Isolation and Purification of *Lactobacillus Acidophilus* and Analyzing its Influence on Effluent Treatment

K.Vellingiri[1,*], T.Ramachandran[2], A. Thirugnanasambantham[3]

[1]Department of Textile Technology, PSG College of Technology, Coimbatore, Tamil Nadu, India

[2]Karpagam Institute of Technology, Coimbatore, Tamil Nadu, India

[3]PSG Polytechnic College, Coimbatore, Tamil Nadu, India

Received 12 June 2014; received in revised form 06 October 2014; accepted 26 November 2014

Abstract

Out of various activities in textile industry, wet processing produces about 70% of the effluents. Of late textile industry is giving importance for the eco-friendly processes to protect the environment. The effluents degrade the quality of water and cause injury to the existing organisms and aquatic life. When biological treatments are given to the textile effluents it results in significant reduction in the effluent characteristics and the resultant becomes environmental friendly. This successful bio-culture treatment uses aquatic organisms to purify effluent and refresh the water. A number of bio-cultural species are widely used in the treatment of effluents. *Lactobacillus acidophilus* is one such bacterium used in the effluent purification. *Lactobacillus acidophilus* has the ability to remove, assimilate and decompose the biodegradable organic matters present in the effluents. In addition to these, the present research study attempts to control the levels of total suspended solids (TSS), improve the dissolved oxygen content, reduce the chemical oxygen demand (COD) and biological oxygen demand (BOD).

Keywords: *Lactobacillus Acidophilus*, chemical oxygen demand, biological oxygen demand, total dissolved solids, Total suspended solids, PH.

1. Introduction

The increasing occurrence of many synthetic and natural organic substances in natural water lead to the importance of using the adsorption technique as one of the most effective methods of removing impurities from wastewater [1]. Environmental problems of the textile industries are mainly caused by discharges of wastewater. The textile sector has a high water demand. Its biggest impact on the environment is related to primary water consumption (80–100 m^3/ton of finished textile) and waste water discharge (115–175 kg of COD/ton of finished textile, a large range of organic chemicals, low biodegradability, colour, and salinity). Therefore, reuse of the effluents represents an economical and ecological challenge for the overall sector. Major pollutants in textile wastewaters are high suspended solids, chemical oxygen demand, heat, colour, acidity, and other soluble substances [2-8]. To use microalgae for wastewater treatment is an old idea, and several researchers have developed techniques for exploiting the algae's fast growth and nutrient removal capacity [9-12].

The nutrient removal is basically an effect of assimilation of nutrients as the algae grow, but other nutrient stripping phenomena also occur, e.g. ammonia volatilization and phosphorus precipitation as a result of the high pH induced by the

* Corresponding author. E-mail address: rkv@fas.psgtech.ac.in
Tel.:0422-2572177 ; Fax: 0422-2573833

algae. Compared to bacterial systems, bioreactors utilizing a symbiotic association between algae and bacteria have been reported to yield higher treatment efficiency [5, 13-14]. Various conventional methods are in practice for purification of water and removing the pollutant contaminants, but most of them are costly and non-eco friendly. One of the promising ways for improving water quality of rivers and lakes is the effective microorganism (EM) technology which has been much appreciated comparative to other conventional methods because of its eco- friendly nature, and requires less inputs, cost and capital [15-16]. Strictly speaking, contaminant treatment can be defined as the complete degradation or mineralization of the contaminants. However, the photo catalytic degradation is suitable for treating hazardous organic pollutants. Generally, biological treatment is the most economical treatment option and the most compatible with the environment when feasible. Though the feasibility of adopting the photo catalytic oxidation is much explored they are not adopted practically, due to the problem of surplus power needed for generation of UV) addresses this issue radiation [17-18]. But in the present years ferrite doped titanium dioxide (TiO_2 and, it also enhances the reusability of the catalyst. Hence it could be an ideal treatment to transform the bio-persistent compounds for an effective treatment system with sustainability. They also address the green technology, by utilizing sunlight as their source of excitation. This could be further eliminates or reduces the production of sludge's, a secondary pollutant. In near future the practice of biological methods could be replaced by the heterogeneous oxidation process. Such replacement will lead pathway to a green technology for the sustainable development [19].

Prevention and treatment of dyeing wastewater pollution are complementary. We can both use preventive measures as well as a variety of methods to control the wastes and make use of treated water. This will not only reduce water consumption, but also effectively reduce the pollution of the printing and dyeing wastewater and achieve sustainable development of society [20]. Many harmless or non pathogenic microbes are used as probiotics microbes for good health, as scavengers in aquaculture practices and in the prevention of diseases. Extensive research has been carried out in exploiting the uses of probiotics. Daily intake of probiotic supplements will vehemently improve the health of human beings. Probiotic organisms are therefore very much essential for the continued existence of life on earth [21].

The survival of probiotic bacteria in refrigerated conditions for at least 21 days were in number of greater than 10^8 cfu•mLG which is essential if a product should have probiotic properties. It is important to emphasize that all the beverages produced possessed excellent stability during 21 days of storage. The sensory scores of the beverages were high and acceptance. From the foregoing results it can be concluded that permeate can be successfully used in the preparation of nutritive beverages. Addition of strawberry or mango may produce more acceptable probiotic permeate beverages [22-23].

Thermophilic Anoxybacillus rupiensis isolated from hot water springs of Unharmed (Dhapoli region of Ratnagiri), Maharashtra showed remarkable decolonization through degradation in stationary cultures at 60^0 C possibly maximum percent decolourisation of textile effluent could have been achieved under submerged cultures. This research work can be further extended to study exactly the types and origin of enzymes involved in decolourization process. Growth conditions can be modified to accelerate the percent decolonization. Therefore the selected thermopile appeared to be an efficient organism for the treatment of textile effluents containing mixture of dyes [24].

The removal of colour from textile wastewater using whole bacterial cells demonstrates the great potential of treatment systems incorporating whole bacterial cells to metabolise the azo dyes, present in coloured, aqueous wastewater from the textile coloration industry [25]. Under anaerobic conditions, these systems can achieve total colour removal with short exposure times. However, as the knowledge base and the funding in this area of research increases, the use of bio processing will become the predominant solution to the problem of colored wastewater in the textile coloration industry. Many dyes and their break down products may be toxic for living organisms [26].colour removal was extensively studied with physico-chemical methods as coagulation, ultra-filtration, electro-chemical adsorption and photo-oxidation [27]. Recently different approaches have been discussed to tackle man made environmental hazards. Clean technology, eco-mark and green chemistry are some of the most highlighted practices in preventing and or reducing the adverse effect on our surroundings [28-30].

This paper presents the results of applying *Lactobacillus* culture to treat textile effluent. The study was conducted in a textile wet processing unit at Erode, Tamilnadu district. The results compared the efficiency of *Lactobacillus* culture in terms of the removal of all contaminants in the selected textile effluents.

2. Materials and methods

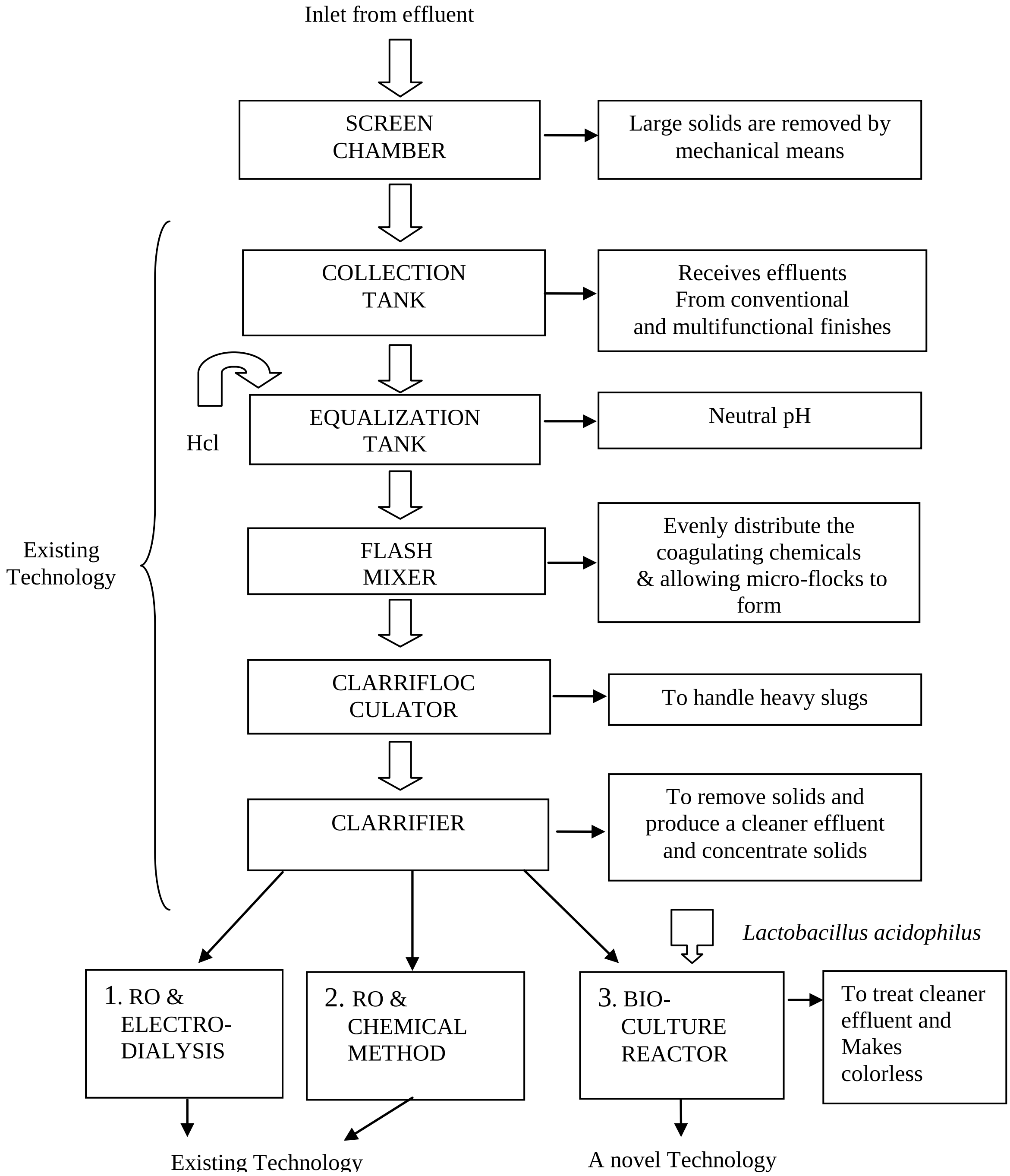


Fig. 1 Flow Chart for Effluent Treatment using *Lactobacillus acidophilus* bacterium

(1 - Novel Technology & 2, 3 Existing Technology)

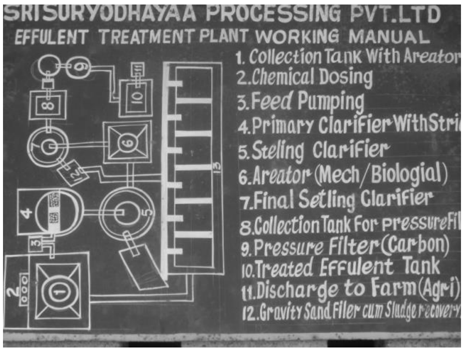
2.1. Materials- Isolation and purification of Lactobacillus acidophilus

In the present research study, *Lactobacillus acidophilus* (MTCC 5462) was developed using the byproducts of milk. Milk, curd, palm jaggery and cow dung were used as inoculums to grow lactobacilli. 12% (v/v) of the byproducts of milk was closely packed and stored in airtight containers at room temperature for 28 days. After 28 days of incubation under anaerobic condition, the culture was sent to a microbiology laboratory for isolation, characterization and confirmation of *Lactobacillus acidophilus.* The culture was found to be *Lactobacillus acidophilus* (MTCC 5462). This culture was used further in the effluent treatments.

2.2. Methodology adopted for biological treatments

The method adopted for biological treatment using *Lactobacillus acidophilus* is given in the Figure 1. The treatment involved a primary treatment to remove larger solid organic and inorganic materials. Secondary treatments with *Lactobacillus acidophilus* breaks down dissolved and suspended organic solids and reduce pathogens. The various stages followed for treating effluent samples adopted at PSG College of Technology, Coimbatore, Tamilnadu, India are given in Figure 1 below. The Figure 2 show the plant process flow chart using biological method adopted in M/s. Sri Suryodhayaa Processing Private Limited* Company, Erode, Tamilnadu, India.

In the Figure 1, the clarifier zone, all the larger solids and all sorts of refuse that has arrived with the effluents are removed. Then, the collection tank receives the effluents and in an equalization tank a pH value of 7 was maintained by adding hydrochloric acid to the effluent. Flash mixing is used to evenly distribute coagulating chemicals in the effluent, allowing micro-flocks to form. As the precursor to flocculation, flash mixing increases the efficiency of flocculation and reduces chemical wastage. Then, the clariflocculators are used for chemical primary treatment and enables to handle heavy slugs while delivering shortest flock settling time. Clarifiers are used for reducing suspended solids load by coagulation and settling method. The purpose of a clarifier is to remove solids, produce a cleaner effluent and concentrate solids were shown in Figure 2 Plant process flowchart using Biological methods (Figures 2a to Figure 2l). Finally *Lactobacillus acidophilus* was used to treat the cleaner effluent and makes it coluorless with significant reduction of treated effluents.



(a) A Process Flowchart at the plant

(b) Bleaching Collection Tank Biological methods

(c) Overall Collection Tank

(d) Equalization and neutralization tank

Fig. 2 Plant Process Flowchart Using Biological Methods

(e) Aeration Tank

(f) Primary Clarifier

(g) Secondary Clarifier

(h) Tertiary Treatment

(i) Treated effluent before sludge formation

(j) Before Sludge Formation

(k) Sludge Formed

(l) Water before and after Pre-treatment

Fig. 2 Plant Process Flowchart Using Biological Methods

In the Figure 2f and Figure 2g the clarifier zone, all the larger solids and all sorts of refuse that has arrived with the effluents are removed. Then, the collection tank receives the effluents and in an equalization tank a pH value of 7 was maintained by adding hydrochloric acid to the effluent. Flash mixing is used to evenly distribute coagulating chemicals in the effluent, allowing micro-flocks to form. As the precursor to flocculation, flash mixing increases the efficiency of flocculation and reduces chemical wastage. Then, the clariflocculators are used for chemical primary treatment and enables to handle heavy slugs while delivering shortest flock settling time. Clarifiers are used for reducing suspended solids load by coagulation and settling method. The purpose of a clarifier is to remove solids, produce a cleaner effluent and concentrate solids. Finally *Lactobacillus acidophilus* was used to treat the cleaner effluent and makes it coluorless with significant reduction of treated effluents.

2.3. Influence of lactobacillus acidophilus (organism) on the effluents

Effluent samples collected from the laboratory were kept for 4 h to reach room temperature. A pH value of 7 was maintained by adding hydrochloric acid to the effluent. *Lactobacillus acidophilus* was added to the effluent was measured in the ratio of 1:100 (v/v) and the samples were stored for 4 days. Figure 3a and 3b show the culture at the starting stage and final stage respectively.

(a) Culture Formation – Starting Stage

(b) Culture Formation – Final Stage

Fig. 3 Isolation, characterization and confirmation of Lactobacillus acidophilus

The Figure 3a shows the measured quantity of 50 litres of collected effluents with a bio culture formation at the beginning stage. The Figure 3b shows the final stage of a bio culture. After the bio culture treatment, all the characteristics of effluents was assessed by the standards and procedures suggested by APHA, AWWA, WEF, 1998; Kenneth, 1990. The characterization of effluents test was carried out at Intertek Company, Tirupur. Table 1 shows characteristics of the effect of biological treatment on effluents.

3. Results and Discussions

The effluent sample of 50 liters was collected directly into containers. After adding 10 ml of Magnesium sulfate solution to fix the dissolved oxygen and it was stored at room temperature. They were preserved to inhibit bio degradation and then analyzed for their characteristics. The characteristics of effluents were given in Table 1.

Table 1: Test results of Effluent characteristics before and after a bio-culture treatment

S. No	Characteristics	Effluent properties (before treatment)	After Biological Treatment properties	% of Reduction	Norms recommended by Central Pollution Control Board-INDIA(CPCB)
1	pH value	9-11 (alkaline)	7 (neutral)	22.22	5.5-9
2	Total suspended solids, mg l^{-1}	1030	94	91	100
3	Total dissolved solids, mg l^{-1}	7750	2000	74.2	2000
4	BOD (5 days at 20^{o}c), mg l^{-1}	300	20	93.3	30
5	COD (5 days at 20^{o}c), mg l^{-1}	1100	70	96.3	250
6	Colour in *Hazen units (Platinum- Cobalt Scale)	1000 Variable dark	300 Pale yellow to Colorless	70	Max-300

It is evident from the results shown in Table 1, that there is an improvement in the effluent characteristics due to biological treatment. The pH value of the untreated effluent is very high as the incoming effluent is highly alkaline in nature. The pH correction is done with the help of hydrochloric acid (HCl) and to bring down to neutral pH which is favorable for the biological treatment. Total Suspended Solids (TSS) is reduced to the extent of 91%. Total Dissolved Solids (TDS) is composed mainly of carbonates, bicarbonates, chlorides, phosphates and nitrates, calcium, magnesium, potassium and manganese, organic matter salts and other particles.TDS of effluent detected could be treated by biological (*Lactobacillus acidophilus)* method. TDS detected could be attributed to the high color from the various treatments being used at PSG College of Technology, Coimbatore. The TDS was reduced to the extent of 74.2 % after the treatment. The higher values of COD and BOD in untreated effluent attributed to the presence of chemical substances and breakdown of raw material used for preparation of fibre respectively. The COD and BOD of treated effluents were reduced to the extent of 96.3% and 93.3 % respectively which are very significant due to the biological treatment process. The color of the effluent was Variable dark and 1000. The colors of the effluent in Platinum- Cobalt Scale were reduced to the extent of 70% after the treatment. This is due to adsorption and degradation of culture. This research work confirms that biological effluent treatment leads to color removal from effluent to an acceptable level for recycling of water. The characteristics of effluents after treatment were compared with general standards for discharged environmental pollutants recommended by the Central Pollution and Control Board (CPCB), INDIA. All the characteristics of effluents after treatment are highly commendable value when compared to the norms recommended by the Central Pollution and Control Board (CPCB), INDIA. So, it indicates the research work on the influence of *Lactobacillus acidophilus* culture is fit for treatment of effluents. Hence, this is an Eco-friendly treatment and safe guards the environment.

4. Conclusion

Lactobacillus acidophilus culture was developed from the byproducts of milk to treat the effluents. The discharges from conventional method and multifunctional methods were treated by *Lactobacillus acidophilus.* A satisfactory result was achieved with biological treatment. The textile effluents contribute more COD and BOD load on the environment which is costlier for reprocessing treatment. The COD and BOD of treated effluents were reduced significantly due to the biological treatment process. The removal of all impurities and toxic substances in the effluent makes the process eco friendly. The effluent recycling process will save water and energy consumption in the textile industry.

References

[1] K. Lacasse, W. Baumann, "Textile Chemicals. Environmental Data and Facts," Springer-Verlag, Heidelberg, Germany, pp. 415-417, 2004.

[2] I.O. Asia and E. Akporhonor, "Characterization and Physicochemical Treatment of Wastewater from Rubber Processing Factory," International Journal of Physical Sciences, vol. 2, no. 3, pp. 61-67, 2007.

[3] B.V. Babu, H.T. Rana, V. Ramakrishna and M. Sharma, "C.O.D. Reduction of Reactive Dyeing Effluent from Cotton Textile Industry," Journal of the Institution of Public Health Engineers India, 4, pp. 5-11, 2000.

[4] P.A. Desai and V.S. Kore, "Performance Evaluation of Effluent Treatment Plant for Textile Industry in Kolhapur of Maharashtra," Universal Journal of Environmental Research and Technology, vol. 1, no. 4, pp. 560-565, 2011.

[5] Y. Fu and T. Viraraghavan, "Fungal decolorization of dye wastewaters: a review," Bio resource Technology, vol.79, pp. 251-262, 2001.

[6] G. McMullan ,C. Meehan , A. Conneely, N. Kirby, T. Robinson, P. Nigam, I.M. Banat, R. Marchant and W.F. Smyth, "Microbial decolourisation and degradation of textiles dyes," Application Microbial Biotechnology, vol. 56, pp. 81-87, 2001.

[7] M.C. Venceslau, S. Tom, and J.J. Simon, "Characterization of textile wastewaters-a review," Environmental Technology, vol. 15, pp. 917-929, 1994.

[8] World Bank, (2007), "Environmental, Health, and Safety Guidelines for Textile Manufacturing, International Finance Corporation," http://www.elaw.org/system/files/FinalTextilesManufacturing_0.pdf, December, 2007.

[9] P. Anam, Gomes F, Xaviermalcata and Franka M. Klaver, "Growth Enhancement of Bifidobacterium lactis Bo Lactobacillus acidophilus Kiby Milk Hydrolyzates," Journal of Dairy Science, vol. 81, pp. 2817-2825, 1996.

[10] M. Ghoreishi and R. Haghighi, "Chemical catalytic reaction and biological oxidation for treatment of non-biodegradable textile effluent," Chemical Engineering Journal, vol. 95, pp. 163-169, 2003.

[11] M.N.V. Kumar, T.R. Sridhari, K.D. Bhavani, P.K. Dutta, "Trends in color removal from textile mill effluents." Bio resource. Technol, vol. 77, pp. 25-34, 1998.

[12] V. Kumar, L. Wati, P. Nigam, I.M. Banat, B.S. Yadav, D. Singh and R. Marchant, "Decolorization and biodegradation of an aerobically digested sugarcane molasses spent wash effluent from bio methanation plants by white-rot fungi," Process Biochemistry, vol. 33, pp. 83-88, 1998.

[13] L. Radhakrishnan, "Removal of priority pollutant nitrobenzene by algal bacterial system in rotating biological reactor," M. Tech. Thesis, IIT, Bombay, 1997.

[14] N. Sahu, "Bio degradation of trichloroethylene by algal –bacterial system in rotating biological contactor," Ph.D. Thesis, IIT, Bombay, 2000.

[15] O. Li Rosi, M. Casarci, I.D. Mattiol, L. De Florio, "Best available technique for water reuse in textile SMEs (BATTLE LIFE Project)," Desalination, vol. 206, pp. 614-619. 2007.

[16] Zuraini Zakaria , Sanjay Gairola and Noresah Mohd Shariff (2010), "Effective Microorganisms (EM) Technology for Water Quality Restoration and Potential for Sustainable Water Resources and Management," International Environmental Modelling and Software Society (iEMSs), International Congress on Environmental Modeling and Software , Modeling for Environment's Sake, Fifth Biennial Meeting, Ottawa, Canada, pp. 1-8.

[17] M. Marcucci, G. Nosenzo, G. Capannelli, I. Ciabatti, D. Corrieri, and G. Ciardelli, "Treatment and reuse of textile effluents based on new ultra filtration and other membrane technologies," Desalination, vol. 138, pp. 75-82, 2001.

[18] Maria Sareela, Gunnar Mogensen, Rangne Fonden, Jaana Matto, Tiina Mattila Sandholm, "Probiotic bacteria: Safety, Functional and Technological properties," Journal of Biotechnology, vol. 84, pp. 197-215, 2000.

[19] Cheng Chee Kaan, Azrina Abd Aziz, Shaliza Ibrahim, Manickam Matheswaran and Pichiah Saravanan, "Heterogeneous Photocatalytic Oxidation an Effective Tool for Wastewater Treatment – A Review," www.intechopen.com, pp. 219-232, 2011.

[20] Zongping Wang, Miaomiao Xue, Kai Huang and Zizheng Liu, "Textile Dyeing Wastewater Treatment," www.intechopen.com, pp. 91-116, 2011.

[21] Naga Deepthi Chettipalli, Phani Santosh and Prithvi Raj (2011), "Evaluation of various uses of microorganisms with emphasis on probiotics," J Microbial Biochem Technol R1:004. doi:10.4172/1948-5948.R1-004, 2011.

[22] Mohammad hossein Marhamatizadeh, Elham Ehsandoost, Paria Gholami, Hanieh Moshiri and Mina Nazemi, "Effect of Permeate on Growth and Survival of Lactobacillus acidophilus and Bifidobacterium bifidum for Production of Probiotic Nutritive Beverages," World Applied Sciences Journal, vol. 18 , no. 1, pp. 1389-1393, 2012.

[23] Muhammad Masud Aslam, Baig M. A, Ishtiaq Hassan, Ishtiaq A. Qazi, Murtaza Malik., Haroon Saeed. (2004), Textile Wastewater Characterization and Reduction of its Cod & Bod by Oxidation, M.S. Environmental Engineering. Institute of Environmental Sciences & Engineering (IESE), 2004.

[24] Y.H. Gursahani and SG. Gupta, "Decolourization of Textile effluent by a thermophilic bacteria Anoxybacillus rupiens," J Pet Environ Biotechnology, vol. 2, no. 2, pp. 1-4, 2011.

[25] C.I. Pearce, J.R. Lloyd, J.T. Guthrie, "The removal of colour from textiles wastewater using whole bacteria cells: a review," Dyes and Pigments, vol. 58, pp. 179-196, 2003.

[26] N. Murugalatha, A. Mohankumar, A. Sankaravadivoo and C. Rajesh, "Textile effluent treatment by Bacillus species isolated from processed food," African Journal of Microbiology Research, vol. 4, no. 20, pp. 2122-2126, 2010.

[27] K.G. Bhattacharyya and A. Sharma, "Adsorption characteristics of the dye, Brilliant Green, on Neem Leaf Powder," Dyes and Pigments, 57, pp. 211-222, 2003.

[28] Nazan Erdumlu, Bulent Ozipek, Goncagul Yilmaz and Ziynet Topatan, "Reuse of Effluent Water Obtained in Different Textile Finishing Processes," AUTEX Research Journal, vol. 12, no. 1, 2012.

[29] F. Ricca, "Adsorption-Desorption Phenomena," Academic Press, New York, pp. 19-32, 1972.

[30] O. Hammouda, A. Gaber, and N. Abdel-Raouf, "Microalgae and wastewater treatment," Ecotoxicol. Environ. Saf, vol. 31, pp. 205–210, 1994.