CHARACTERIZATION AND ANAEROBIC TREATMENT OF BIOLOGICAL AND PHYSICOCHEMICAL SLUDGE FROM A MOROCCAN DAIRY INDUSTRY

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SUMMARY

The physicochemical and biological sludge (PCS and BS) from a Moroccan dairy industry, constitute a source of organic matter that is ranging respectively between 58.67 to 90.6% and 18.85 to 92.5%. The composition of these sludges is very specific because they are derived from an effluent mixture (milk, orange, whey, ...) which varied according to production of this industry. As results, the physicochemical and microbiological characteristics of these sludges fluctuate according to time. The pH of biological sludge varied between 7.35 to 8.85 while the pH of physicochemical sludge fluctuated between 5.32 to 7.5. In addition the microbial germs is represented by total coliforms (3.95.10^7 and 2.02.10^11 UFC.ml^-1), total germs (1.61.10^9 and 2.36.10^12 UFC.ml^-1), sulphate-reducing bacteria (1.55.10^8 and 5.72.10^9 UFC.ml^-1), lactobacillus (2.15.10^8 and 9.37.10^11 UFC.ml^-1) and streptococcus (1.05.10^9 and 2.46.10^12 UFC.ml^-1) (respectively for BS and PCS). Assessment of anaerobic digestion for treating these wastes in batch reactor at mesophilic condition (38°C) show the promising results in terms of methane production and organic pollution removal. Indeed, the methane yields were 5.601 and 20.79 mlSTP.gVS^-1 gave respectively by BS and PCS for the maximal load (100% of substrate) which is the optimal load. The total solid and volatile solid reduction reached 79.63 and 83.60% for biological sludge while it reached 87.06% and 89.16% for physicochemical sludge for the same load (100% of sludge).

Keywords: biological sludge, physicochemical sludge, organic loads, methane yield, pollution removal.

1. INTRODUCTION

The activated sludge process is widely used for the treatment of urban and industrial waste waters. This technique can reduce organic pollution contained in these waters, but it generates an excess of sludge, in which the management is a serious challenge. The knowledge of the physicochemical and bacteriological quality of this sludge 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 (Jardé, 2002; Durand et al., 2004). The reincarnation of the anaerobic digestion as a sludge stabilization technology, energy production (biogas) and reduction of microbial load (Kalloum et al., 2011; Skiadas et al., 2005) is very interesting. Indeed anaerobic digestion stabilizes the organic matter in wastes and reduces the total solids by converting part of the volatile solids fraction into biogas (Merlin Christy et al., 2014). This biogas is a source of renewable energy because it’s constituted principally by methane. In Morocco, the quantities of sludge produced are estimated over than 60 000 t/year. This production will know,
Characterization and anaerobic treatment of biological and physicochemical sludge from a Moroccan dairy industry

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 (National Observatory of the Environment, 2015). In this regard, this study is conducted to enhance the treatment of the sludge from dairy effluent (who’s the attention is multiplied in recent decades because they show very high COD (22,000 mgO₂/l) and constitute a source of renewable energy (Uma Rani et al., 2012; Uma Rani et al., 2013; Uma Rani et al., 2014).

The objective of this work is to contribute to the qualitative and quantitative characterization of biological sludge (BS) and physicochemical sludge (PCS) generated by a Moroccan dairy industry and to evaluate how these characteristics fluctuated according to time. In the other hand, anaerobic digestion was assessed for treating these wastes according to three organic loads. The effect of these organic loads in methane production and organic pollution removal was also evaluated.

2. MATERIAL AND METHODS

2.1 Samples and sampling areas

The samples are from a Moroccan cooperative, located at 7 km south of Taroudant (Morocco). The cooperative has a waste water treatment plants (WWTPs) (situated at 1 km of the industry) in which 41,333 m³ (in average) of effluent is treated monthly using two methods. The first is physical-chemical treatment and leads to the production of physicochemical sludge, while the latter was biological treatment which generates biological sludge. The amount of organic matter (OM) produced in these wastes is 1,188 t/year (Lhanafi et al., 2014); this makes them a serious environmental problem for this industry. The sample of BS was performed at 40 m³ tank wherein the sludge is stored, while the PCS is taken at flocculation/flotation tank (Fig.1).

![Fig.1: Sludge sampling sites: A) tank of 40 m³ in which the BS was taken B) flocculation/flotation tank in which the PCS was collected.](image)

2.2 Characterization of BS and PCS

The diversity of products manufactured by the industry (cheese, milk, butter, orange juice, caramel flan, chocolate dessert, ...), leads to variation in the characteristics of the overall effluent generated by this cooperative (Table 1) and consequently, the composition and coloring of the produced sludge which change according to the presence and/or absence of the whey (W) and orange press effluents (Fig.2). Therefore, this study was conducted to determine the fluctuation of these wastes according to time.
Table 1: The overall effluent’s composition giving the sludge.

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>sanitary water</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Effluent release from orange</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>W</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>-</td>
</tr>
</tbody>
</table>

+: présent ; - : absent ; W : whey

2.3 Analytical method

The samples were taken monthly for a year (from 10/2014 to 09/2015) and transported at 4°C. The analyses were carried within 24 hours maximum (AFNOR NF EN 25667 (ISO 5667)). The physicochemical parameters monitored during this work were pH, alkalinity, Ca$^{2+}$, TP, TS, VS, COD and BOD5 (Rodier et al., 2009; Tremblav, 2006). The microbiological analyses were focused on five bacteria. Total coliforms (TC) were counted on tergitol 7 lactose agar and TTC; the total germs (TG) on PCA; the sulphate-reducing bacteria (SRB) on TSC and lactobacillus (LB) on MRS in anaerobic condition and streptococcus (Str) on M17 (Dib et al., 2012). The lactic acid bacteria was identified by Gram staining and catalase test (Da Silva Ferrari et al., 2016).

2.4 Anaerobic digestion

2.4.1 Inoculum

The inoculum was taken at an anaerobic settling tank of the WWTP of M’Zar (Agadir, Morocco). This inoculum was humidified and sieved before been diluted in distilled water to form the inoculum used in the inoculation of digesters. Its main features were: pH 6.43; Ca$^{2+}$ 561.12mg.l$^{-1}$; TP 33.46 mg.l$^{-1}$; TS 2.15%; VS 66.25%; TC 3.49.10$^7$; TG 4.24.10$^7$ and SRB 2.60.10$^6$ CFU.ml$^{-1}$.

2.4.2 Experimental approach

The anaerobic digestion (AD) was performed in batch reactors composed of the serum bottles with 200 ml of capacity (Fig. 3). In each reactor, 40 ml of inoculum is added to each sample, and then the
reactor was incubated at 38 ± 1°C with a discontinuous stirring 2 times a day. Three organic loads were evaluated 100%, 50% and 25% of substrates; these loads correspond respectively to substrate/inoculum ratios of 3, 1.5 and 0.75. A control was carried in the same working conditions to determine the amount of endogenous gases in the inoculum. The initial pH was adjusted to around neutrality (7.36 ± 0.01) with NaOH 2N and H₂SO₄ (0.1N). The amount of methane produced was measured by the water displacement method after elimination of CO₂ by a bath of NaOH 9N. Methane production gave in the standard conditions of temperature (0°C) and pressure (1 atm) (STP). The tests were duplicated and only the maximum of methane production was reported.

Fig.3: 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.

3. RESULTS AND DISCUSSION

3.1 Characterization of BS and PCS

The Analysis carried during a year, show a fluctuation in the parameters measured for BS and PCS. Indeed, the BS has a pollution load rates especially in terms of COD and BOD₅ which varied respectively between 1346-8790 mgO₂/l and 221-2850 mgO₂/l, depending on the production of the industry and the effluent type drained to the WWTP (Table 1). In addition, the variation in pH shows two zones: the first in which the pH is greater than 8, while it is between 7 and 8 in the second zone (Fig. 4). The decrease in pH in this range is probably due to drainage of Whey (which is acid) to the WWTP. However, the pH value in BS remains favorable for anaerobic digestion because it’s in the zone of pH recommended in bibliography (pH between 6.8 and 8.8) (Rajesh Banu et al., 2008). The same remark was observed in the alkalinity that exceeds 1.000 mg CaCO₃/l (Table 2). The concentration of Ca²⁺ and TP (elements highly represented in milk) showed a fluctuation following the month, but it didn’t inhibit anaerobic digestion (Ca²⁺< 2.5 g/l) (René, 2012). The amount of TP is highly (TP>10mg/l) and will cause the eutrophication of the environment in case of direct discharge of this waste (Fig.5). The %VS contained in the BS varies by month, but it is still exceeding the 50% except the months of October and November (Fig.6) caused probably by the amount of products that were produced by the industry.
Furthermore, the pH variation in the PCS follows the same zone as in the BS. Thus, two pH ranges were observed: the first is highly to 7 and the second is less than 7 (Fig.7). These variations were caused by the initial composition of the total effluent (presence of the W and/or orange press effluent). Anaerobic digestion of this sludge situated in the second area will require a pH adjustment. In additionally, the PCS are very rich in OM that varies between 58.67 to 90.6% (Fig.8).

Microbiological analysis shows that the number of germs is very important in PCS compared with the BS (Table 2). The TC, TG and SRB accounts respectively $3.95 \times 10^7$, $1.61 \times 10^9$, $1.55 \times 10^8$ CFU.ml$^{-1}$ and $2.02 \times 10^{11}$, $2.36 \times 10^{12}$, $5.72 \times 10^9$ CFU.ml$^{-1}$ in BS and PCS. Lactic acid bacteria are also present in
large numbers in the PCS. Indeed, $9.37 \times 10^{12}$ of LB and $2.46 \times 10^{11}$ CFU ml$^{-1}$ of Str are counted in the PCS in contrast to BS in which the number of these bacteria reached respectively $2.15 \times 10^9$ and $1.05 \times 10^9$ CFU ml$^{-1}$.

![Fig.7: pH variation in the physicochemical sludge.](image1)

![Fig.8: Variation in VS and TS in the PCS.](image2)

Table 2: Physicochemical and microbiological characterization of BS and PCS.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CaCO$_3$ mg/l</th>
<th>BOD$_5$ mgO$_2$/l</th>
<th>COD mgO$_2$/l</th>
<th>Moisture %</th>
<th>TC CFU.ml$^{-1}$</th>
<th>TG CFU.ml$^{-1}$</th>
<th>SRB CFU.ml$^{-1}$</th>
<th>LB CFU.ml$^{-1}$</th>
<th>Str CFU.ml$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS</td>
<td>3500</td>
<td>2850</td>
<td>8790</td>
<td>99.81</td>
<td>$2.72 \times 10^{9}$</td>
<td>$9.44 \times 10^{9}$</td>
<td>$1.24 \times 10^9$</td>
<td>$1.56 \times 10^9$</td>
<td>$7.43 \times 10^9$</td>
</tr>
<tr>
<td>PCS</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>96.14</td>
<td>$1.76 \times 10^{12}$</td>
<td>$1.85 \times 10^{13}$</td>
<td>$4.50 \times 10^{10}$</td>
<td>$8.43 \times 10^{12}$</td>
<td>$1.67 \times 10^{12}$</td>
</tr>
<tr>
<td>Min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$1.34 \times 10^4$</td>
<td>$2.24 \times 10^9$</td>
<td>0.00</td>
<td>$2.58 \times 10^7$</td>
<td>$2.49 \times 10^8$</td>
</tr>
<tr>
<td>BS</td>
<td>1000</td>
<td>221</td>
<td>1346</td>
<td>96.12</td>
<td>$2.10^{10}$</td>
<td>$9.55 \times 10^9$</td>
<td>0.00</td>
<td>$9.09 \times 10^7$</td>
<td>$8.10^9$</td>
</tr>
<tr>
<td>PCS</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>88.56</td>
<td>$2.10^{10}$</td>
<td>$9.55 \times 10^9$</td>
<td>0.00</td>
<td>$9.09 \times 10^7$</td>
<td>$8.10^9$</td>
</tr>
<tr>
<td>Average</td>
<td>BS</td>
<td>2058.33</td>
<td>1269.33</td>
<td>6872.7</td>
<td>$3.95 \times 10^7$</td>
<td>$1.61 \times 10^7$</td>
<td>$1.55 \times 10^8$</td>
<td>$2.15 \times 10^7$</td>
<td>$1.05 \times 10^7$</td>
</tr>
<tr>
<td>PCS</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>93.05</td>
<td>$2.02 \times 10^{11}$</td>
<td>$2.36 \times 10^{12}$</td>
<td>$5.72 \times 10^7$</td>
<td>$9.37 \times 10^7$</td>
<td>$2.46 \times 10^7$</td>
</tr>
</tbody>
</table>

Max: maximum; Min: minimum; NA: not available
3.2 Methane production

The bio-methanogenic potential (BMP) assays were carried in mesophilic conditions (38°C ± 1) with adjustment of the pH. Indeed, the BMP of BS was 5.601 ml_{STP}.gVS^{-1} which was given by the load 100% of substrate introduced in the reactor, whereas the potential was zero for the two other loads (50 and 25%). However, the BMP of PCS - taking account to acid pH of the substrate - gave a maximum yield of CH_{4} 20.79 ml_{STP}.gVS^{-1} for 100% load. Although the loads of 50% and 25% gave 3.14 ml and 0 ml CH_{4} gVS^{-1} respectively (Fig.9). So the higher load was the optimum load to produce the maximum of methane for both of sludges.

The methane produced by these sludges remained less if it’s compared with those found in another research (Moletta 2011; Serrano et al., 2014). This is due to their composition which was very different (this study). Moreover, the presence of lactic acid bacteria in these sludges (table 2) can decrease methane production. Indeed, in literature, lactic acid bacteria are able to convert sugars into lactic acid, which is 10 times acidic (pKa 3.8) than volatile fatty acid (VFA) (acetic, propionic and butyric acid (pKa 4.7-4.8)) (Elmer Marth and Steele James, 2001). This acidity affects negatively the methanogenesis step because the methanogenic archaea are sensitive to decrease in pH. Moreover, the use of homo and hetero-fermented 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 (Vervaeren et al., 2010). Furthermore, sludge used in this study contained dairy and orange waste. The waste of oranges has a large methanogenic coefficient which is in order of 0.27-0.29 L_{STP}.CH_{4}.gDCO^{-1} added. Moreover, the orange waste contains an essential oil (D-limonene) which is antimicrobial (Martín et al., 2010). This oil is extracted from the orange waste of this industry and excluded the hypothesis of inhibition of methanogenic bacteria by this oil. On the other hand, the synergy between the orange waste and residual glycerol derived from the biodiesel industries during co-digestion is remarkable and allowed to produce up to 330±51 ml_{STP}.CH_{4}.gSV^{-1} added (Martín et al., 2013). Dairy effluent and orange waste don’t present probably the same synergy. However, a significant synergy is observed between dairy effluent, sludge and inoculum (Kothari et al., 2011).

Fig.9: methane production yield by BPC according to the loads.

![Graph showing methane production yield by BPC according to the loads.](image)

3.3 Organic matter removal

After anaerobic digestion the TS and VS reduction on BS reached 79.63 and 83.60% for 100% load, while this reduction exceeded 70% of the both TS and VS for the average load 50%, while it was over than 50 and 70% for the low charge. However, %VS and %TS abatement was very important and represented 89.16 and 87.06% for the higher load (100%), while this reduction reached 87.16% and 84.43% for the load of 50% and surpassed 70% for
the both TS and VS in the load 25% for PCS (Table 3). These reductions are higher than those reported in bibliography (45-50% of solid matter) (Marche, 2001). High organic matter reduction and less methane production observed in this work were explained by the presence of the lactic acid bacteria (this study). These bacteria converted the organic matter into lactic acid (lactic fermentation), and entered in competition with the methanogenesis bacteria (especially for the fermentation of lactose) in typical pH conditions for methanogenic reactors (pH around neutrality) (Vasileios et al., 2014).

The retention times (RT) varied from load to load. In load of 100% substrate of PCS, the digestion took 8 day (d) while for the two other loads it took only 5d. However, the digestion of BS took only 3d for the maximum load and 1 to 2d respectively to the load of 25% and 50%. In addition the final pH of BS (7.23 ± 0.16) is higher than PCS (6.59 ± 0.16) and remains favorable for digestion (Table 4).

### Table 3: Abatement of VS and TS and variation of RT.

<table>
<thead>
<tr>
<th>Loads</th>
<th>Abatement TS%</th>
<th>Abatement VS%</th>
<th>Retention times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical chemical sludge</td>
<td>100%</td>
<td>87.06</td>
<td>85.17</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>84.43</td>
<td>87.16</td>
</tr>
<tr>
<td></td>
<td>25%</td>
<td>79.91</td>
<td>83.60</td>
</tr>
<tr>
<td>Biological sludge</td>
<td>100%</td>
<td>79.63</td>
<td>74.98</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>71.30</td>
<td>71.20</td>
</tr>
<tr>
<td></td>
<td>25%</td>
<td>55.56</td>
<td>71.20</td>
</tr>
</tbody>
</table>

### Table 4: Initial and final pH value during the tests

<table>
<thead>
<tr>
<th>Physical chemical sludge</th>
<th>Biological sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial pH</td>
<td>Final pH</td>
</tr>
<tr>
<td>100%</td>
<td>7.36</td>
</tr>
<tr>
<td>50%</td>
<td>7.37</td>
</tr>
<tr>
<td>25%</td>
<td>7.38</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>6.59±0.16</strong></td>
</tr>
</tbody>
</table>

### 4. CONCLUSION

The biological and the physicochemical sludge from a Moroccan dairy industry were characterized and their treatment by anaerobic digestion was assessed. Physicochemical and microbiological characteristics showed that these sludges are a source of organic matter and germs. Indeed, the organic matter is varied respectively between 58.67 to 90.6% and 18.85 to 92.5% for physical chemical sludge and biological sludge. In addition, these sludges are rich in germs as total coliforms, total germs, sulphate-reducing bacteria, *lactobacillus* and *streptococcus*. As results, the Physicochemical and microbiological characteristics of these sludges fluctuate according to the effluents produced by this industry. In another hind, the anaerobic treatment of these wastes according to three organic loads show a promising result in terms of methane production and organic pollution removal. Indeed, the methane yield reached 5.601 and 20.79 ml(STP)-gVS⁻¹ respectively for biological and physical chemical sludge at the maximal load (100% of substrate) which was the optimum loads for producing the maximum of methane. Moreover, the organic pollution was reduced to 83.60% and 89.161%
respectively for biological and physical chemical sludge.

ACKNOWLEDGMENT

We thank the managers and employees of the industry for their support for the success of this work.

REFERENCES