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Anaerobic treatment and methane production of two dairy wastes in a Moroccan cooperative

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ABSTRACT

This study is focused on anaerobic treatment and methane production of two effluents generated by a dairy cooperative from Morocco: Pure Whey (PW) and Loss in Dairy Product (LDP). Physicochemical characterization showed the richness of these effluents in organic matter that reached 97% for LDP and 87% for PW. In addition microbiological analysis showed that the both effluents contained several microorganisms (total germs and lactic acid bacteria). However, the total coliforms were presented only in LDP and the Sulphate-reducing bacteria were absent in both substrates. The Biochemical Methane Potential (BMP) assay was done according to three organic loads (100%, 50% and 25% corresponding to 120, 60 and 30ml of substrate) at 38 ± 1°C. The results showed that the low loads (25% and 50%) gave respectively the most important CH_4 yields for the two substrates PW: 25.546 ml_{STP}/gVS and LDP: 79.1 ml_{STP}/gVS. The reduction of the volatile solid (VS) and total solid (TS) was over than 80% for these two effluents. This reduction implicates a decrease of organic pollution contained in these wastes.

1. Introduction

Whey (W) is the by-product of cheese manufacturing. It is considered as a casein and fat free milk [1]. Despite this, it is rich in organic matter (OM) with chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) reach respectively 50-102g/l and 27-60g/l, due essentially to the presence of 45-50g/l of lactose [2]. This charge in OM combined with the large volume product (9kg of W for 1kg the cheese formed) and treatment options are limited in number and efficiencies; make the whey incontestably harmful to the ecosystem in which it was rejected [2].

Anaerobic digestion (AD) is a technology for organic matter treatment, and energy production (electricity and heat). Moreover, it provides some additional advantages over other treatments technologies, such as the possibility of working at different temperature ranges, high organic load rate and the hygienization of the final effluent [3].

The whey digestion was widely investigated in last decades [4]. Because it has a very interesting methanogenic potential that can reach "theoretically" 350LCH_4/kgDCO. Nevertheless, the experimental AD of the undiluted whey constitutes a real problem because of the fast acidification (~ 50 meq alkalinity/l) [5], caused by bacteria instead of yeasts [6]. The CH_4 yields vary between studies depending on the conditions that BMP assay was performed (Temperature, ratio inoculum/substrate, whey composition, pH, dilution, stirred, pretreatment ... etc.) [2, 6-10].

The purpose of this work is to contribute to the qualitative and quantitative characterization of Pure Whey (PW) and Loss in Dairy Product (LDP) from the Moroccan cooperative in one hand, and to evaluate in laboratory scale the optimal conditions for digestion of these effluents in the second hand. The optimal condition was studied in order to improve methane production and organic pollution degradation.

2. Experimental details

2.1. Characterization of samples and sampling areas

The samples are collected from the cooperative, located at 7 km south of Taroudant in the region of Ait Izza (Morocco). This cooperative was activating in several sectors ranging from agricultural production to process and therefore different fermentable resources were available throughout the year [11]. Thus, two of the most generated waste was chosen for the evaluation and the characterization: whey and LDP.

2.1.1. The whey

In average 20 000 l/day of milk are destined to the cheese manufacturing. Approximately, two thirds of this account was releasing as whey (Figure 1a). This waste which was generated in large quantities by the cooperative (19000 t/year equivalent to 940 t OM/year) was treated by two energy techniques.

The sample which was collected in cheese separation unit formed the pure whey (PW), whiles the second sample taken at the drying unit, formed the limed whey (LW).

2.1.2. The loss in dairy product

The LDP was collected from the washing unit and included all unsold and expired dairy, the loss of machines and laboratories of analysis (milk, yogurt, fresh cheese, flan, chocolate dessert... etc.). This substrate had a white color (Figure 1b), and the composition changed according to discarded products. It was characterized by rapid fermentation mainly caused by lactic acid bacteria (LAB) contained in the primary commodities, which compound the LDP [12].

a) **b)**

Figure 1: Color and appearance of substrate sample: a) Whey; b) Loss in dairy product

2.2. Analytical method

The samples were taken periodically once a month for a year (from 10/2014 to 09/2015) and transported at 4 °C (AFNOR NF EN 25667 (ISO 5667)).

The physicochemical parameters analyzed were: pH measured with a pH meter (probe HI 1332 AD 1030), alkalinity by H_2SO_4 0.1N to pH 4.5. The Ca^{2+} was measured by EDTA 0.02N and the TP was measured by a spectrophotometer after mineralization with concentrated nitric and sulfuric acid [13]. The TS was determined after drying a substrate bulk to 105°C, and then calcined at 550°C to get the VS rate [14]. The COD was measured by a COD-meter (CR.2200), while the BOD_5 was measured by the respirometric method [13].

The microbiological analysis was focused on five kinds of bacteria (Table 1). Decimal dilutions were prepared (10^{-5} to 10^{-9}) in buffered peptone water. The identification of lactic acid bacteria (LAB) was done by Gram staining and catalase test.

2.3. Methane production

2.3.1. The inoculum

The inoculum (In) used in BMPs assay constituted of stabilized sludge taking from an anaerobic settling tank of the wastewater treatment plants (WWTPs) M'zar (Agadir, Morocco). This plant treated a domestic and a

fraction of industrial wastewaters. The In was collected in the dry state, and then moistened and sieved (Figure 2). Only the particles <2 mm were taken and diluted in distilled water to form the inoculum used in inoculation of digesters. The In was characterized by a pH = 6.43; TS =2.15%; VS= 66.25%; TC= 3.49 10^7 CFU/ml; TG 4.24 10^7 CFU/ml and the SRB 2.60 10^6 colony-forming unit/ml (CFU/ml).

Table1: Microbiological parameters and analyses conditions.

Bacteria	Culture medium	Incubation Condition	Seeding technique	Ref
Total coliforms (TC)	Lactose Agar with tergitol 7 and TTC (Biomark)	37°C for 24h	Seeding in surface 0.1 ml of the sample or one of these dilutions	[15]
Total germs (TG)	PCA (Biokar)	37°C for 24h		
Sulphate-reducing bacteria (SRB)	Agar TSC (Oxoid)	37°C for 24 hours in anaerobic jar		
Lactobacillus (LB)	MRS (Biokar)	37°C for 72 hours in anaerobic jar		[16]
Streptococcus (Str)	M17 (Biokar)	37°C for 48 hours in aerobic		

a) b)

Figure 2: The stabilized sludge used as inoculum a) before humidification; b) after humidification.

2.3.2. Experimental approach

All the BMPs tests were performed on fresh samples undergoing any pretreatment, to get real results [17]. The main characteristic of PW and LDP used were: pH 4.73, alkalinity 1125 mg $CaCO_3$/l, Ca^{2+} 1042.08 mg/l, PT 0.27 mg/l, COD 29200 mgO_2/l, BOD_5 10940 mgO_2/l, %VS 82.46 mg/l and %ST 5.835 mg/l for PW and pH 4.18, alkalinity 0 mg $CaCO_3$/l, Ca^{2+} 881.76 mg/l, PT 151.11mg/l, COD 30000mgO_2/l, BOD_5 3344 mgO_2/l, %VS 95.92 and %ST 16.88 for LDP. The batch reactors constituted by serum bottles of 200 ml capacity, were incubating at 38±1°C. The stirring of these reactors were discontinuously 2 times a day for 5 min. In each reactor, 40 ml of inoculum were added to three organic loads of each sample (100%, 50% and 25% corresponding to 120, 60 and 30ml of substrate). The final volumes of digesters were completed to 160ml by distilled water. The control assay performed in the same working conditions to determine amount of endogenous gas from the In. indeed 40ml of inoculum was added to 120ml of distilled water and incubated in 38°C. The

value will be subtracted to the amount of methane produced by the substrate [14]. Methane production was given in the Standard conditions of Temperature and Pressure (STP) [19]. Moreover, the initial pH was adjusted to around neutrality (7.36 ± 0.01) with NaOH (2N). The amount of methane produced was measured -after CO_2 capture by a NaOH 9N bath- by the water displacement method [20]. The tests were duplicated and the maximum of methane produced was reported in the graphs.

2.4. Organic pollution abatement

To determinate the VS and TS abatement, the samples were taken in the beginning and in the end of digestion. The abatement was calculated with the following formula.

VS abatement% = (VSb-VSf) *100/VSb (1)
TS abatement % = (TSb-TSf) *100/TSb (2)
With b: beginning
f: final

3. Results and Discussion

3.1. Substrate characterization

3.1.1. Whey

The results of the physicochemical and microbiological analysis of the whey -collected from the cooperative- were presented in Table 2. The results showed that the pollution load in the two Whey was very large with COD and BOD_5 which were respectively 20700-30400 and 6690-19660 mgO_2/l in the PW and LW. However, the percentage of VS in PW was more important and oscillates between 77.41- 87.50%, unlike the LW in which the amount of VS varied between 56.56 and 81.04%. In addition, the enumeration of bacteria in the LW showed a high number of TC 10^2-510^5 CFU/ml and SRB 0-10^6CFU/ml - absent in PW-. The lactic acid bacteria (LB and Str) at LW showed a decrease resulting of their demanding nature. The pH was around neutrality (6.26 to 7.94), and high alkalinity that exceeded 1000 $mgCaCO_3/l$ (minimum value recommended [21]), presented more advantage for AD of LW compared to PW. Despite this, the choice of LW for methanisation may disrupt the function of digesters, because the effect of lime on methanogenic bacteria was unknown. In another hand, the increase of Ca^{2+} concentration (1643.28-3847.68 mg/l) can stop the digestion because it reached in some months the inhibitory concentrations 2.5g/l to 4.5g/l [21]. For these reasons, the methanogenic potential of PW was assessed.

3.1.2. Loss in dairy product

The cooperative produced in average 26188T/month of dairy products. However, the quantity of products intended for laboratories for quality determination, in addition to the loss of market and the loss caused by machinery formed the LDP. This loss of product quantified from 9 to 14 t/d, was characterized by an acidic pH (4.02-5.58) and an alkalinity ranging between 0-14000 $mgCaCO_3/l$, depending to discard dairy products. The amount of VS in the LDP exceeded 94%, while the amount of Ca^{2+} presented did not inhibit AD. Furthermore, the microbiological analysis showed a diverse microbial load with an amount of TG which varied between 3.60 10^8- 4.22 10^8 CFU/ ml. Indeed, the number of TC, LB and Str counted at LDP was respectively 1.82 10^6-3.71 10^7, 1.04 10^7- 3.36 10^8 and 7.09 10^7- 9.04 10^8 CFU/ml (table 2).

3.2. Methane production

The results of PBMs assay carried in mesophilic conditions (38°C ± 1) with pH of 7.36 ± 0.01 were presented in figure 3 and 4. The methane accumulation was higher in the middle load (50%) for the two effluents (figure 3) compared to 100 and 25% loads. But the digestion of PW gave a maximum of CH_4 yield equal to 25.546 ml $_{STP}$/gVS (Figure 4) at the minimum load (25%) which was the optimal load. While the yields obtained for the loads 50% and 100% were respectively 16.185 and 5.53 $mlCH_{4STP}$/gVS for the retention time that didn't reach 8 day (d).

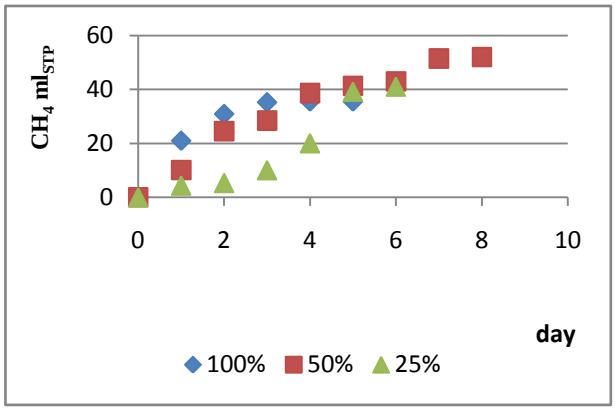
On another part, the LDP methanisation showed a very high CH_4 production compared to the PW (Figure 4). Indeed, the methane yield was 79.1 $mlCH_{4STP}$/gVS for 50%, which was the optimal load. While the yield was about 25.86 $mlCH_{4STP}$/gVS for 25% load, unlike the 100% load which gave a very small amount of methane. In addition the RT differs between loads and doesn't reach 5d for loads of 25 and 100%, whereas it reached 8 d to the 50% load.

Table 2: Physicochemical and microbiological characterization of PW, LW and LD

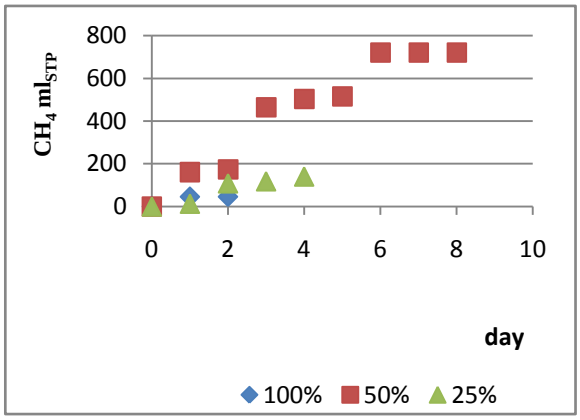
parameter	pH	$CaCO_3$ mg/l	Ca^{2+} mg/l	TP mg/l	COD mgO_2/l	BOD_5 mgO_2/l	COD/ BOD_5	TS %	VS%	Moisture %	TC CFU/ml	TG CFU/ml	SRB CFU/ml	LB CFU/ml	Str CFU/ml
PW	4.38-5.4	0-2500	801.6-1683.36	0.044 – 1.42	20700-30400	6690-13090	2.67-4.37	5.58-6.17	77.41-87.50	93.83-94.42	00	$2\ 10^3$-$1.1\ 10^9$	00	$2.4\ 10^6$ - $6.45\ 10^6$	$3.64\ 10^3$- $5.6\ 10^8$
LW	6.26-7.94	3000-7000	1643.28-3847.68	0.264-3.52	28600-29800	12030-19660	1.46-2.37	6.01-9.14	56.56-81.04	90.86-93.99	10^2 - $5\ 10^5$	$2.02\ 10^4$-$1.06\ 10^7$	0 - 10^6	$2.20\ 10^3$ - $1.4\ 10^6$	10- $3.4\ 10^6$
LDP	4.02-5.58	0-14000	801.6-1402.8	10.56-438.48	30000-60000	1510-3344	8.97-39.74	15.19-18.57	94.3 - 97.54	84.81-81.43	$1.82\ 10^6$-$3.71\ 10^7$	$3.60\ 10^8$-$4.22\ 10^8$	00	$1.04\ 10^7$-$3.36\ 10^8$	$7.09\ 10^7$-$9.04\ 10^8$

CFU/ml: colony-forming unit; TP: total phosphorus

The methane yield obtained was very less if it was compared with the OM contained in these effluents (respectively 77.41- 87.50 and 94.3 -97.54% for PW and LDP). This was explained with the presence of LAB in these effluents (table 2) which transformed the OM to CO_2 [12]. Consequently, OM was reduced but the methane production was less. However, making digestion under two types of digesters or used some pretreatment subsequently to AD led to considerable production [6, 10]. But these solutions are expensive for the cooperative to which this study is done.



A



B

Figure 3: methane accumulation according to the loads A: LSP; B: LDP

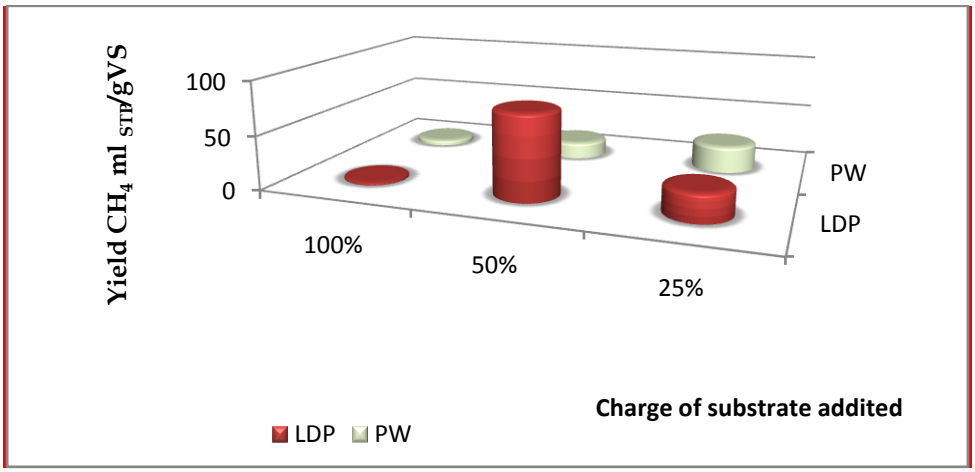


Figure 4: Methane yield given by the PW and LDP according to the loads on substrate**.**

3.1.Organic matter reduction

Anaerobic treatment of PW and LDP led to decreasing in organic pollution by it converting at CH_4. Indeed the VS and TS reduction in PW were respectively 89.54 and 84.75% for the optimum load (25%). While the reduction in VS and TS was in the order of 84.90 and 80.66% for the 50% load and in order of 87.04 and 85.02% for 100% load of substrate introduced. However, the reduction of TS and VS was above 90% for the 50% load for LDP. While abatement of VS and TS achieved 68.11 and 35.76% for the 25% load (table 3). These abatements were very important if they compared with these obtained in AD of dairy wastewater (80%) [8]. Indeed, the charge introduced in digester in our study was higher than this used by this authors. The presence of the LAB (present study) in these effluents used the lactose in competition with microorganisms involved in AD [6]. Consequently, they reduced the OM contained in the effluent.

Table 3: Abatement of the total solid (TS) and volatile solid (VS)

	PW		LDP	
Loads	VS%	TS%	VS%	TS%
100%	87.04	85.02	85.45	72.7
50%	84.90	80.66	94.31	91.29
25%	89.54	84.75	68.10	35.76

3.2.Final pH

In the end of digestion the final pH decreased to 4.18 ± 0.07 for PW and to 5.65 ± 0.59 for LDP. This decreasing was probably caused by the accumulation of H_2S due to the richness of dairy effluent in sulfur. In addition, the lactic fermentation done in conjunction with the stage of acidogenesis, caused degradation of the OM and accumulation of volatile fatty acids (VFA) and therefore the decreases in the final pH led to the stopping of the anaerobic digestion process [6, 22].

Conclusions

The physicochemical and microbiological characterization of dairy waste from a cooperative of Morocco gave further information about composition of dairy waste in Morocco. PW and LDP analyzed in this work presented a higher concentration in organic matter that reached respectively 87% and 97%. In addition, the concentration of Ca^{2+} didn't inhibit the anaerobic digestion. The microbiological analysis showed several microorganisms which found in substrates analyzed (TC, TG, RSB and LAB). Methane production of these dairy wastes showed a considerable potential of methane. Indeed, PW produced 25.546 mL_{STP}/gVS gave by 25% of substrate and LDP produced 79.1 mL_{STP}/gVS obtained by load contained 50% of substrate. Therefore the low load constituted the optimal load for giving the important production of methane compared to higher load (100% of substrate). In addition the reduction in organic pollution was over than 80%. However, other studies are needed to elucidate the relationship between the microbial community inside the digesters in the one hand, and the relationship between these microorganisms and the substrate on the other hand.

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