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Improved anaerobic digestion performances of whey in a batch reactor

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ABSTRACT/RESUME

Abstract: *Biogas and methane yields, under different operating conditions, and chemical oxygen demand (COD) removal efficiencies were investigated in a mesophilic batch reactor (38°C) for the mixtures of dairy manure (DM) and cheese whey (CW) at 7% or 9% of total solids (TS). Biogas production of 401 L/kg of volatile solids (VS) and methane yield of 215.3 L/kg of VS was obtained after an operating time of 100 days for the mixture containing 7% of TS. A maximum increase in biogas production of 92% compared to the start-up phase was achieved with a volume of 3.6 L of biogas on day 55. The corresponding methane yield reached a maximum value of 80%. The removal efficiency of the COD was 73%. Results show that anaerobic co-digestion of cheese whey and dairy manure with 7% of TS gives better results compared to 9% of TS.*

I. Introduction

Due to the implementation of a support program for agricultural sectors involved in milk production, dairy industries have recorded considerable growth during the last decade [1]. If we consider the FAO statistics, the Algerian cheese production (all kinds of cheeses) in 2018 reached 27 634 t, generating about 41 162 t of whey [2]. This amount of whey could generate as much as 1.41 million m3 of methane, which is equivalent to 3.97 GWh of electricity. Whey is a rich by-product that contains about 55% of the initial dairy nutrients [3]. The most abundant of these nutrients are lactose (4.5–5% w/v), soluble proteins (0.6–0.8% w/v), lipids (0.4–0.5% w/v) and mineral salts (8–10% of dried extract) [4, 5] Whey also contains appreciable quantities of other components, such as lactic and citric acids, non-protein nitrogen compounds and B group vitamins. According to the procedure used for casein precipitation, there are two main varieties of whey: acid whey (pH <5), resulting from the production of fresh or soft cheeses, and sweet whey (pH 6-7), resulting from hard cheeses [4- 8]. In terms of pollutant load, one liter of treated milk generates about 50 g of COD in whey and 10 g of COD in white water [9]. So, because of its high organic load, whey is a highly polluting effluent. The methanisation of this substrate is therefore an interesting alternative to the existing management/treatment process because, in addition to the environmental aspects, it is possible to convert a readily available source of organic matter into renewable energy, through the production of biogas. Therefore, anaerobic whey treatment offers a dual benefit of reducing pollution potential and biogas production, which can be an additional source of income when properly converted to heat and electricity. The produced bioenergy could be directly used to reduce fuel consumption in cheese processing plants. However, according to previous studies already undertaken [10], anaerobic digestion of whey alone results in an instability of the process due mainly to the acidification of the reaction medium; which may cause the inhibition of the methanogenic flora and the cessation of the methanation process. Its association with substrates with buffering capacity such as sludge, manure, or crop residues could be an alternative to avoid this possible inhibition risk [11]. In the present work, the mesophilic anaerobic co-digestion of cheese whey and dairy manure has been investigated in two batch reactors at 38 °C. A few studies have investigated the co-digestion of whey and the corresponding methane production potential [12-19].

However, the reported results show a real disparity that can be mainly caused by different parameters such as operating conditions, the substrate to inoculum ratio, and other parameters. We focus our study on mesophilic anaerobic co-digestion of cheese whey generated from one of the largest dairy industry in Algeria (Laiterie Fromagerie de Boudouaou) and the performances of each digester in terms of methane production, pollution reduction.

II. Materials and methods

II.1. Substrates

The effluent studied is the cheese whey (the soft whey) generated by the cheese dairy of Boudouaou, located in Boumerdes located in Algiers. It is made from a pressed cheese (Edam) and is still very rich in nutrients. Samples were taken from the coagulation tank (Figure 1) and stored in 2.5 L plastic jerricans at 4°C until analyzed and processed in the laboratory.

Figure1. *Coagulation tank containing sweet whey.*

Before each experiment, the substrate is removed from the freezer and defrost at room temperature for 24 h. The dairy manure used in this study comes from a private farm located in the commune of Bouzareah (in Algiers). The samples were made on fresh cow dung and were sent directly to the CDER laboratory. The dairy manure was directly weighed and put in the digesters. It is considered as an inoculum because it contains the microorganisms that will be used to degrade the nutrients contained in the whey. The main characteristics of the cheese whey and the dairy manure are reported in Table 1.

According to this table, the whey contains a very high pollutant load, represented by a COD of 134 g of O_2/L and has a fairly high level of fermentable organic solids, represented by the VS which is 98.3% (% MS); which makes it a good substrate for producing biogas.

On the other hand, the whey being acidic (pH 6.4) and the dairy manure being basic (pH 8.1) and of very high alkalinity. Their codigestion can improve the pH of the digester because of the buffering capacity of the dairy manure.

Table 1*. Physicochemical characteristics of the cheese whey and the dairy manure*

Parameter	CW	DM
pH	6.4	8.1
E_h (mV)	23.1	-69.1
TS %	7	16
VS (% of TS)	98.3	86.7
COD (g L^{-1})	134	17.4
SS (g L^{-1})	62.5	61
A_T (mg L^{-1})	0	1040
VFA (mgL^{-1})	37	11

**CW=cheese whey, DM=dairy manure, TS= total solids, VS=volatil solids, E_h=potential reduction, COD= chemical oxygen demand, SS=suspended solids, A_T=total alkalinity, VFA=volatil fatty acids*

II.2. Analytical methods

The system performance was tested by measuring biogas and methane productions, COD reductions, total solids (TS), volatile solids (VS), total alkalinity AT, pH, potential reduction (Eh), and volatile fatty acids (VFA) concentrations. The influent and effluent pHs were measured from samples with a glass electrode pH meter (WTW InoLab pH Level 1). The total alkalinity (AT) and the volatile fatty acids (VFA) were determined by titration at a pH of 4 and 3.5, respectively, according to the method described by Anderson [20]. All of the other analyses (TS, VS, and COD) were performed according to Standard Methods [21-23]. Biogas production of the system was determined daily by water displacement gas meter designed as a scaled measuring cylinder. Methane content biogas was measured using a gas analyzer Multitec 540.

II.3. Experimental set-up

Experimental studies were performed in the anaerobic reactor with a total volume of 2 L (Figure 2). The reactor was heated in a water bath equipped with a temperature controller (LAUDA E200) to maintain a constant temperature of 38 ° C. After that cheese whey and dairy manure was filled to the reactor, the reactor inlet was closed to prevent air leakage. The biogas produced is measured with a

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gas meter and stored in a special bag for analysis by a biogas analyzer.

1: Anaerobic digester
2: Thermo regulator
3: Bathwater
4: Gas meter
5: Gasbag
6: Syringe
7: Valve

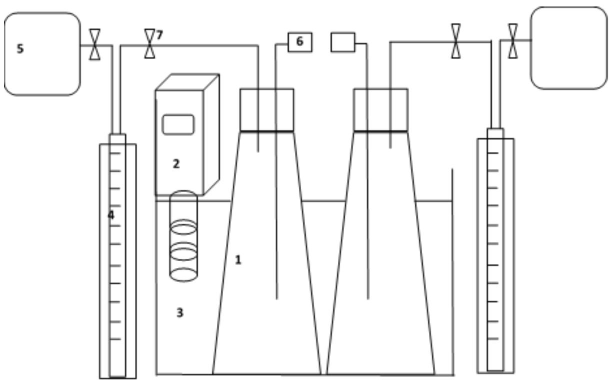


***Figure 2.** Experimental set-up: two anaerobic digesters in a heated bath, two gasometers and storing bags*

II.4. Digester startup

The physicochemical characteristics of the two substrates before starting the digesters I and II are given in Table 2. The two experiments were, carried out to study the effects of TS on the reactor performances.

***Table 2**. Main characteristics of digesters (I) and digester (II).*

Parameter	Digester I	Digester II
pH	6.9	7
E_h (mV)	-1.8	-8.7
TS %	9	7
VS (% of TS)	87.3	87.1
COD (g L^{-1})	88	66.4
A_T (mg L^{-1})	1600	1400
VFA (mgL^{-1})	30	10

III. Results and discussion

III.1. pH control

pH is the main control parameter of the anaerobic co-digestion process. To avoid acid inhibition of acetogenic and methanogenic bacteria, it is suggested to work at pH values between 6.5 and 8.5, since the acidic pH has a negative influence on methanation [24].
The pH was monitored to obtain maximum biogas production in a batch reactor by regular measurements, and sodium bicarbonate (1M) solution was used to adjust the pH to a required level. Figure 3 shows the evolution of pH for both anaerobic co-digestion processes (DI with 7% of TS and DII with 9% of TS). At startup, the pH values in D I and D II were 6.9 and 7.03, respectively. After 7 days of anaerobic co-digestion, the pHs dropped to 3.99 and 4.17 for DI and DII, respectively.
This is hydrolysis and acidogenesis phases. After adjustment with sodium carbonate (1M), pH increase up to 6.2 and 6.3 in DI and DII, respectively, after 15 days corresponding to acetogenic phase. During processes, pH values tend to stabilize around 6.2 and 6.4 for DI, and 6.6 and 7.73 for DII. pH values of DII correspond to the optimal pH range given according to Moletta [24].

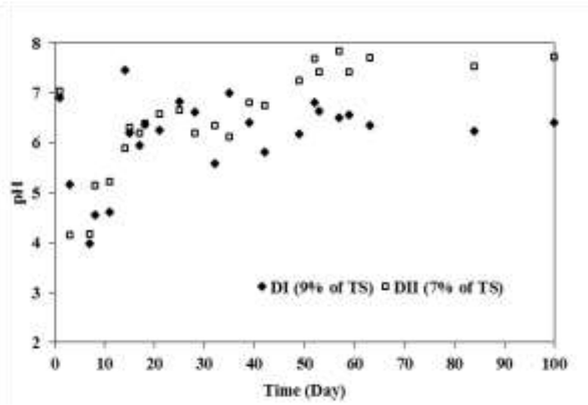


***Figure 3.** pH behavior during anaerobic co-digestion of dairy manure and cheese whey at 9% and 7% of TS.*

III.2. Potential reduction evolution

Figure 4 shows the evolution of potential reduction in two anaerobic digesters DI and DII. At start-up, reduction potentials (Eh) were -1.8 mV and -8.7 mV for DI and DII, respectively, and after 7 days of anaerobic co-digestion, potentials were up to 162.7 and 154.9 mV for DI and DII, respectively. After adjustment, the potentials decrease reaching an optimum of -28.8 for the DI and -69.1 for the DII. Those values indicate a correct bacteria activity in the digester. According to the literature reduction potentials from 50 to 0 mV are generally measured for digesters in full activity [25].

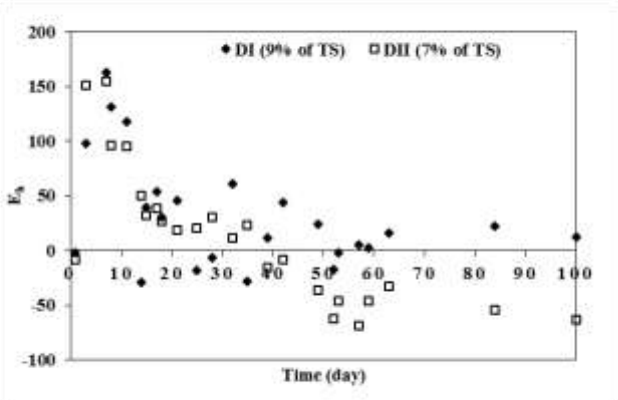


Figure 4. *Eh behavior during anaerobic co-digestion of dairy manure and cheese whey at 9% and 7% of TS.*

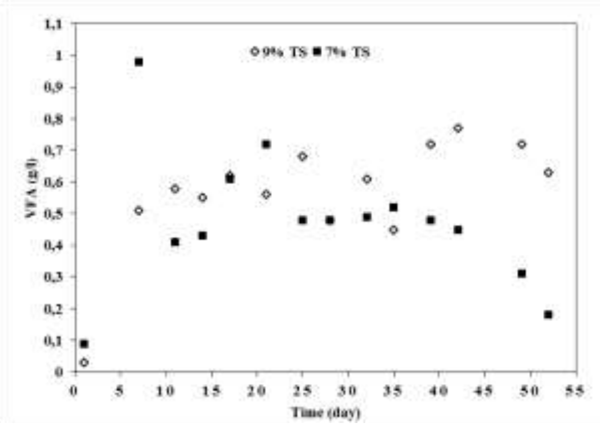


Figure 5. *Eh behavior during anaerobic co-digestion of dairy manure and cheese whey at 9% and 7% of TS*

III.3. VFA behavior

Monitoring the concentration of volatile fatty acids helps to ensure that the bioreactions are proceeding correctly. Indeed, the main cause of acidification of the environment is the accumulation of volatile fatty acids, which must be avoided. Figure 5 illustrates volatile fatty acids concentration evolution (expressed in g/L) as a function of the co-digestion time. The evolution curve of the AGVs in the digester I, can be divided into two phases:

Phase 1: observed during the first 11 days of digestion, with high production of AGV from 30 mg/L on the first day to 580 mg/L on the 11th day. This phase corresponds to the hydrolysis and acidogenesis phase.

Phase 2: During the phase, the AGV values remained too high, around 580 and 630 with a maximum of 770 mg/L. This accumulation of AGV has slowed down the activity of acetogenic bacteria according to Delfosse [26], which is reflected in a slowdown in the production of biogas and CH_4 (Figure 7).

While the evolution of AGV in the digester II, can be divided into three phases:

Phase 1: observed during the first 21 days since the beginning of digestion, with high production of AGV (from 90 mg/L on the first day to 720 mg/L on 21st day with a maximum of 980 mg/L) is recorded. This phase corresponds to hydrolysis and acidogenesis reactions.

Phase 2: This phase begins from 25th day to 42nd day when stabilization of AGV concentrations around 480 and 450 mg/L is noted. Unlike digester 1, there is no accumulation of AGV because they were converted to acetates, it is acetogenesis phase.

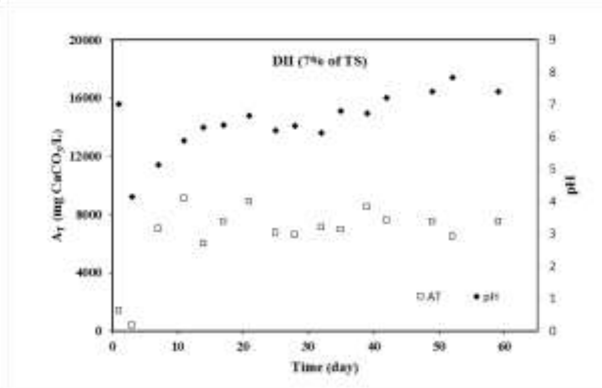
Phase 3: From day 42, there is a decrease in AGV concentrations from 450 to 180 g/L. This phase corresponds to the beginning of methane production (Figure 8); This is the methanogenesis phase.

III.4. Total alkalinity

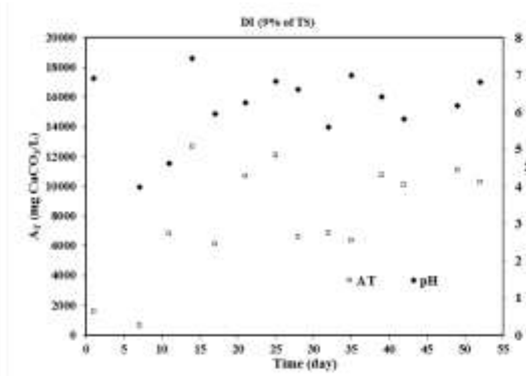
The digestion alkalinity represented by calcium bicarbonates concentration must be relatively high for the process to work It is generally considered that it is necessary to have at least 1000 mg $CaCO_3$/L alkalinity in a well functioning reactor [27].

Figure 6.a shows the evolution of alkalinity in digester I. Total alkalinity was 1600 mg of $CaCO_3$/L on the first day of co-digestion. With decreasing pH up to 3.99, alkalinity decreased to 631 mg $CaCO_3$/L. However, to ensure optimal functioning of digestion, it is recommended that alkalinity is between 1000 and 3000 mg/L $CaCO_3$ [24], below this level, corrective action must be taken, hence the interest of adjustments. After adjusting and increasing pH, alkalinity increased to 12 700 mg of $CaCO_3$/L on the 14th day. This may inhibit the process of anaerobic digestion. For, according to Grady [28] the use of the base for pH adjustment can lead to toxic effects that inhibit microorganisms if the concentration is greater than 8000 mg $CaCO_3$/L.

Figure 6.b illustrates the evolution of alkalinity in the second digester where total alkalinity was 1400 mg $CaCO_3$/L at startup. After adjustment, the alkalinity reached 9 100 mg of $CaCO_3$/L. Alkalinity can be disturbed by additions of sodium carbonate during adjustments, which explains these high values. With advancing anaerobic digestion, alkalinity values tend to stabilize at values below 8000 mg $CaCO_3$/L.



a.



b.

Figure 6. *A_T and pH behavior during anaerobic co-digestion of dairy manure and cheese whey at 9%TS in (a) and 7% TS in (b).*

III.5. Biogas production

Figure 7 shows the cumulative volume of biogas produced by digesters I and II during the 100 days of anaerobic co-digestion. It is clear that the total production obtained by the digester II is higher than that obtained by the digester I. Actually, it reached 42.2 L for digester II, and 9.1 L for digester I. This difference is due to the different digester behaviours regrading pH, A_T and COD reduction.

III.6. Methane production

During the first 20 days of digestion, a very low level of methane was observed (6%). After 24 days, the analyzed methane content is 36.7%, for a cumulative production of 5.4 L, the methane rate drops from 29% (the 46th day) to 36.5% (the 88th day), with a cumulative volume of 8.2 L.

According to (Fig.8.a.), the digester I remained in the starting phase during 100 days, because of the absence of water in the digester which is a very important factor in the rapidity of the methanation process. In fact, due to the high rate of dilution in digester II the hydrolysis phase is more efficient comparing to digester I. (Fig.8.b.) indicates that during the first 40 days of digestion, methane rates of 8% and 25% were obtained in the volumes analyzed. The methane levels rise from 50% to 77% for cumulated biogas of 37 L.

The optimum methane rate was reached on the 53rd day (80% of CH_4). After that, the methane rate decreased from 68% to 45% for a cumulative volume of 42 L. Unlike digester I, digester II operated correctly by including the four phases of the methanation process. This is mainly due to the small TS characterizing this digester.

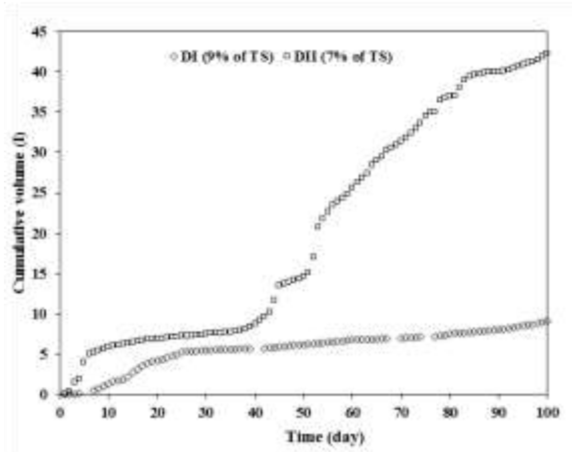


Figure 7. *Evolution of biogas produced by digesters I and II*

III.7. Biomethane potential and depollution performances

The table below includes the initial and final characteristics of the substrates in digester I and II in terms of pH and COD, as well as the performance of both digesters for 100 days of anaerobic co-digestion

In the second digester, the concentration of the COD at the inlet was 66 g of O_2/L, and at the outlet 17.8 g of O_2/L. This gives us a reduction in COD of 73%, and corresponds to a considerable decrease in the organic matter while in the first digester, the COD abatement was only 48%. Another very important parameter for calculating the performance of a digester is the specific methane yield, also called "methane potential", expressed in L of CH_4/Kg of VS. According to Table 3, the anaerobic co-digestion performed for 100 days, without agitation, resulted in methane yields of 14.2 and 215.3 L of CH_4/Kg of VS for digester I and II, respectively. Anaerobic co-digestion in digester II followed the expected methanation steps and the trend in biogas production was similar to those observed previously [16, 13, 19]

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a. **b**

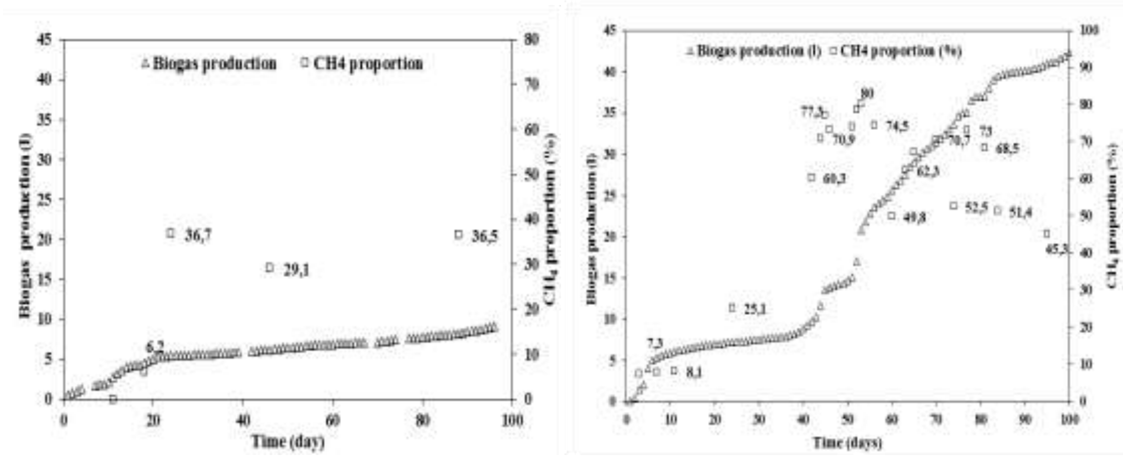

Figure 8. *Process performance during anaerobic co-digestion of dairy manure and cheese whey at 9% TS in (a) and 7% TS in (b)*

III.8. Comparison with anaerobic co-digestion processes of cheese whey and dairy manure

Several studies have attempted to address anaerobic co-digestion of whey and dairy manure. A comparison of the results of this work with other work on anaerobic co-digestion of whey and dairy manure is presented in Table 4.

Rico [12] tested the process with CW fractions higher than 50% in the feed mixture but obtained a very low methanogenic yield (182 L CH_4/KgVS). Comino [14] reported that anaerobic co-digestion of CW and dairy manure was possible with CW fraction up to 65%, but the best yields were obtained with a 50 % fraction in the feed, with 211.4 and 621 L CH_4/Kg VS in [16] and [14] respectively. Bertin [13] performed a two-stage anaerobic co-digestion process with a 50% CW fraction in the feed and achieved 258 L CH_4/Kg VS of methane yield. These authors did not attempt the process with more CW fractions because they observed acidification with fractions of CW higher than 60%. On the other hand, Labatut [19] achieved an attractive yield (252.4 L CH_4/Kg VS) with 75% CW in the feed. The methanogenic yield obtained by the present study is fairly close to those obtained by Bertin 2013, Labatut [19] and Comino [16], and quite high that obtained by Rico [12].

Table 3. COD reduction efficiency and methane yield for both digesters

	Effluent		**Influent after 100 days**		**COD abatement (%)**	**Methane yield (L-CH_4/ Kg-VS)**
	pH	DCO (g of O_2 / L)	pH	DCO (g of O_2 / L)		
Digester I	6.9	88	6.5	45.5	48.3	14.2
Digester II	7.0	66.4	7.7	17.8	73.2	215.3

Table 4. Operational features of successful continuous anaerobic co-digestion systems for cheese whey and dairy manure.

Substrates	**Ratio**	**Reactor type**	**T (°C)**	**CH_4 in biogas (%)**	**Methane yields (L CH_4/Kg VS)**	**References**
CW:DM	50:50	One-stage CSTR	35	51,4	211,4	Comino et al. (2009)
CW:DM	50:50	One-stage CSTR	35	55	621	Comino et al. (2012)
CW:DM	50:50	Two-stage CSTR	35	60	258	Bertin et al. (2013)
CW:DM	50:50	One-stage batch reactor	38	70	215,3	This study
CW:sDM	65:35	One-stage CSTR	35	53	182	Rico et al. (2015)
CW:DM	75:25	BMP assay	35	NR	252,4	Labatut et al. (2011)
CW:sDM	85:15	One-stage CSTR	35	57	182	Rico et al. (2015)

CW: cheese whey; DM: dairy manure; sDM: screened dairy manure; NR: not reported.

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IV. Conclusion

Methanisation of cheese whey generated by one of the most important milk industries in Algeria showed how it was possible to produce valuable renewable energy while reducing in parallel its very high organic pollution. The comparative study of two anaerobic co-digestion by monitoring the biogas production for 100 days reveals that digester containing 50/50 whey and dairy manure with 7% of TS gives better results compared to the digester with 9% of TS.

The biogas production reached 401 L/kg of volatile solids with an equivalent methane yield of 54%. The AGV control indicated that hydrolysis and acidogenisis reactions take place rapidly in this case. They have also a small impact on methanogenic bacteria and result in an effective methane production comparing to digester with a high solid rate. Additionally, the buffering capacity of dairy manure was ensured during the operating time.

The corresponding removal efficiency of the effluent COD was more than 73%. Thus, the use of anaerobic co-digestion for cheese whey recovery seems to be an optimal way to ensure milk industry energy consumption and to remove the effluent pollution continuously generated

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