



# Optimization of biogas production by co-digesting whey with diluted poultry manure

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## Abstract

A series of laboratory experiments were performed in continuously stirred tank reactors at mesophilic conditions, fed semi-continuously with various mixtures of diluted poultry manure and whey. Co-digestion of whey with manure was proved to be possible without any need of chemical addition up to 50% participation of whey (by volume) to the daily feed mixture. Up to this point, specific biogas production ( $L/kg VS_m$ ) remained roughly unchanged at the various whey fractions added in the feed mixture, mainly due to the lower chemical oxygen demand (COD) of whey compared to that of manure. At whey fractions above 50%, the reactor turned to be unstable, as shown by the considerable decrease in pH and biogas production. The experiments were scaled up to a continuously stirred pilot tank reactor, which had previously been acclimated to poultry manure digestion. Whey was gradually introduced in the feed, at increasing rates, replacing equivalent volumes of manure, in such a way, that total COD of the feed remained constant. For an hydraulic retention time of 18 days at 35 °C and organic loading rate of 4.9 g COD/L<sub>R</sub> d, it was found that biogas production increased from 1.5 to 2.2 L/L<sub>R</sub> d (almost 40%). This could be mainly attributed to the higher biodegradability of carbohydrates (main constituent of whey) compared to lipids (main constituent of manure) and to the correction (increase) of C:N ratio. © 2007 Elsevier Ltd. All rights reserved.

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## 1. Introduction

Cheese factories produce two streams of wastes: the washing water of the floor and equipment (mixed with detergents and remnants of milk in pipes, machines, etc.) and the cheese whey which actually is the liquid remaining after the precipitation and removal of milk casein during the cheese-making process. The wastewaters of the first stream are of low organic load and are usually treated on site in aerobic treatment installations. Whey, although in much less volume (about  $\frac{1}{3}$  that of the wastewaters), is of very high organic load and consequently, has a very strong polluting potential to be allowed for direct disposal on land or water courses.

Several possibilities have been assayed for whey exploitation over the last 50 years. Nevertheless, cheese producing units usually do not proceed with investments for recovery of the valuable constituents (like casein) contained in whey and so approximately half of world whey production is not treated, but discarded as waste effluent. But even after the exploitation of whey to recover proteins, the waste stream remaining has also a very high pollution load (mainly lactose contained in the permeate) and thus its direct disposal still constitutes a major environmental problem. Since whey has a very high biological oxygen demand (BOD), usually 30–50,000 mg/L, and a typical chemical oxygen demand (COD) value of 60–100,000 mg/L, anaerobic digestion is the only viable biological method for treating (or at least pre-treating) this wastewater. In addition to the environmental advantages of the method, anaerobic digestion can produce biogas, which can be used to cover thermal energy required for steam production during the cheese-making process. Alternatively, it could be used, within limits, to cover electrical power used for the aerobic treatment of the low organic waste stream, the washing waters.

Various types of anaerobic reactors have been used in laboratories to anaerobically treat whey, ranging from Upflow Anaerobic Sludge Blanket (UASB) to the simplest Plug Flow (PF) reactor type. Most of the anaerobic reactor types tried have achieved quite satisfactory removals of COD. Lo and Liao [1] achieved more than 75% COD removal in lab-scale rotating biological (anaerobic) contact reactors for hydraulic retention times (HRT) down to 5 days. Yan et al. [2] achieved even higher COD removal (over 98%) in a 17.5 L lab-scale UASB reactor. A little bit earlier, Lo and Liao again [3] had examined a two-phase system, consisted of a completely mixed reactor (for the acidogenic phase) and a rotating biological (anaerobic) contact reactor (for the methanogenic phase), and concluded an increase in methane production and a higher decrease in COD compared to one-phase systems. At the same time, Canovas-Diaz and Howell [4] investigated the anaerobic digestion of deproteinized whey in a down-flow fixed-film anaerobic reactor. Comparing fully to partly flooded operation they found that under organic or hydraulic overloading the former had higher levels of volatile acids, while the latter produced slightly higher methane at lower hydraulic retention times. Patel et al. [5] examined the application of upflow fixed film anaerobic reactor with high COD in the influent (70 g/L) and achieved a removal of up to 81%. Malaspina et al. [6] achieved a much higher COD removal (up to 98%) in a hybrid reactor with a down-flow (pre-acidification) and an up-flow chamber (for biomethanation).

Whey contains a significant amount of carbohydrates (typically 4–5%), mainly lactose  $C_{12}H_{22}O_{11}$ , which consists of equal amounts of glucose and lactose. It also contains proteins not exceeding 1%, fats at about 0.4–0.5%, lactic acid less than 1%, salts that may range from 1% up to 3%, amounts of milk minerals and all the water-soluble vitamins.

The pH of whey is close to 4.5 or lower and its alkalinity roughly exceeds 50 meq/L. Although it has a very high biodegradability (close to 99%), it constitutes a difficult substrate to treat (especially in highly loaded reactors) due to its high organic content and its low bicarbonate alkalinity. Indeed, the high level of carbohydrates in it promotes the growth of acid forming bacteria, but have negative effect on methane-producing bacteria [7]. In addition, in some cases, whey might contain an increased concentration of  $\text{Na}^+$  ions, which may become detrimental to the anaerobic reactor efficient operation.

To cope with the above problems, co-digestion of whey with livestock and poultry wastes has been investigated. Lo and Liao [3] shown that co-digestion with dairy manure provides the necessary nutrients and buffer capacity. As a result, they obtained, in a two stage system, an overall COD reduction over 46%. Ghaly [8] examined a two-stage, two-phase, unmixed anaerobic reactor of 155 L to treat whey with dairy manure and concluded that pH should be controlled at the methanogenic phase, otherwise production of biogas was not possible. Another source of manure is poultry manure, which could increase the nitrogen content of the reactor liquid and thus support the growth of methanogens. According to Desai et al. [9], the combination of whey and poultry manure had been found to be capable of maintaining the proper C/N ratio in the reactor. It has been shown that the digestion of the mixture of these wastes was more efficient in producing methane than of each material individually. Although previous studies have shown in lab-scale reactors that co-digestion of whey with manure can be advantageous, no optimization of the co-digestion process has been tried until now, taking into account that whey is a rather seasonal by-product (in Greece for example, sheep milk is processed only from October to June). In this study, the optimization of co-digested mixtures of diluted poultry manure with whey, in lab-scale anaerobic reactors has been investigated. The outcome of the co-digestion was then further tested and confirmed in a pilot-scale anaerobic reactor.

## 2. Materials and methods

A series of 4 lab-scale CSTR reactors and a pilot tank reactor, of the same type, were used in this work for both, the laboratory and the pilot-scale experiments. The wastes were treated without any dilution (COD values above 40,000 mg/L) and at this state, CSTRs are preferable due to their simplicity [10]. For the lab-scale experiments a series of four cylindrical metallic anaerobic reactors were used, with a working volume of 25 L each. The influent mixture of manure and whey, has been added on a 48-h basis to each reactor and processed anaerobically under mesophilic conditions (temperature set at  $35 \pm 2$  °C) and hydraulic retention time 20 days. At the beginning, all reactors were fed with manure only, until steady-state conditions were achieved (shown by constant pH and biogas production rates). Then whey had been added to the influent manure, replacing an equal volume of manure, so that the total influent volume to remain constant, as determined by the retention time. Whey was added at slowly increasing rates to avoid any adverse effect to reactors stability, until its fraction in the influent had reached the predetermined levels of 15%, 25%, 35% and 50% (v/v), correspondingly.

The pilot-scale experiment was carried out in a fully automated unit, manufactured by Didacta Italia Srl. The unit had a 100-L working volume equipped with a heating and agitation system, an arrangement for separation and recycling of sludge and a biogas cleaning and upgrading system. The reactor was set to operate in the mesophilic temperature range and at a hydraulic retention time of 20 days. Biogas production had

been measured with a diaphragm-type volume-meter automatically adjusted for temperature.

Standard methods [11] were applied for pH measurement and the determination of total solids (TS), volatile solids (VS), COD, alkalinity, ammonia nitrogen, total nitrogen, phosphorous, phenols and lipids. Proteins were calculated by multiplying the organic bound nitrogen (assumed to be equal to total nitrogen–ammonia nitrogen) by 6.25. Methane content had been determined volumetrically by passing biogas through a strong NaOH solution for CO<sub>2</sub> absorption. Volatile acids were estimated by esterification with ethylene glycol and then esters determination by the ferric hydroxamate reaction [12]. Carbohydrates were determined as the fraction of VS remained after subtraction of proteins, lipids and VFA.

### 3. Results and discussion

#### 3.1. Waste characterization

Whey had been supplied by a cheese manufacturing unit in Argos area (Southern Greece), and kept in a freezer until its use. Manure had been obtained from poultry manure at a semi-solid state (TS content of about 25% by weight), which was then diluted with tap water up to a TS content below 6% and a COD value around 100 g/L. Diluted manure was then screened for removal of the gross inert material and then, in the case of the pilot experiment only, it was further blended for homogenization to avoid clogging of the inlet pipes. Characteristic values of chemical analysis of the manure and whey used are presented in Table 1. Carbon content in the waste (used to estimate C:N ratio) was predicted, based on typical chemical formulas for carbohydrates (C<sub>6</sub>H<sub>10</sub>O<sub>5</sub>)<sub>n</sub>, proteins C<sub>5</sub>H<sub>7</sub>NO<sub>2</sub> and lipids C<sub>57</sub>H<sub>104</sub>O<sub>6</sub>, according to Angelidaki and Sanders [13].

Table 1  
Characteristics of diluted poultry manure and whey used in the experiments

Parameter	Manure	Whey
pH (at 22 °C)	7.4	3.5
Total solids (% w/w)	6.3	7.8
Volatile solids (% w/w)	3.7	4.8
COD (g O <sub>2</sub> /L)	103.6	74.9
Ash (% w/w)	2.4	2.8
Ammonia nitrogen (g/L NH <sub>3</sub> -N)	5.55	0.06
Total nitrogen (g/L N)	6.81	1.02
Alkalinity (g/L)	22.6	0
Proteins (% w/w)	0.8	0.6
Oil and grease (% w/w)	2.7	0.7
Carbohydrates (% w/w)	0.3	3.6
Phenols C <sub>6</sub> H <sub>5</sub> OH (mg/L)	137	0.5
Phosphorous (g/L)	1.5	0.3
COD:N ratio	15.2	73.4
C:N ratio <sup>a</sup>	3.9	24.1

<sup>a</sup>Carbon content estimated, based on typical chemical formulas for proteins, lipids and carbohydrates, according to [13].

From the data of Table 1 it becomes obvious that manure had a high level of ammonia-nitrogen (5.55 g/L), while whey, a much lower one (60 mg/L). Similarly, manure had a high alkalinity (22.6 g/L), while whey almost zero. The pH of whey was as low as 3.5. Finally, manure had a high content of lipids (more than 70% of VS), while whey had a high content of easily biodegradable carbohydrates (almost 75% of VS). From the above comparison it could be concluded that co-digestion of these two wastes is advantageous than processing each one separately. This can be explained by the availability of the necessary nitrogen and organic carbon to bacteria from manure and whey, respectively. In addition the alkalinity created by nitrogen presence enhances the stability of the process.

### 3.2. Laboratory experiments

The four lab-scale reactors were fed with the aforementioned mixtures of whey in manure (15%, 25%, 35% and 50% v/v) until steady-state conditions were established and then, effluent samples for 3 consecutive sampling periods were analyzed for TS, VS, pH and methane content in biogas. Volumetric biogas production was also measured and presented in Fig. 1 for all reactors studied, together with the variability of VS in the manure and whey influent mixture. According to Fig. 1 it becomes obvious that optimum biogas production resulted at whey fraction of 35% v/v in the feed mixture. At a fraction of 50% v/v the system collapsed. Due to the variability of VS in the raw materials (in both manure and whey), it is not clear whether the increase in biogas production caused by the replacement of manure with whey in the influent mixture or by the total increase of VS at the different whey fractions. In order to evaluate the effect of VS variation, the specific biogas production in L/g VS<sub>IN</sub> has been plotted against the variation of whey VS fractions (these were higher than corresponding volumetric fractions, due to the higher organic content of whey), as shown in Figs. 2–5. A steady-state line indicating stabilization level is additionally presented in the figures. Steady state was assumed to be reached when the measured parameters were not changing more than 10% of the estimated mean value of the steady-state period.

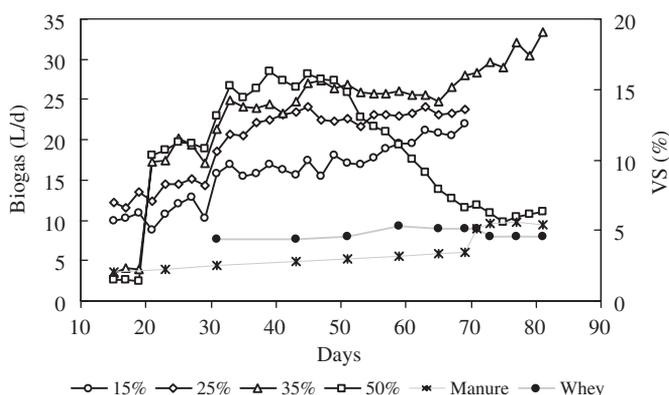


Fig. 1. Daily biogas production from the four lab-scale reactors processing manure/whey mixtures with 15%, 25%, 35% and 50% whey fractions (v/v), respectively. The two lower curves present the variation of VS (%) in each waste.

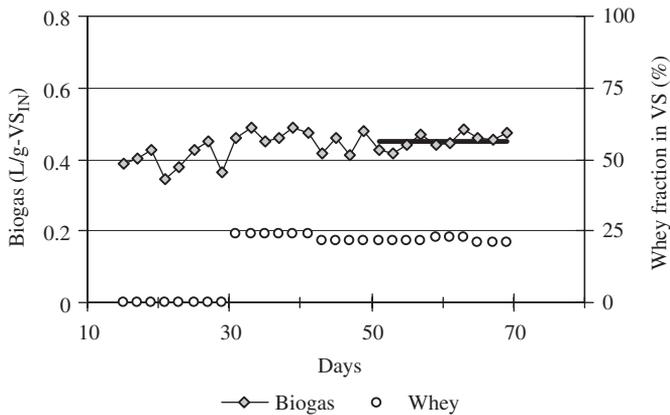


Fig. 2. Variation of whey fraction in the feed (by VS) and specific biogas production (L/g VS<sub>IN</sub>), in the first lab-scale reactor where volumetric fraction of whey in the feed reached 15% (v/v).

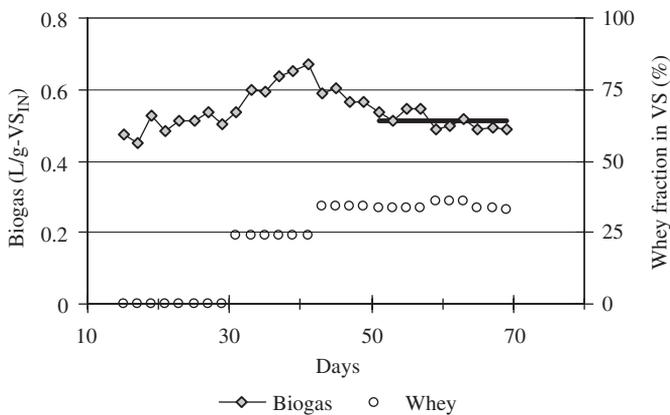


Fig. 3. Variation of whey fraction in the feed (by VS) and specific biogas production (L/g VS<sub>IN</sub>), in the second lab-scale reactor where volumetric fraction of whey in the feed reached 25% (v/v).

It should be noticed that with the only exemption of the reactor that was fed with 50% whey, the pH in all other three reactors remained alkaline. A slight pH decrease when co-treating whey in comparison to processing manure only, is attributed to the lower alkalinity and ammonium content of whey, as compared to manure. From Figs. 2–5 it becomes apparent that in steady state the specific biogas production was practically the same, at about 0.5 L/(g VS<sub>IN</sub>) (considering the theoretical biogas potential of 1. L(g VS<sub>destroyed</sub>), the above means 50% destruction of VS). However, a trend to decrease is obvious when whey fraction exceeds 50% (Fig. 4) and becomes very clear in Fig. 5, where whey fraction exceeds 60%. On the other hand, temporary surpassing of the above steady-state value—up to 0.7 L/(g VS<sub>IN</sub>)—may occur due to previously accumulated potential that came later in a form of overshoot.

The almost stable specific biogas production rate at the various influent mixtures applied, seems to be in contradiction with their energy content. Indeed, manure has a high

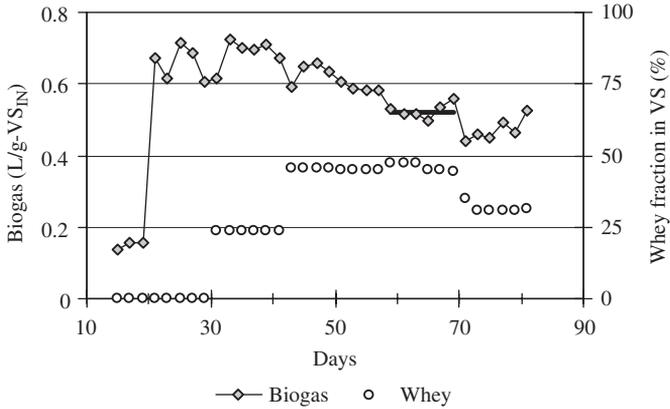


Fig. 4. Variation of whey fraction in the feed (by VS) and specific biogas production (L/g VS<sub>IN</sub>), in the third lab-scale reactor where volumetric fraction of whey in the feed reached 35% (v/v).

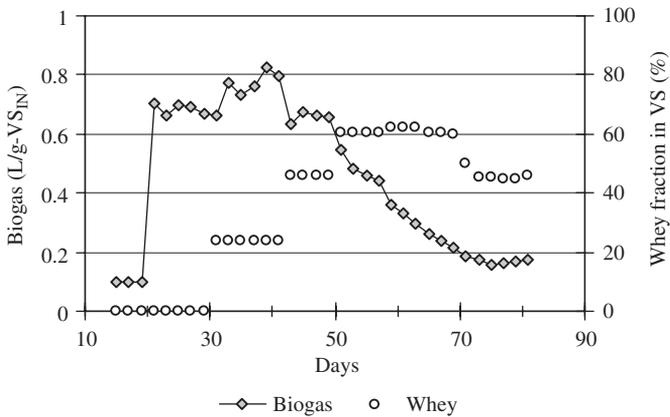
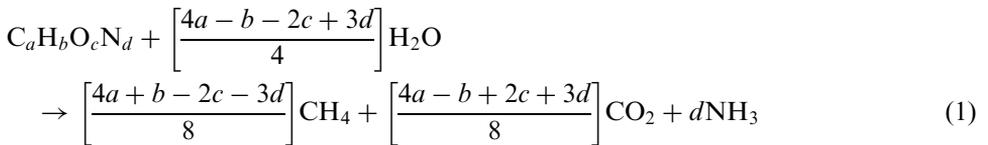


Fig. 5. Variation of whey fraction in the feed (by VS) and specific biogas production (L/g VS<sub>IN</sub>), in the fourth lab-scale reactor where volumetric fraction of whey in the feed reached 50% (v/v).

lipid content, while whey has a high percentage of carbohydrates. According to the general form of anaerobic digestion:



and the typical chemical formulas of the above constituents (see Section 3.1), it is concluded that when lipids are fully degraded they lead to a biogas production of 1.45 L/g VS<sub>destroyed</sub>, while carbohydrates lead to production of 0.83 L/g VS<sub>destroyed</sub> only (proteins produce 0.99 L/g VS<sub>destroyed</sub>). The fact that biogas production remained almost stable with

Table 2

Calculations for the estimation of specific biogas production from manure or from whey

	Manure	Whey
Dry solids (DS)	6.3% or 63 kg DS/t	7.8% or 78 kg DS/t
Volatile dry solids (VDS)	3.8% or 38 kg VDS/t	4.9% or 49 kg VDS/t
Carbohydrates/proteins/lipids	0.3/0.8/2.7% or 3/8/27 kg/t	3.6/0.6/0.7% or 36/6/7 kg/t
Destruction of carbohydrates/proteins/lipids	97/97/60%	97/97/60%
Biogas from each constituent	2.4/7.7/23.5 m <sup>3</sup>	29.0/5.8/6.1 m <sup>3</sup>
Total biogas production	33 m <sup>3</sup> /t	40.8 m <sup>3</sup> /t
Specific biogas production	0.88 m <sup>3</sup> /kg VDS	0.83 m <sup>3</sup> /kg DVS

the addition of whey to manure (for the same influent VS) could be justified by the partial degradation of lipids to biogas (e.g. in the range of about 60%) instead of the almost complete transformation of the easily biodegradable carbohydrates of whey. Really, by assuming 60% destruction for the lipids and 97% for carbohydrates (and the same for proteins), we estimate—according to the calculations of Table 2—specific biogas production per influent VS 0.88 L/g VS<sub>IN</sub> for the manure, and 0.83 L/g VS<sub>IN</sub> for the whey. Similarly, for the 35% v/v mixture of whey in manure we calculate specific biogas production at 0.86 L/g VS<sub>IN</sub>, which is indeed very close to the value estimated for manure alone.

The irrelevance of biogas production with whey fraction in a manure-based feed, that is concluded here, is in agreement with previous published results. Desai et al. [9] examined co-digestion of various mixtures of cattle dung and poultry waste, keeping unchanged the TS of the feed. Although the researchers had suggested an optimum mixture, by concisely presenting the results here, as per Fig. 6, no clear trend in biogas production is recognized when whey was added to the manure based feed.

### 3.3. Pilot plant experiment

Prior to addition of whey to the influent of the pilot plant, the unit has been processing manure only and had reached a steady-state operation at a pH = 7.7 (alkalinity 18 g CaCO<sub>3</sub>/L), OLR = 4.85 g COD/L<sub>R</sub> d, influent COD = 88 g/L and HRT = 20 days. The volumetric biogas production rate was 1.5 L/L<sub>R</sub> d and the COD removal about 70%. Whey was stepwise added to the feed up to a maximum percentage of almost 40% by COD, replacing an equivalent quantity of manure to keep the influent COD constant. The evolution of biogas production rate from the pilot reactor during the transient period changing from manure only to manure/whey mixture is shown in Fig. 7, together with the variation of whey fraction in the feed. From this graph becomes obvious that a significant increase in the biogas production rate from 150 to 220 L/d (increase about 40%) occurs, following the addition of whey in the feed. The possible reasons for this increase could be the following:

- (a) Improvement of COD:N and C/N ratios due to the addition of whey to manure. Initially COD:N ratio was very low (15:1) and when whey added at 35% v/v to the

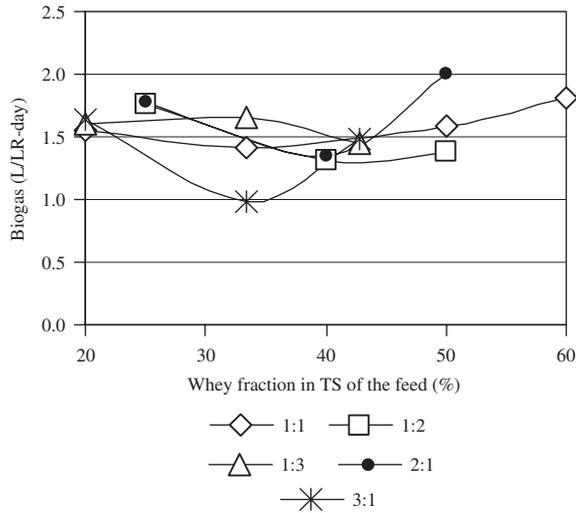


Fig. 6. Variation of volumetric biogas production (L/L<sub>R</sub>-d), as a function of whey fractions in the TS of the influent feed, for various mixtures of cattle manure (CM) and poultry waste (PW) (CM:PW ratios of the initial feed mixtures-to which whey was added-are expressed in the note by volume). In all cases, TS of the constituents and the influent feed was 6% (results based on the work of Desai et al. [9]).

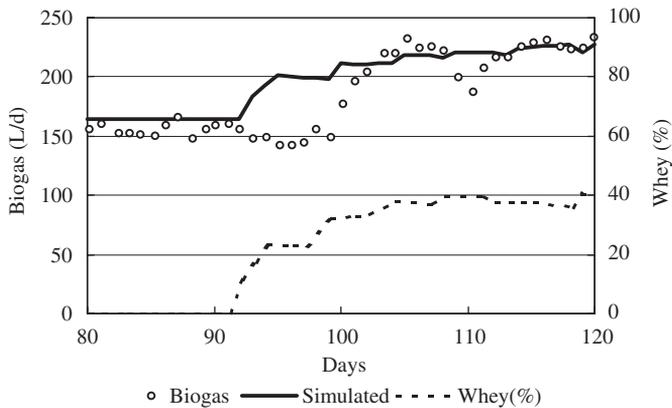


Fig. 7. Biogas production rate from the pilot plant and simulation results. The whey fraction in the influent feed mixture (as COD) is also presented.

influent mixture this ratio increased to:

$$\frac{0.65 \times 103.6 + 0.35 \times 74.9}{0.65 \times 6.81 + 0.35 \times 1.02} = 20 : 1.$$

Similarly, C:N ratio increased from approximately 4:1 (with manure, only) to about 5.5:1 when whey was added, and so it still remained quite lower from the optimum

suggested range of 13–28/1 [14]. As a result, only a very slight increase in biogas production rate could be attributed to C:N ratio increase.

- (b) Dilution of manure caused by adding whey and the decrease of nitrogen content of the initial feed, which could have a beneficial effect to biogas production rate. Actually, ammonia in manure was high, exceeding 4 g N/L, a value reported as inhibitory for the anaerobic digestion of cattle manure [15]. A threshold limit of free ammonia inhibition in swine manure digestion of 1.1 g N/L has also been reported [16]. In the present co-digestion experiments, addition of whey to the manure restricted total ammonia to 3.2 g N/L and free ammonia to 0.2 g N/L (estimated for 35 °C, pH = 7.8), values that are lower than the above limits. On the other hand, concentrations of free ammonia between 0.05 and 0.2 g/L were shown to be beneficial for bacterial growth [17]. So, it is probable that addition of whey may have alleviated any potential inhibitory effect of the initially high nitrogen content. At the same time, however, dilution with whey slightly decreased the free ammonia needed by the bacteria. Hence, the increase in biogas production rate hardly could be attributed to this factor.
- (c) Change in feed composition following the addition of whey to manure, from a waste rich in lipids to a mixture with an increased fraction of carbohydrates. Again, from the data presented by Angelidaki and Sanders [13] it can be concluded that, when fully degraded, carbohydrates produce biogas 0.7 L/g COD<sub>destroyed</sub>, while on the other hand lipids produce 0.5 L/g COD<sub>destroyed</sub>. This means that carbohydrates may lead to 40% higher gas production than lipids do. In our experiment, however, neither the initial feed was consisted only of lipids nor it was entirely changed to carbohydrates, and so the above argument, although more sound than the above two, may still only partly explain the 40% increase in biogas production.
- (d) Higher biodegradability of whey when compared to manure. As it has already mentioned, whey can be completely biodegraded while manure only at a percentage of about 70%. Indeed, manure has in general low biodegradability due to content of biofibers that contain a large proportion of lignin [18,19], while on the other hand whey contains mainly readily degradable organics. Hence, whey may lead to a 40% higher biogas production rate than an equivalent (same total COD) quantity of manure. Taking into consideration that whey was introduced in the feed at about 40% in terms of COD, it can be concluded that about 15% higher biogas production rate should be expected due to the complete biodegradation of whey.

As a conclusion, none of the above arguments seems to be able to explain adequately by itself the significant increase in biogas production rate resulted from the co-digestion of whey and manure. This increase is rather due to the combined effect of all above arguments, with the last two, however, being the most important.

### 3.4. Simulation results

In order to better evaluate our work, we proceeded with the simulation of the pilot-scale experiment. For this aim we applied a detailed model that takes into consideration the specific composition of the feed, as distinguished to proteins, lipids and carbohydrates. The model regards six biological processes namely hydrolysis (of proteins, lipids and cellulose), fermentation (of sugars and aminoacids), anaerobic oxidation (of long chain fatty acids), acetogenesis, acetoclastic methanogenesis and hydrogenotrophic methanogenesis. The

Table 3  
Main reactions and constants assumed in the model

<i>Hydrolysis of proteins</i>	
Proteins (1) <sup>a</sup> → amino acids (1)	$K_H = 0.104 \text{ d}^{-1}$
<i>Hydrolysis of lipids</i>	
Lipids (1) → long chain fatty acids (1)	$K_H = 0.118 \text{ d}^{-1}$
<i>Hydrolysis of cellulose</i>	
Cellulose (1) → sugars (1)	$K_H = 0.146 \text{ d}^{-1}$
<i>Fermentation (with acetate inhibition)</i>	
Sugar and aminoacids (66) → propionate (20) + acetate (35) + hydrogen (11)	$\mu_{MAX} = 5.559 \text{ d}^{-1}$ $K_S = 28 \text{ gCOD/m}^3$ $K_I = 604 \text{ gCOD/m}^3$ $Y = 0.043 \text{ gVSS/gCOD}$
<i>Anaerobic oxidation</i>	
Long chain fatty acids (34) → acetate (23) + hydrogen (11)	$\mu_{MAX} = 0.382 \text{ d}^{-1}$ $K_S = 1.816 \text{ gCOD/m}^3$ $Y = 0.11 \text{ gVSS/gCOD}$
<i>Acetogenesis (with acetate inhibition)</i>	
Propionate (7) → acetate (4) + hydrogen (3)	$\mu_{MAX} = 0.111 \text{ d}^{-1}$ $K_S = 247 \text{ gCOD/m}^3$ $K_I = 181 \text{ gCOD/m}^3$ $Y = 0.018 \text{ gVSS/gCOD}$
<i>Aceticlastic methanogenesis</i>	
Acetate (1) → methane (1)	$\mu_{MAX} = 0.167 \text{ d}^{-1}$ $K_S = 56 \text{ gCOD/m}^3$ $Y = 0.026 \text{ gVSS/gCOD}$
<i>Hydrogenotrophic methanogenesis</i>	
Hydrogen (1) → methane (1)	$\mu_{MAX} = 0.695 \text{ d}^{-1}$ $K_S = 0.13 \text{ gCOD/m}^3$ $Y = 0.018 \text{ gVSS/gCOD}$

$K_S$  = saturation concentration (half-velocity concentration),  $K_H$  = hydrolysis constant,  $K_I$  = inhibition constant,  $\mu_{MAX}$  = maximum reaction velocity,  $Y$  = biomass yield coefficient.

<sup>a</sup>In parentheses ( ) are the coefficients for COD transformation.

simplified expressions of the biochemical reactions of the model—with their stoichiometry, as expressed in COD—and the values of its parameters (taken from [20] and adjusted for mesophilic conditions according to Arrhenius-type temperature dependence) are presented in Table 3. Hydrolysis reactions were assumed to follow first order kinetics, while the Monod equation was applied to all other reactions. The predictions of the model concerning the biogas released are also presented in Fig. 7 together with the experimental data.

The slightly delayed (almost 10 days) response of the reactor to the participation of whey in the feed may be justified by the time required for adaptation of microorganisms to the new feed. According to that, the change of the feed based on manure to a mixture containing whey resulted in an increase of acidogenic bacteria in the reactor, a change, however, that took place quite quickly and without introducing any instability to the anaerobic digestion process. Such a behavior was also observed by Lyberatos et al. [21] in a theoretical study investigating—among other—the change of feed of a reactor from pig and olive mill wastes to pig and dairy wastes. Actually, they had found that, at a HRT of 13 days, a change to an influent mixture of 67% pig and 33% dairy wastes (v/v) resulted to

Table 4

Microorganisms concentrations and biogas production characteristics before and after the feed change from manure to manure/whey mixture, as estimated by the model

	Manure	Manure/whey
<i>Microorganisms, in gVSS/m<sup>3</sup></i>		
Fermentors	446.6	1355.0
Oxidizers	6051.6	4447.1
Acetogens	52.0	167.3
Acetoclastic methanogens	1153.2	1283.0
Hydrogenotrophic methanogens	373.8	401.7
Methane production (L/L <sub>R</sub> d)	1.14	1.25
COD removal efficiency (%)	69.8	76.8
Carbon dioxide (L/L <sub>R</sub> d)	0.60	1.06
Biogas production (L/d)	164	221

almost doubling acidogenic population within 20 days. The predictions of the model concerning the microorganisms concentration in the reactor and biogas production characteristics are shown in Table 4. From this Table it becomes apparent an increase of fermentative and acetogenic bacteria compared to other microbial populations, which have remained unchanged. From Fig. 7 and Table 4 it is concluded that a model with the capability of distinguishing the specific synthesis of the feed, can still predict quite well the significant increase of biogas production rate, when co-digestion of manure and whey is taking place, although ammonia inhibition or nitrogen deficiency criteria were not included. This findings may confirm that the higher biogas production rate was mainly caused by the increased biodegradability of whey and its greater productivity in carbon dioxide.

### 3.5. Comparison between laboratory and pilot-scale experiments

For both experiments the same experimental conditions were used. From the lab-scale experiments it was concluded that by adding whey to manure, the specific biogas production (L/g VS<sub>in</sub>) remains almost unaffected. From the pilot-scale experiment instead, it was concluded that specific biogas production rate has significantly been increased if expressed in terms of influent COD. These conclusions are explained by the common fact for both cases: whey has a lower specific energy potential (expressed in terms of VS) mainly due to his higher content of carbohydrates compared to lipids. At the same time, it is more easily biodegradable. More specifically:

- (i) From both experiments it was proved that it is easy to change from processing manure only to a mixture of manure/whey. In addition, this feed change, even though it had been done fast enough, did not stimulate any instability of the process.
- (ii) The lab-scale experiments have shown that whey can participate in the feed to a percentage lower than 50% on a VS basis. In the same frame, pilot plant experiments were successful with a whey participation up to 40% on a COD basis.
- (iii) In the pilot experiment (where influent COD was attempted to be kept constant) it has clearly been apparent that specific biogas production (expressed in terms of COD

entering the reactor,  $L$  biogas/g COD<sub>IN</sub>) was significantly increased following the addition of whey to manure. The same conclusion, however, arises from the lab-scale experiments, where influent VS were rather constant. Actually, the steady-state specific biogas production ( $L$  biogas/g VS<sub>IN</sub>) in the lab-scale experiments remained unaffected from the addition of whey. However, for the same VS content in the feed mixture, COD decreases when whey replaces manure, as carbohydrates contain less COD (mg/L), than lipids. This can satisfactorily explain the increase in specific biogas production, when expressed in terms of influent COD.

#### 4. Conclusions

Whey was quantitatively degraded to biogas when co-digested with diluted poultry manure (COD removal increased from 70% to 77% when whey added to the manure), at mesophilic conditions in a CSTR, without addition of any chemicals. Biogas production rate remained actually stable with the increase of whey fraction—at a constant total VS content in the influent mixture—up to a whey fraction of 35%. At the fraction of 50% the stability of the reactor was adversely affected and a dramatic decrease in biogas production was obtained.

The safe limit of 50% whey fraction (based on VS) has been confirmed in a pilot plant reactor of 100 L, fed with a whey/manure influent mixture containing a whey fraction of 40% in terms of COD (equivalent to that of 50% in terms of VS). COD in the feed was 91 g/L, OLR 5 g/L<sub>R</sub> d and HRT 20 days. The reactor operated with no problem reaching quite soon stable conditions and producing biogas at the rate of 2.2 L/L<sub>R</sub> d.

Due to its high organic content and biodegradability, the most appropriate treatment method for whey is anaerobic digestion and it can be applied to existing facilities, already used for poultry manure digestion alone. As a result, co-digestion of whey with manure is a sustainable and environmentally attractive method to treat and simultaneously convert such a waste mixture to a useful energy source.

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