Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2018.23161

132 (2018) 274–280
November

Treatment of lipid-rich wastewater using a mixture of free or immobilized bioemulsifier and hydrolytic enzymes from indigenous bacterial isolates

Adegoke Isiaka Adetunji, Ademola Olufolahan Olaniran*

Discipline of Microbiology, School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal (Westville campus), Private Bag X54001, Durban 4000, South Africa, Tel. +27(0)616540408, email: adegokeadetunji8@gmail.com (A.I. Adetunji), Tel. +27 31 260 7400, Fax +27 31 260 7809, email: olanirana@ukzn.ac.za (A.O. Olaniran)

Received 22 November 2017; Accepted 19 September 2018

ABSTRACT

The combined use of free or immobilized bioemulsifier produced by *Acinetobacter* sp. and enzyme pool (protease and lipase) from *Bacillus aryabhattai* in the reduction of organic pollutants present in lipid-rich wastewater was investigated. Physicochemical characterization of the raw wastewater revealed high pollutant load in poultry processing wastewater in comparison to dairy wastewater. Biodegradability of the wastewater was assessed by measuring the reduction of COD and lipid contents at time intervals under varying process conditions. In dairy wastewater treated at 37°C without pH adjustment, maximum COD (61 and 65%) and lipid (48 and 64%) reduction efficiencies were recorded at 120 h using free and immobilized bioproducts, respectively. However, under these conditions, maximum COD (86 and 94%) and lipid (52 and 69%) removal efficiencies of poultry processing wastewater were observed at 120 h when treated with free and immobilized bioproducts, respectively. At temperature of 50°C and pH 8.0, there was enhanced reduction of organic pollutants, with maximum COD (66 and 78%) and lipid (55 and 71%) removal efficiencies obtained in dairy wastewater at 72 h when using free and immobilized bioproducts, respectively. In the case of poultry processing wastewater, optimum COD (90 and 95%) and lipid (63 and 77%) removal was recorded at 72 h when treated with free and immobilized bioproducts, respectively. Reusability studies suggest that the immobilized bioproducts could be reused for up to six and seven times for the treatment of dairy and poultry processing wastewater, respectively. This study suggests the efficient and synergistic application of the developed immobilized bioemulsifier and hydrolytic enzymes in the treatment of high fat-containing wastewater.

Keywords: Lipid-rich wastewater; Bioproducts; Biodegradation; Organic pollutants; Hydrolytic enzymes

1. Introduction

Lipid-rich wastewater is defined as a wastewater that consists of lipids along with vast array of dissolved organic and/or inorganic substances in suspension at high concentrations [1]. It is characterized by high chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS) and other toxic compounds [2,3]. Lipid-contaminated wastewater is discharged from a wide variety of commercial establishments including slaughterhouses, restaurants, fish processing industries, dairy industries, leather industries, edible oil refineries etc. [4–8]. These industries consume large amounts of water for various processes, equipment and washing facilities, resulting in the release of huge volume of wastewater, which, if left untreated, could lead to increased disposal and severe pollution problems, thereby creating environmental hazards and hampering the normal operations of the ecosystems [8].

Due to increase in the production and discharge of raw and poorly treated lipid-rich wastewater coupled with stringent regulations for effluent discharge and increasing drive for re-use of treated wastewater, treatment of lipid-rich wastewater has become an issue of great necessity [9,10]. Various physicochemical methods including chemical coagulation, gravity separation etc. have been

*Corresponding author.

1944-3994 / 1944-3986

employed for the treatment of lipid-rich wastewater [11,12]. However, these techniques remain unsatisfactory due to low treatment efficiency in the removal of dissolved and/or emulsified fats, operational difficulties, high operational costs, generation of secondary pollutants, amongst others [13]. Hence, the need for adoption of biological technologies involving application of bioproducts for an efficient, cost-effective, sustainable and eco-friendly treatment of high fat-containing wastewater [14]. The use of biosurfactants facilitates enzymatic activity and degradation of lipids by accelerating their solubility and bioavailability, thus eliminating the need for additional processes [15–17]. In addition, application of hydrolytic enzymes reduces organic pollutant load in the effluent *via* enzymatic catalysis and further enhances performance of microbial population at a later phase of biological treatment [1,18–23].

Several studies on the combined use of free bioemulsifier and enzyme pool for oily wastewater treatment have been reported [4,16,24,25]. However, to the best of our knowledge, no studies have been reported on the comparative study of a mixture of free or immobilized bioemulsifier and hydrolytic enzymes (protease, lipase) for lipid-rich wastewater treatment. Therefore, this study investigates the synergistic effects of free or immobilized extracellular glycoprotein bioemulsifier secreted from *Acinetobacter* sp. Ab9-ES and hydrolytic enzymes (protease and lipase) produced from *Bacillus aryabhattai* in the reduction of organic pollutants from two different lipid-rich wastewater types.

2. Materials and methods

2.1. Materials

The bioproducts (hydrolytic enzymes and bioemulsifier) used in this experiment were produced by submerged fermentation from *Bacillus aryabhattai* and *Acinetobacter* sp. Ab9-ES, respectively. They exhibited robust activities at optimum pH and temperature of 8.0 and 50°C (protease), 9.5 and 60°C (lipase) and 7.0 and 50°C (bioemulsifier). Sodium alginate used as support material for immobilization was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Extraction of bioproducts

The bioemulsifier was extracted from bacterial culture supernatants by precipitation using three volumes of cold ethanol followed by incubation overnight at –20ºC. Thereafter, the precipitated bioemulsifier was re-dissolved in distilled water, dialyzed in cellulose tube membrane (cut-off: 12.4 KDa) (Sigma-Aldrich, USA) for 24 h against distilled water (1%, w/v) and then lyophilized [26,27]. The hydrolytic enzymes (protease and lipase) were recovered by ammonium sulphate precipitation of the obtained cell free culture supernatant, followed by dialysis [28,29].

2.3. Immobilization protocol

The bioproducts were immobilized separately by entrapment method using the modified method of Guleria et al. [30] and Ferhat et al. [31]. Bioproduct solution was added to a suitable amount of 2% (w/v) sodium alginate suspension. The mixture was extruded dropwise through a syringe into a $CaCl_2$ solution. Alginate drops solidified when in contact with $CaCl_2$ solution, forming beads and thus entrapping the bioproduct. The obtained beads were allowed to harden for 1 h and washed with sterile distilled water for the removal of excess Ca^{2+} ions.

2.4. Collection of raw lipid-rich wastewater

Raw lipid-rich wastewater samples were collected from two different sources namely, dairy and poultry processing industries, all in the KwaZulu-Natal province of South Africa, into separate sterile 1 L Schott bottles. The wastewater was generated mainly from production line, equipment, and floor cleaning operations.

2.5. Physicochemical characterization of lipid-rich wastewater

Raw lipid-rich wastewater samples were characterized for BOD, COD, TSS, total dissolved solid (TDS), turbidity, resistivity, salinity, electrical conductivity (EC), total nitrogen (TN), total phosphorus (TP), pH and temperature in accordance with standard methods [32,33]. The BOD of the diluted wastewater was measured using HACH LD 101 probe following incubation at 20°C for 5 d while COD, TN and TP were measured by chromosulfuric acid oxidation and persulfate digestion method, respectively using Spetroquant NOVA 60 [34]. The pH and temperature of the wastewater were determined by direct measurement using a bench pH meter (HI 2002–01, Hanna Instruments, Woonsocket, RI, USA) and glass thermometer, respectively. In addition, salinity, TDS and EC of the wastewater samples were measured directly using CDC401 probe while turbidity was measured by nephelometric method using turbidimeter 2100P. TSS was determined by gravimetric method, in which aliquot of the wastewater was transferred through a pre-weighed filter paper, dried at 103°C for 1 h. The obtained residues were then weighed. The lipid content was determined using a partition gravimetric method of Kirschman and Pomeroy [35] while protein concentration was determined according to the method of Lowry et al. [36] using bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) as a standard. All analyses were performed in triplicate.

2.6. Batch biodegradation of lipid-rich wastewater

The synergistic effects of bioemulsifier and enzyme pool in the biodegradation of pollutants present in lipid-rich wastewater were investigated in 1 L Erlenmeyer flasks containing 500 ml sterile lipid-rich wastewater. Lipid-rich wastewater sample was treated by addition of 5% (v/v) each of the free bioproducts or 2.5, 3 and 3.75 g of immobilized protease, lipase and bioemulsifier, respectively. These bioproducts had enzymatic (protease and lipase) and emulsifying activities of 151.14 U/ml, 81.43 U/ml and 83.3%, respectively, obtained under standard assay conditions using the method of [37] (protease), [38] (lipase) and [39] (bioemulsifier). The flasks were incubated separately and simultaneously at 150 rpm for 120 h in a shaking incubator under two different experimental conditions: 37°C without adjustment of pH of the wastewater samples and 50°C by adjusting the pH of the wastewater to 8.0. A control flask without enzyme or bioemulsifier preparation was kept under similar conditions. Biodegradability of the lipid-rich wastewater samples was assessed by determining percentage removal efficiencies of COD and lipid contents at 24, 72 and 120 h using the formula shown in Eq. (1) [40]. All analyses were conducted in triplicate.

$$\text{Removal efficiency}(\%) = \frac{C_{control} - C_f}{C_{control}} \times 100 \qquad (1)$$

where $C_{control}$ is the value of COD or lipid contents of the control lipid-rich wastewater, and C_f is the amount of these parameters after treatment.

2.7. Reusability of immobilized protease, lipase and bioemulsifier

Batch biodegradation of lipid-rich wastewater was repeated several times using a combination of immobilized protease, lipase and bioemulsifier at 50°C, pH 8.0 for 72 h, since considerable biodegradability was recorded under these optimum conditions. After each cycle, the beads were separated, washed with sterile distilled water and then added into a fresh wastewater sample. Removal efficiencies of COD and lipid contents were determined after every cycle. All experiments were carried out in triplicate.

Table 1
Physicochemical characteristics of raw dairy and poultry processing wastewater

Parameter	Dairy wastewater	Poultry processing wastewater
Color	Whitish	Brownish
Odor	Unpleasant	Unpleasant
Temperature (°C)	24.7 ± 1.5	22.6 ± 2.0
pH	12.6 ± 0.1	6.6 ± 0.1
COD (mg/L)	5693 ± 17	7518 ± 378
BOD_5 (mg/L)	691± 16	707 ± 13
TSS (mg/L)	2333 ± 1528	4667 ± 2887
TDS (mg/L)	5700 ± 0.00	807000 ± 0.00
Salinity (‰)	5.88 ± 0.00	0.81 ± 0.00
Resistivity (Ω·cm)	96 ± 0.00	620 ± 0.00
Total nitrogen (mg/L)	129 ± 3	79 ± 11
Total phosphorus (mg/L)	27.2 ± 1.3	24.3 ± 0.9
Lipid content (mg/L)	53367 ± 4306	88900 ± 10468
Turbidity (NTU)	1962 ± 7	2077 ± 83
Electrical conductivity (mS/cm)	10.42 ± 0.00	1614 ± 0.58
Protein content (mg/L)	785 ± 96	507 ± 40

*All values are expressed in mean; ± indicates SD.

3. Results and discussion

3.1. Characterization of lipid-rich wastewater

The physicochemical characteristics of dairy and poultry processing wastewater were determined using standard procedures, and the mean values of the parameters are shown in Table 1. Both wastewater types are characterized with unpleasant odor coupled with whitish (dairy wastewater) and brownish (poultry processing wastewater) appearance. Furthermore, wastewater from poultry processing industry contained high concentrations of organic matter in form of lipids (88900 ± 10468 mg/L), COD (7518 ± 378 mg/L), BOD_5 (707 ± 13 mg/L), TDS (807000 ± 0.00 mg/L) and TSS (4667 ± 2887 mg/L) in comparison to dairy industry wastewater with lipid contents (53367 ± 4306 mg/L), COD (5693 ± 17 mg/L), BOD_5 (691 ± 16 mg/L), TDS (5700 ± 0.00 mg/L) and TSS (2333 ± 1528 mg/L). However, in both wastewater samples, the COD values were found to be higher than those of BOD, suggesting slow biodegradation of organic compounds in the wastewater [41]. Similar characteristics of poultry and dairy wastewater with high pollutant load have been reported in literature [4,16,18,42–44].

In addition, pH of poultry processing wastewater was found to be acidic while that of dairy wastewater was extremely alkaline, probably due to the presence of acidic and caustic cleaning agents, respectively, in the wastewater [45–47]. Furthermore, high salinity of dairy wastewater (5.88 ± 0.00‰) which is about 7-fold higher than that of poultry processing wastewater may be due to the presence of high concentrations of Na and Cl, resulting from the use of large amounts of alkaline cleaners in the dairy plant [48]. The EC of poultry processing wastewater was 155-fold higher than that of dairy wastewater, an indication of the presence of high ionic substances in the wastewater [49]. The higher turbidity of poultry processing wastewater (2077 ± 83 NTU) in comparison to that of dairy wastewater may probably be due to the presence of residual blood, fats and intestinal content in the wastewater. The high nutrient levels in dairy wastewater as compared to that of poultry processing wastewater is indicated by high TN (129 ± 3 mg/L) and TP (27.2 ± 1.3 mg/L) values, suggesting increased eutrophication risk of the receiving water bodies [46]. In general, variation in the physicochemical properties of the wastewater to previously reported studies is a function of the type of products being produced and different operating procedures adopted at each plant [47]. The pollutant load of these wastewater types was found to exceed the South African guidelines for effluent discharge, thus necessitating treatment before disposal [50].

3.2. Simultaneous treatment of lipid-rich wastewater using a mixture of free or immobilized bioemulsifier and enzyme pool

In the first set of experiment, there was a significant reduction in the COD and lipid contents of the wastewater in comparison to control during treatment with free or immobilized bioproducts for 120 h. Maximum COD (61 and 65%) and lipid (48 and 64%) reduction efficiencies were recorded after 120 h in dairy wastewater when treated with the free and immobilized bioproducts, respectively (Figs. 1A and B). These results were found to be lower

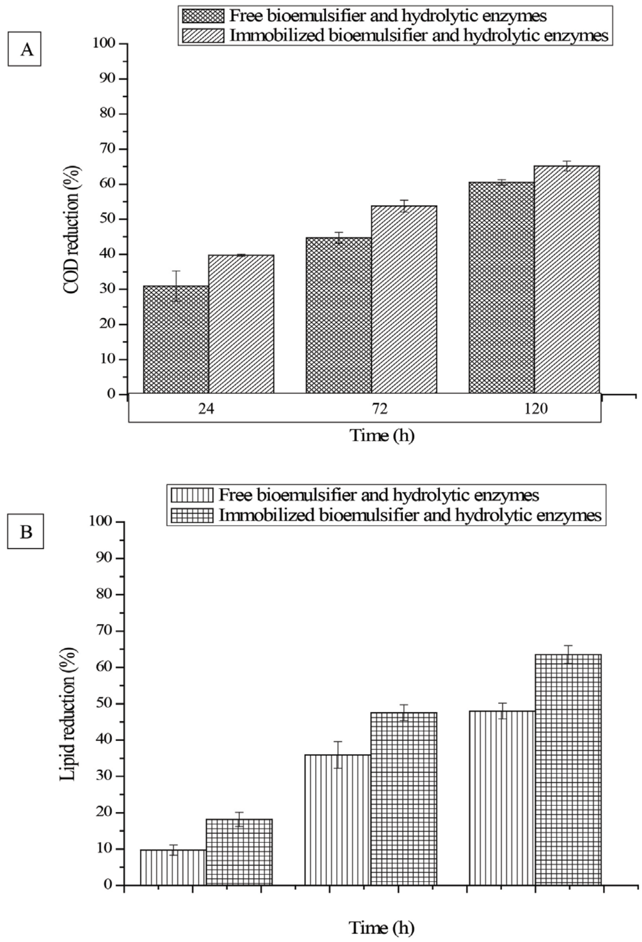

Fig. 1. Reduction in (A) COD and (B) lipid content during treatment of dairy wastewater with free or immobilized bioemulsifier and hydrolytic enzymes. The wastewater was treated at 37°C without pH adjustment. Values indicate the average of triplicate values while the error bars represent the standard deviation.

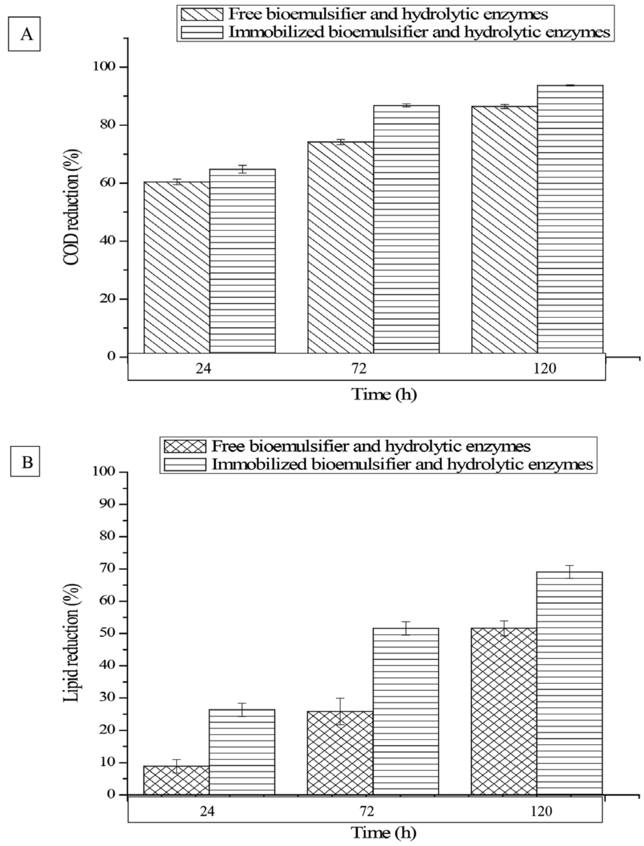

Fig. 2. Reduction in (A) COD and (B) lipid content during treatment of poultry processing wastewater with free or immobilized bioemulsifier and hydrolytic enzymes. The wastewater was treated at 37°C without pH adjustment. Values indicate the average of triplicate values while the error bars represent the standard deviation.

when compared to COD (86 and 94%) and lipid (52 and 69%) removal efficiencies obtained after 120 h during treatment of poultry processing wastewater using free and immobilized bioproducts, respectively (Figs. 2A and B). The lower degradation of dairy wastewater compared to poultry processing wastewater suggests higher affinity of the bioproducts to poultry effluent constituents [51]. This may probably be due to the presence of high salt content inhibiting the degradation of pollutants in the dairy wastewater [52].

In the second set of experiment, during 120 h treatment of dairy wastewater, maximum reduction efficiencies of COD (66 and 78%) and lipid contents (55 and 71%) were recorded at 72 h using free and immobilized bioproducts, respectively (Figs. 3A and B). In contrast to dairy wastewater, there was enhanced reduction of organic matter in poultry processing wastewater, with maximum COD (90 and 95%) and lipid (63 and 77%) removal efficiencies obtained at 72 h when treated with free and immobilized bioproducts, respectively (Figs. 4A and B). The observed decrease in the removal efficiencies of both wastewater types after 72 h incubation may be attributed to reduction in the activities of the bioproducts at longer treatment period, thus rendering them ineffective towards the bioconversion reactions [53]. This corroborates the findings of Jacobucci et al. [53] where a decrease in the degradation of oily wastewater was reported between 72–120 h during 5 d treatment with bioproduct produced from *Planococcus citreus*.

In general, the combined application of the bioemulsifier and enzyme pool was found to be more effective in the reduction of COD and lipids from poultry processing wastewater in comparison to dairy wastewater. In addition, the immobilized bioproducts recorded higher pollutant removal efficiencies in contrast to the free counterparts, an indication of protective role provided by the support material, thus stabilizing the bioproducts and, as a consequence maintain their activities [54,55]. Such cocktail application of biosurfactant and enzyme pool has been reported for enhanced removal of COD and lipids from lipid-rich wastewater [4,16,25]. Furthermore, the degradation potential of the bioproducts increased when applied for the treatment of wastewater at 50°C and pH 8.0 in comparison to wastewater treated at 37°C without pH adjustment, an indication that temperature and pH had effects on the physical nature and chemical composition of the oily wastewater constituents as well as on the rate of

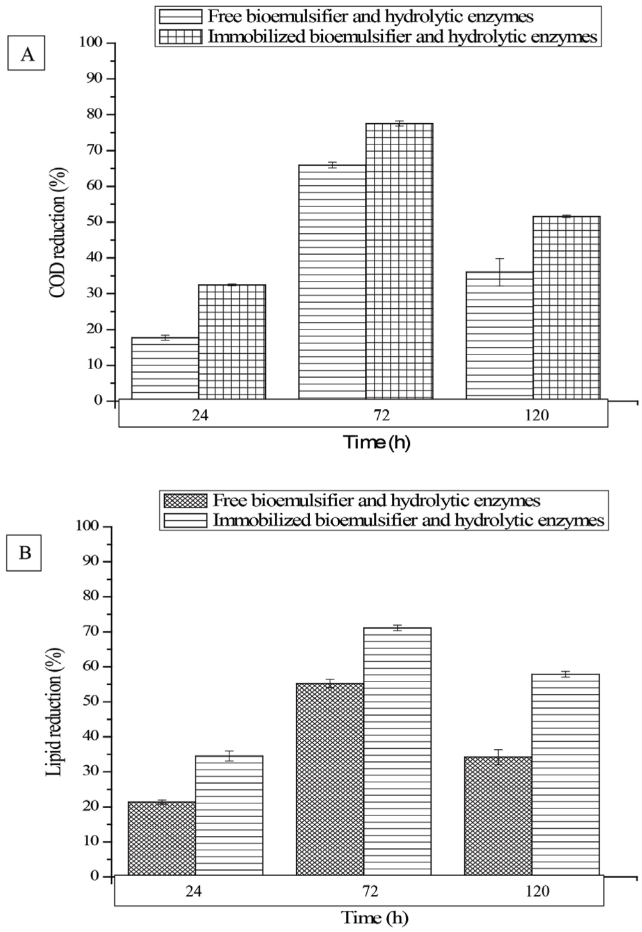

Fig. 3. Reduction in (A) COD and (B) lipid content during treatment of dairy wastewater with free or immobilized bioemulsifier and hydrolytic enzymes. The wastewater was treated at 50°C and pH 8.0. Values indicate the average of triplicate values while the error bars represent the standard deviation.

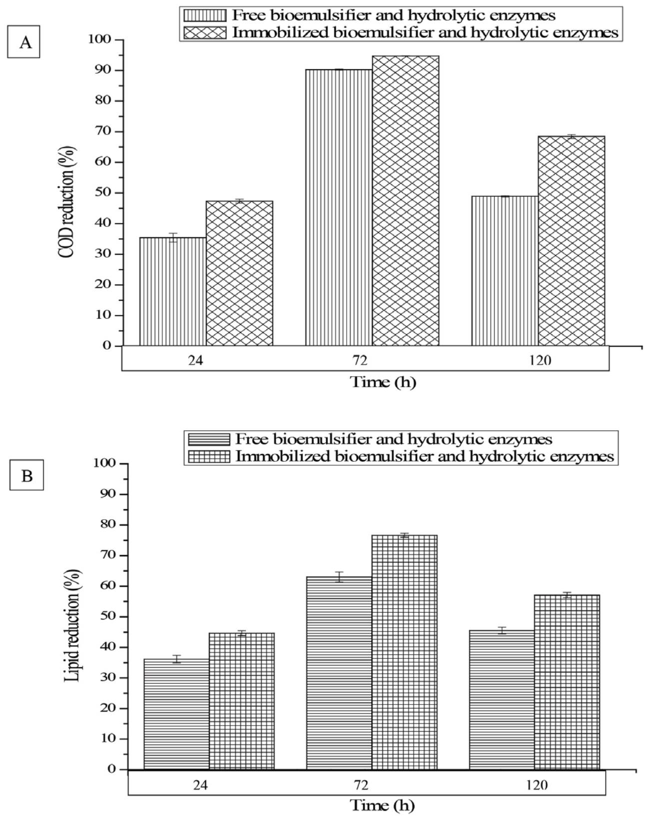

Fig. 4. Reduction in (A) COD and (B) lipid content during treatment of poultry processing wastewater with free or immobilized bioemulsifier and hydrolytic enzymes. The wastewater was treated at 50°C and pH 8.0. Values indicate the average of triplicate values while the error bars represent the standard deviation.

pollutants degradation by the bioproducts [56,57]. Batch treatment of coconut mill effluent using celite-immobilized lipase from *Staphylococcus pasteuri* COM-4A at 50°C and pH 9.0 has been reported [58]. Results showed removal efficiencies of COD (29%) and O and G (45%).

3.3. Reusability potential of immobilized bioproducts

The reusability of immobilized bioemulsifier and enzymes is a key factor for a cost-effective application of the bioproducts in the degradation of pollutants present in lipid-rich wastewater. In this study, cycle number affected the reduction of both COD and lipid contents in the wastewater by the immobilized bioproducts. In dairy wastewater, the immobilized bioproducts could be reused for a maximum of six cycles for the reduction of COD and lipid contents, retaining 22% and 18% removal efficiencies, respectively at sixth cycle (Fig. 5A). In the case of poultry processing wastewater, the immobilized bioproducts could be reused repeatedly for up to seven batches for the removal of COD and lipids, with reduction efficiencies of 40% and 30%, respectively recorded at the seventh cycle (Fig. 5B). The decreased degradation potential of the immobilized bioproducts in both wastewater types, after every cycle, may be due to leakage of the bioproducts from the beads or its inhibition by substrate/product molecules [58,59]. This supports the findings of Kanmani et al. [58], in which lipase immobilized in celite beads was repeatedly used for up to seven cycles during treatment of coconut mill effluent. Similarly, *Candida rugosa* lipase entrapped in alginate beads has been reported to be reused for up to four batches during enzymatic treatment of high strength lipid-rich wastewater [59].

4. Conclusions

Mixture of free or immobilized bioemulsifier and hydrolytic enzymes was employed for the degradation of organic pollutants present in dairy and poultry processing wastewater. Despite the high pollutant load observed in poultry processing wastewater, higher COD and lipid removal efficiencies were recorded at all the treatment conditions in comparison to dairy wastewater. The immobilized bioproducts were found to be more efficient in the degradation of organic pollutants (COD and lipid contents) when compared to the free mixtures. In addition, temperature, pH and incubation time seemed to be crucial

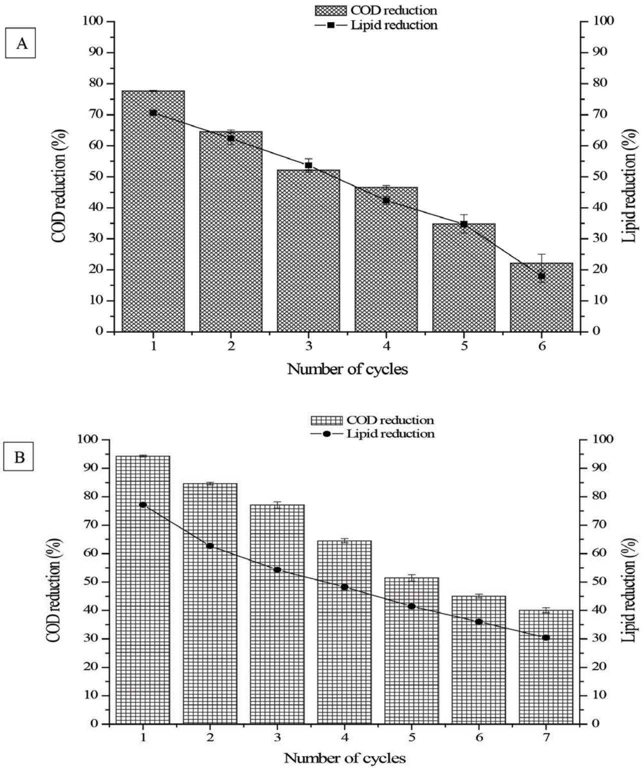

Fig. 5. Reusability potential of immobilized bioemulsifier and hydrolytic enzymes in the treatment of (A) dairy and (B) poultry processing wastewaters. Values indicate the average of triplicate values while the error bars represent the standard deviation.

factors influencing reduction of COD and lipid contents from the wastewater, with the treatment conditions of 50°C, pH 8.0 and 72 h showing higher removal efficiencies (in terms of COD and lipid contents) when compared to 37°C without pH adjustment for 120 h. The poultry processing wastewater, when treated with a mixture of immobilized bioemulsifier and hydrolytic enzymes at 50°C and pH 8.0 gave removal efficiencies of 95% COD and 77% lipids. Meanwhile, under similar process conditions, dairy wastewater gave removal efficiencies of 78% COD and 71% lipids when treated with a mixture of immobilized bioemulsifier and hydrolytic enzymes. Based on these findings, the combined use of bioemulsifier and hydrolytic enzymes should be applied as alternative remedy in the treatment of high strength fat-containing wastewater.

Acknowledgments

The financial support of the National Research Foundation (NRF) of South Africa towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the NRF.

Conflict of interest

The authors declare no conflict of interest.

References

[1] T.V. Adulkar, V.K. Rathod, Pre-treatment of high fat content dairy wastewater using different commercial lipases, Desal. Water Treat., 53 (2015) 2450–2455.
[2] B. Farizoglu, S. Uzuner, The investigation of dairy industry wastewater treatment in a biological high performance membrane system, Biochem. Eng. J., 57 (2011) 46–54.
[3] C.S. Couras, V.L. Louros, T. Gameiro, N. Alves, A. Silva, M.I. Capela, L.M. Arroja, H. Nadais, Anaerobic degradation of dairy wastewater in intermittent UASB reactors: Influence of effluent recirculation, Environ. Technol., 36 (2015) 2227–2238.
[4] P. Chowdhury, T. Viraraghavan, A. Srinivasan, Biological treatment processes for fish processing wastewater-a review. Bioresour. Technol., 101 (2010) 439–449.
[5] M. Chowdhury, M.G. Mostafa, T.K. Biswas, A.K. Saha, Treatment of leather industrial effluents by filtration and coagulation processes, Water Res. Ind., 3 (2013) 11–22.
[6] F.R.C. Damasceno, D.M.G. Freire, M.C. Cammarota, Assessing a mixture of biosurfactant and enzyme pools in the anaerobic biological treatment of wastewater with high fat content, Environ. Technol., 35 (2014) 2035–2045.
[7] J.D. Bala, J. Lalung, N. Ismail, Studies on the reduction of organic load from palm oil mill effluent (POME) by bacterial strains, Int. J. Recycl. Org. Waste Agric., 4 (2015) 1–10.
[8] H.J. Porwal, A.V. Mane, S.G. Velhal, Biodegradation of dairy effluent by using microbial isolates obtained from activated sludge, Water Resour. Ind., 9 (2015) 1–15.
[9] J.J. Qin, M.H. Oo, G. Tao, K.A. Kekre, Feasibility study on petrochemical wastewater treatment and reuse using submerged MBR, J. Membr. Sci., 293 (2007) 161–166.
[10] I.E. Affandi, N.H. Suratman, S. Abdullah, W.A. Ahmad, Z.A. Zakaria, Degradation of oil and grease from high-strength industrial effluents using locally isolated aerobic biosurfactant-producing bacteria, Int. Biodeter. Biodegrad., 30 (2014) 1–8.
[11] A. Rodrigues, S. Xavier, F. Bernardes, Application of photoelectrochemical-electro-dialysis treatment for the recovery and reuse of water from tannery effluents, J. Clean. Prod., 10 (2007) 1–7.
[12] J.P. Kushwaha, V.C. Srivastava, I.D. Mall, An overview of various technologies for the treatment of dairy wastewaters, Crit. Rev. Food Sci. Nutri., 51 (2011) 442–452.
[13] J. Guolin, L. Mingming, C. Tingting, Prospects for development of oily sludge treatment, Chem. Technol. Fuels Oils, 47 (2011) 312–326.
[14] D. Alberton, A.D. Mitchell, J. Cordova, P. Peralta-Zamora, N. Krieger, Production of fermented solid containing lipases of *Rhizopus microsporus* and its application in the pre-hydrolysis of a high-fat dairy wastewater, Food Technol. Biotechnol., 48 (2010) 28–35.
[15] A. Daverey, K. Pakshirajan, Pretreatment of synthetic dairy wastewater using the sophorolipid-producing yeast *Candida bombicola*, Appl. Biochem. Biotechnol., 163 (2011) 720–728.
[16] F.R.C. Damasceno, M.C. Cammarota, D.M.G. Freire, The combined use of a biosurfactant and an enzyme preparation to treat an effluent with high fat content, Coll. Surf. B: Biointerfaces, 95 (2012) 241–246.
[17] N.J. Cosmann, B.M. Gomes, S.D. Gomes, A.P.R. Simiqueli, G.M. Pastore, Use of biosurfactant surfactin produced from cassava wastewater for anaerobic treatment of effluent from a poultry slaughterhouse, Afr. J. Biotechnol., 16 (2017) 224–231.
[18] B.A. Mahdi, A. Bhattacharya, A. Gupta, Enhanced lipase production from *Aeromonas* sp. S1 using Sal deoiled seed cake as novel natural substrate for potential application in dairy wastewater treatment, J. Chem. Technol. Biotechnol., 87 (2012) 418–426.
[19] M.C. Cammarota, D.M.G Freire, A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content, Bioresour. Technol., 97 (2006) 2195–3010.
[20] J. Jeganathan, G. Nakhla, A. Bassi, Hydrolytic pretreatment of oily wastewater by immobilized lipase, J. Hazard. Mater., 145 (2007) 127–135.

[21] D.R. Rosa, I.C.S. Duarte, N.K. Saavedra, M.B. Varesche, M. Zaiat, M.C. Cammarota, D.M.G. Freire, Performance and molecular evaluation of an anaerobic system with suspended biomass for treating wastewater with high fat content after enzymatic hydrolysis, Bioresour. Technol., 100 (2009) 6170–6176.

[22] V.M.F Alexandre, A.M. Valente, M.C. Cammarota, D.M.G. Freire, Performance of anaerobic bioreactor treating fish-processing plant wastewater pre-hydrolyzed with a solid-enzyme pool, Renew. Energy, 36 (2011) 3439–3444.

[23] F.D.A. Facchini, A.C. Vici, M.G. Pereira, J.A. Jorge, M.L.T.M. Polizeli, Enhanced lipase production of *Fusarium verticillioides* by using response surface methodology and wastewater pretreatment application, J. Biochem. Tech., 6 (2015) 996–1002.

[24] R. Tarntip, T. Sirichom, Isolation of proteolytic, lipolytic, and bioemulsifying bacteria for improvement of the aerobic treatment of poultry processing wastewater, Afr. J. Microbiol. Res., 5 (2011) 5493–5497.

[25] J. do N Silva, M.L.E. Gutarra, D.M.G. Freire, M.C. Cammarota, Application of home-made enzyme and biosurfactant in the anaerobic treatment of effluent with high fat content, J. Bioprocess. Biotech., 3 (2013) 139.

[26] S. Joshi, C. Bharucha, A.J. Desai, Production of biosurfactant and antifungal compound by fermented food isolate *Bacillus subtilis* 20B, Bioresour. Technol., 99 (2008) 4603–4608.

[27] E.J. Gudiña, J.F.B. Pereira, R. Costa, D.V. Evtuguin, J.A.P. Coutinho, J.A. Teixeira, L.R. Rodrigues, Novel bioemulsifier produced by a *Paenibacillus* strain isolated from crude oil, Microb. Cell Fact., 14 (2015) 14.

[28] R. Boran, A. Ugur, Partial purification and characterization of the organic solvent-tolerant lipase produced by *Pseudomonas fluorescens* RB02–3 isolated from milk, Prep. Biochem. Biotechnol., 40 (2010) 229–241.

[29] T. Mothe, V.R. Sultanpuran, Purification and characterization of a thermotolerant alkaline serine protease from a novel species of *Bacillus caseinilyticus*, 3 Biotech, 6 (2016) 53.

[30] S. Guleria, A. Walia, A. Chauhan, C.K. Shirkot, Immobilization of *Bacillus amyloliquefaciens* SP1 and its alkaline protease in various matrices for effective hydrolysis of casein, 3 Biotech., 6 (2016) 208.

[31] S. Ferhat, R. Alouaoui, A. Badis, N. Moulai-Mostefa, Production and characterization of biosurfactant by free and immobilized cells from *Ochrobactrum intermedium* isolated from the soil of southern Algeria with a view to environmental application, Biotechnol. Biotechnol. Equip., 31 (2017) 733–742.

[32] A.E. Greenberg, L.S. Clesceri, A.D. Eaton, Standard methods for the examination of water and wastewater, American Public Health Association/American Water Works Association/Water Pollution Control Federation, Washington DC, 2005.

[33] E.W. Rice, R.B. Baird, A.D. Eaton, Standard methods for the examination of water and wastewater, 23rd edn.; American Public Health Association: Washington DC, USA; American Water Works Association: Denver, CO, USA; Water Environment Federation: Alexandria, VA, USA, (2017).

[34] M. Hosomi, R. Sudo, Simultaneous determination of total nitrogen and total phosphorus in freshwater samples using persulfate digestion, Int. J. Environ. Stud., 27 (2007) 267–275.

[35] H.D. Kirschman, R. Pomeroy, Determination of oil in oil field wastewater, Anal. Chem., 21 (1949) 793.

[36] O.H. Lowry, N.J. Rosebrough, A.I. Farr, R.J. Randall, Protein measurement with Folin phenol reagent, J. Biol. Chem., 193 (1951) 265–275.

[37] P. Secades, J.A. Guijarro, Purification and characterization of an extracellular protease from the fish pathogen *Yersinia ruckeri* and effect of culture conditions on production, Appl. Environ. Microbiol., 65 (1999) 3969–3975.

[38] U.K. Winkler, M. Stuckmann, Glocogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*, J. Bacteriol., 138 (1979) 663–670.

[39] D.G. Cooper, B.G. Goldenberg, Surface-active agents from two *Bacillus* species, Appl. Environ. Microbiol., 53 (1987) 224–229.

[40] P. Piro, M. Carbone, G. Tomei, Assessing settleability of dry and wet weather flows in an urban area serviced by combined sewer, Water Air Soil Pollut., 214 (2011) 107–117.

[41] W. Qasim, A.V. Mane, Characterization and treatment of selected food industrial effluents by coagulation and adsorption techniques, Water Res. Ind., 4 (2013) 1–12.

[42] A.A. Mendes, E.B. Pereira, A. Jr. Furigo, F. de Castro, Anaerobic biodegradability of dairy wastewater pretreated with porcine pancreas lipase, Braz. Arch. Biol. Technol., 53 (2010) 1279–1284.

[43] G. Dors, A.A. Mendes, E.B. Pereira, H.F. de Castro, A. Jr Furigo, Simultaneous enzymatic hydrolysis and anaerobic biodegradation of lipid-rich wastewater from poultry industry, Appl. Water Sci., 3 (2013) 343–349.

[44] U. Pathak, P. Das, P. Banerjee, S. Datta, Treatment of wastewater from a dairy industry using rice husk as adsorbent: Treatment efficiency, isotherm, thermodynamics, and kinetics modelling, J. Thermodyn., 2016 (2016) 1–7.

[45] A.K. Slavov, General characteristics and treatment possibilities of dairy wastewater- a review, Food Technol. Biotechnol., 55 (2017) 14–28.

[46] S. Bayar, Y.S. Yildiz, A.E. Yilmaz, S. Irdemez, The effect of stirring speed and current density on removal efficiency of poultry slaughterhouse wastewater by electrocoagulation method, Desalination, 280 (2011) 103–107.

[47] Y.-Y. Liu, R.J. Haynes, Origin, nature, and treatment of effluents from dairy and meat processing factories and the effects of their irrigation on the quality of agricultural soils, Critical Rev. Environ. Sci. Technol., 41 (2011) 1531–1599.

[48] B. Demirel, O. Yenigun, T.T. Onay, Anaerobic treatment of dairy wastewater: A review, Proc. Biochem., 40 (2005) 2583–2595.

[49] J.N. Edokpayi, J.O. Odiyo, T.A.M. Msagati, E.O. Popoola, Removal efficiency of faecal indicator organisms, nutrients and heavy metals from a peri-urban wastewater treatment plant in Thohoyandou, Limpopo province, South Africa, Int. J. Environ. Res. Public Health, 12 (2015) 7300–7320.

[50] Government Gazette. No. 36820 (2013), Wastewater values applicable to the irrigation of any land or property up to 2000 cubic meters; Minister of Water and Environmental Affairs: South Africa.

[51] A.B.G. Valladão, M.C. Cammarota, A.G. Torres, D.M.G. Freire, Profiles of fatty acids and triacylglycerols and their influence on the anaerobic biodegradability of effluents from poultry slaughterhouse, Bioresour. Technol., 102 (2011) 7043–7050.

[52] O. Lefebvre, S. Quentin, M. Torrijos, J.J. Godon, J.P. Delgenès, R. Moletta, Impact of increasing NaCl concentrations on the performance and community composition of two anaerobic reactors, Appl. Microbiol. Biotechnol., 75 (2007) 61–69.

[53] D.F.C. Jacobucci, M.R.G. Oriani, L.R. Durrant, Reducing COD level on oily effluent by utilizing biosurfactant-producing bacteria, Braz. Arch. Biol. Technol., 52 (2009) 1037–1042.

[54] V. Minovska, E. Winkelhausen, S. Kuzmanova, Lipase immobilized by different techniques on various support materials applied in oil hydrolysis, J. Serb. Chem. Soc., 70 (2005) 609–624.

[55] A.A. Homaei, R. Sariri, F. Vianello, R. Stevanato, Enzyme immobilization: an update, J. Chem. Biol., 6 (2013) 185–205.

[56] C.S. Karigar, S.S. Rao, Role of microbial enzymes in the bioremediation of pollutants: A review, Enzyme Res., 1 (2011) 11.

[57] Q. Chen, J. Li, M. Liu, H. Sun, M. Bao, Study on the biodegradation of crude oil by free and immobilized bacterial consortium in marine environment, PLos One, 12 (2017) e0174445.

[58] P. Kanmani, K. Kumaresan, J. Aravind, Pretreatment of coconut mill effluent using celite-immobilized hydrolytic enzyme preparation from *Staphylococcus pasteuri* and its impact on anaerobic digestion, Biotechnol. Prog., 31 (2015) 1249–1258.

[59] J. Jeganathan, A. Bassi, G. Nakhla, Pre-treatment of high oil and grease pet food industrial wastewaters using immobilized lipase hydroxylation, J. Hazard. Mater. B137, (2006) 121–128.