UNIVERSITI PUTRA MALAYSIA

CHEMICAL OXYGEN DEMAND REMOVAL USING ARTIFICIALLY CONSTRUCTED BIOFILM BY DIELECTROPHORESIS ON WIRECLOTH ELECTRODE

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By

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Chairman: Associate Prof. Zurina Zainal Abidin, PhD

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Artificial structured biofilm constructed (ASB) by dielectrophoresis (DEP) principle using wirecloth electrode was explored for its potential in wastewater treatment. The wirecloth produced by adopting textile technology, utilised 100 µm stainless steel wire and 83 decitex polyester yarns. The wirecloth is highly flexible and able to be fixed in most treatment system. The ASB was then immobilized using polyethylenimine (PEI) solution. The biofilm formation time was greatly shortened as the DEP attraction can result in an effective immobilization process. *Micrococcus sp.*, *Rhodococcus sp. and Bacillus sp.* isolated from pharmaceutical wastewater was used. A lab scale reactor was used to determine the optimum conditions and the ability of DEP-constructed biofilm in wastewater treatment. The experiment parameters included wirecloth surface area, pH, temperature and HRT. 2 types of medium; low strength and high strength synthetic wastewater were used in the experiments. The treatment process showed a good potential in treating both type of synthetic wastewater where almost 90% COD reduction was achieved at the optimum conditions for each parameter. The larger the wirecloth surface area used the better the COD reduction can be achieved. The restriction may only cause by insufficient of nutrient in a given medium's volume. The performance of the artificial biofilm was dependent on the pH value. The optimum pH value for both mediums was found to be pH 8. Lower pH and higher pH value tend to inhibit the activity of microorganisms and decrease the COD reduction to 62.4% in low strength and 75.1% in high strength synthetic wastewater, respectively. The best temperature chosen was 40 ℃. This is due to higher COD removal rate achieved and the COD reduction was nearly 90% for both mediums. The suitable HRT for low strength synthetic wastewater are around 1 to 3 days and the HRT for high strength synthetic wastewater was 4 to 7 days. As the COD value was higher, shorter retention time will result in an incomplete treatment where COD reduction was just 56.3%. The built up of artificial biofilm (increase of active biomass) can eventually shorten the treatment time which shown in the experiment results of HRT. ESEM and SEM images revealed the surface morphology

of ASB. The layers of EPS proved to be interconnected and support the nutrient diffussion and the build up of microcolonies for a better COD removal.

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PENGYINGKIRAN PERMINTAAN OKSIGEN KIMIA (COD) DENGAN MENGGUNAKAN KONSTRUKSI BIOFILEM BUATAN YANG DIBINA DARIPADA PROSES DEP PADA TENUNAN KAIN WAYAR

Oleh

WAI YORK CHOW

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Biofilem berstruktur tiruan (ASB) dibina dengan prinsip dielectrophoresis (DEP) dengan menggunakan kain tenunan wayar telah diterokai untuk mengetahui potensinya dalam rawatan air sisa. Cara in dapat dicapai dengan menyekat gerakan mikroorganisma tertentu pada kain tenunan wayar dan memulakan proses pembentukan biofilem buatan. Kain tenunan wayar ini dihasilkan oleh teknologi tekstil dengan menggunakan wayar berdiameter 100 µm dan benang poliester 83 desiteks. Kain tenunan wayar ini sangat fleksibel dan dapat digunakan dalam kebanyakan sistem rawatan. Seterusnya, penyekatan gerakan mikroorganisma dapat dihasil dengan menggunakan polyethylenimine (PEI). Masa pembentukan biofilem dapat dikurangkan dengan ketara kerana tarikan DEP dapat menghasilkan proses penyekatan yang lebih sempurna. Mikroorganisma yang tumbuh dalam air sisa farmaseutikal telah diasingkan dengan kaedah mengkultur dan dikenal pasti sebagai *Micrococcus sp., Rhodococcus sp. dan Bacillus sp.* Sebuah reactor yang berskala makmal telah dibina untuk menentukan keadaan yang optima dan keupayaan biofilem konstruksi-DEP semasa rawatan. Parameter eksperimen termasuk luas kawasan permukaan, pH, suhu dan HRT. Perantara yang digunakan dalam eksperimen ini ialah sisa kumbahan sintetik kekuatan tinggi dan kekuatan rendah. Proses rawatan menunjukkan potensi yang baik semasa merawat kedua-dua jenis air sisa sintetik di mana hampir 90% pengurangan COD dapat dicapai bagi keadaan-keadaan optimum dalam setiap parameter. Kawasan permukaan kain tenunan wayar yang lebih besar adalah lebih berkesan dalam rawatan air sisa sintetik. Sebab ini adalah tidak benar apabila tiada nutrien yang mencukupi dalam air sisa sintetik tersebut. Nilai pH yang optima bagi kedua-dua medium adalah pH 8. pH yang rendah dan pH yang tinggi dapat menghalang aktiviti mikroorganisma dan mengurangkan COD kepada 62.4% dalam air sisa sintetik berkekuatan rendah dan 75.1% dalam air sintetik berkekuatan tinggi. Suhu terbaik yang dipilih adalah 40 ℃. Ini adalah kerana kadar keturunan COD yang lebih tinggi dan pengurangan COD dicapai pada hampir 90% bagi kedua-dua medium. Dalam parameter HRT, masa yang sesuai untuk air sisa sintetik

berkekuatan rendah adalah sekitar 1 hingga 3 hari, dan HRT untuk air sisa sintetik berkekuatan tinggi adalah 4 hingga 7 hari. COD yang tinggi dan masa penahanan yang pendek akan menyebabkan rawatan yang tidak lengkap di mana hanya 56.3% COD dapat disinkirkan. Pembinaan biofilem tiruan (peningkatan biomas aktif) boleh memendekkan masa rawatan seperti mana yang ditunjukkan dalam keputusan eksperimen HRT. Imej daripada ESEM dan SEM mendedahkan morfologi permukaan biofilm buatan dengan DEP proses. Lapisan EPS adalah saling bergabung dan dapat menyokong diffussi nutrien dan membina microcolonies dengan lebih baik serta meningkakan kecekapan rawatan air sisa.

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I certify that a Thesis Examination Committee has met on [date] to conduct the final examination of Wai York Chow on his thesis entitled “Chemical Oxygen Demand Removal Using Artificially Constructed Biofilm by Dielectrophoresis on Wirecloth Electrode” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the degree of Master of Science.

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LIST OF ABBREVIATIONS

ω	Angular Frequency
ΔE^2	Square of the Electric Field Gradient
ASB	Artificial Structured Biofilm
ASMC	Artificial Structured Microbial Consortia
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
CSLM	Confocal Scanning Laser Microscopy
DEP	Dielectrophoresis
EPS	*Extracellular polymeric substances*
ESEM	Environment Scanning Electron Microscope
ε	Permittivity
ε_m	Medium’s conductivity
ε_o	Free Space Permittivity
FEMLAB	Comsol Multiphysics
GDD	Gaseous Detection Device
HRT	Hydraulic Retention Time
OD	Optical Density
ORP	Oxidation Reduction Potential
PEI	Polyethylenimine
pk-pk	Peak to Peak
r	Radius
$R_e(f_{cm})$	Clausius-Mossotti Factor
r_o	Radius Vector
SA	Surface Area
SEM	Scanning Electron Microscope

SS	Suspended Solids
TDS	Total Dissolved Substance
TOC	Total Organic Carbon
TSS	Total Suspended Solid
V	Potential
VOC	Volatile Organic Compounds
VSS	Volatile Suspended Substance
α	Particle's Efective Polarisability

CHAPTER 1

INTRODUCTION

1.1 Background

Water is one of the most important elements to support almost all of the living creatures, hence, wastewater treatment has become a crucial topic which gains attention from human beings. Water contamination has become a major issue in the global context as a result of globalization, population growth, urbanization, industrialization and more extravagant life.

Due to increasing industrial activity from all over the world, discharging of untreated wastewater has become a significant issue. The wastewater effluent can pollute surface, and underground water and soil. Hence, the discharging of produced water on environment has become a crucial challenge that need to be solved immediately. The impacts of wastewater need to be reduced and this results in a more stringent regulatory standard for discharging wastewater. In Malaysia, national water quality standards can be divided to 5 classes and the value of some parameters such as COD, BOD, pH, TDS are strictly controlled before the wastewater can be discharged into the river (Environmental Quality Reports, 2011). For example, discharging limit of COD and BOD in class IIA are 25 and 3 mg/L.

Environmental microbiology can be related to the studies of municipal waste treatment and waste degradation and during the last ten years biofilms have become an important subject in microbiological inquiry. This can be considered as a critical element in the preservation of water quality systems as well as a key component of biological reactions in wastewater treatment (Flemming et al., 2000). Many natural and engineered systems are influenced by biofilms such as microorganisms firmly attached to surfaces (Lewandowski and Beyenal, 2007). The effects of biofilms may be vary from desirable, through undesirable, even to a bad situation, depending on the specific locations where the biofilms are deposited (International Water Assn, 2008).

One of the significant elements in biofilms is naturally occurred microbial consortia where it is used widely in industry, for example in wastewater treatment, bioremediation, metal leaching, silage production and various food fermentations (Rebac et al., 1995; James, 1993). Biofilm treatment process can act as a safer biological control method when we compare to a chemically modification method (Woolard, 1997). Microbial consortia in nature tend to exist in highly organized macroscopic structures with extensive internal organization where the macroscopic structuring also provides protection against exogenous toxic substrates (Alp et al., 2002).

To treat wastewater using biofilms, different methods have been studied including aerobic submerged biofilm (González-Martínez and Duque-Luciano, 1992), multistage biofilm reactors (Ghaniyari-Benis et al., 2009), immobilization of bacteria (Li et al., 2013), and biofilm hybrid system (Qi et al., 2007). Most of the biofilm formations are time dependent and require a suitable amount of nutrients to initiate the growth. However, a better and more efficient wastewater treatment method should be developed in order to turn those disadvantages into benefits.

1.2 Problem statement

Severe waterborne diseases such as cholera and typhoid fever were discovered and spread through contaminated water (Yates et al., 2004). This posed a threat to human beings which greatly increased the environmental attention and resulted in the construction of sewer system as well as the needs of wastewater treatment. The emerging knowledge of wastewater treatment resulted in the development of many new treatment methods include anaerobic digestion, trickling filter, biological treatment and etc. (Metcalf et al., 1979). Despite recent studies advance in wastewater treatment, the development of new and better method is still a big concern. In this study, a new method of artificial constructed biofilm using DEP is able to open a new chapter in wastewater treatment process.

In previous studies, some problems occurred while using DEP processes such as the difficulties in producing large surface area microelectrodes and the electrodes are less flexible to fix in selected treatment system. From the development of a novel microelectrode by using weaving technology (Abidin et al., 2007), the application of DEP can be further developed such as the construction of biofilm in wastewater treatment. Biofilm is a biological treatment method which is environmental friendly and efficient. This method can significantly reduce the chemical usage that needed by other wastewater treatment methods.

Time is an important parameter in biological fix-film treatment system as natural biofilm takes time to develop; i.e. a few weeks or even months need to be used to develop biofilm which also means a higher preparation cost. However, the construction of biofilm using DEP technique can provide a better and faster way of biofilm formation which significantly reduces the waiting time of natural biofilm formation. This is because the DEP technique can efficiently attract the microorganisms on the wirecloth electrode and form an effective bonding after the immobilization process.

The flexibility of biofilm's attached medium is always a great concern as the treatment tank varies in different sizes. Wirecloth constructed using weaving technology enables one to fold it into various type of shape in order to fix in required pattern. The additional advantage of using wirecloth is that it provides a larger surface area which also theoretically means a higher active biomass generation in biofilm construction. During wastewater treatment, greater treatment efficiency can be achieved if the active biomass (biofilm) is higher. The flexibility is due to the elasticity of polyester yarns and the larger surface area is generated by using weaved microelectrodes.

In order to treat wastewater, the removal of organic and inorganic constituents is needed. Suitable parameter should be chosen in order to determine the wastewater treatment efficiency. The value of COD and BOD has been used as an important indicator to show the evidence for biodegradability. There are several factors that limit the biodegradation process, including nutrient availability and chemical composition of selected wastewater (Metcalf et al., 1979). Biodegradation patterns can fluctuate over the long term. Sometimes the value of COD and BOD might fall within certain limits, then suddenly spike up again (Leeson and Hinchee, 1997).

Due to the rising concern about environmental issues, stringent effluent limitations have been set up to control wastewater treatment plants. Thus, finding a new environmental friendly in a most effective and less time consuming way which can treat wastewater efficiently is crucial in this research.

1.3 Objectives of the Study

The main goal of this research is to use wirecloth as a matrix to form artificial structured biofilm (ASB) using dielectrophoresis (DEP) technique and utilized this ASB for wastewater treatment. Specific objectives of the research are listed as below:

a. To construct and morphologically characterize artificial constructed biofilm on wirecloth electrode using DEP and also immobilization.
b. To investigate and evaluate the efficiency of the immobilized constructed biofilm for COD removal of the synthetic wastewater based on different operating parameters, such as surface area of wirecloth, pH, temperature and HRT.

1.4 Scope of the study

The fabrication of wirecloth electrode was done by research teammate by using weaving machine at Swiftech Sdn. Bhd. The wirecloth was weaved using polyester yarn and stainless steel wire in plain weave pattern. The diameter of stainless steel wires were 100 µm and the diameter of polyester yarn was 83 decitex.

The selected microorganisms were separated by culture method and the microorganisms were cultured by incubating in an incubator shaker and separated using centrifugation method. Further details and method were discussed in chapter 3.

The synthetic wastewater for wastewater treatment was prepared freshly when needed. Two types of synthetic wastewater are prepared which are low strength and high strength synthetic wastewater. Whereas for artificial bioflm construction, the selected microorganisms were separated out from inoculum, mixed together and ready to use on following process. The wirecloth electrode was connected by a frequency generator and the mixed microorganisms were introduced on the surface of wirecloth. Once the frequency generator was turned on, the microorganisms were be attracted towards the wirecloth by DEP process, and forming artificial consortia which later developed into an artificial biofilm.

During the immobilizing process, the attracted microorganisms were immobilized on wirecloth by using polyethylenimine (PEI). After the process was completed, the wirecloth was ready to use for wastewater treatment where the artificial biofilms develop on the wirecloth in the wastewater.

Performance and evaluation of the constructed biofilm were based on the initial and final values of COD that were calculated in order to observe the treatment efficiency. The selected parameters were, surface area, pH, temperature and HRT. After the treatment ended, the biofilm was send to Institute of Bioscience to obtain the image of ESEM and SEM.

REFERENCES

Abdullah, A. R. (1995). Environmental pollution in Malaysia: trends and prospects. *TrAC Trends in Analytical Chemistry*. 14(5): 191-198.

Abidin, Z. Z., Downes, L., & Markx, G. H. (2007a). Large scale dielectrophoretic construction of biofilms using textile technology. *Biotechnology and Bioengineering*. 96(6): 1222-1225.

Abidin, Z. Z., Downes, L., & Markx, G. H. (2007b). Novel electrode structures for large scale dielectrophoretic separations based on textile technology. *Journal of Biotechnology* 130(2): 183-187.

Abidin, Z. Z., & Markx, G. H. (2005). High-gradient electric field system for the dielectrophoretic separation of cells. *Journal of Electrostatics*. 63(6–10): 823-830.

Alimahmoodi, M., Yerushalmi, L., & Mulligan, C. N. (2012). Development of biofilm on geotextile in a new multi-zone wastewater treatment system for simultaneous removal of COD, nitrogen and phosphorus. *Bioresource Technology*. 107(0): 78-86.

Alp, B., Andrews, J. S., Mason, V. P., Thompson, I. P., Wolowacz, R., & Markx, G. H. (2003). Building structured biomaterials using AC electrokinetics. *Engineering in Medicine and Biology Magazine, IEEE*. 22(6): 91-97.

Alp, B., Stephens, G. M., & Markx, G. H. (2002). Formation of artificial, structured microbial consortia (ASMC) by dielectrophoresis. *Enzyme and Microbial Technology*. 31(1–2): 35-43.

Andrews, J. S., Mason, V. P., Thompson, I. P., Stephens, G. M., & Markx, G. H. (2006). Construction of artificially structured microbial consortia (ASMC) using dielectrophoresis: Examining bacterial interactions via metabolic intermediates within environmental biofilms. *Journal of Microbiological Methods*. 64(1): 96-106.

Appels, L., Baeyens, J., Degrève, J., & Dewil, R. (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science*. 34(6): 755-781.

Bai, W., Zhao, K. S., & Asami, K. (2006). Dielectric properties of E. coli cell as simulated by the three-shell spheroidal model. *Biophysical Chemistry*. 122(2): 136-142.

Banks, M. K., & Bryers, J. D. (1991). Bacterial species dominance within a binary culture biofilm. *Applied and Environmental Microbiology*. 57(7): 1974-1979.

Benoit, S., Taouji, S. d., Benachour, A., & Hartke, A. (2000). Resistance of Rhodococcus equi to acid pH. *International Journal of Food Microbiology*. 55(1–3): 295-298.

Biological Wastewater Treatment: Principles, Modelling and Design. International Water Assn, 2008.

Borja, R., & Banks, C. J. (1995). Response of an anaerobic fluidized bed reactor treating ice-cream wastewater to organic, hydraulic, temperature and pH shocks. *Journal of Biotechnology*. 39(3): 251-259.

Brodelius, P., & Mosbach, K. (1987). Overview. In M. Klaus (Ed.), *Methods in enzymology* (pp. 173-175). Academic Press.

Bryers, J., & Characklis, W. (1981). Early fouling biofilm formation in a turbulent flow system: overall kinetics. *Water Research*. 15(4): 483-491.

C.R, Woolard (1997). The advantages of periodically operated biofilm reactors for the treatment of highly variable wastewater. *Water Science and Technology*. 35(1): 199-206.

Cairncross, S., & Feachem, R. G. (1993). *Environmental health engineering in the tropics: an introductory text*: J. Wiley.

Caldwell, D. E., Korber, D. R., & Lawrence, J. R. (1992). Confocal laser microscopy and digital image analysis in microbial ecology. *Advances in microbial ecology* (pp. 1-67). Springer.

Cánovas, M., García-Cases, L., & Iborra, J. (1998). Limonin consumption at acidic pH values and absence of aeration by Rhodococcus fascians cells in batch and immobilized continuous systems. *Enzyme and Microbial Technology*. 22(2): 111-116.

Carpenter, W. L., Vamvakias, J. G., & Gellman, I. (1968). Temperature relationships in aerobic treatment and disposal of pulp and paper wastes. *Journal (Water Pollution Control Federation)*. 733-740.

Castillo, A., Cecchi, F., & Mata-Alvarez, J. (1997). A combined anaerobic-aerobic system to treat domestic sewage in coastal areas. *Water Research*. 31(12): 3057-3063.

Chan, Y. J., Chong, M. F., Law, C. L., & Hassell, D. G. (2009). A review on anaerobic–aerobic treatment of industrial and municipal wastewater. *Chemical Engineering Journal*. 155(1–2): 1-18.

Chang, H., & Rittmann, B. (1986). Biofilm loss during sample preparation for scanning electron microscopy. *Water Research*. 20(11): 1451-1456.

Cheetham, P., & Bucke, C. (1984). Microbiological Methods For Environmental Biotechnology. *Microbiological methods for environmental biotechnology*. 19: 219.

Chen, H., Liu, Y., Zhang, H., Yu, L., Zhu, Y., & Li, D. (2010). Separation and Manipulation of Rare-earth Oxide Particles by Dielectrophoresis. *Chinese Journal of Chemical Engineering*. 18(6): 1034-1037.

Chen, K.-C., Lee, S.-C., Chin, S.-C., & Houng, J.-Y. (1998). Simultaneous carbon-nitrogen removal in wastewater using phosphorylated PVA-immobilized microorganisms. *Enzyme and Microbial Technology*. 23(5): 311-320.

Chua, H., Hu, W., Yu, P., & Cheung, M. (1997). Responses of an anaerobic fixed-film reactor to hydraulic shock loadings. *Bioresource Technology*. 61(1): 79-83.

D Goldfarb, S., & A Chase, H. (1996). The Estimation of the optimum amount of solid support in an immobilized cell bioreactor. *Progress in Biotechnology*. 11: 524-531.

Dauphin, S., Joannis, C., Deguin, A., Bridoux, G., Ruban, G., & Aumond, M. (1998). Influent flow control to increase the pollution load treated during rainy periods. *Water science and technology*. 37(12): 131-139.

De Beer, D., Stoodley, P., Roe, F., & Lewandowski, Z. (1994). Effects of biofilm structures on oxygen distribution and mass transport. *Biotechnology and Bioengineering*. 43(11): 1131-1138.

Delatolla, R., Tufenkji, N., Comeau, Y., Lamarre, D., Gadbois, A., & Berk, D. (2009). In situ characterization of nitrifying biofilm: Minimizing biomass loss and preserving perspective. *Water Research*. 43(6): 1775-1787.

Deletic, A. (1998). The first flush load of urban surface runoff. *Water Research*. 32(8): 2462-2470.

Du, L.-N., Zhao, M., Li, G., Xu, F.-C., Chen, W.-H., & Zhao, Y.-H. (2013). Biodegradation of malachite green by Micrococcus sp. strain BD15: Biodegradation pathway and enzyme analysis. *International Biodeterioration & Biodegradation*. 78(0): 108-116.

Dunne, W. M. (2002). Bacterial adhesion: seen any good biofilms lately? *Clinical microbiology reviews*. 15(2): 155-166.

Eding, E. H., Kamstra, A., Verreth, J. A. J., Huisman, E. A., & Klapwijk, A. (2006). Design and operation of nitrifying trickling filters in recirculating aquaculture: A review. *Aquacultural Engineering*. 34(3): 234-260.

Eighmy, T., Maratea, D., & Bishop, P. (1983). Electron microscopic examination of wastewater biofilm formation and structural components. *Applied and Environmental Microbiology*. 45(6): 1921-1931.

Environmental Quality Report; Kuala Lumpur, 2011.

Filipe, C. D. M., & Grady, C. P. L. (1998). *Biological Wastewater Treatment, Second Edition, Revised and Expanded*. Taylor & Francis.

Flemming, H.-C., Szewzyk, U., & Griebe, T. (2000). *Biofilms Investigative Methods & Application*. United states of America: Technomic Publishing Company, Inc.

Fletcher, M. (1976). The effects of proteins on bacterial attachment to polystyrene. *Journal of general microbiology*. 94(2): 400-404.

Foster, K. R., & Sowers, A. E. (1995). Dielectrophoretic forces and potentials induced on pairs of cells in an electric field. *Biophysical Journal*. 69(3): 777-784.

Ghaniyari-Benis, S., Borja, R., Monemian, S. A., & Goodarzi, V. (2009). Anaerobic treatment of synthetic medium-strength wastewater using a multistage biofilm reactor. *Bioresource Technology*. 100(5): 1740-1745.

Gianni, M. (2007). Lab on a Chip for Live-Cell Manipulation, *24*, 26-36.

González-Brambila, M., Monroy, O., & López-Isunza, F. (2006). Experimental and theoretical study of membrane-aerated biofilm reactor behavior under different modes of oxygen supply for the treatment of synthetic wastewater. *Chemical Engineering Science*. 61(16): 5268-5281.

González-Martínez, S., & Duque-Luciano, J. (1992). Aerobic submerged biofilm reactors for wastewater treatment. *Water Research*. 26(6): 825-833.

Gottenbos, B., Van der Mei, H. C., & Busscher, H. J. (1999). Models for studying initial adhesion and surface growth in biofilm formation on surfaces. *Methods in enzymology*. 310: 523-534.

Graves, D. J. (1975). Biochemical reactors, B. Atkinson, Pion Limited, London (1974). 267 pages. £5.00. *AIChE Journal*. 21(5): 1037-1037.

Hafifudin, N. b. (2013). *Construction of Artificial Consortia of Microoganism Using Dielectrophoresis for Wastewater Treatment.* Universiti Putra Malaysia (UPM).

Hall-Stoodley, L., Costerton, J. W., & Stoodley, P. (2004). Bacterial biofilms: from the natural environment to infectious diseases. *Nature Reviews Microbiology*. 2(2): 95-108.

Hao, R., Li, S., Li, J., & Meng, C. (2013). Denitrification of simulated municipal wastewater treatment plant effluent using a three-dimensional biofilm-electrode reactor: Operating performance and bacterial community. *Bioresource Technology*. 143(0): 178-186.

Henze, M. (1992). Characterization of wastewater for modelling of activated sludge processes. *Water Science & Technology*. 25(6): 1-15.

Horath, T., Neu, T. R., & Bachofen, R. (2006). An endolithic microbial community in dolomite rock in Central Switzerland: Characterization by reflection spectroscopy, pigment analyses, scanning electron microscopy, and laser scanning microscopy. *Microbial Ecology*. 51(3): 353-364.

Hsieh, B.-C., Cheng, T.-J., Shih, S.-H., & Chen, R. L. C. (2011). Pencil lead microelectrode and the application on cell dielectrophoresis. *Electrochimica Acta*. 56(27): 9916-9920.

Huang, Z., Ong, S. L., & Ng, H. Y. (2011). Submerged anaerobic membrane bioreactor for low-strength wastewater treatment: Effect of HRT and SRT on treatment performance and membrane fouling. *Water Research*. 45(2): 705-713.

Imam, S. H., & Gould, J. M. (1990). Adhesion of an amylolytic Arthrobacter sp. to starch-containing plastic films. *Applied and Environmental Microbiology*. 56(4): 872-876.

Ingmar Nopens, C. C., Peter A. Vanrolleghem. (2001). *Stability analysis of a synthetic municipal wastewater*. Gent: Universiteit Gent.

Jamal Khan, S., Ilyas, S., Javid, S., Visvanathan, C., & Jegatheesan, V. (2011). Performance of suspended and attached growth MBR systems in treating high strength synthetic wastewater. *Bioresource Technology*. 102(9): 5331-5336.

James, A. (1982). The electrical properties and topochemistry of bacterial cells. *Adv. Colloid Interface Sci.* 15: 171-221.

James D, B. (1993). Bacterial biofilms. *Current Opinion in Biotechnology*. 4(2): 197-204.

Jung, Y., Koh, H., Shin, W., & Sung, N. (2005). Wastewater Treatment using Combination of MBR Equipped with Non-Woven Fabric Filter and Oyster-Zeolite Column. *Environmental Engineering Research Seoul*. 10(5): 247.

Jurecska, L., Barkács, K., Kiss, É., Gyulai, G., Felföldi, T., Törő, B., et al. (2013). Intensification of wastewater treatment with polymer fiber-based biofilm carriers. *Microchemical Journal*. 107(0): 108-114.

Karamanev, D. G., & Nikolov, L. N. (1988). Influence Of Some Physicochemical Parameters On Bacterial Activity Of Biofilm: Ferrous Iron Oxidation By Thiobacillus Ferrooxidans. *Biotechnology and Bioengineering*. 31(4): 295-299.

Kariminiaae-Hamedaani, H.-R., Kanda, K., & Kato, F. (2003). Wastewater treatment with bacteria immobilized onto a ceramic carrier in an aerated system. *Journal of Bioscience and Bioengineering*. 95(2): 128-132.

Kinner, N. E., Balkwill, D. L., & Bishop, P. L. (1983). Light and electron microscopic studies of microorganisms growing in rotating biological contactor biofilms. *Applied and Environmental Microbiology*. 45(5): 1659-1669.

Kondo, A., Murakami, F., & Higashitani, K. (1992). Circular dichroism studies on conformational changes in protein molecules upon adsorption on ultrafine polystyrene particles. *Biotechnology and Bioengineering*. 40(8): 889-894.

Kornaros, M., & Lyberatos, G. (2006). Biological treatment of wastewaters from a dye manufacturing company using a trickling filter. *Journal of Hazardous Materials*. 136(1): 95-102.

Kristensen, G. H., & Christensen, F. R. (1982). Application of cryo-cut method for measurements of biofilm thickness. *Water Research*. 16(12): 1619-1621.

La Motta, E. J. (1976). Kinetics of growth and substrate uptake in a biological film system. *Applied and Environmental Microbiology*. 31(2): 286-293.

LaPara, T., Konopka, A., Nakatsu, C., & Alleman, J. (2000). Effects of elevated temperature on bacterial community structure and function in bioreactors treating a synthetic wastewater. *Journal of Industrial Microbiology and Biotechnology*. 24(2): 140-145.

LaPara, T. M., & Alleman, J. E. (1999). Thermophilic aerobic biological wastewater treatment. *Water Research*. 33(4): 895-908.

LaPara, T. M., Nakatsu, C. H., Pantea, L. M., & Alleman, J. E. (2001). Aerobic Biological Treatment of a Pharmaceutical Wastewater:: Effect of Temperature on COD Removal and Bacterial Community Development. *Water Research*. 35(18): 4417-4425.

Lapizco-Encinas, B. H., Simmons, B. A., Cummings, E. B., & Fintschenko, Y. (2004). Insulator‐based dielectrophoresis for the selective concentration and separation of live bacteria in water. *Electrophoresis*. 25(10-11): 1695-1704.

Lazarova, V., Capdeville, B., & Nikolov, L. (1992). Biofilm performance of a fluidized bed biofilm reactor for drinking water denitrification. *Water Science & Technology*. 26(3-4): 555-566.

Lazarova, V., & Manem, J. (1995). Biofilm characterization and activity analysis in water and wastewater treatment. *Water Research*. 29(10): 2227-2245.

Leeson, A., & Hinchee, R. E. (1997). *Soil Bioventing: Principles and Practice*. CRC/Lewis Publishers.

Leitão, R. C., van Haandel, A. C., Zeeman, G., & Lettinga, G. (2006). The effects of operational and environmental variations on anaerobic wastewater treatment systems: A review. *Bioresource Technology*. 97(9): 1105-1118.

Lens, P. (2003). *Biofilms in Medicine, Industry and Environmental Biotechnology: Characteristics, Analysis and Control*. International Water Assn.

Lewandowski, Z., & Beyenal, H. (2007). *Fundamentals of Biofilm Research*. United States of America: Taylor & Francis.

Lewandowski, Z., & Beyenal, H. (2007). *Fundamentals of biofilm research*. CRC/Taylor&Francis.

Li, C., Li, Y., Cheng, X., Feng, L., Xi, C., & Zhang, Y. (2013). Immobilization of Rhodococcus rhodochrous BX2 (an acetonitrile-degrading bacterium) with biofilm-forming bacteria for wastewater treatment. *Bioresource Technology*. 131(0): 390-396.

Liu, G.-h., Ye, Z., Li, H., Che, R., & Cui, L. (2012). Biological treatment of hexanitrostilbene (HNS) produced wastewater using an anaerobic–aerobic immobilized microbial system. *Chemical Engineering Journal*. 213(0): 118-124.

Liu, G.-h., Ye, Z., Tong, K., & Zhang, Y.-h. (2013). Biotreatment of heavy oil wastewater by combined upflow anaerobic sludge blanket and immobilized biological aerated filter in a pilot-scale test. *Biochemical Engineering Journal*. 72(0): 48-53.

Macaskie, L. E., Bonthrone, K. M., Hewitt, C. J., Paterson, M., & Kennedy, J. F. (1996). Use of immobilized microorganisms for bioremediation of acidic mine wastewater. *International Biodeterioration & Biodegradation*. 37(3–4): 238.

Mara, D. D. (1977). Wastewater treatment in hot climates. *Water, wastes and health in hot climates*.

Mark, J. E. (1999). *Polymer Data Handbook*. Oxford University Press Incorporated.

Markx, G. H., & Davey, C. L. (1999). The dielectric properties of biological cells at radiofrequencies: applications in biotechnology. *Enzyme and Microbial Technology*. 25(3–5): 161-171.

Markx, G. H., Huang, Y., Zhou, X.-F., & Pethig, R. (1994). Dielectrophoretic characterization and separation of micro-organisms. *Microbiology*. 140(3): 585-591.

Markx, G. H., Talary, M. S., & Pethig, R. (1994). Separation of viable and non-viable yeast using dielectrophoresis. *Journal of Biotechnology*. 32(1): 29-37.

Metcalf, Eddy, & Tchobanoglous, G. (1979). *Wastewater engineering: treatment disposal reuse*. McGraw-Hill.

Micro-fluidics cut cancer test from a day to an hour. (2009). Retrieved from http://www.electronicsweekly.com/news/components/programmable-logic-and-asic/micro-fluidics-cut-cancer-test-from-a-day-to-an-hour-imec-tech-forum-2009-10/

Mohan, S. V., Rao, N. C., & Sarma, P. N. (2007). Low-biodegradable composite chemical wastewater treatment by biofilm configured sequencing batch reactor (SBBR). *Journal of Hazardous Materials*. 144(1–2): 108-117.

Morgan-Sagastume, F., & Allen, D. G. (2003). Effects of temperature transient conditions on aerobic biological treatment of wastewater. *Water Research*. 37(15): 3590-3601.

Najafpour, G. D., Zinatizadeh, A. A. L., & Lee, L. K. (2006). Performance of a three-stage aerobic RBC reactor in food canning wastewater treatment. *Biochemical Engineering Journal*. 30(3): 297-302.

Nakashima, Y., Hata, S., & Yasuda, T. (2010). Blood plasma separation and extraction from a minute amount of blood using dielectrophoretic and capillary forces. *Sensors and Actuators B: Chemical*. 145(1): 561-569.

Neijssel, O. M., van der Meer, R. R., Luyben, K. C. A. M., Society, N. B., & Biotechnology, E. F. O. (1987). Proceedings of the 4th European Congress on Biotechnology, 1987, Amsterdam, June 14-19, 1987. Elsevier.

Nemerow, N. L. (1971). *Industrial water pollution: origins, characteristics, and treatment*. Addison-Wesley Pub. Co.

Neumann, E., Sowers, A. E., & Jordan, C. A. (1989). *Electroporation and Electrofusion in Cell Biology*. Springer.

Olem, H., & Unz, R. F. (1980). Rotating-disc biological treatment of acid mine drainage. *Journal of the Water Pollution Control Federation*. 52(2): 257-269.

Oller, I., Malato, S., & Sánchez-Pérez, J. A. (2011). Combination of Advanced Oxidation Processes and biological treatments for wastewater decontamination-A review. *Science of The Total Environment*. 409(20): 4141-4166.

Patrick, K., Young, J., & Ferguson, K. (1994). Closing the loop: the effluent-free pulp and paper mill. *Pulp and paper*. 68.

Pedersen, K. (1990). Biofilm development on stainless steel and PVC surfaces in drinking water. *Water Research*. 24(2): 239-243.

Pethig, R. (1979). *Dielectric and electronic properties of biological materials*. New York: John Willey & Sons.

Pethig, R. (2010). Review article-dielectrophoresis: status of the theory, technology, and applications. *Biomicrofluidics*. 4: 022811.

Pethig, R., Huang, Y., Wang, X.-b., & Burt, J. P. (1992). Positive and negative dielectrophoretic collection of colloidal particles using interdigitated castellated microelectrodes. *Journal of Physics D: Applied Physics*. 25(5): 881.

Pohl, H. A. (1951). The motion and precipitation of suspensoids in divergent electric fields. *Journal of Applied Physics*. 22(7): 869-871.

Pohl, H. A. (1978). *Dielectrophoresis: The Behavior of Matter in Non-uniform Electric Fields.* Cambridge University Press Cambridge.

Pohl, H. A., & Hawk, I. (1966). Separation of living and dead cells by dielectrophoresis. *Science, 152*(3722), 647-649.

Pohl, H. A., & Pollock, K. (1978). Electrode geometries for various dielectrophoretic force laws. *Journal of Electrostatics*. 5(0): 337-342.

Puñal, A., & Lema, J. (1999). Anaerobic treatment of wastewater from a fish-canning factory in a full-scale upflow anaerobic sludge blanket (UASB) reactor. *Water science and technology*. 40(8): 57-62.

Qasim, S. R. (1999). *Wastewater treatment plants: planning, design, and operation*. Technomic Publishing Company.

Qi, R., Yang, K., & Yu, Z.-x. (2007). Treatment of coke plant wastewater by SND fixed biofilm hybrid system. *Journal of Environmental Sciences*. 19(2): 153-159.

Rajaraman, S., Noh, H.-s., Hesketh, P. J., & Gottfried, D. S. (2006). Rapid, low cost microfabrication technologies toward realization of devices for dielectrophoretic manipulation of particles and nanowires. *Sensors and Actuators B: Chemical*. 114(1): 392-401.

Ramos, A. (2011). *Electrokinetics and Electrohydrodynamics In Microsystems*. New York: Springer Wien.

Rebac, S., Ruskova, J., Gerbens, S., van Lier, J. B., Stams, A. J. M., & Lettinga, G. (1995). High-rate anaerobic treatment of wastewater under psychrophilic conditions. *Journal of Fermentation and Bioengineering*. 80(5): 499-506.

Richards, S., & Turner, R. (1984). A comparative study of techniques for the examination of biofilms by scanning electron microscopy. *Water Research*. 18(6): 767-773.

Saito, M., Schwan, H., & Schwarz, G. (1966). Response of nonspherical biological particles to alternating electric fields. *Biophysical Journal*. 6(3): 313-327.

Samuelsson, M.-O., & Kirchman, D. L. (1990). Degradation of adsorbed protein by attached bacteria in relationship to surface hydrophobicity. *Applied and Environmental Microbiology*. 56(12): 3643-3648.

Sancho, M., Martínez, G., & Martín, C. (2003). Accurate dielectric modelling of shelled particles and cells. *Journal of Electrostatics*. 57(2): 143-156.

Schwan, H. (1982). Nonthermal cellular effects of electromagnetic fields AC-field induced ponderomotoric forces. *The British Journal of Cancer. Supplement*. 5: 220.

Schwan, H. P. (1957). Electrical properties of tissue and cell suspensions. *Advances in biological and medical physics*. 5: 147.

Schwartz, T., Jungfer, C., Heißler, S., Friedrich, F., Faubel, W., & Obst, U. (2009). Combined use of molecular biology taxonomy, Raman spectrometry, and ESEM imaging to study natural biofilms grown on filter materials at waterworks. *Chemosphere*. 77(2): 249-257.

Sekaran, G., Karthikeyan, S., Gupta, V. K., Boopathy, R., & Maharaja, P. (2013). Immobilization of Bacillus sp. in mesoporous activated carbon for degradation of sulphonated phenolic compound in wastewater. *Materials Science and Engineering*. 33(2): 735-745.

Shrove, G. S., Olsen, R. H., & Vogel, T. M. (1991). Development of pure culture biofilms of P. putida on solid supports. *Biotechnology and Bioengineering*. 37(6): 512-518.

Sieracki, M. E., Johnson, P. W., & Sieburth, J. (1985). Detection, enumeration, and sizing of planktonic bacteria by image-analyzed epifluorescence microscopy. *Applied and Environmental Microbiology*. 49(4): 799-810.

Silva, R. A., Grossi, V., Olivera, N. L., & Alvarez, H. M. (2010). Characterization of indigenous Rhodococcus sp. 602, a strain able to accumulate triacylglycerides from naphthyl compounds under nitrogen-starved conditions. *Research in Microbiology*. 161(3): 198-207.

Singh, R., Paul, D., & Jain, R. K. (2006). Biofilms: implications in bioremediation. *TRENDS in Microbiology*. 14(9): 389-397.

Sreekrishnan, T., Ramachandran, K., & Ghosh, P. (1991). Effect of operating variables on biofilm formation and performance of an anaerobic fluidized-bed bioreactor. *Biotechnology and Bioengineering*. 37(6): 557-566.

Stover, E. (1999). *Aerobic autoheated thermophilic treatment process for high strength industrial waste residuals*. Paper presented at the Industrial Wastes Technical Conference.

Sukhorukov, V. L., Meedt, G., Kürschner, M., & Zimmermann, U. (2001). A single-shell model for biological cells extended to account for the dielectric anisotropy of the plasma membrane. *Journal of Electrostatics*. 50(3): 191-204.

Sumbali, M. A. (2009). *Principles Of Microbiology: M&S*. McGraw-Hill Education (India) Pvt Limited.

Trueba, F., & Woldringh, C. (1980). Changes in cell diameter during the division cycle of Escherichia coli. *Journal of bacteriology*. 142(3): 869-878.

Visvanathan, C., & Nhien, T. T. H. (1995). Study on Aerated Biofilter Process under High Temperature Conditions. *Environmental Technology*. 16(4): 301-314.

Von Hippel, A. R. (1954). *Dielectric Materials and Applications*. New York: 22 Contributors.

Water a shared responsibilities. (2006).

Wilén, B.-M., Lund Nielsen, J., Keiding, K., & Nielsen, P. H. (2000). Influence of microbial activity on the stability of activated sludge flocs. *Colloids and Surfaces B: Biointerfaces*. 18(2): 145-156.

Wulfhorst, B., Gries, T., & Veit, D. (2006). *Textile Technology*. Hanser Publishers.

Yates, M. V., Percival, S. L., Embrey, M., Williams, D., Hunter, P., Chalmers, R., et al. (2004). *Microbiology of Waterborne Diseases: Microbiological Aspects and Risks*. Elsevier Science.

Zhang, J., Zhou, J., Liu, Y., & Fane, A. G. (2010). A comparison of membrane fouling under constant and variable organic loadings in submerge membrane bioreactors. *Water Research*. 44(18): 5407-5413.

Zhang, L., Lee, Y.-W., & Jahng, D. (2011). Anaerobic co-digestion of food waste and piggery wastewater: Focusing on the role of trace elements. *Bioresource Technology*. 102(8): 5048-5059.

APPENDICES

Appendix A

Wirecloth's Gap Calculation and Reagents' Preparation

1. Calculation of Gap Between Electrodes

80 picks per inch = 80 wires in inch (length og wire cloth), 79 gaps

Total 80 wires with the diameter 71 µm = 80 x 71 µm = 5680 µm

1 inch = 0.0254 m = 5680 µm + total length of gaps between electrodes

Gap between each electrode,

$$= \frac{0.0254m - 0.00568m}{79}$$

$= 250 \times 10^{-6}$ µm

2. BOD and COD Reagent Preparation

Iodine Azide Solution

500 g of Sodium Hydroxide, NaOH + 150 g of Potassium Iodide KI + 10 g of Sodium Azide, NaN_3

Mix all and let them dissolve in 1000 ml of distilled water.

Starch Indicator

Use either an aqueous solution or soluble starch powder mixtures. To prepare an aqueous solution , dissolve 2g laboratory-grade soluble starch and 0.2g salicylic and as a preservative, in 100ml hot distilled water.

Standard Sodium Thiosulfat Titrant

Dissolve 6.205g Sodium thiosulfate $Na_2S_2O_3.5H_2O$ in distilled water.
Add 1.5 ml 6N Sodium hydroxide NaOH or 0.4 g solid NaOH and dilute to 1000ml.
Standardize with bi-iodite solution, 0.0021M;
Dissolve 812.4 mg Potassium hydride $KH(IO_3)_2$ in distilled water and dilute to 1000ml.

Manganous Sulfate Solution

Dissolve 480g $MnSO_4.4H_2O$, 400g $MnSO_4.2H_2O$ OR 364g $MnSO_4.H_20$ in distilled water, filter and dilute to 1L. The $MnSO_4$ solution should not give a colour with starch when added to an acidified potassium iodide (KI) Solution.

Preparation of Dilution Water

For 8 liter distilled water

8ml Magnesium Sulphate, $MgSO_4.7H_2O$

8ml Calcium Chloride, $CaCl_2$

8ml Ferum Chloride, $FeCl_3.6H_2O$

8ml Phosphate buffer solution

Aerate the dilution water for minimum 8 hours.

Preparation:

a. $MgSO_4.7H_2O$ (Manganese sulfate solution)
   Dissolve 22.5g $MgSO_4.7H_20$ in distilled water and dilute until 1 liter.
b. $CaCl_2$ (Calcium chloride solution)
   Dissolve 27.5g $CaCl_2$ in distilled water and dilute to 1 liter.
c. $FeCl_3.6H_2O$ (Ferric chloride solution)
   Dissolve 0.25g $FeCl_3.6H_2O$ in distilled water and dilute to 1 liter.
d. Phosphate buffer solution

Dissolve	8.5g	KH_2PO_4
	21.75g	K_2HPO_4
	33.4g	$Na_2.HPO_4.7H_2O$
	1.7g	NH_4Cl

For Phosphate buffer solution, dissolve all in 500 ml of distilled water.

Preparation of Sulfuric Acid Reagent

Dissolve 5.5g of Argentum Sulphate, $AgSO_4$ in each kg of concentrate sulphuric acid.

This means, 1000g H_2SO_4 will need to use 5.5g Silver Sulphate.

In standard solution of H_2SO_4, 1L = 1000kg

So, for 1.84kg H_2SO_4, use = 1.84/1 x 5.5g $AgSO_4$

= 10.12g $AgSO_4$

So, 10.12g $AgSO_4$ in 1L H2SO4 (concentrate)

Preparation of Standard Ferrous Ammonium Sulfate Titrant (FAS) (0.10M)

Dissolve 39.2g Ferrous Ammonium Sulfate, $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water.

Add 20ml concentrated H_2SO_4, Cool and dilute to 1000ml.

Standardize solution daily against standard $K_2Cr_2O_7$ digestion solution as follow:

-Pipette 5ml digestion solution into a small beaker.

-Add 10ml reagent water to substitute for sample.

-Cool to room temperature

-Add 1 to 2 drops diluted ferroin indicator and titrate with FAS titrant.

Preparation of Standard Potassium Dichromate Digestion Solution 0.01667M

Add 500ml distilled water to 4.903g $K_2Cr_2O_7$ (primary standard grade), which dried at 150 ℃ for 2 hours. Next, add in 167 ml concentration H_2SO_4 and 33.3 g $HgSO_4$. Dissolve, cool to room temperature and dilute to 1000ml.

Appendix B

COD Profiles

Table B1 Effect of PEI in COD reduction for low strength synthetic wastewater

Set	Avg. used FAS, ml	COD value, mg/L
blank	1.50	246
0	0.70	246
1	0.70	246
2	0.70	246
3	0.70	246
4	0.60	276
5	0.60	276
6	0.60	276
7	0.50	307
8	0.50	307
9	0.50	307
10	0.45	323

Table B2 Effect of PEI in COD reduction for high strength synthetic wastewater

Set	Avg. Used FAS, ml	COD value, mg/L
blank	1.50	1230
0	1.10	1230
1	1.10	1230
2	1.10	1230
3	1.10	1230
4	1.10	1230
5	1.10	1230
6	1.10	1230
7	1.10	1230
8	1.10	1230
9	1.10	1230
10	1.10	1230

Table B3 Growth rate curve, OD600

Time, hrs	Type C	Type B	type A
0	0.021	0.021	0.021
3	0.085	0.055	0.055
6	0.102	0.067	0.068
9	0.244	0.102	0.092
12	0.382	0.168	0.162
15	0.487	0.214	0.283
18	0.576	0.284	0.364
21	0.674	0.316	0.451
24	0.776	0.387	0.495
27	0.871	0.445	0.525
30	0.976	0.582	0.589
33	0.988	0.684	0.647
36	0.988	0.809	0.759
39	0.986	0.818	0.874
42	0.976	0.811	0.901
45	0.978	0.828	0.911
48	0.979	0.826	0.924
51	0.956	0.821	0.928
54	0.950	0.802	0.928
57	0.945	0.791	0.928
60	0.945	0.786	0.899
63	0.943	0.779	0.886
66	0.940	0.775	0.877
69	0.937	0.770	0.870
72	0.935	0.770	0.870

Table B4 Wastewater treatment ability test for selected microorganisms

Time, days	Type A	Type B	Type C
	COD removal, %	COD removal, %	COD removal, %
0	0	0	0
1	18.10	18.11	18.11
2	30.10	30.1	24.58
3	55.45	55.45	55.45
4	67.05	65.45	67.05
5	76.75	74.21	74.21
6	80.41	80.41	82.1
7	80.41	82.32	82.1
8	88.76	88.76	85.21
9	88.76	88.76	89.89
10	88.76	88.76	89.89
11	88.76	88.76	89.89
12	88.76	88.76	89.89
13	88.76	88.76	89.89
14	88.76	88.76	89.89

Table B5 Effect of SA for low strength synthetic wastewater

Time, day	SA, 64 cm2		SA, 56 cm2		SA, 48 cm2		SA, 40 cm2	
	COD, mg/L	Reduction %	COD, mg/L	Reduction %	COD mg/L	Reduction %	COD mg/L	Reduction %
0	237	0	237	0	237	0	237	0
1	59	75.11	59	75.11	88	62.87	118	50.21
2	29	87.76	29	87.76	59	75.11	88	62.87
3	29	87.76	29	87.76	29	87.76	29	87.76
4	29	87.76	29	87.76	29	87.76	29	87.76
5	29	87.76	29	87.76	29	87.76	29	87.76
6	29	87.76	29	87.76	29	87.76	29	87.76
7	29	87.76	29	87.76	29	87.76	29	87.76

Table B6 Effect of SA for low strength synthetic wastewater

Time, day	SA, 64 cm2		SA, 56 cm2		SA, 48 cm2		SA, 40 cm2	
	COD, mg/L	Reduction %	COD, mg/L	Reduction %	COD mg/L	Reduction %	COD mg/L	Reduction %
0	1259	0	1259	0	1259	0	1259	0
1	1027	18.43	1027	18.43	1173	6.83	1173	6.83
2	880	30.10	880	30.10	1027	12.45	1173	6.83
3	733	41.78	733	41.78	880	24.98	880	24.98
4	586	53.45	586	53.45	586	53.45	586	53.45
5	440	65.05	440	65.05	440	65.05	586	53.45
6	440	65.05	440	65.05	440	65.05	440	65.05
7	146	88.4	176	86.02	293	76.73	440	65.05
8	117	90.7	117	90.7	293	76.73	293	76.73
9	117	90.7	117	90.7	234	81.41	234	81.41
10	117	90.7	117	90.7	176	86.02	205	83.72
11	146	88.4	117	90.7	146	88.4	146	88.4
12	117	90.7	146	88.4	146	88.4	146	88.4
13	117	90.7	117	90.7	117	90.7	117	90.7
14	117	90.7	117	90.7	117	90.7	117	90.7
15	117	90.7	117	90.7	146	88.4	146	88.4
16	117	90.7	146	88.4	117	90.7	117	90.7
17	117	90.7	117	90.7	117	90.7	117	90.7
18	117	90.7	117	90.7	117	90.7	117	90.7
19	117	90.7	117	90.7	117	90.7	117	90.7
20	117	90.7	117	90.7	117	90.7	117	90.7

Table B7 Effect of pH for low strength synthetic wastewater

Day	pH2		pH4		pH6		pH8		pH10	
	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %
0	250	0	250	0	250	0	250	0	250	0
1	250	0.00	250	0.00	250	0	109	56.4	250	0
2	250	0.00	188	24.80	125	50.00	31	87.60	220	12
3	250	0.00	156	37.60	31	87.60	31	87.60	156	37.6
4	125	50.00	94	62.40	31	87.60	62	75.20	125	50.00
5	94	62.40	94	62.40	62	75.20	31	87.60	125	50.00
6	94	62.40	62	75.20	31	87.60	31	87.60	94	62.40
7	94	62.40	31	87.60	31	87.60	31	87.60	94	62.40
8	94	62.40	31	87.60	31	87.60	31	87.60	62	75.20
9	94	62.40	31	87.60	31	87.60	31	87.60	62	75.20
10	94	62.40	31	87.60	31	87.60	31	87.60	62	75.20

Table B8 Effect of pH for high strength synthetic wastewater

Day	pH2		pH4		pH6		pH8		pH10	
	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %
0	1207	0	1207	0	1207	0	1207	0	1207	0
1	1207	0.00	1207	0	1207	0	1207	0.00	1207	0
2	1056	12.51	1056	12.51	1056	12.51	1056	12.51	1056	12.51
3	1056	12.51	1056	12.51	754	37.53	754	37.53	1056	12.51
4	1056	12.51	1056	12.51	754	37.53	754	37.53	1056	12.51
5	1056	12.51	603	50.04	452	62.55	301	75.06	754	37.53
6	1056	12.51	603	50.04	452	62.55	120	90.05	603	50.04
7	1056	12.51	301	75.06	150	87.57	120	90.05	301	75.06
8	754	37.52	150	87.57	150	87.57	120	90.05	301	75.06
9	301	75.06	150	87.57	120	90.05	120	90.05	301	75.06
10	301	75.06	150	87.57	150	87.57	150	87.57	301	75.06
11	301	75.06	150	87.57	120	90.05	120	90.05	301	75.06
12	301	75.06	150	87.57	120	90.05	120	90.05	301	75.06
13	301	75.06	150	87.57	120	90.05	120	90.05	301	75.06
14	301	75.06	150	87.57	120	90.05	120	90.05	301	75.06

Table B9 Effect of Temperature for low strength synthetic wastewater

Day	26 ℃		40 ℃		50 ℃		60 ℃		70 ℃	
	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %
0	261	0	261	0	261	0	261	0	261	0
1	232	11.11	87	66.67	232	11.11	261	0	261	0
2	174	33.33	29	88.89	189	27.58	261	0	261	0
3	58	77.77	29	88.89	145	44.44	261	0	261	0
4	29	88.89	29	88.89	145	44.44	261	0	261	0
5	29	88.89	29	88.89	145	44.44	261	0	261	0
6	29	88.89	29	88.89	145	44.44	261	0	261	0
7	29	88.89	29	88.89	145	44.44	261	0	261	0

Table B10 Effect of Temperature for high strength synthetic wastewater

Day	26 ℃		40 ℃		50 ℃		60 ℃		70 ℃	
	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %	COD, mg/L	Reduction, %
0	1305	0	1305	0	1305	0	1305	0	1305	0
1	1305	0.00	1160	11.11	1305	0	1305	0	1305	0
2	1160	11.11	870	33.33	1305	0.00	1305	0	1305	0
3	1160	11.11	870	33.33	870	33.33	1305	0	1305	0
4	870	33.33	586	55.56	580	55.56	1305	0	1305	0
5	725	44.44	435	66.67	870	33.33	1305	0	1305	0
6	580	55.56	290	77.78	580	55.56	1305	0	1305	0
7	580	55.56	145	88.89	870	33.33	1305	0	1305	0
8	290	77.78	145	88.89	580	33.33	1305	0	1305	0
9	290	77.78	145	88.89	580	55.56	1305	0	1305	0
10	145	88.89	145	88.89	580	55.56	1305	0	1305	0
11	145	88.89	145	88.89	870	33.33	1305	0	1305	0
12	145	88.89	145	88.89	580	55.56	1305	0	1305	0
13	145	88.89	145	88.89	580	55.56	1305	0	1305	0
14	145	88.89	145	88.89	580	55.56	1305	0	1305	0
15	145	88.89	145	88.89	580	55.56	1305	0	1305	0
16	145	88.89	145	88.89	580	55.56	1305	0	1305	0
17	145	88.89	145	88.89	580	33.33	1305	0	1305	0
18	145	88.89	145	88.89	580	55.56	1305	0	1305	0
19	145	88.89	145	88.89	580	55.56	1305	0	1305	0
20	145	88.89	145	88.89	580	55.56	1305	0	1305	0

Table B11 Effect of HRT for low strength synthetic wastewater

Time, day	COD, mg/L	Reduction. %
0	276	0
1	123	55.43
2	61	77.89
3	30	89.13
4	30	89.13
5	30	89.13
6	30	89.13
7	30	89.13
8	30	89.13
9	30	89.13
10	30	89.13
12	30	89.13
14	30	89.13
16	30	89.13
18	30	89.13
20	30	89.13
22	30	89.13
24	30	89.13
26	30	89.13
28	30	89.13
30	30	89.13
33	30	89.13
36	61	77.89
39	30	89.13
42	30	89.13
45	61	77.89

Time, day	COD, mg/L	Reduction. %
48	61	89.13
51	30	89.13
54	30	89.13
57	30	89.13
60	30	89.13
64	30	89.13
68	123	55.43
72	184	33.33
76	123	55.43
80	215	22.10
84	184	33.33
88	184	33.33
92	184	33.33
96	215	22.10
100	123	55.43
105	215	22.10
110	215	22.10
115	184	33.33
120	246	10.87
125	215	22.10
130	184	33.33
135	215	22.10
140	123	55.43
145	184	33.33
150	215	22.10

Table B12 Effect of HRT for high strength synthetic wastewater

Time, day	COD, mg/L	Reduction, %
0	1230	0
3	923	24.95
6	538	56.26
9	461	62.52
12	538	56.26
15	538	56.26
18	538	56.26
21	538	56.26
25	538	56.26
29	538	56.26
33	153	87.56
37	153	87.56
41	153	87.56
45	153	87.56
49	153	87.56
54	153	87.56
59	307	75.04
64	153	87.56

Time. day	COD, mg/L	Reduction, %
69	153	87.56
74	153	87.56
79	153	87.56
84	153	87.56
90	153	87.56
96	307	75.04
102	307	75.04
108	153	87.56
114	153	87.56
120	153	87.56
126	153	87.56
133	307	75.04
140	307	75.04
147	153	87.56
154	307	75.04
161	153	87.56
168	153	87.56
175	153	87.56

BIODATA OF STUDENT

Wai York Chow was born in Kluang on 09th April 1988. He completed his primary education at SJK (C) Layang-Layang at the year of 2000. Thus, He furthered his secondary education at SMK Layang-Layang at where he accomplished his SPM (Sijil Pelajaran Malaysia) examination at the year of 2006. Then, he decided to take a pre-university study, STPM (Sijil Tinggi Persekolahan Malaysia) at Sekolah Tinggi Kluang. At 2009, he enrolled in the degree course of Industrial Chemistry (Honors) in Universiti Putra Malaysia (UPM), and graduated with upper second-class honours degree at 2011. At the same year, he entered his degree of Master in Science to further his postgraduate life in UPM under supervise of Assoc. Prof. Dr Zurina Zainal Abidin.

PUBLICATIONS

Book Chapter (Published)

Abidin, Z. Z., Wai, Y. C., Haffifudin, N., & Ahmadun, F. (2013). Construction of ASMC by Dielectrophoresis Using Wirecloth Electrode for the Treatment of Wastewater. In R. Pogaku, A. Bono & C. Chu (Eds.). *Developments in Sustainable Chemical and Bioprocess Technology* (pp. 65-71). Springer US.