Effect of Surfactant and Natural Coagulant on Biodegrading Capability of Bacterial Isolates from Dairy Effluent

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Abstract: The present study investigates the effect of surfactant and natural coagulant i.e. nirmali seeds on dairy effluent biodegradation. Forty bacteria from dairy effluent sample of two different seasons from Common Dairy, Mohali, India, were isolated by spread plating and streaking method. Bacterial isolates with degrading efficiency were identified as *Bacillus sp.*(B_1), *E.coli*.(B_3), *Staphylococcus sp.*(B_8), *Enterobacter* (B_{10}), *Staphylococcus sp.*(B_{16}), *Streptococcus sp.*(B_{20}), *Staphylococcus sp.*(B_{23}), *E.coli*.(B_{24}), *Bacillus sp.*(B_{27}), *Citrobacter freundii* (B_{30}), *Tatumella morbirosei* (B_{32}) and *Staphylococcus sp.* (B_{34}) on the basis of morphological and biochemical characteristics. Degrading efficient strains were identified on the basis of reduction in COD value. Variable amount of reduction in COD was observed in dairy effluentafter the treatment with free and immobilized identified twelve bacterial strains. Maximum biodegradation was shown by immobilized cells of B_3, B_8, $B_{10}$and B_{16} and by free cells of $B_3$and B_{10}. The goal of this work was to obtain insight into how surfactant and nirmali seeds affect biodegradation of dairy effluent by different bacterial isolates. Surfactant was found to have stimulatory effect on B_3, B_{10}, $B_{20}$and B_{16} and nirmali seeds have stimulatory effect on all strains**.** Along with bacterial strains, surfactant can achieve maximum 85.8% reduction in COD value while with natural coagulant 79.41% reduction can be achieved.Fluctuating results were observed with combined treatment of dairy effluent with surfactant and free cells and with natural coagulant and free cells. Our results indicate that action of surfactant and natural coagulant is species specific and influenced by the season.

Keywords: *Surfactants, Biodegradation, Dairy Effluent, COD.*

1. INTRODUCTION

Dairy wastewater is enriched in organic matter (about 45-72 g/l, COD) and also contains biodegradable carbohydrates [7].Aerobic treatment of liquid waste produced from food industries and animals is evolving as one of pretreatment option to reduce chemical oxygen demand, biological oxygen demand and odor problems [9]. Aerobic conditions and appropriate microorganisms are necessary for an optimal rate of bioremediation. Bioremediation is the naturally occurring process by which microorganisms either stop or renovate environmental contaminants to inoffensive end products [8]. Biological treatment is necessary if organic matter is to be removed from water. Nonetheless, biological treatment offers an economical alternative to physical and chemical treatment methods. The mechanism underlying biological treatment is the decomposition of finely dispersed matter, colloidal and dissolved substances by metabolism of aerobic microorganisms [5].

Many of the most persistent contaminants exhibit low water solubility and hence, bioavailability of contaminants can often be improved by addition of emulsifiers [19]. One biological strategy that can enhance contact between bacteria and water-insoluble organic content is emulsification of the organic content. It has been assumed that surfactants would enhance the bioavailability of organic compounds.

By reducing surface and interfacial tension between liquids, solids and gases, allowing them to disperse readily as emulsions, chemical or biological surfactants may have variable effects on contaminant biodegradation [3].Surfactants can interact with microbial proteins and can be manipulated to modify enzyme conformation in a manner that alters enzyme activity, stability and specificity [12].

The number of studies dedicated to evaluating the influence of biosurfactants on bioremediation efficiency is constantly growing [14]. For a bioremediation application, solubilization efficiency is a prior criterion for the selection of a surfactant. However, its biodegradability and toxicity to the microorganism have to be considered to ensure an efficient remediation and the environmentally friendly application of the surfactant. However, bioavailability and biodegradation kinetics of the hydrophobic pollutants are affected variably by the surfactants. Both stimulating and inhibiting effects of surfactants on bioremediation of pollutants are known depending on the chemical characteristics of the surfactant, pollutant and physiology of the microorganism [19]. Surfactants with moderate biodegradability to the microorganisms should be considered. To make the surfactant-mediated bioremediation a cost effective technique, efforts should be taken on the development of synthetic surfactants that biologically compatible with cell.

Coagulation and flocculation are commonly used methods for water turbidity removal, and are usually conducted byadding

chemicals such as salts of aluminum and iron and polyelectrolytes [4]. Recently researchers have shown tremendous interest in using natural coagulants [9].The history of the use of natural coagulants is long. Natural organic polymers have been used for more than 2000 years in India, Africa, and China as effective coagulants and coagulant aids at high water turbidities. They may be manufactured from plant seeds, leaves, and roots. There is no human health danger and the cost of these natural coagulants would be less expensive than the conventional chemicals alike [2].Coagulants should be biodegradable and are presumed to be safe for human health. In addition, natural coagulants produce readily biodegradable and less voluminous sludge that amounts only 20– 30% that of alum treated counterpart. Nirmali seed extracts are anionic polyelectrolytes that destabilize particles in water by means of interparticle bridging [22].

Biodegradation of dairy effluent was studied in terms of reduction of COD. Dairy effluent has high organic loads as milk is its basic constituent with high levels of chemical oxygen demand [15]. Aerobic treatment of liquid waste produced from food industries and animals is evolving as one of pretreatment option to reduce chemical oxygen demand [9]. Objective of present work was to identify some new active strains from the dairy effluent which can bring about fast biodegradation of the organic compounds in the dairy effluent. Another objective is to determine the effect of surfactant and effect of nirmali seeds as a natural coagulant on dairy effluent biodegradation.

II. MATERIAL AND METHODS:

1.1 Effluent Sample

For the present study the effluent samples were collected in different months i.e. August, November, February and May in sterile plastic container from common dairy, Mohali, India.

1.2 Media

For the isolation of the microorganisms from the effluent following used medias were purchased from SRL (sisco research laboratories):- Nutrient agar, MacConkey Agar, Eosin methylene blue agar and Czapek-Dox agar and King's B and OF Basal medium were purchased from HIMEDIA. The Nutrient agar medium had the following composition (g/l): agar 15.00, peptone 5.00, sodium chloride 5.00, yeast extract 2.00, beef extract 1.00. MacConkey agar medium contained the following ingredients (g/l): peptic digest of animal tissue 17.00, agar 13.50, lactose 10.00. sodium chloride 5.00, bile salts 1.50, proteose peptone 3.00, neutral red 0.03, crystal violet 0.001. The Eosin methylene blue agar medium had the following composition (g/l): peptone 10.00, agar 13.50, lactose 5.00, sucrose 5.00, dipotassium hydrogen phosphate 2.00, eosin Y 0.40, and methylene blue 0.065. King's medium B agar medium contained the following ingredients (g/l): proteose peptone no.3 20.00, dipotassium hydrogen phosphate 1.50, magnesium sulphate, $7H_2O$ 1.50, agar 20.00. OF basal medium contained the following ingredients (g/l): casein enzymic hydrolysate 2.00, sodium chloride 5.00, dipotassium phosphate 0.30, bromo thymol blue 0.08, and agar 2.00.

1.3 Isolation of the microorganism and characterization of the isolated strain

0.1ml of the given effluent was spread on to the solidified Nutrient Agar medium, EMB, MacConkey, King's B medium and incubated at 37ºC for 48 hours. Based on the morphological and biochemical test isolates were identified.

1.4 Preparation of seed culture (Inoculum)

Cells from bacterial isolates B_1, B_3, B_8, B_{10}, B_{16}, B_{20}, B_{23}, B_{24}, B_{27}, B_{30}, B_{32}, and B_{34} were inoculated into 50 ml of LB medium and incubated in a rotary shaker at 37°C for 24 hours.

1.5 Preparation of free cells

100 ml of LB medium was inoculated with 1 ml of respective seed culture and incubated at 37˚C in a rotary shaker for 24hrs. Fully grown cells harvested by centrifuging at 5000 rpm for 15 min. Washings were given to the cell pellet with 50ml of autoclaved distilled water (D.W.) twice. The specific bacterial pellet was resuspended in 10ml of autoclaved D.W. out of which 2.5ml of suspension was used as free cells.

2.6 Preparation of immobilized cells

Agar Solution and Inoculi were prepared separately. Fifty milliliter of each of the inoculi was prepared and incubated for 24 hours. A solution containing 3% bacteriological agar (100ml) in a 250ml Erlenmeyer flask was sterilized and cooled to 40-45ºC. The inoculi with OD at 600nm= 0.1 which correspond to 10^9 CFU/ml was mixed with the prepared agar plates and solidification occurred after 10 minutes. The solidified agar block was cut into equal size cubes, then added to sterile 0.1ml phosphate buffer (pH 7.0), and kept in the refrigerator (1hour) for curing. Phosphate buffer was decanted and the cubes were washed with sterile distilled water 3 to 4 times before use [1]. Four 2.5gms immobilized bacterial cells beads were added to the effluent and COD was determined to monitor the progress of biodegradation.

2.7 Biodegradation using free and immobilized cells in shaker flasks

Degradation of dairy wastewater was conducted in Erlenmeyer flasks using free and immobilized bacterial cells [16].

2.8 Estimation of chemical oxygen demand (COD)

The COD of the samples was determined using titration method [6].

2.9 Determination of surfactant effect

Tween 80 and Tween 20 are polysorbates which are amphipathic, nonionic surfactants composed of fatty acid esters of polyoxyethylene sorbitan [13]. Tween 80 and Tween 20 are non-ionic surfactant. Nonionic surfactants are less toxic to microorganisms than ionic surfactants.An experiment for shake flask biodegradation of dairy effluent was performed with tween 80 (2%) and tween 20 (2%) to study the effect of surfactants [20]. samples were collected after 24hrs incubation. Biodegradation was checked by determination of COD values of the processed samples.

2.10 Preparation of Moringa oleifera seeds powder:

The Nirmali dry pods were obtained from a field located 90 km away from Chandigarh. High quality pods, those which were new and not infected with disease, were selected. Seeds were opened and from pods and then dried sunlight 48 hr. Hulls and wings from the kernels were removed manually. The kernels were crushed and ground to a medium fine powder in grinder. The powder was sieved using 0.45mm mesh and the powder was stored in a container in refrigerator to avoid loss of its activity [21] .The fine powder was used as coagulant for analysis [9].

2.11 *Determination of Natural Coagulant effect*

Nirmali Seed is a natural coagulant material with polyelectrolytes. These polyelectrolytes are responsible for coagulation property of Nirmali seeds [17]. Nirmali seed powder was weighed in to 0.1, 0.3, 0.5, 0.7 gm and was added to 4 different conical flasks respectively and 100ml of dairy wastewater (DWW) sample was added to each. The samples were rotated on a shaker at 120 rpm for 24hrs and after 24hrs the samples were allowed to settle for 30 minutes and were filtered through normal filter paper and the change in COD value was Determined [9].

III. RESULTS

3.1 Identification of effluent biodegrading strain from the dairy effluent

The 40 strains were selected by spread plate method and isolated using streaking method. Among all, twelve strains B_1 (*Bacillus sp*), B_3(*E.coli.*), B_8 (*Staphylococcus sp).*,B_{10} (*Enterobacter*), B_{16}(*Staphylococcus sp.*), B_{20} (*Streptococcus sp.*) B_{23} (*Staphylococcus sp.*) B_{24} (*E.coli),*B_{27}(*Bacillus sp.),* B_{30} (*Citrobacter freundii*), B_{32} (*Tatumella morbirosei.*), and B_{34} (*Staphylococcus* sp.) had shown maximum biodegrading capacity and those were characterized by various morphological and biochemical tests (table 1) and also confirmed by online ABIS software.

Table I: Different Bacterial strains isolated by Biochemical Tests

Months →	August				November		February				May	
BIOCHEMICAL CHARACATERSTICS	B_1	B 3	B 8	B 10	B 16	B 20	B 23	B 24	B 27	B 30	B 32	B 34
GRAM STAINING	+	-	+	-	+	+	+	-	-	-	-	+
CATALASE	-	+	+	+	+	-	+	+	+	-	+	+
CITRATE	-	-	-	+	-	-	-	-	+	+	+	+
MR	-	-	-	+	+	+	+	+	+	+	+	-
VP	-	+	+	-	-	-	-	-	+	-	+	+
INDOLE	-	+	-	-	+	+	-	+	-	+	-	-
NITRATE	+	+	+	+	+	+	+	+	+	+	+	+
H2S	-	-	-	-	-	+	-	-	+	+	-	-
STARCH HYDROLYSIS	+	-	+	+	-	-	-	-	+	-	+	-
GLUCOSE	-	+	+	+	-	+	+	+	+	+	-	-
SUCROSE	-	+	-	+	-	+	-	+	+	+	-	+
LACTOSE	-	+	-	+	-	-	-	+	+	+	-	-

3.2 Biodegradation using Immobilized, Free Cells and Free Cells and Surfactant and Free Cells and Natural Coagulant in Shake Flasks

Immobilized cells and free cells were prepared for the biodegradation studies. COD of dairy effluent treated with immobilized and free cells of isolated bacterial strains had shown variable results. Comparative COD value of effluent samples before and after 24hrs treatment with free cells, immobilized cells and combined treatment with free cells and 2% tween 20 and tween 80 (surfactant) and combined treatment with free cells and Natural coagulant were shown in Table 2.

Table II: COD values of Dairy Effluent before and after treatment with various methods

Sample COD mg/l	Month of sample collection	Bacterial strain	COD after immobilization Treatment (mg/l)	COD after free cell treatment (mg/l)	COD after combined treatment of free cell and surfactant treatment (mg/l)	COD after combined treatment of free cell and natural coagulant(0.3gm) (mg/l)
272	August'12	B1	206.4	208	360	172
		B3	43.2	62.4	38.4	56
		B8	48	112	185.6	102.5
		B10	68.8	67.2	38.4	64
262.4	November'12	B16	54.4	115.2	72	96
		B20	94.4	115.2	54.4	116
260	February'13	B23	169.6	136	112	104
		B24	160	192	216	188
		B27	208	184	384	176.4
		B30	123.2	83.2	72	82.8
168	May'13	B32	134.4	132.8	272	130.6
		B34	110.4	123.2	136	112

From the comparison of the results it was found that B_3 (*E.coli*) and B_8 (*Staphylococcus sp.*)free cells and immobilized B_3 beads of summer season improved the quality of dairy effluent effectively whereas from the winter season free cells of B_{30} (*Citrobacter freundii*) and immobilized cells of B_{16} (*Staphylococcus sp.*) shows highest biodegradation capacity among all the strains. The decrease in level of COD indicates the reduction of biologically oxidisable and inert organic materials as result of the degradation by the bacterial isolates [10].

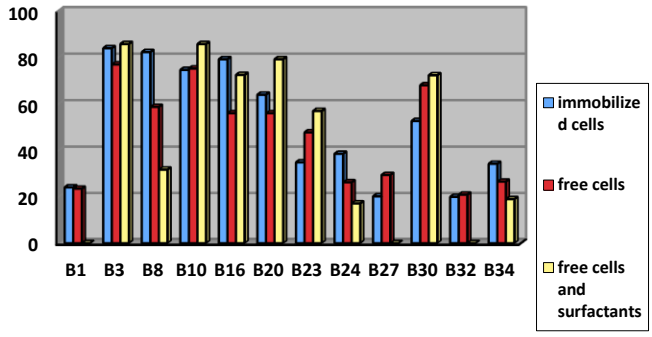

Fig 1: Dairy effluent COD Percentage Reduction by free cells, immobilized cells and with surfactant and free cells

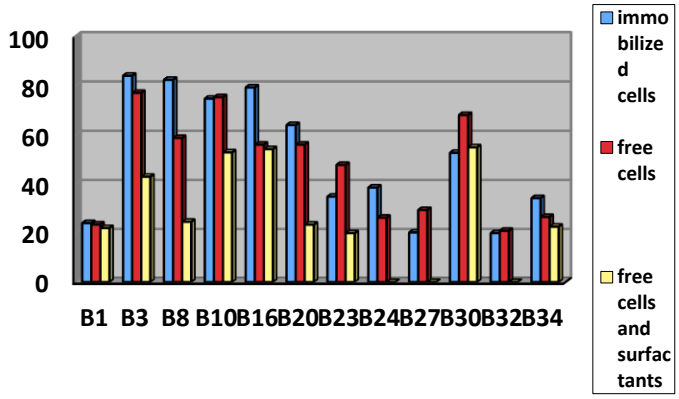

Fig 2: Dairy effluent COD Percentage Reduction by free cells, immobilized cells and with natural coagulant and free cells

Highest reduction (percentage) in COD value after 24hrs treatment was shown by immobilized *E.coli. (B_3)*(84%)and *Staphylococcus sp. (B_8)*(82.35%)of summer season and *Staphylococcus sp. (B_{16})*(79.26%) of winter season. Highest COD reductions (%) were shown by *E.coli. (B_3)*(77.05%) and *Enterobacter (B_{10})*(75.29%) free cells of summer season. *Bacillus sp*. (B_1),*Bacillus sp. (B_{27})*and *Tatumella morbirosei* (B_{32})had shown least biodegradation capacity both in free cell and immobilized form. Free cells have low degrading capacity as compared to immobilized cells and reason may be entrapment of bacterial cells in agar and agar may enhance the activity of degrading bacteria.

In the present study Tween 20 and Tween 80 were used as a surfactant. The synthetic surfactant at low concentrations may be useful for bioremediation of sites contaminated with hydrophobic pollutants [11]. Generally, nonionic surfactants are less toxic to microorganisms than ionic surfactants. The negatively charged surface of bacterial cells makes the cells more sensitive to the introduction of charged surfactants, especially positively charged cationic surfactants. Surfactant has enhanced the biodegrading capacity of B_{16}, B_{20}, B_{23}, B_{30}, B_3 and B_{10}. *Bacillus sp*. (B_{27}), *Tatumella morbirosei* (B_{32}) and *Bacillus sp*. (B_1) had shown no biodegradation process along with surfactants. Surfactants with respective bacterial free cells B_{16}, B_{20}, B_{23}, B_{30}, B_3 and B_{10} had shown increase in the biodegradation rate up to i.e. 16.47%, 23.17%, 9.2%, 4.3%, 8.75%, 10.51% whereas respective immobilized cells of B_{20}, B_{23}, B_{30}, B_3 and B_{10} strains had shown increment in COD reduction upto 15.24%, 22.15%, 19.63%, 1.8% and 15.24%. From the figure 1 it is depicted that B_8 in free cell has shown COD reduction of 82.35% but surfactant has adversely affected this strain by decreasing it efficiency by 40.59%.

Surfactants used in this study (Table 3) were non-ionic. The positive effects are generally attributed to the increased solubility/dissolution these compounds by surfactants which enhances their bioavailability. The negative effects are contributed by a variety of factors, which include toxicity of surfactants to microorganism, preferential degradation of surfactants and limited bioavailibity of substrate micelles. Nonionic surfactants are normally less toxic to microorganisms than ionic surfactants due to the weaker interactions between the neutral surfactant molecules and charged cell membrane.

A comparison of the effects of surfactants on the biodegradation of dairy effluent by different bacterial isolates shows that surfactants and natural coagulant stimulated the biodegradation of dairy effluent to a greater extent with some bacterial strains. However, the effects of surfactants on bioremediation cannot be predicted in the absence of empirical evidence because surfactants sometimes stimulate bioremediation and sometimes inhibit it [19]. Because nirmali seeds have potential to enhance the biodegrading capacity of bacterial strains thus it can be used in biodegradation treatment of wastewater.

Table III: Effects of Surfactant on biodegradation of dairy effluent.

Month of sample collection	Microorganism	Effects	Explanation
August	*Bacillus sp.*(B_1)	-	Surfactant was negative due to its preferable degradation by bacteria
	E.coli.(B_3)	+	Surfactant enhanced dissolution
	Staphylococcus sp.(B_8)	-	Surfactant was negative due to its preferable degradation by bacteria
	Enterobacter (B_{10})	+	Surfactant enhanced dissolution
November	*Staphylococcus sp.*(B_{16})	+	Surfactant enhanced dissolution
	Streptococcus sp.(B_{20})	+	Surfactant enhanced dissolution
February	*Staphylococcus sp.*(B_{23})	+	Surfactant enhanced dissolution
	E.coli.(B_{24})	-	Surfactant was negative due to its preferable degradation by bacteria
	Bacillus sp.(B_{27})	-	Surfactant was negative due to its preferable degradation by bacteria
	Citrobacter freundii (B_{30})	+	Surfactant enhanced dissolution
May	*Tatumella morbirosei* (B_{32})	-	Surfactant was negative due to its preferable degradation by bacteria
	Staphylococcus sp. (B_{34})	-	Surfactant enhanced dissolution

IV. CONCLUSION

The present study had isolated bacterial strains having the capacity to degrade dairy effluent. Biodegrading capacity of bacterial strains showed different results in free, immobilized state and combination of free cell with surfactant and combination of free cell with natural coagulant. Degradation of dairy effluent was detected by comparing COD value of effluent before and after the respective treatment process. Degrading efficiency of nine strains could be improved by natural coagulant along with free cell while five strains by surfactant along with free cell. Same bacterial strain isolated in different season had shown contrast behavior in their degrading property. Degrading efficiency of bacterial strains can be enhanced by addition of surfactant and natural coagulant. Some strains are unaffected by combined treatment of surfactant and free cells, natural coagulant and free cells. Our study concluded that effect of tween 20 and tween 80 and of natural coagulant is species specific and greatly influenced by summer season and winter season.

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REFERENCES:

[1]. A. T. Ajao, G. B.Adebayo, and S. E. Yakubu,"Bioremediation of Textile Industrial Effluent using mixed culture of *Pseudomonas aeruginosa* and *Bacillus subtilis* immobilized on agar in a Bioreactor,"J. of Microbiology and Biotech. Research, vol.1 (3), pp. 50-56, 2011.

[2]. Md. Asrafuzzaman, M. N. A. Fakhruddin, andA. Md. Hossain,"Reduction of Turbidity of Water Using LocallyAvailable Natural Coagulants,"Inter. national Scholarly Research Network ISRN Microbiology, vol. 2011, pp. 1-6, 2011.

[3]. I.M. Banat, R.S. Makkar, andS.S. Cameotra, (2000). "Potential commercial applications ofmicrobial surfactants," Appl Microbial Biotechnol, vol.53, pp. 495–508, 2000.

[4]. S. Choubey, K. S. Rajput, andN .K. Bapat,"Comparison of Efficiency of some Natural Coagulants-Bioremediation," International Journal of Emerging Technology and Advanced Engineering, vol. 2(10), pp. 429-434, 2012.

[5]. P. Dhall,R. Kumar, and A. Kumar, "Biodegradation of Sewage Wastewater Using Autochthonous Bacteria" The Scientific World J. vol.2012, pp. 1-8, 2012.

[6]. R. C. Dubey, andD. K. Mahashwari,"Practical microbiology," Rajendra Ravindra Printers (Pvt.) Ltd. Ram Nagar,New Delhi, pp. 326-327, 2007.

[7]. A. Ebrahimi, andM. Asadi, **"**Dairy wastewater treatment using three-stage rotating biological contactor (NRBC)," Int. J. of Eng, vol.22 (2), pp. 107-114, 2009.

[8]. A. Hamid, Z. Bibi, I. Ghafoor, Z. Anwar, K. Khan, andJ. Zaman,**"**Approach of Bioremediation in Olive Oil and Dairy Industry: A Review," Research J. of Applied Sci. vol. 5(6), pp. 2255-2263, 2013.

[9]. D. P. Harush, U. S. Hampannavar, andM. E. Mallikarjunaswami, "Treatment of dairy wastewater using aerobic biodegradation and coagulation,"InternationalJ.of Env. Sci. & Research, vol.1(1), pp. 23-26**,** 2011.

[10]. P. Ingole, S.Kakde, U. Aruna, and C. R. Maggirwar, "Biodegradation of tannery effluent by using tannery effluent isolate,"*Int. Multidisciplinary Research J*. vol. 2(3), pp. 43-44, 2012.

[11]. R. Jayashree, andN. Vasudevan, "Effect of tween 80 and moisture regimes on endosulfan degradation by pseudomonas aeruginosa," Applied ecology and environmental research, vol.7(1),pp.35-44**,** 2009.

[12]. N. Kamiya, M. Inoue, M. Goto, N. Nakamura, andY. Naruta, "Catalytic and structural properties of surfactant-horseradish peroxidase complexin organic media," Biotechnol Prog, vol.16, pp. 52–8, 2000.

[13]. B. A. Kerwin, "Polysorbates 20 and 80 used in the formulation of protein bio therapeutics: structure and degradation pathways," J Pharm Sci., vol. 97(8), pp. 2924-35, 2008.

[14]. L. Lawniczak, R. Marecik, andL. Chrzanowski, "Contributions of biosurfactants to natural or induced bioremediation," Appl Microbiol Biotechnol., vol.97(6), pp. 2327-39, 2013.

[15]. K. J. Singh, L. R. Meshram, andS. D. Ramteke, "Production of Single cell protein and removal of 'COD' from dairy waste water,"European J. of Experimental Bio, vol. 1 (3), pp. 209-215, 2011.

[16]. R. Manogari, D. Daniel, andA. Krastanov, (2008). "Biodegradation of rice mill effluent by immobilized pseudomonas sp. cells," Ecological eng. and env.protection, vol. 1, pp. 30-35, 2008.

[17]. A. Y. Maruthi, S. A. Dadhich, andK. Hossain, "Nirmali Seed as a Natural Biosorbent; Evaluation of its Potential for Iron (II) Removal from Steel Plant Effluents and Sewage Disinfecting Capacity," European Journal of Sustainable Development, vol.2 (3), pp. 77-84, 2013.

[18]. O. Obire, andS. A. Wemedo, "Seasonal effect on the bacterial and fungal population of an oilfield wastewater polluted soil in Nigeria," Journal Applied science environment management,vol.6 (2), pp. 17-21, 2002.

[19]. A. Singh, V. Hamme, D. Jonathan, P. Owen, andO. Ward, "Surfactants in microbiology and biotechnology: Part 2," Application aspects Biotechnology advances, Elsevier, pp. 1-23, 2005.

[20]. H. Ullah, S. A. Ali, F. Hasan, andA. Hameed, "Biodegradation of Trinitrotoluene by Immobilized Bacillus sp. YRE1," Pak. J. Bot., vol.42(5), pp. 3357-3367, 2010.

[21]. .S. Vieira, A. Marquetotti, F. Marcelo, V. Silva, F. Gabriel, A. Araújo, et al."Use of Moringa oleifera Seed as a Natural Adsorbent for Wastewater Treatment," Water Air Soil Pollution Brazil,vol. 206, pp. 273–281, 2010.

[22]. G. Vijayaraghavan, T. Siva Kumar, V. A. Kumar, "Application of plant based coagulants for waste water treatment," International Journal of Advanced Engineering Research and Studies, vol. 1(1), pp. 88-92, 2011.

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