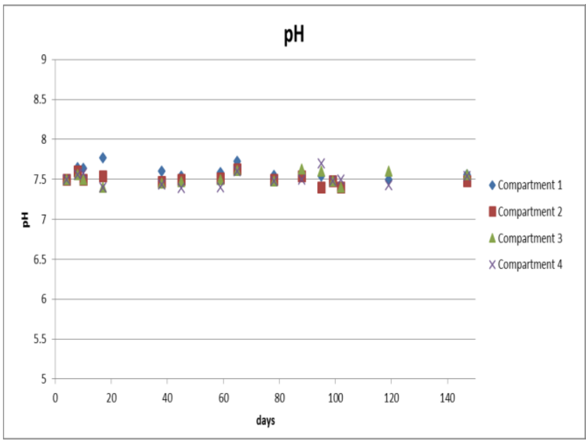

Figure 1 pH

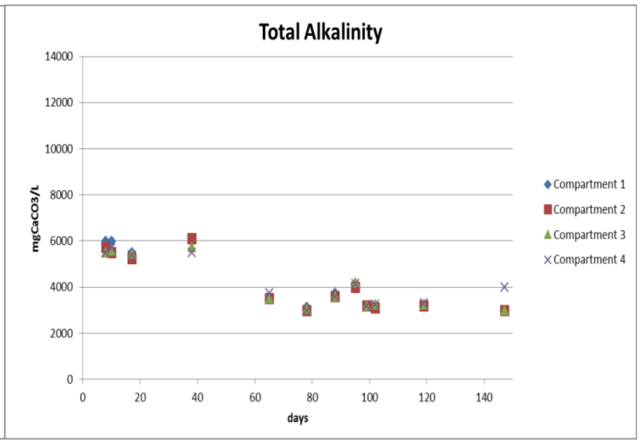

Figure 2 Total Alkalinity

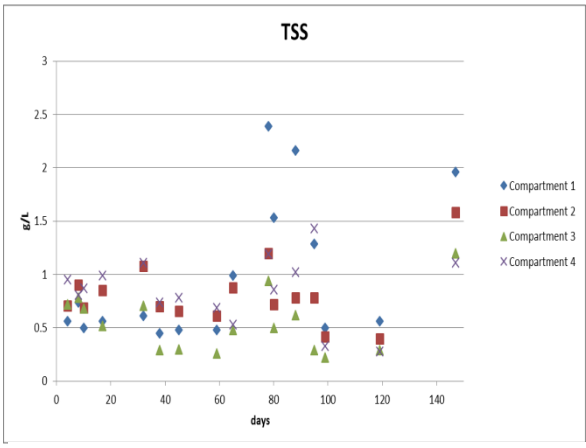

Fig. 3 Total Suspended Solids

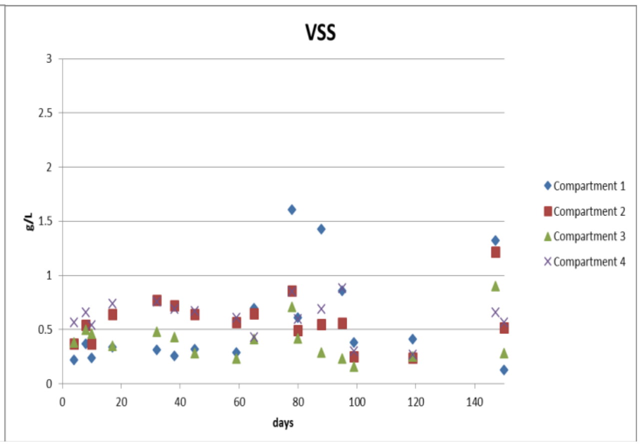

Fig. 4 Volatile Suspended Solids

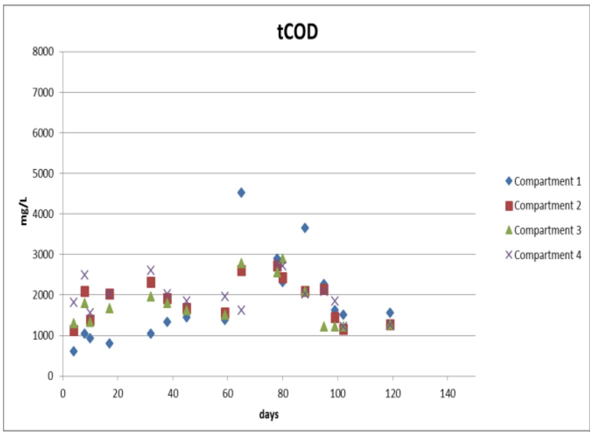

Fig. 5 Total COD

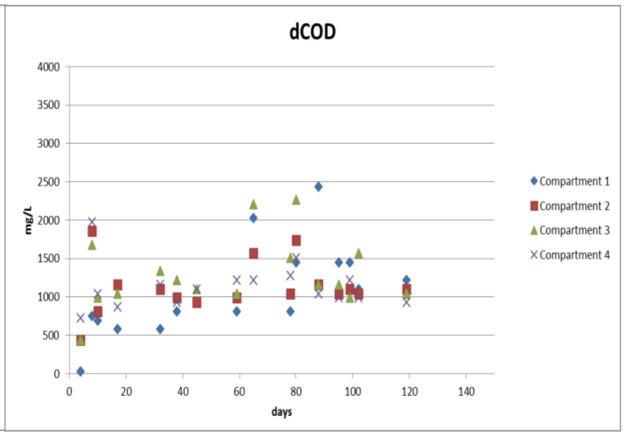

Fig. 6 Dissolved COD

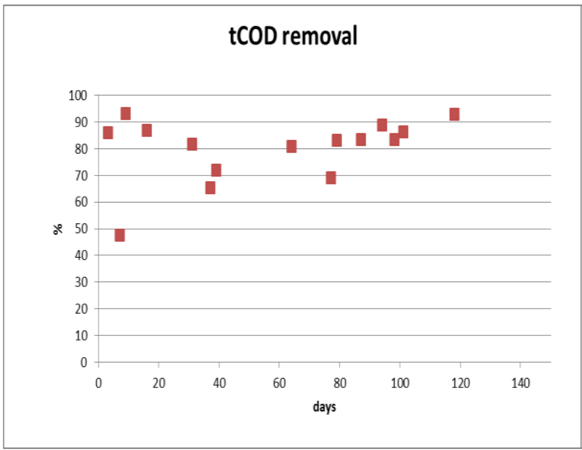

Fig. 7 **%**total COD Removal

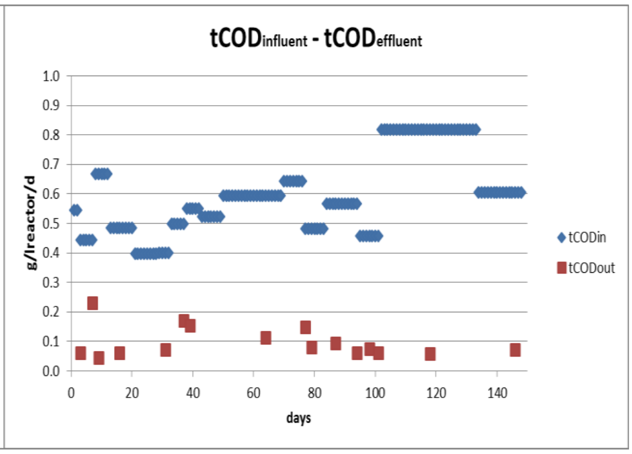

Fig. 8 tCODinffluent - tCODeffluent

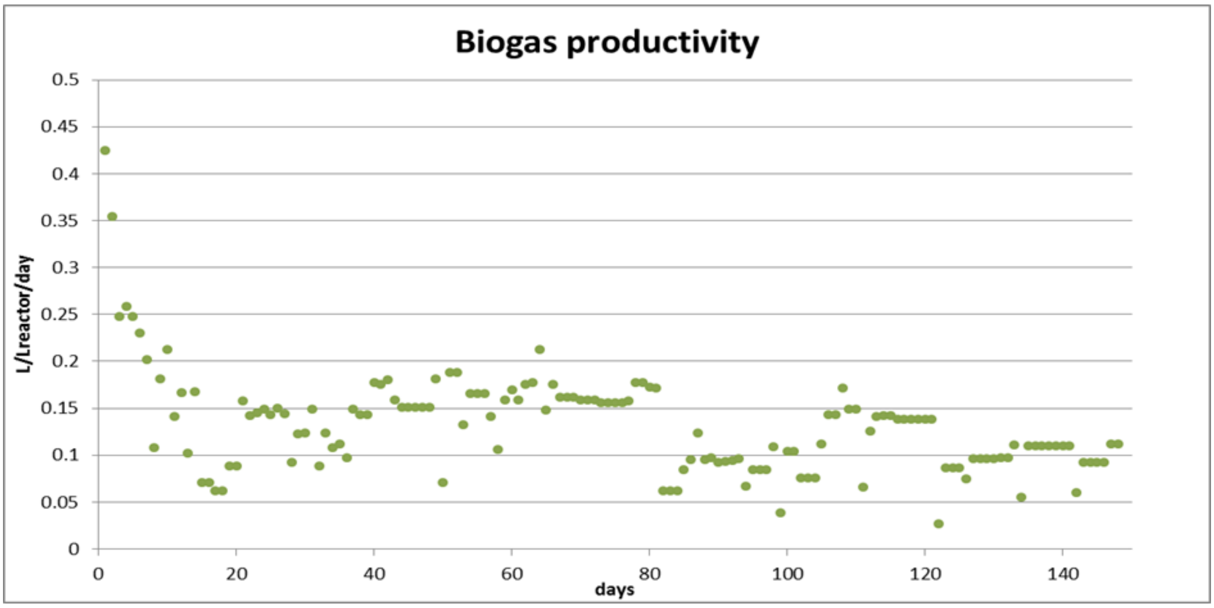

Fig. 9 Biogas productivity

The simple model was used to predict the behavior of a continuous, high rate anaerobic bioreactor, a PABR, which –as mentioned- fed with substrate coming from livestock manure, at the HRT of 22.3 d. The retaining factor concept described [3] was used. The retaining factor R_b was estimated through fitting of the COD data for a 102 days transient operation as the PABR was already operating in a "steady-state". The best value found was R_b= 0.465. As for the Monod kinetics parameters, the best values estimated were: K_s=0.1034, μ_{max}= 0.0732. To conclude, the initial biomass fraction of the specific operational period of the PABR was estimated: XB_{ini}= 0.126g/L.

The model simulation as well as the experimental data for the dCOD in the four compartments of the PABR is presented in Fig. 10, 11, 12 and 13:

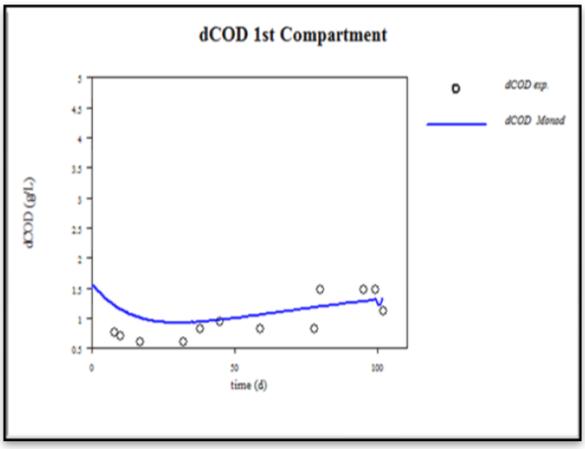

Fig. 10 dCOD Compartment 1 (experimental-model)

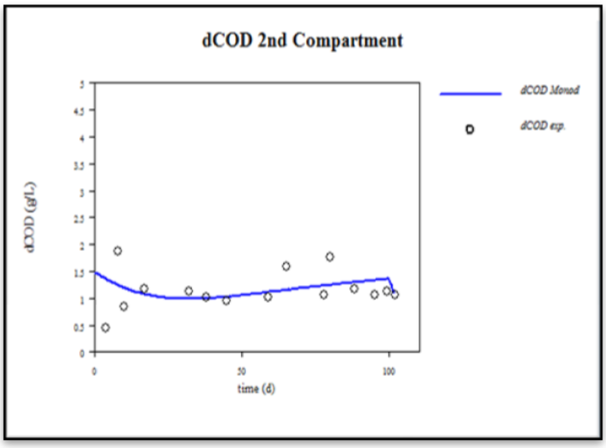

Fig. 11 dCOD Compartment 2 (experimental-model)

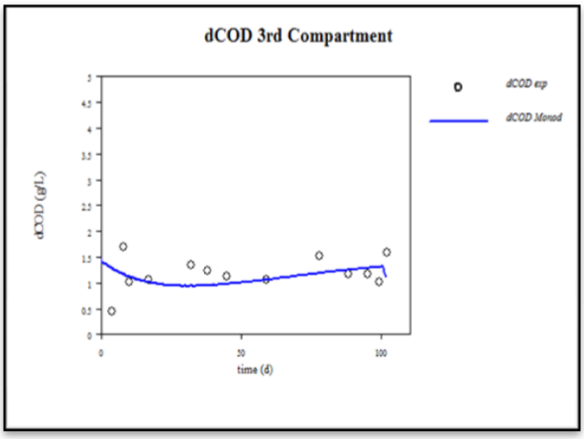

Fig. 12 dCOD Compartment 3 (experimental-model)

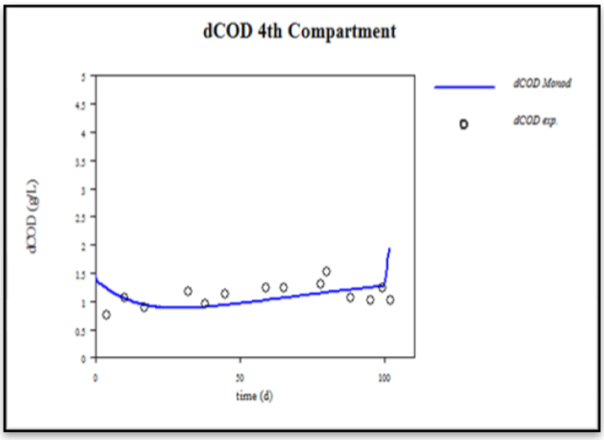

Fig. 13 dCOD Compartment 4 (experimental-model)

It is clear that the simple Monod kinetics model was able to satisfactorily describe the behavior of the PABR in terms of dCOD, while the values of the estimated parameters are reasonable.

Electricity production with MFC

After the startup of the MFC, using glucose as substrate the synthetic wastewater was replaced by the solid-free diluted livestock waste and whey supplemented with nutrients at the final concentration of 0.4 g dCOD/L. After the fresh wastewater addition into the anodic compartment, the MFC voltage increased rapidly reaching a practically constant value of 275 mV within only a few hours and after 68 h of operation the voltage dropped to zero (Fig. 14). The livestock waste and whey concentration was then sequentially increased, by addition in the anolyte of the proper amount of solid-free diluted livestock waste and whey after the end of each operation cycle, marked by the abrupt decrease of the MFC potential to practically zero. The operation cycles with initial concentrations 0.4, 0.8, 1.5 g dCOD/L were conducted in duplicate. The MFC voltage (R_{ext} = 1 kΩ) and the COD consumption versus time for the different initial concentrations are shown in Fig.14.

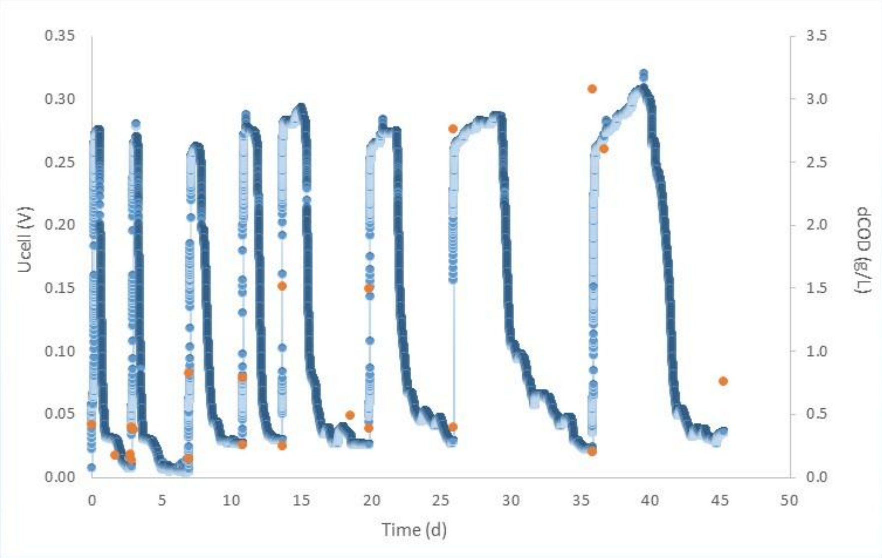

Fig. 14 MFC voltage U_{cell} and dCOD consumption versus time using the pretreated and filtered livestock waste and whey as substrate at different initial concentrations (1^{st} and 2^{nd} cycle = 0.4 g dCOD/L, 3^{rd} and 4^{th} cycle = 0.8 g dCOD/L, 5^{th} and 6^{th} cycle = 1.5 g dCOD/L, 7^{th} cycle = 2.8 g dCOD/L, 8^{th} cycle = 3.1 g dCOD/L. External resistance R_{ext} = 1kΩ).

As depicted in Fig. 14, the duration of the cycles increased with increasing the initial concentration of the substrate [7, 8, 14]. Fig. 15 shows the dependence of each cycle duration on the initial dCOD concentration. It is observed that there is a linear relationship between the duration of each cycle operation with the initial concentration according to the following equation: **y = 92.379 *x.** The dCOD removal efficiency remained practically constant (67-75%) for all the concentrations tested. This result indicates that the MFC could operate at higher wastewater concentrations, given the time needed for the substrate to be degraded.

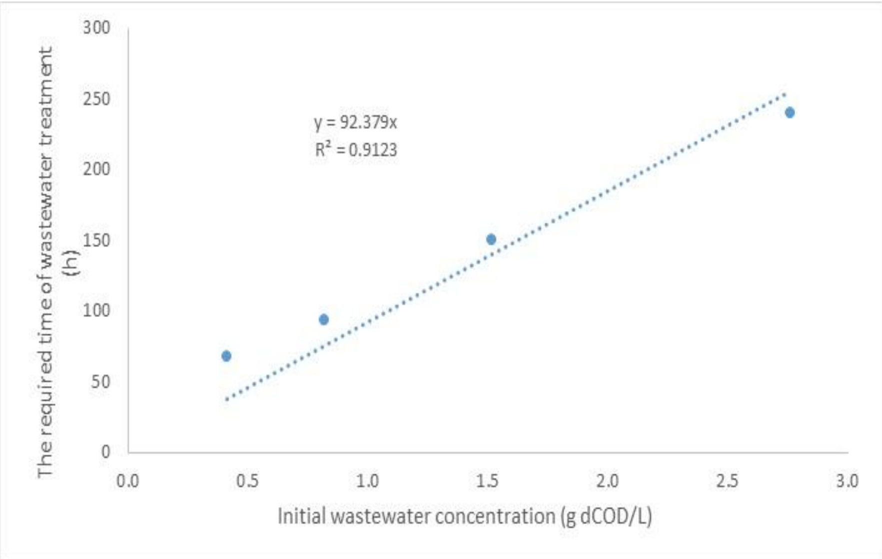

Fig. 15 Relationship between the required duration of each cycle operation with the initial concentration of the pretreated and filtered livestock waste and whey.

The experimental results show that the pretreated and filtered livestock waste and whey, with a relatively high strength (at least as high as 3.1 g dCOD/L), may be treated efficiently using a dual chamber MFC. The CE of the last cycle (cycle with the highest initial concentration) was approximately 2.1% which is quite low [7]. The low CE implies that most of the dCOD was removed by methanogens or other non-electrogenic microbes established in the anode rather than by electron transfer bacteria [7, 8, 15]. Relatively high COD removal efficiencies in MFC systems accompanied with low CE have also been reported for other wastewaters/substrates [16, 17].

Fig. 16 shows the dependence of the MFC voltage, U_{cell}, and the produced power density, P, on the current density passing through the MFC, at different initial wastewater concentrations. The data were obtained after the MFC voltage, for R_{ext} = 1 kΩ, had leveled off to a practically constant value, following the addition of the substrate in the anolyte (Fig. 14). As shown in Fig. 16, the maximum power density remains practically constant (close to 50 mW/m^2) for all cycles. The effect of substrate concentration on the maximum power density obtained in an MFC has been reported for substrates different than livestock waste and whey. However the conclusions drawn were not in the same direction in all cases, depending on the conditions [8, 18, 19]. Tremouli et al [8] studied the effect of substrate concentration on the maximum power density obtained in an MFC by working with a dual chamber MFC (R_{ext}=100 Ω) and was observed practically no change in the maximum power density for initial cheese whey concentration ranging from 0.35 to 6.7 g COD/L. On the contrary, an increase of the maximum power density (19.5% increase) with increasing the conductivity of the anolyte with the highest concentration (6.7 g COD/L) by duplicating the quantity of the phosphate buffer, was observed. The maximum power densities obtained in the present work are similar to those obtained in earlier studies using dual-chamber MFC systems with different organic substrates [14, 20, 21, 22]. The observation that similar maximum power densities were obtained with various substrates, in different concentration ranges, is consistent with previous findings that power generation in two-chambered MFCs is limited by neither the wastewater strength nor the reactions carried out in the anodic compartment. In this type of MFC, power generation is generally limited by the ohmic resistance which is high [8, 23] and is not affected by the bacteria or the specific substrates used [7, 8, 14, 24]. The almost constant slope of the polarization curves (Fig. 16) confirms the very significant contribution of ohmic losses in the dual chamber MFC of the present study.

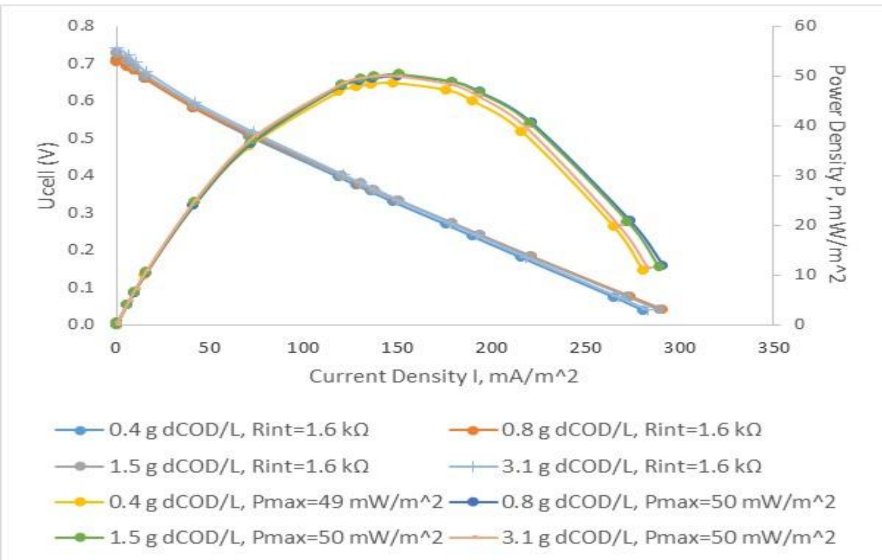

Fig. 16 MFC voltage Ucell and power density versus current density when using the pretreated and filtered livestock manure and whey as substrate at initial concentrations 0.4, 0.8, 1.5 and 3.1 g dCOD/L respectively.

4. CONCLUSIONS

In the present study, a simple Monod-kinetics model was developed in order to simulate and predict the anaerobic co-digestion of the liquid fraction of a mixture of various types of livestock manure and whey in a pilot scale Periodic Anaerobic Baffled Reactor. The inclusion of a biomass retaining factor R_b was important for the model to be able to describe the operation of the PABR, since its behavior regarding the relationship between the HRT and the solids retention time differs from the CSTR-type digesters. The simple model was able to adequately predict the COD concentration in each PABR compartment in comparison with the experimental data.

It was also shown that power generation is possible using filtered pretreated livestock waste and cheese whey as substrate (electron donor) in a two-chamber MFC. Relatively high dCOD removal and power density were achieved for the concentrations tested. The highest value of coulombic efficiency was obtained at the highest dCOD concentration but was in low levels, which could be attributed to the biochemical oxidation of the organic substrate by nonelectrogenic microbes contained in the livestock and cheese whey wastewater. The experiments showed that the period of time needed to degrade the substrate increases linearly with the substrate concentration. In addition, the dCOD removal efficiency as well as the maximum power density seems not to be affected by wastewater strength. Further study is needed in order to elucidate the impact of specific wastewater characteristics and to optimize the MFCs performance.

5. REFERENCES

1. Michalopoulos, I., Mathioudakis, D., Chatzikonstantinou, D., Papadopoulou, K., Lyberatos, G.: Anaerobic Co-digestion in a pilot-scale periodic anaerobic baffled reactor (PABR) and composting of animal by-products and whey. Albi : 6th International Conference on Engineering for Waste and Biomass Valorization, 2016.

2. Reicher, P.: User Manual of Aquasim for the Identification and Simulation of Aquatic Systems. Dubendorf: Swiss Federal Instityte for Environmental Science and Technology (1998)

3. Stamatelatou, K., Kopsahelis, A., Blika, P., Lyberatos, G.: Anaerobic Digestion of Olive Mill Wastewater in a Periodic Anaerobic Baffled Reactor (PABR). Patras: 4th BioRemediation Conference (2009)

4. Skiadas, I.V., Gavala, H.N., Lyberatos, G.: Modelling of the periodic anaerobic baffled reactor (PABR) based on the retaining factor concept. Water Research, Patras: Elsevier, Vol. 34 (2000)

5. Antonopoulou, G., Alexandropoulou, M., Lytras, C., Lyberatos, G.: Modeling of Anaerobic Digestion of Food Industry Wastes in Different Bioreactor Types. Waste and Biomass Valorization. Vol. 6 (2015)

6. Hernandez-Fernandez, F.J., Perez de los Rios, A., Salar-Garcia, M.J., Ortiz-Martinez, V.M., Lozano-Bianco, L.J., Godinez, C., Tomas-Alonso, F., Quesada-Medina, J.: Recent progress and perspectives in microbial fuel cell for bioenergy generation and wastewater treatment. Fuel Processing Technology. Vol. 138 (2015)

7. Stamatelatou, K., Antonopoulou, G., Tremouli, A., Lyberatos, G.: Production of Gaseous Biofuels and Electricity from Cheese Whey. Ind. Eng. Chem. Res. 2011, Vol. 50.

8. Tremouli, A., Antonopoulou, G., Bebelis, S., Lyberatos, G.: Operation and characterization of a microbial fuel cell fed with pretreated cheese whey at different organic loads. Bioresource Technology. Vol. 131 (2013)

9. Tremouli, A., Intzes, A., Intzes, P., Bebelis, S., Lyberatos, G.: Effect of periodic complete anolyte replacement on the long-term performance of a four air cathodes single chamber microbial fuel cell. J Appl. Electrochem. Vol. 45 (2015)

10. Oh, S.-E., Min, B., Logan, B.E.: Cathode performance as a factor in electricity generation in microbial fuel cells. Environ. Sci. Technol. 38 (18), 4900-4904 (2004)

11. APHA, AWWA, WEF: Standard Methods for the Examination of Water and Wastewater. Washington, DC: American Public Health Association (1995)

12. Josefsson, B.: Rapid spectrophotometric determination of total carbohydrates – from Methods of Seawater Analysis. In: Grasshoff, K., Ehrhardt M., Krenlig, K. (Eds) Verlag Chemie GmbH, pp 340-342. Weinheim Germany (1983)

13. Skiadas, I.V., Lyberatos, G.: The periodic anaerobic baffled reactor. Water Sci. Technol. Vol. 38, Issues 8-9, 401-408 (1998)

14. Min, B., Kim, J.R., Oh, S.E., Regan, J.M., Logan, B.E.: Electricity generation from swine wastewater using microbial fuel cells. Water Res. 39, 4961-4968 (2005)

15. He, Z., Huang, Y., Manohar, A.K., Mansfeld, F.: Effect of electrolyte pH on the rate of the anodic and cathodic reactions in an air-cathode microbial fuel cell. Bioelectrochemistry. Vol. 74 (2008)

16. He, Z., Minteer, S., Angenet, L.: Electricity generation from artificial wastewater using an upflow microbial fuel cell. Environ. Sci. Technol. 39, 5262-5267 (2005)

17. Jadhav, G.S., Ghangrekar, M.M.: Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration. Bioresour. Technol. 100, 717-723 (2009)

18. Liu, H., Cheng, S., Logan, B.E.: Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. Environ. Sci. Technol. 39, 658-662 (2005)

19. Cheng, S., Logan, B.E.: Increasing power generation for scaling up single-chamber air cathode microbial fuel cell. Bioresour. Technol. 102, 4468-4473 (2011)

20. Kim, J.R., Min, B., Logan, B.E.: Evaluation of procedures to acclimate a microbial fuel cell for electricity production. Appl. Microbiol. Biotechnol. 68, 23-30 (2005)

21. Logan, B.E., Murano, C., Scott, K., Gray, N.D., Head, I.M.: Electricity generation from cysteine in a microbial fuel cell. Water Res. 39, 942-952 (2005)

22. Bond, D.R., Lovley, D.R.: Electricity production by Geobacter sulfurreducens attached to electrodes. Appl. Environ. Microbiol. 69, 1548-1555 (2003)

23. Antonopoulou, G., Stamatelatou, K., Bebelis, S., Lyberatos, G.: Electricity generation from synthetic substrates and cheese whey using a two chamber microbial fuel cell. Biochem. Eng. J. 50, 10-15 (2010)

24. Min, B., Cheng, S., Logan, B.E.: Electricity generation using membrane and salt bridge microbial fuel cells. Water Res. 39, 1675-1686 (2005a)