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Methane production by sludge from a Moroccan dairy industry under anaerobic treatment

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Sludge from a Moroccan dairy industry, constitute a source of organic matter ranging between 74 to 95%. Assessment of methane production in batch reactor at 38°C in laboratory scale according to three dilutions showed promising results. The co-digestion gave 11 times more methane production for BS and 3 times for PCS given by the mixture M_3 at dilution 4 compared to mono-digestion that gave respectively 5.6 and 20.79mlCH_{4STP}/gVS in substrate without dilution. Significant synergy between BS, PW and LDP was observed. In contrast, PCS showed a low synergy with these co-substrates. The TS and VS abatement exceeds 83% and 86% respectively for M_3.

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Introduction

In Morocco, the quantities of sludge produced by activated sludge process are estimated over than 60 000 t/year. This production will know, on the horizon of 2030, a significant increase and will reach a total, globalizing in both urban and industrial sludge, more than 600 000 tons of dry matter/year [1]. The management of these quantities is a serious challenge. Knowledge of the physicochemical and bacteriological quality of this waste is necessary before suggesting any possibility of treatment. Indeed, the bacterial load contained in the wastewater as well as all other elements (heavy metals, organic micropolluant -phthalates, PCBs, PAHs-) is always eliminated and concentrated in sludge, which represents a potential risk for possible valorization in agriculture [2-3]. The reincarnation of the anaerobic digestion (AD) as a sludge stabilization technology, energy production (biogas) and reduction of microbial load [4-5] is very interesting. Indeed, anaerobic digestion stabilizes the organic matter in wastes, reduces pathogens and odors and reduces the total solids by converting part of the volatile solids fraction into biogas [6]. Consequently, AD was used for valorizing many wastes such as manure, agricultural waste, sewage sludge, domestic waste…etc. Moreover, it provides some additional advantages over other treatment technologies, such as the possibility of working at different temperature ranges, high organic load rates and the hygienization of the final effluent [7]. The AD process occurs in four mainly steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis [8]. However, the refractory structure of the sludge and the low-carbon compared to nitrogen and phosphorus (COD/N/P varies from 50/4/1 to 350/5/1) requires pretreatment, or looking for co-substrates in synergy to increase the amount of methane produced [9-12].

In this regard, this study conducted to enhance the treatment of the sludge from dairy effluent.
The objective of this work is to assess in laboratory scale the methane production of two sludges from a Moroccan dairy industry in mono and co-digestion. Especially, in terms of methane production, optimal dilution and synergy and/or antagonisms between the substrates studied.

Materials and methods

Samples

The samples were taking from a Moroccan dairy industry. The biological sludge (BS) and physicochemical sludge (PCS) were taking at wastewater treatment plants of the same industry in which 41 333 m^3 (in average) of effluent is treated per month. The amount of OM locked in these waste is 1188 t/year [13], and they makes them a serious environmental problem for this industry. On another hind, pure whey (PW) and loss in dairy product (LDP) used in co-digestion with the sludge, were collected respectively in the separation unit and in the washing unit. The mixtures were reported in table 1 and the physicochemical characteristics were presented in table 2.

Table 1: Substrates used in biological Methane Potential tests in mono and co-digestion.

Mixture	Index
100% BS	MBS
100% PCS	MBPC
50% LDP+ 50% BS	M1
50% LDP+50% PCS	M2
25% LDP+ 25% BS+ 25% PW+ 25% PCS	M3
50% PW+50% BS	M4
50% PW+ 50% PCS	M5
50% BS+50% PCS	M6

Table 2: Physicochemical characterization of substrates assessed in biological Methane Potential

Parameter	pH	$CaCO_3$ mg/l	Ca^{2+} mg/l	TP mg/l	TS%	VS%
M_{BS}	7.49	2250	160.32	563.50	1.55	74.41
M_{PCS}	6.28	NA	NA	NA	6.96	81.96
M_1	4.8	1125	841.68	208.67	9.012	95.43
M_2	4.94	4000	541.08	363.42	11.78	90.22
M_3	5.14	1625	761.52	376.07	6.78	89.47
M_4	5.8	1500	1042.08	192.60	3.71	86.32
M_5	5.8	2000	1282.56	335.68	5.84	85.3
M_6	6.71	3000	1442.88	297.16	4.03	83.41

NA: not available

Analytical method

The physicochemical parameters monitored during this work are pH, alkalinity, Ca^{2+}, TP, TS, VS, COD and BOD_5 [13-15].

The biological Methane Potential *tests*

Methane production was assessed by evaluation of biological methane potential (BMP). These tests were performed in batch reactors composed by serum bottles with 200 ml capacity. In each reactor, 40 ml of inoculum were adding to samples in mono and co-digestion. The main features of inoculum were: pH 6.43; Ca^{2+} 561.12mg/l; TP 33.46 mg/l; TS 2.15%; VS 66.25%. The reactor was incubated at 38±1°C with discontinuous stirring. Three dilutions of substrate added were evaluated (without dilution (D0), dilution 2 times (D2) and dilution 4 times (D4)). A control was carried in the same working conditions to determine the amount of endogenous CH_4 from the inoculum. The initial pH was adjusted around neutrality (7.36 ± 0.01) with NaOH 2N and H_2SO_4 0.1N. The amount of methane produced was measured by the water displacement method after elimination of CO_2 by a bath of NaOH 9N (fig 1). Methane production was giving in the standard conditions of temperature (0°C) and pressure (1atm) (STP) [9-10]. The BMP tests were duplicated and the maximum of methane production were reported.

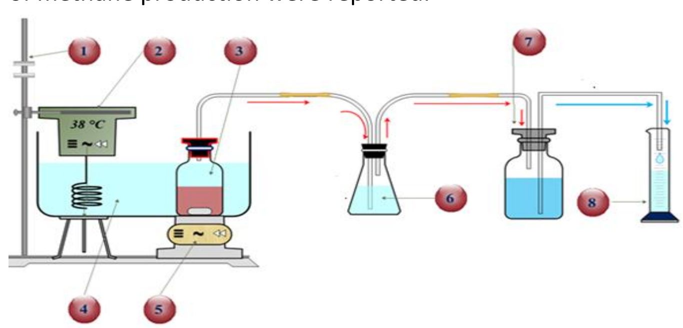

Figure 1: Experimental device used in PBM tests. 1: support; 2: thermostat; 3: reactor in batch; 4: bain marie; 5: agitator; 6: NaOH (9N); 7: gasometer; 8: tube for measurement.

Results and discussion

Methane production

In mono-digestion, BS (M_{BS}) showed a very low methanogenic potential with maximum methane 5.601ml$_{STP}$/gVS gave by substrate without dilution (D0) introduced in the reactor, whereas the potential was zero in the two other dilutions (2 and 4) (fig 2). While the PCS (M_{PCS}) gave a maximum yield of

CH_4 20.79ml$_{STP}$/gVS when substrate was introduced in reactor without dilution (D0). Although the dilution of 2 and 4 gave respectively 3.14 ml and 0 ml CH_{4STP}/gVS (Fig 2). So the substrate without dilution was the optimum to produce the maximum of methane in mono-AD of these sludges. However, these productions were less if compared by those found in literature. Indeed, the methane production varied between studies according to composition of sludge and pretreatment used (thermic, ultrasonic, microwave…etc) [9-12, 16, 17].

In our study, the composition of sludge is specific and didn't cite before in literature. These sludges are deriving under treatment of effluent mixture produced by this industry (dairy, orange…etc). In another hind, applicate pretreatment to improve methane production of sludge of this industry isn't a good idea, because the cost of treatment will be very expensive. For that, co-digestion approach is used to increase methane production. The results of co-digestion are presented in fig.3.

The mixture M_3 gave the maximum of CH_4 (62.85 ml$_{STP}$/gVS) at dilution 4. In addition, the Mixtures M_1 and M_2 produced the maximum methane yields at the same dilution of substrate. Contrary to mixtures, M_5 and M_6 whose maximum yields were observed respectively in medium and higher dilution (2 and 4). However, M_4 showed a yield (CH_4 50 ml$_{STP}$/gVS) which was almost the same for both dilution (2 and 4). Mixtures between BS and co-substrates tested increased the methane yield in all dilution with 11 times higher (relative to M_{BS}) given by M_3 and over +8 and +9 times by M_4 (table 3). This production was higher than that found by Li et al., 2013 (+0.33 compared to control) after dilution and post treatment of 80% primary sludge and 20% biofilm sludge [18]. However, the production of methane gave by M_6 was zero or low for higher and medium dilution. Synergy between the co-substrates (PW and LDP) and the BS was very important whatever the applied dilution, while the PCS must be applied without dilution with the BS and showed the least synergy with the BS (M_6) (table 4). The synergy existing between certain substrates outcome from dairy industries has been demonstrated by the results of Kothari et al [19]. However, the synergy was less important between PCS and co-substrates used, in particular in mixtures M_2, M_5 and M_6. Indeed, CH_4 production doesn't exceed a lot the value obtained by the control M_{PCS} (+1.04 to +1.79 times). So the mixture between PCS, LDP and PW, must be done at higher dilution for the first and at medium dilution for the second. The maximum value was obtained by M_3 with a methane yield multiplied to +3 times compared to M_{PCS}. The mixture (M_6) composed of 50% BS and 50% PCS didn't produce methane advantage if compared with the yield of mono-digestion PCS contrary to the BS. However, the mixture of the four components BS, PCS, PW and LDP (M_3) with a higher dilution (4) provided the maximum methane (table 3). In general PCS was in less synergy with LDP and PW, while an antagonism was between PCS and BS (table 4). To be in synergy or antagonism is currently observed in co-digestion of sewage sludge with bio-waste in different ratios [20]. These results are in relation with the composition of sludge and bio-waste used in co-digestion. However, 50% of studies pay attention to co-digestion because it overcomes the difficulties occurred during mono-digestion [20]. Indeed, co-digestion improved the nutritional balance, the synergetic effect of microorganisms, biogas production (and methane) and diluted the toxic compounds [21-23]. It is therefore imperative to choose the best co-substrate and the best mixing ratio to improve positive interactions and avoid inhibitions [24-26].

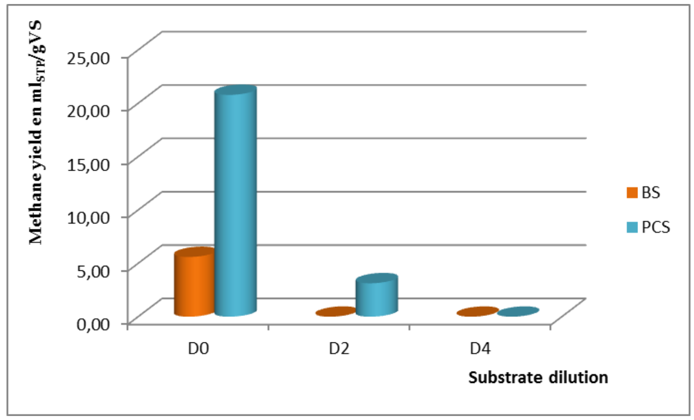

Figure 2 : methane yield of BS and PCS

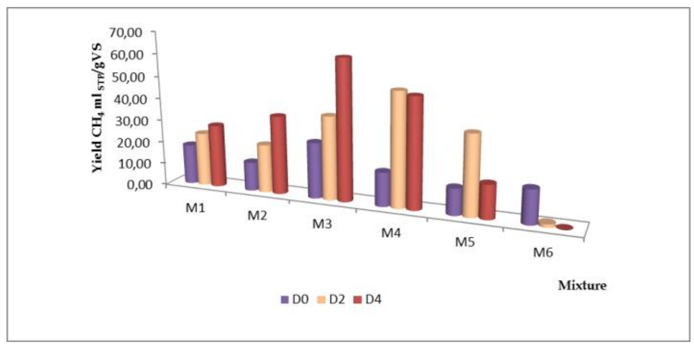

Figure 3 : methane yield of mixture

Table 3: methane production rate compared to control

	BS			PCS		
	4	2	0	4	2	0
M_1	+5.04	+4.28	+3.20			
M_2				+1.69	+1.04	-0.4
M_3	+11.22	+6.64	+4.47	+3.02	+1.79	+1.20
M_4	+8.75	+9.04	+2.71			
M_5				-0.27	+1.72	-0.43
M_6	-1	-0.73	+2.72	-1	-0.93	-0.27

Table 4: Summary table of relationships between different substrates

	BS	PCS	LDP	PW
BS	C	-/+	+	+
PCS	-	C	+/-	+/-

C : Control ; - : Antagonism ; + : Synergy

Organic matter removal

TS and VS reduction in M_{BS} reached respectively 79.63 and 83.60% in substrate without dilution (D0). While these reduction exceeded 70% for dilution 2 (D2) and was over than 50 and 70% in higher dilution (D4). However, %VS and %TS abatement was very important and represented 89.161 and 87.064% in M_{PCS} without dilution (D0). While this reduction reached 87.16 and 84.43% for the medium dilution (D2) and surpassed 70% for both TS and VS in higher dilution (D4) (table 5).

In mixtures, TS and VS removal varied from 34.68 to 89.27% and from 61.40 to 92.70% (Table 5). However the maximum reduction in VS and TS were found in the case of M_1 (92.70 and 89.27% respectively), but the maximum yield of methane was given by M_3 with a reduction which reached respectively 86.44 and 83.33%. The reductions observed in this work regardless the load are higher than those cited in bibliography (45-50% of solid matter) [27]. These reductions were explained by the presence of lactic acid bacteria. These bacteria entered in competition with the methanogenesis bacteria (especially for the fermentation of lactose) [28] and degrade the organic matter without methane production. Indeed, the use of lactic acid bacteria for corn silage is very important because they produce a lot of lactic acid which protects of rottenness but restricts the production of biogas when this Waste is digested [29].

Table 5: Abatement of VS and TS

Substrate dilution	Abatement % TS			Abatement % VS		
	D0	D2	D4	D0	D2	D4
PCS	87.06	84.43	79.91	89.16	87.16	85.17
BS	79.63	71.30	55.56	83.60	74.98	71.20
M_1	34.68	41.91	89.27	61.40	72.82	92.70
M_2	87.00	84.20	86.81	87.69	85.51	89.83
M_3	85.62	83.65	83.33	87.38	86.47	86.44
M_4	83.38	81.83	79.61	86.14	85.94	86.94
M_5	73.23	76.55	51.43	87.16	89.46	88.92
M_6	83.33	78.51	74.56	86.65	84.26	81.18

Final pH

Final pH in M_{BS} (7.23 ± 0.16) was higher than in M_{PCS} (6.59 ± 0.16) but it remains favorable for digestion. However, the final pH in mixture (M_1-M_6) was between 4.49 and 6.72 noting an acidification of the digester especially in mixture containing PW and LDP. These substrates were rich in lactose [30] which is fermented in propionate in typical pH conditions for methanogenic reactors (pH around neutrality). Furthermore, lactic acid bacteria are able to convert sugars into lactic acid, which is 10 times acidic (pKa 3.8) than volatile faty acid (VFA) (acetic, propionic and butyric acid (pKa 4.7-4.8)) [31]. As consequence the concentration of volatile fatty acid increased and the pH decreased [28].This acidity affects negatively the methanogenesis step because the methanogenic archaea are sensitive to decrease in pH (pH must be between 6.8 and 8.8 [32]. However, the presence of PW and LDP in co-digestion increases methane production. Kawai et al., 2014 showed also that labile organic fraction in food waste causes acidification, but also contributes to high methane yields [33].

Table 6: Initial and final pH value during the co-digestion

Substrate dilution	D0		D2		D4	
	pHi	pHf	pHi	pHf	pHi	pHf
M_1	7.38	6.27	7.35	6.16	7.36	5.88
M_2	7.33	5.67	7.36	5.96	7.36	5.92
M_3	7.35	5.69	7.39	6.13	7.35	5.95
M_4	7.35	4.49	7.35	6.06	7.35	5.92
M_5	7.38	5.98	7.39	5.95	7.31	6.01
M_6	7.37	6.62	7.36	6.64	7.35	6.72

Conclusions

The optimization of methane production of BS and PCS using the co-substrates (PW and LDP) according to three dilution, leads to conclude that the PCS has less synergy with these co-substrates contrary to the BS which representing more synergy regardless the applied dilution. Although, the mixture of these substrates provides a solution for this cooperative because, it optimizes the methane yield. However, some problem occurred during digestion such as acidification (probably due to the presence of lactic ferments) and required more study to optimize the production of methane.

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