Biodegradation of dairy wastewater using bacterial and fungal local isolates
Raed S. Al-Wasify, Mohamed N. Ali and Shimaa R. Hamed

ABSTRACT

Dairy wastewater contains high levels of organics and other pollutants. The present study was carried out to investigate the biodegradation process of dairy effluents using some locally isolated bacteria and fungi. Four different dairy effluent samples were collected from Obour and 6th October industrial cities, Egypt. Five bacterial species (Pseudomonas aeruginosa, Bacillus subtilis, Lactobacillus delbrueckii, Staphylococcus aureus and Enterococcus hirae) and three fungal strains (Alternaria sp., Fusarium sp. and Aspergillus sp.) were isolated from dairy wastewater samples, identified and used for biodegradation process. Bacterial and fungal consortia were prepared separately in the laboratory. Two-stages (aeration and filtration) laboratory scale model was designed. Rice straw and activated carbon layers were used as filtration media. Results indicated the great ability of both studied bacteria and fungi for removal of organics (biological oxygen demand removal percent were 78.7% and 74.7% for bacteria and fungi, respectively) and the improvement of the physicochemical quality (total suspended solids removal percent were 99.3% and 99.0% for bacteria and fungi, respectively) of the dairy effluent. The addition of rice straw and activated carbon increased removal efficiencies. Biodegradation of dairy wastewater depending on local microorganisms is an effective, cheap and eco-friendly technology.

Key words | bacteria, biodegradation, dairy effluent, fungi

INTRODUCTION

The fast growth of industries does not solely increase the productivity, it also results in unleash of toxic substances that are discharged into the land or water bodies. This in turn destroys the environment and causes serious health hazards for humans (Porwal et al. 2015).

There are many physicochemical methods that have been studied and applied for wastewater treatment. These methods include screening, sedimentation, flotation, filtration, aeration, coagulation, ozonation, chlorination, ion exchange, degasification, neutralization, etc. However, these methods have many limitations such as the use of chemical agents, higher cost, partial treatment, production of secondary pollutants and production of large volumes of solids. Due to these limitations, the application of biological methods was more suitable to be used as an alternative technique (Rodrigues et al. 2008). Produced wastes (sludge and effluents) from food industries, including dairy industry, contain high levels of organic matter, fatty acids, oil and grease (O&G) and notable nitrogenous compounds (Porwal et al. 2015).

Food industry is one of the highest industries in consumption of water and production of effluents per unit of production. Also, the food industries generate large volumes of sludge through the biological treatment process. Sludge production in aerobic systems is about 0.5 kg per kg of removed chemical oxygen demand (COD), while in the anaerobic system, sludge production is about 0.1 kg (Kaur & Chaman 2014). As for milk processing industries, the high load of pollutants in dairy wastewater led to the discharge of partially treated or untreated wastewater which in turn caused serious environmental and public health problems (Kaur & Chaman 2014).

Since water is the major component in the dairy industry, then the safe disposal of the significant effluent volumes that are frequently generated is a real challenge. Dairy industries generate, on average, about 6 to 10 L of wastewater per litre of processed milk (Kolhe & Pawar 2011). Raw dairy wastewater composition varies depending on operations and products. Dairy wastewater contains
large quantities of milk constituents, including lactose, casein, inorganic salts, in addition to sanitizers and detergents used for washing (Kolhe et al. 2009).

Dairy effluents contain fats, dissolved proteins and sugars, and residues of additives. According to the presence of these high organic loads, when dairy effluents discharged into water bodies, these effluents rapidly degraded causing depletion of dissolved oxygen (DO) levels of receiving water bodies. These receiving water bodies then become a propagation place for flies and mosquitoes carrying dangerous diseases such as malaria, chicken guinea, dengue fever and yellow fever. Additionally, dairy wastewater characterized by the strong odour of butyric acid and heavy black flocculated sludge masses (Kumar & Desai 2011). Dairy wastewater effluents can have negative impacts on wastewater treatment systems since these effluents are rich in fats, O&Gs which cause blockage of sewerage pipes and foul odours (Page et al. 2014). Raw milk contains ammonia nitrogen and nitrate which may cause methemoglobinemia if converted to nitrite, which contaminates groundwater (Kushwaha et al. 2011).

Many biological treatment methods are used for dairy wastewater treatment; these methods include aerated lagoons, activated sludge, sequencing batch reactor (SBR), trickling filters, anaerobic filters and anaerobic sludge blanket (UASB) (Demirel et al. 2005). In recent years, a new technology called bioremediation was discovered. This technology depends mainly on using the microorganisms or their enzymes to convert polluted environment into its original condition. This technology is economical (relatively low-cost), uncomplicated (requires low-technology techniques) and eco-friendly (used non-pathogenic microorganisms which have high public acceptance). Microorganisms used during the bioremediation process may be indigenous to the contaminated area or they may be isolated from different sources and brought to the contaminated site. Moreover, to obtain efficient bioremediation, it is very necessary to know the microbial composition of wastewater to be treated, as well as biochemical properties correlated to origin pollutants and the optimum metabolic activity and physico-chemical conditions (Janczukowicz et al. 2008).

Many heterotrophic bacterial species can be found in dairy wastewater such as Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Pseudomonas fluorescens, Escherichia coli, Streptococcus faecalis, Enterobacter, etc. Also, yeasts belonging to genus Candida, Saccharomyces and Cryptococcus are found (Madigan et al. 2000). A laboratory model was designed to study the ability of some well adapted local microorganisms (bacteria and fungi), which isolated from dairy wastewater, to degrade the organic nutrients present in dairy effluent and to improve the physicochemical quality of dairy wastewater. The model was supplemented with a natural filtration media (rice straw and activated carbon) for better treatment.

MATERIALS AND METHODS

Sample collection

Four fresh dairy effluent samples were collected from four different dairy factories located in Obour City and 6th October City industrial zones, Egypt. Samples were collected in sterile 4-L polyethylene containers (Thomas Scientific®, USA). Samples were stored at 4 °C inside an ice box then transferred immediately to the laboratory for further experiments.

Isolation of microorganisms

Dairy effluent samples were serially diluted (10⁻¹–10⁻⁵). One mL of each dilution was inoculated into a 250-mL Erlenmeyer flask containing nutrient broth (for bacteria), and another 1 mL was inoculated into another 250-mL Erlenmeyer flask containing potato dextrose broth (for fungi). Flasks were incubated at room temperature on a rotary shaker at 100 rpm for 24–96 h. A loopful of enriched sample from nutrient broth flask was streaked on nutrient agar Petri dishes and a loopful of enriched sample from potato dextrose broth was streaked on potato dextrose agar Petri dishes. Inoculated nutrient agar Petri dishes were incubated at 35 °C for 24 h, while inoculated potato dextrose Petri dishes were incubated at 25 °C for 5 days. For each medium, the plating was done in triplicate.

Specific culture media

Two different dairy products (milk and yogurt) were used because these two products are the main dairy products consumed by customers in Egypt. Well grown individual bacterial colonies (based on shape and colour) on the surface of nutrient agar Petri dishes were picked up and inoculated into 250-mL Erlenmeyer flasks containing milk broth (peptone; 5 g, yeast extract; 3 g, fresh milk; 10 mL). After inoculation, flasks were incubated at 35 °C on a rotary shaker for 24–48 h. After that, a loopful was streaked on milk agar Petri dishes and incubated at 35 °C for 24 h. After incubation, single pure colonies were suspended in
nutrient broth containing 10% (v/v) glycerol and stored at -80 °C for identification and further experiment. In the same way, individual fungal colonies were selected and inoculated into potato dextrose broth containing 5 mL yogurt then incubated at 25 °C for 5 days. After that, single pure colonies were inoculated into potato dextrose broth containing 10% (v/v) glycerol and stored at -80 °C for identification and further experiment.

Identification of microbial isolates

Different bacterial isolates (n = 16) were identified using Biolog’s microbial identification system (Biolog® Gen III, USA). While fungal isolates (n = 7) were identified depending on colony morphology and microscopic examination using lactophenol cotton blue staining method (Kaur & Chaman 2014).

Inoculum preparation

For each identified bacterial isolate, 0.1 mL of bacterial suspension was inoculated in 100 mL inoculum media (nutrient broth). Inoculated flasks were kept on a rotary shaker at 150 rpm at 35 °C for 24 h. Also, for each identified fungal isolate, a loop was inoculated into 100 mL potato dextrose broth and incubated at 25 °C on a rotary shaker at 120 rpm for 5 days. For studying the biodegradation efficiency of identified bacterial and fungal isolates, the biomass of actively growing cells was prepared. Each bacterial isolate was grown on 50 mL nutrient broth and 50 mL potato dextrose broth for 48 h. After the incubation period, active growing microbial cultures washed three times with sterile deionized water, then centrifuged at 10,000 rpm for 10 min to get wet pellets (Porwal et al. 2015).

Experimental setup

A laboratory two-stage model was set up for studying the biodegradation process of dairy effluent (Figure 1). The model was inspired by the model suggested by Porwal et al. (2015), and modified according to the requirements of the present study. Two rectangle glass tanks of capacity 2.0 L with 50 cm height for each were used. The first tank was considered as the primary tank while the second tank was considered as the secondary tank. Two different models were set up, one for bacterial isolates and the other one was for fungal isolates. The two tanks were sterilized with ethanol, then rinsed with sterile deionized water. An aerator was used for continuous aeration while keeping a DO level above 5 mg/L. At the bottom of the secondary tank, two layers of filtration media were placed, 5 cm height of rice straw on the top while 5 cm height of activated carbon was on the bottom. The first tank was filled with 1.5 L cooled (room temperature) autoclaved untreated dairy effluent. At bacterial model, 10 mL of each identified bacterial culture was inoculated into the primary tank. Similarly, the fungal model was inoculated with 10 mL of each identified fungal culture into the primary tank. Aerator then placed and the primary tank was covered with aluminum foil. Retention time was 48 h in the first tank, and then the dairy effluent was allowed to flow into the secondary tank. Following the complete filtration, the treated dairy effluent was tested physiochemically in the laboratory.

Measured parameters

Some physicochemical parameters were measured for untreated dairy effluent and final filtrate after treatment, according to Standard Methods for the Examination of Water and Wastewater (APHA 2010). These parameters include; pH, colour, electric conductivity (EC), turbidity, total dissolved solids (TDS), total suspended solids (TSS), biological oxygen demand (BOD), COD, O&G, and sulfates.

RESULTS AND DISCUSSION

Characterization of dairy effluent

Table 1 summarizes the obtained results of the physicochemical analysis for untreated dairy wastewater. The obtained values of raw dairy wastewater were in accordance with findings of Vida et al. (2007). pH of dairy effluents depending on the nature of the end-product and can range from 4.7 to 12.2 (Passeggi et al. 2009). The influent dairy wastewater was slightly acidic (6.0 ± 0.152). The acidic pH is attributed to the breakdown of milk lactose into lactic acid (Slavov 2017). Untreated acidic dairy effluent could have harmful effects on soil, water bodies and microflora (Bako et al. 2008). TSS is one of the main parameters of water which used to evaluate and determine the efficiency of treatment processes of wastewater. The influent dairy milk showed high concentrations of TSS (644 ± 11.35). Porwal et al. (2015), reported the same high concentrations of TSS (626.6 ± 8.79 and 601.6 ± 3.46). The presence of high concentrations of TSS can cause some problems for aquatic life. Suspended solids reduce the penetration of
light in receiving water bodies which may clog fish gills (Baruah et al. 1993). Suspended solids in dairy effluent originate from gelatinous milk and flavourings (Brown & Pico 1979). BOD is one of the most widely used indicators of water quality. The influent dairy wastewater showed high concentrations of BOD (1,166 ± 13.22 mgO2/L). The dairy effluents are characterized by high levels of BOD due to the presence of fats, lactose, nutrients, casein, sanitizing agents and inorganic salts (Kolhe et al. 2009).

### Characterization of isolated microorganisms

Sixteen bacterial isolates were subject to identification using Biolog® Gen III identification system. The identified bacterial isolates showed some repeats and finally, five bacterial strains were identified as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Lactobacillus delbrueckii*, *Staphylococcus aureus* and *Enterococcus hirae*. In addition, seven isolated fungal isolates were subject to identification based on colony morphology and microscopic examination. Some repeats were also detected. Three fungal strains were identified as *Alternaria* sp., *Fusarium* sp. and *Aspergillus* sp.

### Characterization of final treated effluent

A mixture of identified five bacterial species (*Pseudomonas aeruginosa*, *Bacillus subtilis*, *Lactobacillus delbrueckii*, *Staphylococcus aureus* and *Enterococcus hirae*) were identified. The final treated effluent was characterized by a decrease in the concentration of turbidity, BOD, TDS, TSS, COD, O&G, and sulfates. The pH and electric conductivity were found to be within the acceptable limits. The characterization of raw dairy effluent is presented in Table 1.

#### Table 1: Characterization of raw dairy effluent

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Average ± standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>–</td>
<td>6.0 ± 0.152</td>
</tr>
<tr>
<td>Colour</td>
<td>–</td>
<td>Milky</td>
</tr>
<tr>
<td>Electric conductivity</td>
<td>μS/cm</td>
<td>450 ± 12.05</td>
</tr>
<tr>
<td>Turbidity</td>
<td>NTU</td>
<td>1,120 ± 27.40</td>
</tr>
<tr>
<td>TDS</td>
<td>mg/L</td>
<td>1,820 ± 27.07</td>
</tr>
<tr>
<td>TSS</td>
<td>mg/L</td>
<td>644 ± 11.35</td>
</tr>
<tr>
<td>BOD</td>
<td>mgO2/L</td>
<td>1,166 ± 13.22</td>
</tr>
<tr>
<td>COD</td>
<td>mg/L</td>
<td>2,248 ± 21.16</td>
</tr>
<tr>
<td>O&amp;G</td>
<td>mg/L</td>
<td>148 ± 7.81</td>
</tr>
<tr>
<td>Sulfates</td>
<td>mg/L</td>
<td>86 ± 7.0</td>
</tr>
</tbody>
</table>

**Figure 1**: Laboratory model for dairy effluent biodegradation.
Staphylococcus aureus and Enterococcus hirae) was prepared with equal percent and used for the treatment process and named as the bacterial model. Similarly, the experiment was carried out separately using three fungal strains that were mixed and used for the treatment process and named as the fungal model. Table 2 shows the values of physicochemical parameters of treated dairy effluent after aeration stage (primary tank) and filtration stage (secondary tank) in addition to the overall removal percent. Results showed that the colour of dairy wastewater has been improved, and it was milky. After complete treatment, it turned clear. This improvement may be attributed to the degradation of organic materials by bacterial and fungal mixtures. Moreover, using rice straw and activated carbon as filter media led to removing more suspended particles and consequently colour improvement (Verma & Madamwar 2002).

**pH**

The obtained results in Table 2 show that pH values moved towards the neutrality in both bacterial and fungal models. It was clear that both aeration stage and filtration stage have the same effect on the changes of pH values. Porwal et al. (2015), studied the biodegradation of dairy wastewater using microbial isolates obtained from activated sludge and they found the same changes in pH values. The change in pH values may be attributed to the ability of microorganisms to accumulate organic acids after the biodegradation process (Kowsalya et al. 2010).

### Electric conductivity (EC)

Electric conductivity is considered as an important parameter which can be used for quantitative measurement of dissolved ionic constituents in water and detection of impurities, which are necessary for cooling water and boiler feed water systems. It was clear, after filtration stage, great reduction in EC values. The bacterial model showed EC removal percent (88.4%) higher than fungal model removal percent (85.7%). Reduction efficiency of EC may be attributed to consumption of ions by bacteria and fungi for their growth and other metabolic activities (Porwal et al. 2015). In addition, the removal efficiency of EC was improved after filtration, and this may be due to the adsorption of ions on the activated carbon layer.

**Turbidity**

Turbidity is a critical parameter for public water supplies, especially for filterability, aesthetics and disinfection processes. After aeration process, it can be seen that turbidity removal efficiency was 45.3% and 37.5% for bacterial model and fungal model, respectively. Turbidity decreased due to consumption of organic materials and suspended particles by bacteria and fungi through growth and survival. In addition, after filtration, the increase in removal efficiencies was significantly observed. The overall removal percent was 99.3% and 99.1% for bacterial model and fungal model, respectively. This observed decrease in turbidity values after filtration stage was a result of using filter materials.

### Table 2 | Treatment of dairy effluent using bacterial and fungal consortia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Bacterial model*</th>
<th>Fungal model*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>After aeration</td>
<td>R (%)</td>
</tr>
<tr>
<td>pH</td>
<td>–</td>
<td>6.5 ± 0.1</td>
<td>–</td>
</tr>
<tr>
<td>Colour</td>
<td>–</td>
<td>Creamy white</td>
<td>–</td>
</tr>
<tr>
<td>EC</td>
<td>µS/cm</td>
<td>236 ± 2.6</td>
<td>47.5</td>
</tr>
<tr>
<td>Turbidity</td>
<td>NTU</td>
<td>612 ± 3.75</td>
<td>45.3</td>
</tr>
<tr>
<td>TDS</td>
<td>mg/L</td>
<td>1,350 ± 19.5</td>
<td>25.8</td>
</tr>
<tr>
<td>TSS</td>
<td>mg/L</td>
<td>428 ± 7.5</td>
<td>33.5</td>
</tr>
<tr>
<td>BOD</td>
<td>mgO₂/L</td>
<td>300 ± 6.0</td>
<td>74.2</td>
</tr>
<tr>
<td>COD</td>
<td>mg/L</td>
<td>620 ± 3.56</td>
<td>72.4</td>
</tr>
<tr>
<td>O&amp;G</td>
<td>mg/L</td>
<td>82 ± 3.72</td>
<td>44.5</td>
</tr>
<tr>
<td>Sulfates</td>
<td>mg/L</td>
<td>74 ± 2.37</td>
<td>13.9</td>
</tr>
</tbody>
</table>

*Average ± standard deviation.
(rice straw and activated carbon) which adsorbed more suspended particulates of dairy effluent. Cosa & Okoh (2014) reported a similar decrease in turbidity (88.3%) using a consortium of marine species.

**Total dissolved solids**

Organic and inorganic matters present in dairy effluent are the main cause of high level of TDS. These dissolved solids such as chlorides, carbonate, bicarbonate, nitrate, phosphate, sulfate, sodium, magnesium, calcium, etc. The presence of high levels of dissolved solids decreases the utility of water for irrigation, drinking and industrial purposes. Obtained results (Table 2) showed important removal rate of TDS, especially after filtration process. Removal percent of TDS was 79.1% and 77.3% for bacterial and fungal models, respectively. The presence of activated carbon as filter media increased the removal efficiency of TDS. Shruthi et al. (2012), used Pseudomonas sp. for treatment of rubber processing effluent and reported a 68.8% reduction in TDS.

**Total suspended solids**

Results in Table 2 indicate the ability of both used bacterial consortia (bacterial model) and fungal consortia (fungal model) for removal of TSS, since the overall removal percent of TSS was 99.3% and 99.0%, respectively. After aeration, removal percent of TSS was not so high; however, after filtration, the maximum removal percentage was reached. Removal efficiency is attributed to the utilization of organics by the microbial consortia in addition to the adsorption capability of used filter materials (rice straw and activated carbon). Gaikwad et al. (2014) reported a maximum reduction in TSS of 79.76% using microbial consortia for treatment of wastewater.

**Biological oxygen demand**

Results in Table 2 indicate that aeration was the main factor in the removal of BOD since many microorganisms require oxygen for simultaneous utilization of organic materials necessary for growth and other activities. The most reduction in BOD occurred during the aeration stage, as removal rates were 74.2% and 71.8% for bacterial and fungal model, respectively. After the filtration stage, there was no high variation in removal percent, since the overall removal percent of BOD was 78.7% and 74.6%, respectively. Das & Santra (2010) reported a considerable reduction of BOD (69.6%) from wastewater using bacterial isolates. Also, same behaviour of BOD removal was reported by Porwal et al. (2015).

**Chemical oxygen demand**

According to obtained results in Table 2, reduction in COD values was as similar as BOD. The observed removal was during aeration stage (72.4% and 69.5% for bacterial and fungal consortia, respectively) and overall removal percentage after filtration was 78.5% and 77.8%, respectively. The reduction in COD values may be due to the presence of high concentrations of nutrients and dissolved organic materials which can be easily used by microorganisms for growth. Obtained results were in accordance with the results obtained by Chatterjee & Pugaht (2013).

**Oil & grease**

Determination of O&G during wastewater treatment is very necessary as they reflect the effectiveness of the tanks settlement. Maximum removal percent of O&G, according to results in Table 2, was observed after the filtration stage (97.9% and 97.2% for bacterial and fungal models, respectively), while after aeration stage, removal percentage was 44.5% and 40.5%, respectively. The presence of rice straw and activated carbon increased removal percent of O&G due to their adsorption abilities. Also, lower removal percentage during aeration stage may be attributed to the difference in degradation power of microorganisms depending on their lipase system and physicochemical properties of substrate (Wakelin & Forster 1997).

**Sulfates**

The presence of sulfates in water with high concentrations can cause odour and corrosion of sewer systems under anaerobic conditions through conversion into hydrogen sulfide. Also, it can form hard scales in heat exchangers and boilers. The overall removal percentage of sulfate was 23.2% and 20.9% for bacterial and fungal models, respectively. Saranraj & Stella (2012) and Porwal et al. (2015), reported similar reductions in sulfates of sugar mill effluent using various bacterial cultures.

**CONCLUSION**

From the obtained results during the present study, the following can be concluded:

- Biodegradation of dairy effluent is an effective treatment technology, especially in the case of using locally isolated bacterial and fungal strains.
• Using bacterial strains was slightly more effective than fungal strains for the biodegradation process of pollutants present in dairy effluent.

• The combination of biological treatment (aeration) and filtration, increased removal percentage of pollutants present in dairy effluent.

REFERENCES


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