Eco-friendly process combining physical–chemical and biological technics for the fermented dairy products waste pretreatment and reuse

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ABSTRACT

Residual fermented dairy products resulting from process defects or from expired shelf life products are considered as waste. Thus, dairies wastewater treatment plants (WWTP) suffer high input effluents polluting load. In this study, fermented residuals separation from the plant wastewater is proposed. In the aim to meet the municipal WWTP input limits, a pretreatment combining physical–chemical and biological processes was investigated to reduce residual fermented dairy products polluting effect. Yoghurt (Y) and fermented milk products (RL) were considered. Raw samples chemical oxygen demand (COD) values were assessed at 152 and 246 g.L\(^{-1}\) for Y and RL products, respectively. Following the thermal coagulation, maximum removal rates were recorded at 80 °C. Resulting whey stabilization contributed to the removal rates enhance to reach 72% and 87% for Y and RL samples; respectively. Residual whey sugar content was fermented using \textit{Candida} strains. Bacterial growth and strains degrading potential were discussed. \textit{C. krusei} strain achieved the most important removal rates of 78% and 85% with Y and RL medium, respectively. Global COD removal rates exceeded 93%.

Key words | \textit{Candida}, dairy residuals, fermentation, pretreatment, thermal coagulation

INTRODUCTION

Dairy industry is a major part of the food industry that generates a high amount of wastewater (Kosseva 2011). In some developing countries, along the marketing chain, products loss is mainly due to spillage and spoilage (Lore \textit{et al.} 2005). Processing losses at the factory level are most likely below 2% (Muriuki 2005). Losses may refer to spillage during industrial treatment and processing (Gustavsson \textit{et al.} 2011). Another important component of food loss is the stock removed from retail shelves because it has reached its ‘sell-by’ date. Such losses chiefly apply to fresh perishable items such as dairy products. Nearly half of these retail losses come from fluid milk and other dairy products (Kantor \textit{et al.} 1997). The operation in global fermented products market provokes frequently the rejection and withdrawal of products out of shelf life (Alonso \textit{et al.} 2011). Limited indications are reported in the literature about food losses. The increasing interest in fermented dairy products has been due to the nutritional value and taste of these products (Samet & Attia 2011). Yoghurt is the best known of all fermented milk products, and the most popular worldwide. Rayeb and Iben are also popular fermented milk products known in the North Africa and the Middle East. Traditionally, rayeb is made from raw milk spontaneously fermented. When butter and buttermilk are separated by churning, the obtained buttermilk is called Iben. In an industrial scale, processing steps are basically the same as those used in the traditional method with the required adjustments for standardization and enhancement of safety and keeping quality of the final products (Benkerroum & Tamime 2004). Figure 1 summarizes the manufacturing stages of rayeb and Iben making.

Lactic acid bacteria (LAB), yeasts and moulds are employed in the manufacture of fermented milk products, which contributes to their organic load increase (Tamime \textit{et al.} 2011). In addition, yoghurt is sweetened with high levels of conventional added sugars such as sucrose and glucose (Tamime & Robinson 2007). The withdrawal of damaged products or those over their sell-by date from the...
market, creates a significant amount of dairy wastes unsuitable for sale. Considering the increasing manufacturing environmental implications, the treatment of residuals has to be considered. Although in some cases they could be used for animal feed (Scholten et al. 1993), their high water content remains a limiting factor (Alonso et al. 2003).

Most of the wastewater volume generated in the dairy industry results from cleaning transport lines and equipment between production cycles, cleaning tank trucks, washing milk silos and equipment malfunctions or operational errors (Baskaran et al. 2003). Hence, by joining the plant wastewater, fermented residuals contribute largely towards their high organic matter and its chemical oxygen demand (COD) rise. Consequently, dairy wastewaters discharging in municipal wastewater treatments is unacceptable. In Tunisia, the discharge standards in the municipal wastewater treatment plant (WWTP) (ONAS) is fixed at 1 g.L⁻¹ (Tunisian Standards NT 106 002). Even though the majority of dairies are equipped with their own WWTP, those receive high loaded rejects and the quality of their effluent is very average. Some of these stations are saturated and have poor performance. Therefore, segregation of polluting effluents from sanitary installations, processing, and cooling systems would facilitate the ability of their treatment and/or recycling (Kasmi et al. 2009). This technique has been demonstrated to be efficient in several food processing plants. In terms of wastewater treatment, some companies achieved annual reductions of 25% in biochemical oxygen demand (BOD₅) and 32% in COD. The improvement was a logical consequence of the reduction of product losses (MedTEST 2012b). Additional environmental benefits have been achieved in terms of wastewater pollution load reductions corresponding, respectively, to 17% BOD₅ and

Figure 1 | Flow chart for the industrial manufacture of Rayeb and Lben using pasteurized milk (Benkerroum & Tamime 2004).
10% COD annual loads, mainly resulting from improved management of residual products. These have reduced the investment and operational costs of the WWTP at design stage (MedTEST 2012a). Furthermore, in the Tunisian dairy industry CLC (Centrale Laitière du Cap bon), the minimization of loss generation efforts have been reduced from 4.5% to 3.2%, a reduction equivalent to annual economic earnings of about $369,310 (MedTEST 2012b).

Otherwise, to meet the stringent effluent discharge criteria, aerobic biological treatments relying on conventional activated sludge plants are generally employed (Tocchi et al. 2013). However, the application of conventional methods on the dairy wastewater proves to be a difficult and complex process, which also consumes a great deal of energy at low efficiency rates (Fergala 1993). Membrane, chemical and simultaneously physical–chemical methods were investigated to reduce the dairy wastewater organic load. However, these processes have an inherent drawback due to the high operating costs from either the use of external acid sources or flocculating agents (Seesuriyachan et al. 2009). Meanwhile, biological processes are suitable cost-effective means of organic removal (Gray 2004). In recent years, several works have focused on the reuse or the recycling of industrial wastes. In a previous study, bio-coagulation of dairy wastewater was performed using Lactobacillus casei for protein recovery (Seesuriyachan et al. 2009). Yoghurt whey from expired products has been fermented using L. casei for lactic acid production (Alonso et al. 2010).

This work proposes an upstream segregation of fermented dairy residuals from the plant wastewater to be treated separately. The reduction of fermented milk wastes polluting load before their entrance into the dairy WWTP is the challenge of this work. Coupled thermal coagulation process and biochemical fermentation are proposed to maximize the generated dairy wastewater COD removal rates. At first, the thermal coagulation was performed to remove protein and fat content. Then, the growth rate and the degrading potential of Candida strains, previously isolated and identified from milk waste, were investigated on the residual recuperated whey.

**MATERIALS AND METHODS**

**Sampling**

Samples of residual fermented dairy products were collected from a nearby dairy industry. Such products were either discarded from the production lines, where they were designated as defective items, or they were withdrawn because of their expired shelf life. Yoghurt and two other Tunisian industrial fermented milk products, rayeb and lben, were considered in this study. Since the fermented milk products have a very similar composition, and the quantity of their residual products is relatively less important, one sample model was performed 50% rayeb and 50% lben. The rayeb and lben sample model will be designed as (RL); the yoghurt sample as (Y). Samples were stored at 4 °C.

**Samples treatment**

The thermal coagulation was realized in glass vessels emerged in a controlled temperature heating bath under a continuous stirring (100 rpm). The heat treatment was performed at different bearing temperatures (20, 30, 40, 50, 60, 70, 80, 90 and 100 °C). Clotting time is indicated as time from the sample exposure to the desired treatment temperature to the formation of the first visible floccules. The heating temperature and the thermal exposure time were considered to optimize the thermal coagulation. Thereafter, samples were decanted in conic devices until a steady pellet settling. Supernatants were collected and centrifuged (3,500 rpm for 15 min) to separate solid–liquid fractions. COD values of the supernatants were assessed.

**Whey preparation: clarification, stabilization**

The recuperated whey was stabilized to remove residual fat and caseins according to the protocol proposed by Moulin et al. (1976). The filtrate pH was adjusted at 4.6 (caseins isoelectric point) with HCl (1N), then it was sterilized for 5 min at 120 °C. After cooling, whey was filtrated (using Whatman filter). Filtration was repeated several times until clear serums were obtained. The filtrate pH was adjusted at 7 using NaOH solution (1N), and then sterilized at 120 °C for 20 min. This same protocol was adopted by Boudjemaa et al. (2009) to manipulate the sweet cheese whey prior its fermentation using Streptococcus thermophilus.

**Microorganism**

Ten milliliters of dairy effluents were enriched in 90 mL of Sabouraud broth (Bio-rad, France) for 48 h at 30 °C. A loopful of the medium was streaked on Chromagar Candida (Chromagar, France), and the different morphotypes obtained were cultured on Sabouraud Chloramphenicol agar (Bio-Rad, Marnes-La-Coquette, France) for 48 h at 30 °C. The identification of Candida species was based on the following: macroscopic characteristics of the colonies; the microscopic examination of the yeast morphology...
(colouration); the chlamydospores formation in Agar Tween (Difco, Paris, France); and the colony colour on Chromagar Candida (Chromagar, France). Strains were stored at 4 °C on Sabouraud dextrose broth (Biol-Rad) supplemented with glycerol at 10% (v/v). Biochemical characteristics were studied using the carbohydrates assimilation tests using both Api Candida and Api 20C systems (bio-Mérieux, Marcy l’Etoile, France). Three Candida strains were isolated and identified from the dairy effluent samples. They were cultivated on Sabouraud medium (50 °C for 48 h) to be inoculated in the fermentation medium.

**Microbial inoculum preparation and culture conditions**

A loopful of Candida strains grown on Sabouraud agar plates was used to inoculate, separately, 100 mL Erlenmeyer flasks containing each one 50 mL of Sabouraud broth media. Flasks were incubated at 37 °C in a thermo-shaker ZHICHENG ZHWY-103B at 150 rpm for 18 h. The obtained active grown cells were used as a seed culture to inoculate 250 mL 6% fermentation medium.

Bacterial growth was monitored through the optical density measure at 600 nm (OD600) using Thermo Spectronic UV1 equipment with VISIONlite™ software. Each culture was centrifuged for 15 min at 4,000 rpm and subsequently the pellet was used to determine the cell dry weight by gravimetric analysis after drying at 105 °C in an oven to constant weight. The cell dry mass is expressed in g.L−1. The total cell count (CFU.mL−1) was determined using a standard agar plate technique. The appropriately diluted samples were plated on MRS (Man-Rogosa-Sharpe) agar beforehand prepared; inoculated plates were incubated at 37 °C for 48 h to get separated colonies.

**Analytical methods**

The pH, conductivity (mS.cm−1) and total dissolved solids (TDS) (g.L−1) of each sample were determined using multi-parameters device Consort C860. To estimate the thermal coagulation efficiency of residual fermented dairy products, the COD of supernatants was measured by the potassium dichromate colorimetric method using an opened reflux system (Rodier et al. 2009). Reducing sugars were quantified using Bertrand method based on a cuprometric titration after the sample defecation (AFNOR 1971). The protein content was obtained using the Kjeldahl nitrogen determination method, using 6.38 as the protein conversion factor (Rombaut & Dewettinck 2007). The titratable acidity was measured by titration with 1N NaOH in the presence of phenolphthalein and it is expressed in the amount of lactic acid (grams) in the sample (AFNOR 1980).

**RESULTS AND DISCUSSION**

**Residual fermented dairy products characterization**

The residual fermented dairy products (Y and RL) characteristics and composition were determined. Table 1 summarizes the obtained results. Since Y and RL samples are fermented milks, their pH is obviously acid. The recorded pH values are very close (3.9 and 3.8; respectively). Conductivity and TDS measured values are more important with Y comparing to RL samples. These values are in proportion with the sugar content present in each sample: 54 g.L−1 for Y and 32 g.L−1 for RL. Such values confirm that yoghurt is supplemented with high level of conventional sugar as reported by Tamime & Robinson (2007). Besides, the mineral content of the fermented products contributes to the augmentation of the dissolved solids concentration. In fact, fermented milks are known for their considerable mineral intake comparing to the drinking milk (Tamang & Kailasapathy 2010; Bergillos-Meca et al. 2015).

Samples polluting charge assessment showed that RL product has an important COD of 246 g.L−1, while Y sample presents only 152 g.L−1. This could be attributed to the action of hydrolytic enzymes facilitating the uptake of lactose, proteins and lipids. The microbial metabolism

<table>
<thead>
<tr>
<th>Yoghurt (Y)</th>
<th>Rayeb &amp; Lben (RL)</th>
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<tbody>
<tr>
<td>pH</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>Conductivity (mS.cm−1)</td>
<td>75.1 ± 0.2</td>
</tr>
<tr>
<td>TDS (g.L−1)</td>
<td>66.8 ± 0.3</td>
</tr>
<tr>
<td>Protein (g.L−1)</td>
<td>33.2 ± 2.3</td>
</tr>
<tr>
<td>Sugar (g.L−1)</td>
<td>54.1 ± 4.0</td>
</tr>
<tr>
<td>Fat (g.L−1)</td>
<td>24.7 ± 3.2</td>
</tr>
<tr>
<td>COD (g.L−1)</td>
<td>152 ± 4</td>
</tr>
</tbody>
</table>
produces volatile carbonyl compounds involved in aromatic rich of RL products: acetaldehyde (ethanal) confers the fruity odor, the acetoin and diacetyl for the cheese smell. These volatile compounds are also found in yoghurts. Nevertheless, in lben, they are associated with the presence of a significant concentration of alcohol (ethanol) that contributes to its typical aroma. Indeed, the bacterial microflora present in these products has a considerable protein fraction, which may explain the recorded protein content (Hanchi et al. 2009; Samet & Attia 2011). Furthermore, it has been reported that the processed milk used for the manufacture of fermented products of rayeb and lben is partially concentrated by boiling before being sown (Tchamba 2007), which contributes to its protein content increase. All those factors seem to be responsible for the important organic polluting load of the residual fermented dairy products.

**Thermal coagulation: pH variation and COD removal**

Heat treatment was performed for the residual fermented dairy samples. Withdrawals were done at each bearing temperature. After decantation and supernatant centrifugation, pH and COD values were measured. Figure 2 illustrates the thermal treatment effect on pH and COD profiles. Samples thermal coagulation in this process was convenient to the pH-T route as described by Vasbinder et al. (2005), where pH was initially low, then, the temperature (T) has been increased. pH values record a slight decrease during the heat treatment for both Y and RL samples. However, a considerable drop in the organic load of the treated samples was noticed at 40 °C where COD values were 104 g.L⁻¹ for the Y sample, and 96 g.L⁻¹ for RL sample. Consequently, COD removal rates were 32% for the Y sample and 61% for the RL sample. Thereafter, samples organic load has continued to decrease moderately until the processing temperature of 80 °C, where removal rates reached 53% for the Y sample and 80% for the RL sample.

Despite their high pollution load, thermal coagulation process seems to be more efficient with RL samples compared to the Y ones. The relative resistance of Y products to the thermal coagulation may be attributed to the performed manufacture process operations. In fact, homogenization is generally combined with heat treatment (pasteurization or UHT) for the milk to be processed in yoghurt in the way to improve the stability of the treated matrix (Singh 2004). The purpose of this treatment is to denature a significant portion of the soluble protein, which has the effect of increasing the water retention capacity of the yoghurt and allow these proteins to bind to the casein. This dual phenomenon modifies the rheological properties of the acidified coagulum resulting from a firmer curd and the trend in serum expulsion during storage is reduced (especially when the product is exposed to high temperature) (FAO 1998).

Nevertheless, a rise of COD values was observed for both samples treated at 90 and 100 °C to meet the recorded removal rates at 60 °C. This behavior may be explained by...
the release of the fat globules initially present in the products. Actually, the emulsion destabilization may cause the release of the unstructured fat globules to the supernatant which contributes to the COD increase of the latter. Such phenomenon has been reported by Fauquant et al. (1985) where the temperature of 80 °C was identified as a threshold beyond which the centrifuged, clarified whey begins to lose its clarity (Fauquant et al. 1985). Apart from a few phospholipids, sterols and fatty acids present in the whey, fat is dispersed in milk in the form of spherical cells limited by a membrane formed of 15 polypeptides, triglycerides and complex lipids (phospholipids, sterols, etc.). The lipoprotein membrane confers the fat globule stability. Due to the membrane fragility, its rupture by agitation, repeated refrigeration or acidification destabilizes the emulsion leading to the fat release. Then, lipoprotein membrane could undergo lipolysis due to the action microbial lipases (Contarini & Povolo 2013). This is supposed to be the case of thermally treated samples.

Another cause that may be responsible for the increase of the supernatant organic load is the lysis of the microbial biomass contained in the fermented products after thermal stress. In fact, the possible lipolytic activity existence in LAB can be confirmed for some species. Streptococcus lactis, a common species of mesophilic starter cultures, has a very low level compared to the lactobacilli. The bacteria produce phosphatases, which, attacking the phospholipids present in the medium, turn them into more assimilated substances for bacteria (Cherkat 1967). The supposed interpretation is based on the internal metabolism of LAB, since the degradation of milk products phospholipids, once absorbed by the bacteria, are transformed back into bacterial phospholipids (Cherkat 1967). After the bacteria lysis, the hydrophilic phospholipid fraction is released into the whey. Most of these phospholipids, nearly 45% of the total, pass into the aqueous phase when the oil-in-water emulsion is destroyed (Rhodes & Lea 1958; Alais 1984). Nevertheless, a considerable proportion of total phospholipids remaining in the supernatant is probably derived from smaller fat globules membranes and lipoprotein particles that escaped the centrifugal separation (Morton 1954).

In conclusion, once treated at 80 °C, thermal coagulation process followed by centrifugation of the fermented dairy products yields maximum COD removal rates of supernatants. The recorded minimal COD values are 48 g.L⁻¹ for Y and 72 g.L⁻¹ for RL samples. Such amounts are still considered important to discharge the resulted whey in that state.

Whey fermentation

Strain isolation and identification

Three morphotypes were obtained on CHROMagar TM Candida based on the colour of the colonies: blue, white, and pink colonies. Three different colonies were purified on Sabouraud Chloramphenicol agar and identified as Candida tropicalis, Candida lusitaniae, and Candida krusei based on the morphological; microscopic; and biochemical characteristics obtained on analytical profile index (API) strips.

Whey clarification: stabilization

The stabilization effect on the reduction of samples COD values was carried out using samples previously treated at 60 °C, 80 °C and 100 °C. Resulting wheys will be designed as follow: Y₆₀, Y₈₀ and Y₁₀₀; RL₆₀, RL₈₀ and RL₁₀₀, for Y and RL whey previously treated at the indicated temperatures, respectively. Obtained results following wheys stabilization are illustrated in Figure 3.

Contrary to the thermal coagulation process, stabilization proves to be more effective with the Y whey than compared to the RL whey at the performed thermal treatment temperatures. The partial reduction rates resulting from wheys stabilization are 20% for Y₆₀ and 15% for RL₆₀, 40% for Y₈₀ and 35% for RL₈₀ and 28% for Y₁₀₀ and 22% for RL₁₀₀. Except that the recorded global removal rates for RL samples (80–87%) remain more interesting
compared to the values obtained with Y samples (54–72%) for all the considered treatment temperatures. The maximum removal rates are recorded for samples previously treated at 80 °C. This conclusion was well illustrated earlier following the heat treatment of this type of dairy waste. The lowest recorded residual COD values for fermented milk products following the physical–chemical treatment are 43.2 and 31.7 g.L⁻¹ for Y₈₀ and RL₈₀ samples, respectively. In fact, such treatments allow the partial removal of the organic load by protein and fat precipitations with different chemical compounds such as aluminium sulphate, ferric chloride and ferrous sulphide (Rusten et al. 1993).

Clarified whey fermentation using candida strains

Considering the resulting performances of the physical–chemical treatment process for the studied fermented dairy products, wheys obtained from the performed thermal treatment at 80 °C will be considered for the biological treatment. At first, the clarified wheys composition has been investigated to determine residual valuable components. Table 2 resumes the acquired results. pH values have been increased after the stabilization process compared to the thermal coagulation resulting pH values. Sugars contents recorded a slight decrease (8–10%), but their concentrations in wheys are considerable (50 and 29 g.L⁻¹). Proteins removal rate exceeded 80%. While fat has been completely eliminated. Nevertheless, residual COD values are still important: 31.7 and 43.2 (g.L⁻¹) for RL₈₀ and Y₈₀ clarified wheys, respectively. Consequently, the resulted liquid fraction needs further treatment.

The degrading potential of Candida strains was evaluated for the recovered wheys. Y₈₀ and RL₈₀ mediums were inoculated separately with the three identified Candida strains. Fermentation was performed in batch for 24 h. Yeast free fermented media COD were assessed. Resulting removal rates are illustrated in Figure 4. Fermented media have presented different reduction rates of the organic load. Fermented RL₈₀ whey COD removal rates vary from 59 to 85%, while the fermented Y₈₀ whey removal values were limited to 11–78%. Except that a reduction rate disparity was noticed for the same strain with different culture media. In fact, the best removal rates were recorded with C. krusei strains (78% and 85%). C. tropicalis strain achieved a polluting load reduction rate of 63% with RL₈₀ and only 11% with Y₈₀. However, C. lusitaniae strain has recorded a COD removal rate of 57% with Y₈₀ whereas its degrading potential was much important with RL₈₀ (59%).

Thus, the degrading potential of Candida strains depends on their affinities with the fermented medium. To maximize the organic material degradation rate, C. krusei strain would be the best suited for Y₈₀ and RL₈₀ medium. Therefore, it is assumed that this strain exhibits a maximum efficiency with the fermented dairy products. Nevertheless, the recorded COD removal rates appear not consistent with the strains bacterial growth. In fact, with Y₈₀ whey C. lusitaniae strain presents the most important bacterial growth (OD₆₅₀ = 8.4), while C. krusei records a less important bacterial concentration (OD₆₅₀ = 7.7).

As a result, it seems that C. tropicalis strain does not exhibit affinity for Y₈₀ medium where its bacterial growth was limited to OD₆₅₀ = 1.9. This may explain its weak degrading potential with this medium, although some old researches confirmed that C. tropicalis strain is among the most effective strains to grow on whey permeate (El-Samragy & Zall 1988). Although, it has been revealed that it is not the case of yoghurt whey. In contrast, with RL whey medium all strains recorded a considerable bacterial growth (OD₆₅₀ > 8) despite the disproportion of their corresponding removal rates. C. krusei strain important degrading potential has been confirmed by Yadav et al. (2012) who added that the strain biomass could be used as protein of unicellular organism (SCP) or as feed for animals since it did not excrete extracellular toxins (Yadav et al. 2012).

Table 2  | Biochemical characteristics of the clarified whey resulted following the physical-chemical treatment of residual dairy fermented products at 80 °C: yoghurt (Y₈₀) and Rayeb & Lben (RL₈₀)

<table>
<thead>
<tr>
<th>Clarified whey</th>
<th>pH</th>
<th>Protein (g.L⁻¹)</th>
<th>Sugar (g.L⁻¹)</th>
<th>Fat (g.L⁻¹)</th>
<th>COD (g.L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y₈₀</td>
<td>6.2 ± 0.2</td>
<td>6.3 ± 0.5</td>
<td>50 ± 2</td>
<td>n.d</td>
<td>43.2 ± 3.7</td>
</tr>
<tr>
<td>RL₈₀</td>
<td>6.4 ± 0.1</td>
<td>4.4 ± 0.2</td>
<td>29 ± 3</td>
<td>n.d</td>
<td>31.7 ± 4.1</td>
</tr>
</tbody>
</table>

n.d: not detected.
Consequently, the best organic load abatement obtained using C. krusei resulted in final residual COD values of 9.5 g.L\(^{-1}\) for Y fermented whey and 4.7 g.L\(^{-1}\) for RL fermented whey. Those values are going to be further reduced under the dilution effect by joining the industry washing waters, which may relieve the dairy WWTP and allow meeting the municipal WWTP input limit.

**CONCLUSION**

Residual fermented dairy products, a waste material containing important concentrations of sugar, proteins, fat and minerals that contribute to its polluting load increase. Y and RL samples have COD values of 152 and 246 g.L\(^{-1}\), respectively. The thermal coagulation treatment at temperatures above 60 °C resulted in important COD removal rates. Although maximum removal values were recorded with samples treated at 80 °C, the thermal coagulation seems to be more efficient with RL samples where organic load reduction was about 80% and only 53% with Y samples. The resulting whey stabilization enhanced removal rates to reach 87% and 72%, respectively. Clarified whey samples have residual COD values of 43.2 and 31.7 g.L\(^{-1}\) for Y\(_{80}\) and RL\(_{40}\) samples, respectively; following physical-chemical treatment. The major component that seems to be responsible for the residual COD values is sugar that could not be removed through the previous treatment. Wheys fermentation using three *Candida* strains, previously isolated and identified from the milk waste, revealed disparate degrading potential with Y and RL media. *C. krusei* achieved the most important removal rates of 78% and 85%, respectively. Therefore, the global polluting load reduction rates are 93% for Y samples and 98% for RL samples. The fermentation contribution to the global effluents COD removal was assessed at 11.2% for RL samples and 23.6% for Y samples. The final residual COD value was limited to 9.5 g.L\(^{-1}\) for Y fermented whey and 4.7 g.L\(^{-1}\) for RL fermented whey following coupled physical-chemical and biological treatments. It is obvious that the biological treatment contribution was moderate compared to the physical-chemical treatment. Multistage fermentation could be performed for better results in term of the polluting load removal.

**REFERENCES**


Benkerroum, N. & Tamime, A. Y. 2004 Technology transfer of some Moroccan traditional dairy products (lben, jben and smen) to small industrial scale. *Food Microbiology* 21 (4), 399–415.


Gray, N. F. 2004 *Biology of Wastewater Treatment*. Imperial College Press, London, UK.


MedTEST 202a Beverage industry, case study FOOD sector—Tunisia. In: Transfer of Environmental Sound Technology in the South Mediterranean Region, MedPartnership UNIDO, Rome, Italy.


Tchamba, C. N. 2007 Caractérisation de la flore lactique des laits fermentes artisanaux au Sénégal: cas de la zone des Niayes Vétérinaire, Université cheikh anta diop de Dakar, Sénégal.


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