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ASSESSMENT OF SOME BACTERIAL AND FUNGAL STRAINS FOR DAIRY WASTEWATER TREATMENT

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ABSTRACT

Microorganisms of the effluents from dairy factories located in 6th October industrial city (Cairo, Egypt) were isolated and screened for their ability to reduce the pollutants found in the dairy wastewater effluent. Five bacterial strains (*Staphylococcus aureus, Pseudomonas aeruginosa Streptococcus thermophilus, Bacillus supergene and Lactobacillus fermentum*) and three fungal strains (*Aspergillus sp., Cladosporium sp. and Fusarium sp*) were isolated to be used in biodegradation. A two stage reactor (incubation then filtration) was used during this study. Two mixtures were prepared (Mix 1: five bacterial isolated strains and Mix 2: three fungal isolated strains). The results showed that bacterial and fungal strains have high efficiency for organic pollutants reduction as the percentage of reduction reached 79.8 % and 72.5 % respectively. TSS for both bacterial and fungal mixtures shows high reduction efficiency with 99.5% and 98.9% respectively, However after incubation the reduction efficiency was not high for both bacterial and fungal mixtures as it increased after filtration Bacterial mixtures was slightly more effective than fungal mixtures during the treatment process. Using activated carbon and sand for filtration after incubation enhances the treatment efficiency of the pollutants present in dairy wastewater.

INTRODUCTION

The wastewater of dairy industries is increasing rapidly due to the increase in production rate. At the last few years industrial wastes generally discharged on lands or into different water sources results in release of toxic substances into the environment, creating health hazards **(porwal *et al*., 2015)**.

In the recent years Egypt ministry of environment has paid a considerable attention to the industrial wastes as a general protocol to protect the environment from pollutants. The most common methods used for treating industrial wastes are physicochemical and biological. Physiochemical methods usually include partial treatment, higher quantity of solids, higher cost and use of chemical agents that's why biological methods are more preferred for the reduction of wastewater pollutants. Recently there is a growing interest in the use of biological methods to treat waste water of dairy industries in due to their advances over physicochemical methods **(Rodrigues *et al.*, 2008)**.

Many types of physicochemical treatment techniques have been studied for their applicability in treatment of wastewaters **(Rodrigues *et al.*, 2008)**. These types of treatments include sedimentation, screening, aeration, filtration, flotation, degassification, chlorination, ozonation, neutralization, coagulation, sorption, ion exchange, etc. Several limitations of physicochemical methods including partial treatment, higher cost, and generation of secondary pollutants, higher quantity solids and use of chemicals agents make the biological methods a favorable alternative for the reduction of pollutants. Pollutants associated with the food industry including the wastes generated by the dairy industry namely sludge, heavy organic matter, fats, oil & grease, fatty acids, nitrogenous compounds are notables **(Healy *et al.*,1995**). Of all industrial activities, the food sector has one of the highest consumptions of water and is one of the biggest producers of effluents per unit of production; in addition, they generate a large volume of sludge in biological treatment **(Ramjeawon, 2000)**.

One of the highest industries in consumption of water and production of effluents per unit of production is food industry. Also, the food industries produce 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 liter of processed milk **(Kolhe & Pawar 2011).**

The process of seeding inoculation of microorganisms for degrading waste materials on streams, rivers and treatment tanks has been rapidly increasing practice in many countries because it is economical and the application is uncomplicated. Bioremediation is any process that uses living microorganisms or their enzymes, to return a polluted environment to its original condition. As such, it uses relatively low-cost, low-technology techniques, such as using environment friendly microorganisms which generally have a high public acceptance and can be used on the site **(Ojo, 2006).** It constitutes the use of natural biota and their processes for pollution reduction and the end products are non-hazardous **(Ahmedna *et al.,* 2004).** The process of biodegradation is a well- established and powerful technique for treating domestic and industrial effluents. The performance of a biological process is often enhanced through bioaugmentation of one or more species of specialized microorganisms **(Sermany *et al.,* 2012)**. Microbial populations have an amazing and extensive capacity to degrade variety of organic compounds. Naturally occurring microorganisms thrive on many of the complex compounds contained in wastewater. Small size, high surface area-to-volume ratio and large contact interfaces with their surrounding environment, are some of the ideal features of microorganisms as bioindicators of chemical pollutants The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. To get an efficient biological wastewater treatment it is very important to know the wastewater microbiota composition and biochemical properties correlated to the origin of pollutants, as well as the optimum metabolic activity and physical-chemical conditions **(Janczukowicz *et al.*, 2007).**

In this study a model was designed to study the ability of a mixture of bacterial and fungal isolates, which are isolated from dairy wastewater, to degrade the organic nutrients found in dairy wastewater and also improving the quality of dairy wastewater. The model was supplemented with a natural filtration media sand and activated carbon for a better treatment.

MATERIALS AND METHODS

Fresh Samples were collected from different dairy effluents treatment factories located in industrial zone of 6^{th} October city in Cairo. The samples were collected in 5 L polyethylene plastic sterilized containers. The samples were transferred to the laboratory immediately and stored at 4˚C to avoid any physicochemical changes in the dairy wastewater effluent.

Isolation of microorganisms

Serial dilution for the dairy effluent samples was done (10^{-1} to 10^{-5}) then in Erlenmeyer flasks containing enrichment cultural media (sterile Nutrient Broth and Sabouraud's Broth) 1ml from each dilution was inoculated respectively. The flasks were kept on rotary shaker at 100 rpm at room temperature for 24–96 h. Then a loopful of enriched sample from Nutrient Broth flasks was streaked on Nutrient Agar petri dishes and another loopful from Sabouraud's Broth was streaked on Sabouraud's Agar petri-dishes. Nutrient Agar petri dishes were incubated for 24 h at 35 °c, while the Sabouraud's Agar plates were incubated for 7 days at 28 °c. Triplicate plating was done for each medium.

Well grown individual bacterial colonies on the surface of nutrient agar petri dishes were picked up and inoculated into 250 ml Erlenmeyer flasks containing milk broth (peptone 5gm, yeast extract 3 gm and 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% (υ/υ) glycerol and stored at – 80 °C for identification and further experiment. The same was done for fungal colonies that was selected and then inoculated at 28 °C for 7 days. After that single pure colonies were inoculated into Sabouraud's Broth containing 10% (υ/υ) glycerol and stored at – 80 °C for identification and further experiment.

For the fourteen bacterial isolates identification was done by using biology system Biolog^{Tm} microplate identification system (Biolog^{Tm} Gen Ш, USA), while for the six fungal isolates identification was done depending on colony morphology and microscopic examination by lactophenol cotton blue staining method **(kaur & Chaman 2014**).

Inoculums preparation

A suspension from each microbial isolate of 0.1 ml was inoculated in 100 ml inoculum medium. The flasks were kept on rotary shaker at 150 rpm for 24 h at 35 °C. also, 0.1 ml of suspension of the fungal isolate was inoculated in 100 ml inoculum medium and kept on rotary shaker at 120 rpm for 5 days at 28 °C. These suspensions were done to study the biodegradation efficiency of the microbial isolates; the activity growing culture of each isolate was then washed with sterile deionized water and centrifuged at 10,000 rpm for 10 minutes to get a wet pellet for each isolate. The pellet was then resuspended in sterile deionized water till turbidity reaches at or above that of McFarland 0.5 standards **(Wayne, 2009).**

Experimental setup and working

Two models of two stages were set up for the experimental treatment of the dairy wastewater effluent **(Fig. 1)** one for bacterial isolates and the other for fungal isolates. The design of this model was obtained from the model suggested by **RamaKrishna and Ligy, (2005) and porwal *et al.*,(2015)** and also from the model suggested by **Arumugam and Sabarethinam,(2008)** for treatment of dairy wastewater. For filtration sand and activated carbon were used during the treatment process.

The tank of the treatment were washed with alcohol to make it sterile and then rinsed with sterile distilled water. In the first model the tankwas fed with 1 L autoclaved untreated dairy wastewater. The autoclaved effluent was cooled to room temperature and then added to the reactor. Then 10 ml of each identified bacterial isolate (bacterial mixture [Mix 1]) was added to the effluent (the same was done in the second model but 10 ml of identified fungal isolates were added [fungal mixture [Mix 2]). An aerator was inserted into the reactor and the open top portion of the reactor was covered. The aerator used maintained the desired level of dissolved oxygen < 5 mg/L in the effluent and to support the survival and growth of the aerobic microorganisms used in this study. The incubation was provided for a period of 48 h. The effluent was given a retention time of 48 h in the primary tank where the microorganisms were allowed to carry out degradation. After 48 h, the aeration was stopped and effluents was allowed to stand for 1 h to allow settling of the sludge formed after that the treated effluent from primary tank was then allowed to flow into the filtration tanks. The filtration was carried out in two tanks one tank contains sand and followed by another tank containing activated carbon.

Analytical methods

All analytical methods used during this study conformed to the **"APHA; 2017".** The colorimetric technique was used for the determination of Chemical oxygen demand (COD), Biological oxygen demand (BOD).The Total Suspended Solids (TSS) was measured by filtration, drying at 105 °C and then combusting at 550 °C.

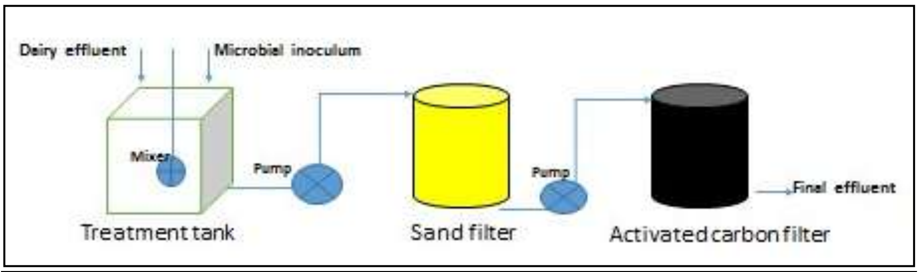

Fig. (1): shape of the model used in biodegradation process.

RESULTS AND DISCUSSION

Table 1 shows the results obtained of the physicochemical analysis for untreated dairy wastewater. The results of raw dairy wastewater were in accordance with the findings of **porwal *et al.* (2015). Passeggi *et al.* (2009)** has reported that the pH of dairy effluents depending on the nature of the end-product and can range from 4.7 to 12.2. In our study the influent of dairy wastewater was slightly acidic (6.01 ± 0.17). The acidic pH is attributed to the breakdown of milk lactose into lactic acid as mentioned by **Slavov, (2017)**.TSS is one of the main parameters of water which used to evaluate and determine the efficiency of treatment processes of wastewater. The dairy wastewater showed high concentrations of TSS (636 ± 11.21). **Porwal *et al.* (2015),** also reported the same high concentrations of TSS (626.6 ± 8.79 and 601.6 ± 3.46). BOD is one of the most widely used indicators of water quality. The influent dairy wastewater showed high concentrations of BOD (1,221 ± 13.01 mg/L). The dairy wastewater are characterized by high levels of BOD due to the presence of lactose, casein, fats, nutrients, sanitizing agents and inorganic salts **(Kolhe *et al.* 2009).**

Table (1): Characterization of dairy wastewater:

parameters	*Average ± standard deviation*	*Unit*
Color	**milky**	-
pH	**6.01 ±0.17**	-
Turbidity	**1133 ±15.33**	NTU
Electrical conductivity	**442 ±9.06**	µS/cm
TSS	**636 ±11.21**	mg/l
TDS	**1790 ±8.22**	mg/l
COD	**2288 ±18.16**	mg/l
BOD	**1221 ±13.01**	mg/l
O&G	**153 ±4.52**	mg/l

Identification of isolated microorganisms:

Fourteen bacterial isolates were identified by using Biolog^{TM} Gen III. The identified bacterial isolates showed some repeats and finally, five bacterial strains were identified as *Staphylococcus aureus, Pseudomonas aeruginosa Streptococcus thermophilus, Bacillus supergene and Lactobacillus fermentum*. Also, six isolated fungal isolates were subject to identification based on colony morphology and microscopic examination. Also some repeats were detected. Three fungal strains were identified as *Aspergillus sp., Cladosporium sp. and Fusarium sp*.

Characterization of the final dairy wastewater after biodegradtion:

A mixture of the identified five bacterial species *(Staphylococcus aureus, Pseudomonas aeruginosa Streptococcus thermophilus, Bacillus* *supergene and Lactobacillus fermentum)* was prepared with equal percent and used for the treatment process and named as the bacterial mixture (Mix 1). The same was carried out separately using three fungal strains *(Aspergillus sp., Cladosporium sp. and Fusarium sp*) that were mixed and used for the treatment process and named as the fungal mixture (Mix 2). **Table 2** shows the values of physicochemical parameters of treated dairy effluent after aeration stage (primary tank) and filtration stage (secondary tank) and also the total reduction percentage. Results showed that the color of dairy wastewater has been improved, as it was milky and after the treatment process, it was clear. This improvement may be due to degradation of organic materials by bacterial and fungal mixtures. Also, using sand and activated carbon as filter media led to removing more suspended particles and consequently color improvement **(Verma & Madam- war 2002**

Table (2): Treatment of Dairy Effluent by using a mixture of bacterial & fungal isolates:

Parameter	*Mix 1 (Bacterial isolate mixture)*				*Mix 2 (Fungal isolates mixture)*				*Unit*
	After incubation	**Reduction (%)**	**After filtration**	**Total reduction (%)**	**After incubation**	**reduction (%)**	**After filtration**	**Total reduction (%)**	
Color	**Creamy white**	-	**clear**	-	**Creamy white**	-	**clear**	-	-
pH	**6.6 ±0.12**	-	**7.3 ±0.11**	-	**6.5 ±0.1**	-	**7.1 ±0.05**	-	-
Turbidity	**623 ±3.9**	**45.0**	**9.0 ±0.23**	**99.2**	**734 ±5.3**	**35.2**	**11.0 ±0.7**	**99.0**	**NTU**
EC	**220 ±2.5**	**50.2**	**61 ±0.72**	**86.1**	**264 ±4.5**	**40.3**	**78 ±1.67**	**82.4**	**µS/ cm**
TSS	**428 ±6.5**	**32.7**	**3.0 ±0.5**	**99.5**	**444 ±5.5**	**30.1**	**7.0 ±0.54**	**98.9**	**mg/l**
TDS	**1343 ±18.5**	**24.9**	**351 ±2.21**	**80.4**	**1487 ±11.6**	**16.9**	**398 ±4.8**	**77.8**	**mg/l**
COD	**660 ±4.2**	**71.2**	**493 ±3.4**	**78.5**	**712 ±5.5**	**68.9**	**523 ±4.5**	**77.1**	**mg/l**
BOD	**328 ±5.0**	**73.1**	**247 ±3.7**	**79.8**	**361 ±4.6**	**70.4**	**336 ±5.5**	**72.5**	**mg/l**
O&G	**87 ±2.61**	**43.1**	**4 ±0.31**	**97.4**	**91 ±3.13**	**40.5**	**6 ±0.51**	**96.1**	**mg/l**

For pH value results shown in **Table 2** that the pH values moved towards the neutrality in both bacterial and fungal mixtures. Also 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**).

As shown in **Table 2** the efficiency of reduction for the turbidity after incubation was 45% and 35.2% for both bacterial mixture and fungal mixture 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 total reduction percent was 99.2% and 99 % for bacterial mixture and fungal mixture respectively. This observed decrease in turbidity values after filtration stage was a result of using filter materials (sand and activated carbon) which absorbed more substances this result was in correspondence with **Porwal *el al.*, 2015).**

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 can be seen that after filtration stage, a great reduction in EC values was detected. The bacterial mixture (Mix 1) showed EC reduction 86.1% while the fungal mixture shows 82.4 %reduction. 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; this may be due to the adsorption of ions on the activated carbon layer.

Concerning TSS for both bacterial and fungal mixtures shows high reduction efficiency with 99.5% and 98.9% respectively as shown in table 2. However after incubation the reduction efficiency was not high for both bacterial and fungal mixtures as it increased after filtration and this results was in correspondence with **Porwal *et al.*, 2015 and Shruthi *et al.*, 2012.**

TDS reduction efficiency was 80.4% and 77.8% for bacterial and fungal mixtures respectively as shown in table 2**. Gaikwad *et al.*, 2014** had also reported a maximum of 74.36% reduction in TDS by using microbial consortia of various bacterial species namely *Pseudomonas, Actinomycetes, Bacillus*, *Staphylococcus and Streptomyces*. The presence of high level of total suspended solids and total dissolved solids is due to organic and inorganic matter present in the effluent. A large number of solids are found dissolved in natural waters, the common ones are bicarbonates, carbonate, phosphates, chlorides, sulfates and nitrates of calcium, magnesium, sodium, potassium, iron, magnesium etc. A high content of TDS reduces the ability to reuse this water for drinking, irrigation and industrial purposes.

For COD the bacterial and fungal mixtures have obtained high reduction efficiency was 71.2% and 68.9% respectively after incubation while after filtration the total reduction efficiency was 78.5% and 77.1% respectively as shown in table 2. Our results are in correspondence with the reduction in COD seen by **Guillen-Jimenez *et al.*, (2000)**. **Chatterjee and Pugaht (2013)** had also reported 67.1% and 48.3% reduction in COD of diary wastewater with use of two bacterial strains namely *Neisseria sp.* and *Citrobacter* sp. The reduction in COD values might be due to more amounts of nutrients present in the form of dissolved and organic nature which is used by microorganisms for their growth.

Concerning BOD reduction efficiency for both bacterial and fungal mixtures high reduction efficiency was observed after incubation. BOD is widely used as an indication of water quality. The significant decrease in BOD values could be associated with consumption of organic material by microorganisms as a source of food. The reduction percentage was 73.1% and 70.4% after incubation for bacterial and fungal mixtures respectively and increased to reach 79.8% and 72.5% respectively after filtration. These results were in correspondence with **Porwal et el (2015) and Das & Santra (2010).**

Oil & grease reduction efficiencies for both bacterial and fungal mixtures was high after filtration as it was 97.4% and 96.1 % respectively while after incubation it was 43.1% and 40.5% respectively as shown in table 2. These results are with correspondence with **Porwal et el 2015.** The presence of sand and activated carbon filter increased reduction percentage of O&G due to their adsorption abilities. Also, lower reduction percentage during incubation 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).**

CONCLUSIONS

From this study we can conclude that the treatment technology of dairy wastewater by using bacterial and fungal strains was very effective as we achieved high reduction efficiency in all the tested parameters.

Bacterial mixtures were slightly more effective than fungal mixtures during the treatment process. Using activated carbon and sand for filtration after incubation enhances the reduction efficiency of the pollutants present in dairy wastewater.

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تقييم بعض السلالات البكتيرية والفطرية لمعالجة مياه الصرف الصحي لمنتجات الألبان

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المركز القومى لبحوث الاسكان و البناء

تم عزل الكائنات الحية الدقيقة من النفايات السائلة من مصانع الألبان التى تقع في مدينة السادس من أكتوبر الصناعية (القاهرة ، مصر) وقياس قدرتها على تقليل الملوثات الموجودة في مياه الصرف لمنتجات الألبان. تم عزل خمس سلالات بكتيرية **Staphylococcus aureus)**

Bacillus و **Pseudomonas aeruginosa**، **Streptococcus thermophilus**، **supergene** و **Lactobacillus fermentum**) وثـلاث سـلالات فطريـة (**Aspergillus sp.** و **Cladosporium sp.** و **Fusarium sp**) لاستخدامها في المعالجة. تم استخدام مفاعل ذو مرحلتين (التحضين ثم ترشيح) خلال هذه الدراسة. تم تحضير خليطين (المزيج ١: خمس سلالات معزولة بكتيرية و المزيج ٢: ثلاث سلالات فطرية معزولة). أوضحت النتائج أن السلالات البكتيرية والفطرية لها كفاءة عالية في تقليل الملوثات العضوية حيث بلغت نسبة تقليل الإزالة ٧٩.٨٪ و ٧٢.٥٪ على التوالي ، وأظهرت النتائج أن السلالات البكتيرية والفطرية ذات كفاءة عالية في إزالة الامـلاح الكليـه العالقه بلغت ٩٩.٥٪ و ٩٨.٩٪ على التوالي. ومع ذلك ، بعد التحضين ، لم تكن كفاءة الإزالة عالية لكل من الخليط البكتيري و الخليط الفطريات حيث زادت بعد الترشيح .كما انه ثبت فى هذه الدراسة ان الخليط البكتيري أكثر فاعلية قليلاً من الخليط الفطري أثناء عملية المعالجة و ايضا إن استخدام الكربون المنشط والرمل للترشيح بعد التحضين يعزز كفاءة معالجة الملوثات الموجودة في مياه الصرف الصحي لمنتجات الألبان.