Single Cultures of *Acinetobacter sp.* and *Cellulosimicrobium sp.* Grown in Pineapple Waste: Adaption Study and Potential in Reducing COD from Real Textile Wastewater

Nor Habibah Mohd Rosli[1,*] and Wan Azlina Ahmad[2]

[1]*Faculty of Applied Sciences, Universiti Teknologi MARA, Pahang Campus, 26400 Bandar Tun Abdul Razak, Pahang, Malaysia.*
[2]*Department of Chemistry, Faculty of Science, UTM, 81310 UTM Skudai, Johor, Malaysia.*
[*]*E-mail: norha505@uitm.edu.my*

Received: 13 February 2018
Accepted: 8 June 2018

ABSTRACT

Wastewater from industrial plants such as textile, electroplating and petroleum refineries contains various substances that tend to increase the chemical oxygen demand (COD) of the wastewater. Therefore, it is desired to develop a process suitable for treating the wastewater to meet the regulatory limits. This work was conducted to investigate the potential of adapted single culture of A. baumannii, A.calcoaceticus and C.cellulans in reducing COD in real textile wastewater. The study was carried out by adapting each single culture (10% inoculums) to increasing concentration (1%, 2.5 %, 5%, 7.5 % and 10%) of textile wastewater. Then it was introduced to the textile effluent without pH adjustment for five days and the COD values were measured. The textile wastewater was supplemented with pineapple waste for bacterial growth and metabolism. Results obtained showed that pineapple waste was a good nutrient supply for the growth of the bacteria and the best concentration of textile wastewater for adaptation was at 2.5%. The results also showed that A.calcoaceticus shows highest COD reduction with 67% removal whereas A. baumannii and C.cellulans with 60% and 58% removal respectively. The outcome supported that the single culture used in this study showed considerably high reduction of COD from real textile wastewater.

Keywords: *COD reduction, textile wastewater, Acinetobacter baumannii, Acinetobacter calcoaceticus genospecies 3, Cellulosimicrobium cellulans*

INTRODUCTION

The worldwide textile consumption keeps rising along with the population growth and general increase of household purchasing power [1]. Thus the disposal and management of textile wastewater have been an issue concern globally. The stages and complicated processes involved in the textile industry produced a complex wastewater containing high concentration of organic dyes, surfactants, phosphates, nitrates and other textile additives [2-7]. Thus, a treatment system for textile wastewater based on a dissolved organic carbon or chemical oxygen demand (COD) measurement should be concerned. The aims of wastewater management strategy nowadays are more to benefit human safety, respect principles of ecology and compatible with other habitability systems [8]. Biological method in the treatment of textile wastewater meets all these requirements over other methods. Moreover, it is said to be an environmental-friendly alternative treatment because the pollutants are converted to non-toxic products such as water and carbon dioxides, easy to use and more cost effective [2,3,9].

Numbers of studies have reported the use of mix cultures as well as single culture in the treatment of textile wastewater via biological method. Strains from *Bacillus, Acinetobacter, Legionella, Staphylococcus, Pseudomonas, Commomonas* and *Sphingomonas* [10-12] are commonly reported bacteria used in biological treatment. However, they are used mostly for the removal of dyes from textile wastewater and study on the use of these strains for COD removal is rare although a few have reported these strains have potential to reduce COD in anaerobic condition [12,13]. Removal of COD by using bacteria in both aerobic and anaerobic processes requires an external carbon source for bacterial growth and metabolism [14]. An agricultural waste can serve this purpose due to its availability and most of agricultural wastes are considered as low value product [15]. In this study, the growth of single culture bacteria of *Acinetobacter baumannii, Acinetobacter calcoaceticus genospecies* 3 and *Cellulosimicrobium cellulans* in pineapple waste taken from local industry will be studied. The capability of the cultures to adapt to the real textile wastewater will be investigated prior to study their potential in reducing COD from real textile.

EXPERIMENTAL

Materials

The cultures used were Acinetobacter baumannii (A.baumannii), Acinetobacter calcoaceticus genospecies 3 (A.calcoaceticus) and Cellulosimicrobium cellulans (C.cellulans) which were locally isolated bacteria as described by Zakaria et. al [14]. The growth medium was a pineapple waste taken from food industry located in Skudai, Johor, Malaysia. Before use, the liquid waste was filtered and centrifuged (B.Braun, SIGMA 4K-15) at 9000 rpm and 4oC for 5 minutes. The textile effluent was obtained from the final discharge section of textile industry located in Senawang, Negeri Sembilan, Malaysia.

Characterization of single culture

The single active cultures (10% inoculum) were grown in a mixture of 80% of neutralized pineapple waste and 10% of textile wastewater. The mixtures were incubated at 30^{o}C for 24 hours with constant shaking at 200 rpm. The samples were complimented with cell-free control set. At various intervals, pH measurement and optical density measurement at 600 nm (OD_{600}) using spectrophotometer (Genesys 20) of the isolated bacteria were determined.

Characterization of untreated textile wastewater

The untreated textile wastewater sample was first analyzed for COD level and pH value after collection. The COD level was measured using spectrophotometer (Hach DR/4000 U) [16]. The pH value was determined using pH meter (Mettler Toledo 320).

Bacteria adaptation study

The overnight active cultures (10% inoculum) were incubated in a mixture containing 89% neutralized pineapple waste and 1% textile wastewater at 30^{o}C with constant shaking at 200 rpm. At various time intervals, the turbid sample was pipetted aseptically for OD_{600} measurements using spectrophotometer (Genesys 20).

When the culture has reached the exponential phase, the culture (10% inoculum) was transferred into mixture containing neutralized pineapple waste and textile wastewater in increasing concentration (1%, 2.5%, 5%, 7.5%, and 10%). Serial dilutions of the bacterial cultures were made and each of the respective cultures was plated onto nutrient agar plates using the spread plate technique. The plates were then incubated at 30°C for 24 hours.

COD reduction study

The experiment was conducted using bacterial cultures adapted to 2.5% textile wastewater. Mixture of adapted bacteria (10% inoculum) and 90% of textile wastewater (pH unmodified) were incubated at room temperature for 5 days with constant shaking at 200 rpm [17]. At various time intervals, COD measurements were taken using spectrophotometer (Hach DR/4000 U) [16]. The diluted samples (2.5 mL) were contacted, in a vial, with mercury sulphate, (3.5 mL) acid silver sulphate and (1.5 mL) standard potassium dichromate which was then held at 150oC for two hours. After cooling the sample was then analyzed in the spectrophotometer at 620 nm [18].

RESULTS AND DISCUSSION

Characterization of single culture

In this experiment, pineapple waste was used as an alternative growth medium due to its availability and abundances in Skudai, Johor. The agricultural waste provides nutrients for biomass maintenance and to enhance biodegradation [13]. Furthermore, pineapple waste is a good carbon source especially for *Acinetobacter sp*. [14]. For the growth profiles of each single culture, samples were monitored at various intervals of the incubation period. Then a graph of optical density measured at 600 nm (OD_{600}) against time was plotted to obtain the growth curve of the bacteria as shown in Figure 1. The growth of *A.baumannii*, *A.calcoaceticus* and *C.cellulans* showed a typical profile consisting of the lag, exponential and stationary phases [19].

This outcome suggested that the pineapple waste provides nutrients for the growth and resilience [20] of the cultures. pH measurement was also taken to characterize the bacteria as shown in Figure 2. In a batch culture, pH can change during growth as a result of metabolic reactions that consume or produce acidic or basic substances as wastes [21]. At the beginning of the growth, all three single cultures showed a decrease in pH value from neutral to acidic environment. This might be due to the production of acetic acid, lactic acid and ethanol from the pineapple waste [21,22]. After 8 hours, the pH value of each single culture remained stationary with an average pH in the range of 4.19-5.15. The pineapple waste was acidic with pH 4.23. It was reported that optimum pH range for bacteria is at pH between 5.0 and 7.5 [23]. Therefore, the pineapple waste was neutralized upon using.

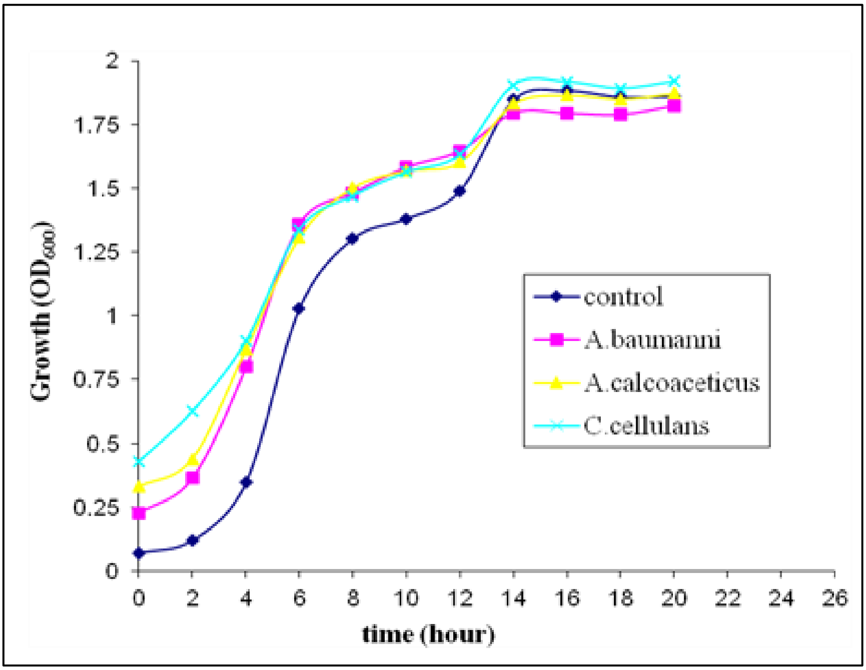

Figure 1: Growth profile of *A.baumannii*, *A.calcoaceticus* and *C.cellulans* as single culture.

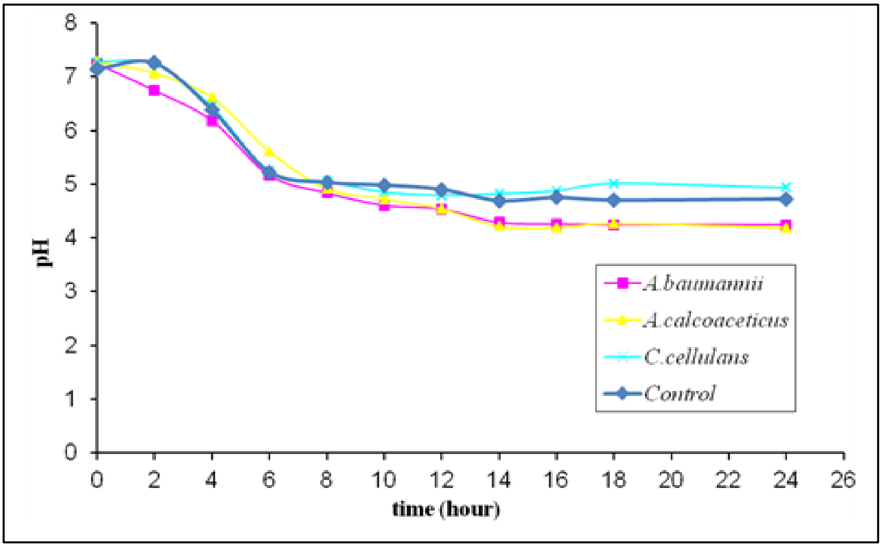

Figure 2: pH profile of *A.baumanni, A.calcoaceticus* and *C.cellulans.*

Characterization of untreated textile wastewater

Real textile wastewater used in this study was taken in Senawang, Negeri Sembilan. Table 1 represents the result obtained for measurements of COD level and pH value. As shown in Table 1, the untreated effluent show alkaline character with pH value of 9.38. The pH of textile wastewater varies depending on the particular dye process going on within the industry. It may be highly alkaline, neutral or acidic depending on the nature of the salts and dyes [3]. As stated in Malaysia Environmental Quality (Sewage and Industrial Effluents) Regulations, 1979, 1999, 2000, the permitted limit of pH value and COD level for standard A effluent were 5.5-9.0 and 100 mg/L respectively [24]. The measured COD value for the untreated textile wastewater was 600 ml/L which exceeded the permitted level. However, the textile wastewater used in this study was classified as low strength wastewaters because the COD concentration was less than 1000 mg/L [25]. Therefore, aerobic treatment process is an appropriate treatment mode to be used in this study because anerobic process is suitable for the treatment of high strength wastewaters (biodegradable COD concentrations over 4000 mg/L) [26]. Furthermore, bacteria used in this study, *A.baumannii* and *A.calcoaceticus*, are aerobic bacteria except *C.cellulans*, an anerobic bacteria.

Table 1: Characteristics of textile wastewater.

Parameter	Unit
pH	9.38
COD	600 mg/L

Bacteria adaptation study

The experiment was conducted prior to the concern of the survival of *A.baumannii*, *A.calcoaceticus* and *C.cellulans* in textile wastewater because only several strains can endure the extreme condition of textile effluent [13]. Upon adaptation, plate count was performed on the nutrient agar plates that had been spread with the samples at various textile wastewater concentrations. All samples were done in duplicate and the results are reported as an average value. The indigenous bacteria from the pineapple waste were observed at 1% and 2.5% textile wastewater while at higher concentration the presence of indigenous bacteria was not observed. This indicated that the indigenous bacteria did not multiply by increasing concentration of textile wastewater [27]. From the plate count as shown in table 2, the highest colony forming unit (CFU) for *A.baumannii* was at 2.5% with 1.0 x 10^9 per mL. *A.calcoaceticus* and *C.cellulans* were also showed the highest CFU at 2.5% with 2.1 x 10^9 per mL and 1.5 x 10^9 per mL respectively. These indicated that the best condition for adaptation was at 2.5% of textile wastewater.

Table 2: Total number of *A.baumannii, A.calcoaceticus* and *C.cellulans* colonies with different concentration of textile wastewater.

Textile wastewater concentration (%)	*A.baumannii* (10^4)/mL	*A.calcoaceticus* (10^4)/mL	*C.cellulans* (10^4)/mL
1.0	200	13000	14000
2.5	10000	21000	15000
5.0	4	22	2300
7.5	1500	23	80
10.0	91	54	63

Based on the results from the plate count in table 2, a graph for screening the best tolerance level based on CFU was plotted as shown in Figure 3. The graph was obtained by plotting OD_{600} against respective concentrations of textile wastewater. Results obtained supported that the best condition for adaptation was at 2.5% textile wastewater. Results obtained also show that the cultures used in this study can survive in the real textile wastewater because even in a low concentration of nutrient, it was sufficient to support the multiplication of bacteria [28].

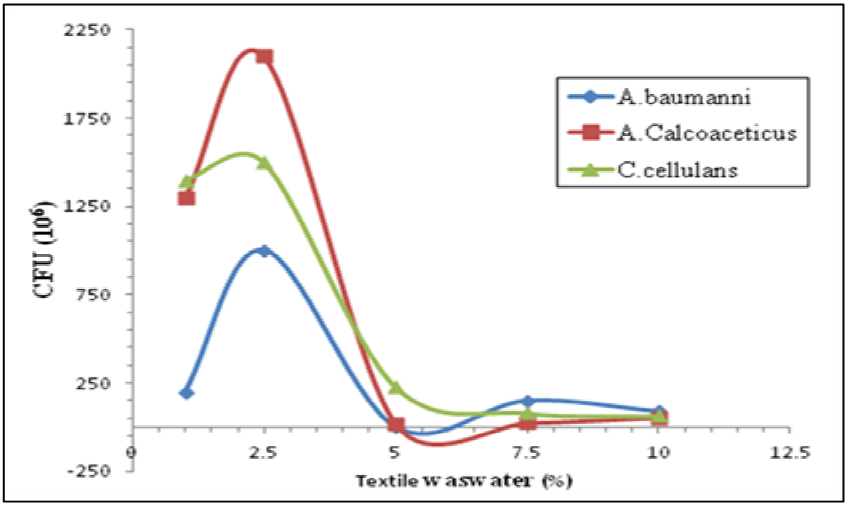

Figure 3: Bacterial tolerance level based on viable cell count.

COD reduction study

Results obtained from bacteria adaptation study were used for COD reduction study. The adapted single culture was introduced to the textile effluent without pH adjustment, and it was conducted under non-sterile condition because textile wastewater can be treated biologically without primary treatment [29]. This is also studied to evaluate whether the bacteria used were able to compete for carbon sources and nutrients with other microorganism [30]. Figure 4 shows the result of COD reduction for the adapted single cultures. A.baumannii, A.calcoaceticus and C.cellulans which were locally isolated microorganisms showed excellent COD removal capabilities where the reduction pattern was increasing during the treatment process. This is because the strains can multiply further during growth [3].

After five days of the treatment process, reduction of COD by A.baumannii, A.calcoaceticus and C.cellulans are more than 50% which shows that adapted bacteria are capable in reducing pollutants more than non-adapted bacteria [13]. The result obtained suggested A.calcoaceticus showed the highest reduction of COD with 67% removal. This is because A.calcoaceticus is a resilient bacterium with a diverse natural habitat [31] therefore it can adapt to the changes of its surrounding. Furthermore, from the adaptation study which was described previously, A. calcoaceticus showed highest survival rate with 2.1 x 109 per mL based on CFU. Therefore, perhaps its survival rate in the real textile effluent is higher than the other two strains. Meanwhile, A. baumanii also demonstrated efficient COD removal with 60% reduction and this might be due to a large metabolic activity of this strain [32] which allows it to grow, reproduce and respond to its environment. An anerobic bacterium C.cellulans also showed considerably high COD removal capability with 58% reduction because C.cellulans adapt themselves to textile wastewater which then convert several organic load into less hazardous forms [2,9]. Results obtained from this study showed that the adapted single cultures used are capable in reducing COD from real textile effluent and supporting that biological treatment is efficient at mineralizing the organic load in wastewater [33].

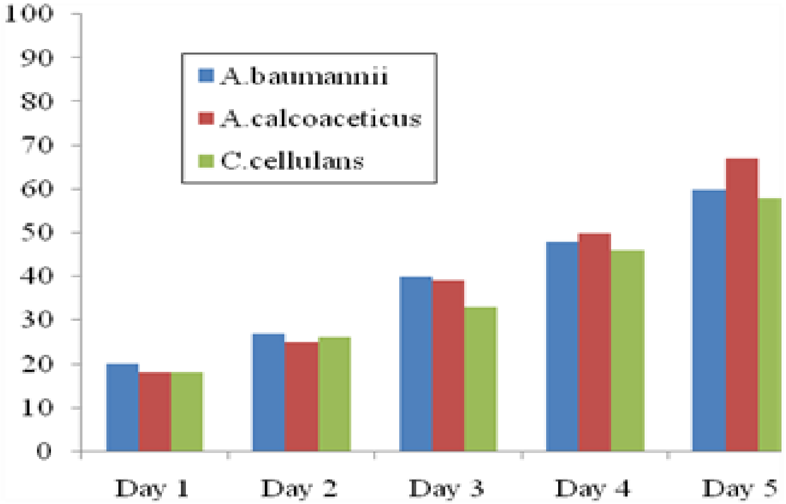

Figure 4: Percentage of COD reduction from textile wastewater using A.baumanni, A.calcoaceticus and C.cellulans as adapted single culture.

CONCLUSION

Acinetobacter baumannii, Acinetobacter calcoaceticus genospecies 3 and Cellulosimicrobium cellulans show typical growth profiles when supplemented with pineapple waste as nutrient. Each single culture was adapted to textile wastewater prior to use to give faster treatment time. The maximal tolerance level was at 2.5% of textile wastewater. An efficient COD reduction was observed in this study, where Acinetobacter calcoaceticus genospecies 3 exhibited highest COD removal with 67% reduction compared to Acinetobacter baumannii and Cellulosimicrobium cellulans with 60% and 58% removal respectively. Nevertheless, the removal efficiency is influenced by the ratio of organic load and the microorganism load, operation temperature, and the oxygen concentration in the system [27]. Thus further study on how these factors affect the percentage of COD removal in textile waste water should be investigated.

ACKNOWLEDGEMENT

Authors would like to acknowledge Department of Science, Universiti Teknologi Malaysia for providing chemicals and apparatus and also to sponsor Yayasan Pahang, for providing essential financial support.

REFERENCES

[1] Y. Hu, C. Du, S.Y. Leu, H. Jing, X. Li, and C.S.K. Li, 2018. Valorisation of Textile Waste by Fungal Solid State Fermentation: An Example of Circular Waste-Based Biorefinery. *Resour. Conserv. Recy.*, vol. 129, pp. 27-35.

[2] C.R. Holkar, A.J. Jadhav, D.V. Pinjari, N.M. Mahamuni, and A.B. Pandit, 2016. A Critical Review on Textile Wastewater Treatments: Possible Approaches. *J. Environ. Manage.*, vol. 182, pp. 351-366.

[3] M. Imran, D.E. Crowley, A. Khalid, S. Hussain, M.W. Mumtaz, and M. Arshad, 2015. Microbial Biotechnology for Decolorization of Textile Wastewaters. *Rev. Environ. Sci. Biotech.*, vol. 14, pp. 73-92.

[4] Z. Dhaouefia, A.T. Cervantes, D. García, A. Bedoui, K. Ghedira, L.C. Ghedira, and R. Muñoza, 2018. Assessing Textile Wastewater Treatment in an Anoxic-Aerobic Photobioreactor and the Potential of the Treated Water for Irrigation. *Algal Res.*, vol. 29, pp. 170-178.

[5] E. Ellouze, N. Tahri, and R.B. Amar, 2012. Enhancement of Textile Wastewater Treatment Process Using Nanofiltration. *Desalination*, vol. 286, pp. 16-23.

[6] K. Pazdzior, J. Wrebiak, A.K. Smolka, M. Gmurek, L. Bilinska, L. Kos, J.S. Ledakowicz, and S. Ledakowicz, 2017. Influence of Ozonation and Biodegradation on Toxicity of Industrial Textile Wastewater. *J. Environ. Manage.*, vol. 195, pp. 166-173.

[7] T. Fazal, A. Mushtaq, F. Rehman, A.U. Khana, N. Rashid, W. Farooq, M.S.U. Rehmana, and X. Jian, 2018. Bioremediation of Textile Wastewater and Successive Biodiesel Production Using Microalgae. *Renew. Sust. Energ. Rev.*, vol. 82, pp. 3107-3126.

[8] V.K. Ilyin, I.A. Smirnov, P.E. Soldatov, I.N. Korniushenkova, A.S. Grinin, I.N. Lykov, and S.A. Safronova, 2004. Microbial Utilisation of Natural Organic Wastes. *Acta Astronautica*, vol. 54, pp 357-361.

[9] M.E. Karim, K. Dhar, and M.T. Hossain, 2018. Article in press, Journal of Genetic Engineering and Biotechnology.

[10] C.I. Pearce, J.R. Lloyd, and J.T. Guthrie, 2003. The Removal of Colour from Textile Wastewater Using Whole Bacterial Cells: A Review. *Dyes Pigments*, vol. 58, pp. 179-196.

[11] T. Robinson, G. McMullan, R. Marchant, and P. Nigam, 2001. Remediation of Dyes in Textile Effluent: A Critical Review on Current Treatment Technologies with a Proposed Alternative. *Bioresour. Technol.*, vol. 77, pp. 247-255.

[12] I.K. Kapdan, and S. Alparslan, 2005. Application of Anaerobic–Aerobic Sequential Treatment System to Real Textile Wastewater for Color and COD Removal. *Enzyme Microb. Technol.*, vol. 36, pp. 273-279.

[13] O.D. Olukanni, A.A. Osuntoki, and G.O. Gbenle, 2006. Textile Effluent Biodegradation Potentials of Textile Effluent-Adapted and Non-Adapted Bacteria. *Afr. J. Biotechnol.*, vol. 5, pp. 1980-1984.

[14] Z.A. Zakaria, Z. Zakaria, S. Surif, and W.A. Ahmad, 2006. Bioremediation of Cr (vi)-containing electroplating wastewater using Acinetobacter sp., In proceeding of International Conference on Environment, Penang, Malaysia.

[15] Z. Aksu, and I.A. Isoglu, 2006. Use of Agricultural Waste Sugar Beet Pulp for the Removal of Gemazol Turquoise Blue-G Reactive Dye from Aqueous Solution. *J. Hazardous Mater.*, B137, pp. 418-430.

[16] D. Georgiou, J. Hatiras, and A. Aivasidis, 2005. Microbial Immobilization in a Two-Stage Fixed-Bed-Reactor Pilot Plant for On-Site Anaerobic Decolorization of Textile Wastewater. *Enzyme Microb. Technol.*, vol. 37, pp. 597-605.

[17] S. Kim, C. Park, T.H. Kim, J.W. Lee, and S.W. Kim, 2003. COD Reduction and Decolorization of Textile Effluent Using a Combined Process. *J. Biosci. Bioeng.,* vol. 95, pp. 102-105.

[18] A.T. Ajao, G.B. Adebayo, and S.E. Yakubu, 2011. Bioremediation of Textile Industrial Effluent Using Mixed Culture of Pseudomonas Aeruginosa and Bacillus subtilis Immobilized on Agaragar In a Bioreactor. *J. Microb. Biotechol. Res*., vol. 1, pp 50-56.

[19] J.P. Harley and L.M. Prescott, 1990. Wm. C. Brow Publisher, Lab Exercise in Microbiology USA.

[20] J. Bhattacharya, S. Dev, and B. Das, 2018: Book chapter in Low Cost Wastewater Bioremediation Technology, Chapter 2 43-75.

[21] M.T. Madigan, J.M. Martinko. and P.J. Brock, 2000.New Jersey: Prentice Hall International, Inc. Biology of Microorganism.

[22] A.H. Jawad, A.F.M. Alkarkhi, O.C. Jason, A.M. Easa, and N.N.A. Nik, 2013. Production of the Lactic Acid from Mango Peel Waste – Factorial Experiment. *J. King Saud Uni.-Sci.,* vol. 25, pp. 39-45.

[23] K. Inoue, 2002. Kanebo Spring Corporation, Textile Dyeing Wastewater Treatment Activated Sludge Treatment Japan.

[24] Malaysia Environmental Quality (Sewage And Industrial Eftluents) Regulations, 1979, 1999, 2000, Page 120. Retrieved Form http://water-treatment.com.cn/resources/discharge-standards/malaysia.htm

[25] A. Torabian, S.M. Abtahi, M.M. Amin, and S.A. Momeni, 2010. Treatment of Low-Strength Industrial Wastewater Using Anaerobic Baffled Reactor. *J. Environ. Health Sci. Eng*., vol. 7, pp. 229-240.

[26] Y.J. Chan, M.F. Chong, C.L. Law, and D.G. Hassell, 2009. A Review on Anaerobic–Aerobic Treatment of Industrial and Municipal Wastewater. *Chem. Eng. J.*, vol. 155, pp. 1-18.

[27] B. Ma, L. Yang, Q. Wang, Z. Yuan, Y. Wang, and Y. Peng, 2017. Inactivation and Adaptation of Ammonia-Oxidizing Bacteria and Nitrite-Oxidizing Bacteria When Exposed To Free Nitrous Acid. *Bioresour. Technol.*, vol. 245, pp. 1266-1270.

[28] J. Hrenovic, I.G. Barisic, S. Kazazic, A. Kovacic, M. Ganjto, and M. Tonkic, 2016, Carbapenem-resistant isolates of Acinetobacter baumannii in a municipal wastewater treatment plant, Croatia, 2014. Euro Surveill. 21.

[29] L.N.A. Ghunmia, and A.I. Jamrah, 2006. Biological Treatment of Textile Wastewater Using Sequencing Batch Reactor Technology. *Environ. Model. Assess.*, vol. 11, pp. 333-343.

[30] F. Spina, A. Anastasi, V. Prigione, V. Tigini, and G.C. Varese, 2012. Biological Treatment of Industrial Wastewaters: A Fungal Approach. *Chem. Eng. Transactions*, vol. 27, pp. 175-180.

[31] D.B. Blossom, and S. Arjun, 2008. Drug-Resistant Scinetobacter Baumannii-Calcoaceticus Complex: An Emerging Nosocomial Pathogen with Few Treatment Options. *Infect. Dis. Clin. Pract.*, vol. 16, pp. 1-3.

[32] S. Constantiniu, A. Romaniuc, L.S. Iancu, R. Filimon, and L. Taraşi, 2004. Cultural and Biochemical Characteristics of *Acinetobacter Spp.* Strains Isolated from Hospital Units. *J. Prev. Med.*, vol. 12, pp. 35-42.

[33] C.Z.A. Abidin, S.A. Fahmi, S.N.N.Ong, Mohd Makhtar, N.R. Rahmat, and R. Ahmad, 2016. Decolourization and COD Reduction of Textile Wastewater by Ozonation in Combination with Biological Treatment. *Int. J. Auto. Mech. Eng.*, vol. 13, 3141-3149.