BIODEGRADATION OF REMAZOL BLACK B BY BACTERIAL CONSORTIUM NAR-2

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Specially dedicated to my beloved Dad and Mom, Reza Kardi and Maria Hadighi .

To my adorable husband
Nima
And
My granny

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ABSTRACT

The ability of the bacterial consortium NAR-2 consisting of A1, C1 and L17 to degrade the model azo dye Remazol Black B (RBB) was studied in batch and in continous systems. Continous decolourisation was performed in a borosilicate glass column (12 mm x 20 mm) packed with Surfactant Modified Clinoptilolite immobilised with bacterial consortium NAR-2. In batch studies, 90.79% decolourisation of RBB was achieved under microaerophilic condition within 80 minutes by inoculating 10% (v/v) of bacterial consortium NAR-2 at a 1:1:1 ratio. This was achieved in modified P5 medium pH 7 and incubated at 45°C under microaerophilic condition. In column bioreactor studies, decolourisation was observed at 45°C and carried out by varying the flow rates and dye concentrations. Flow rate at 0.2, 0.4, 0.6, 0.8, and 1.0 ml/min were tested and dye concentration of 0.1, 0.3, 0.5, 0.7, and 1.0 g/L were used. Almost 95.87% decolourisation of 0.1 g/L RBB was achieved at the flow rate 0.2 ml/min. By fixing 0.2 ml/min as default flow rate, varying concentrations of RBB were examined. Above 90% decolourisation was achieved with 0.1, 0.3 and 0.5 g/L RBB but at 0.7 and 1.0 g/L the percentage drop to 36 and 28%, respectively. Decolourisation percentage began to droped at higher dye concentration. Biomass leached out from the column was determined using viable cell count. From both flow rate and dye concentration experiments, it can be seen that C1 cell wash out was the highest as compared to A1 and L17. Analyses of decolourized and biodegradation products of RBB using total aromatic amines (TAA) showed that reduction of RBB resulted in the formation of aromatic amines. Further aerobic degradation for 15 days showed the amines concentration reduced from an initial of 18 mg/L to 2 mg/L following aerobic treatment in batch whereas in column experiment, the amines concentration dropped significantly from 34 mg/L to 11 mg/L.

ABSTRAK

Keupayaan konsortium bakteria NAR-2 terdiri daripada A1, C1 dan L17 untuk menyahwarnakan model azo pewarna Remazol Black B (RBB) telah dikaji dalam kelompok dan dalam sistem lengkap berterusan. Penyahwarnaan lengkap berterusan dilakukan dengan menggunakan kolum kaca borosilika (12 mm x 20 mm) dimampatkan dengan konsortium bakteria NAR-2 yang disekat gerak di atas clinoptilolite dengan permukaan yang telah diubah suai dengan surfaktan. Dalam eksperimen kelompok, 90.79% penyahwarnaan RBB telah dicapai di bawah keadaan mikroaerofilik dalam tempoh 80 minit dengan menginokulasi 10% (v/v) konsortium bakteria NAR-2 pada nisbah 1:1:1. Ini telah dicapai dalam medium P5 terubah suai pada pH 7 dan dieram pada 45°C di bawah keadaan mikoaerofilik. Dalam eksperimen kolum bioreaktor, penyahwarnaan telah diperhatikan pada 45°C dan dijalankan dengan mengubah kadar alir dan kepekatan pewarna. Kadar alir 0.2, 0.4, 0.6, 0.8, dan 1.0 ml/min, dan kepekatan pewarna 0.1, 0.3, 0.5, 0.7, dan 1.0 g / L telah dikaji. Hampir 95.87% penyahwarnaan 0.1 g/L RBB telah dicapai pada kadar alir 0.2 ml/min. Dengan menetapkan 0.2 ml/min sebagai kadar alir tentu awal, RBB pada kepekatan berbeza diperiksa. Lebih daripada 90% penyahwarnaan dicapai dengan 0.1, 0.3 dan 0.5 g/L RBB tetapi pada kepekatan 0.7 dan 1.0 g/L, peratusan menurun kepada 36 dan 28%, masing-masing. Peratusan penyahwarnaan mula berkurangan pada kepekatan pewarna yang lebih tinggi. Biomas yang terlarut lesap dari kolum ditentukan dengan menggunakan kiraan sel berdaya hidup. Berdasarkan kedua-dua eksperimen kadar alir dan kepekatan pewarna, dapat dilihat bahawa sel C1 yang terlarut resap adalah yang tertinggi berbanding A1 dan L17. Analisis produk ternyahwarna dan biodegradasi RBB menggunakan jumlah amina aromatik (TAA) mengesahkan bahawa penyahwarnaan RBB menghasilkan amina aromatik. Lanjutan degradasi aerobik selama 15 hari menunjukkan kepekatan amina menurun daripada 18 mg/L kepada 2 mg/L dalam eksperimen kelompok manakala dalam eksperimen kolum, kepekatan amina menurun dengan ketara daripada 34 mg/L hingga 11 mg/L.

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LIST OF ABBREVIATIONS

A1	-	*Citrobacter* sp. A1
C1	-	*Enterococcus* sp. C1
CMC	-	Critical micelle concentration
EPS	-	Exopolysaccharide
HCL	-	Hydrochloric acid
HDTMA-Br	-	Hexadecyltrimethylammonium bromide
KH_2PO_4	-	Potassium dihydrogen phosphate
K_2HPO_4	-	Dipotassium hydrogen phosphate
L17	-	*Citrobacter* sp. L17
LPS	-	Lipopolysaccharide
NA	-	Nutrient agar
NAOH	-	Sodium hydroxide
RBB	-	Remazol Black B
SEM	-	Scanning electron microscope
SMC	-	Surfactant modified clinoptilolite
TAA	-	Total aromatic ammines
TAHNDS	-	Triaminohydroxynaphthalene disulphanilic acid
$TAHNDSDP_2$	-	7-amino-8-hydroxy-1,2 naphthaquinone 3,6-disulphonate-1,2-diimine
$TAHNDSDP_3$	-	5,6-Dihydroxy-3-imino-4-oxo-3,4-dihydronaphthalene-2,7-disulphonic acid
$TAHNDSDP_4$	-	4,6-Dihydroxy-3,5-dioxo-3-dihydronaphthalene-2,7-disulphonic acid

LIST OF SYMBOLS

cfu/mL	-	Colony forming units per mL
g/L	-	gram per litre
L	-	Litre
mg/L	-	milligram per Litre
mL	-	millilitre
mm	-	millimeter
mM	-	millimolar
M	-	Molar
µm	-	mcrometer
nm	-	nanometer
OD600	-	Optical density at 600 nm
rpm	-	revolution per minute
v/v	-	volume per volume
w/v	-	weight per volume

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CHAPTER 1

INTRODUCTION

1.1 Introduction and Background

A variety of commercial applications of dyes generate effluent in huge amount that requires professional treatment. Different kinds of dyes are utilized in companies such as paper, textile, pulp, paints, carpet, and printing (Aksu *et al.*, 2005). The emissions which contain dyes are enormously coloured and are among the main causes of ecological pollution (Zouari-Mechichi *et al.*, 2006). Up to 50% of the dyes are vanished after the dyeing process in the textile manufacturing industry, and approximately 10–15% of them are discharged in the effluents (Vaidya and Datye, 1982). Dyes are grouped as triphenylmethane, azo, heterocyclic, anthraquinone, and polymeric dyes depending on the chemical structure of the chromophoric category. The largest and most relevant of the dyes used are azo dyes. They are carcinogenic and mutagenic material which may cause cancer to human being and are not fully eliminate through traditional wastewater treatment mechanism (Wong and Yuen, 1996). Azo dyes are branded by the existence of one or more azo category (-N=N-) and negative sulfonate family (-SO3-). Azo dyes itself is dangerous; though when the azo bonds are condensed, it produces amines which are more carcinogenic than the parent arrangement (Hong *et al.*, 2007).

The physical and chemical treatment of azo dyes in wastewater entails chemical corrosion, electrochemical active carbon adsorption, reverse osmosis, anion exchange resins, irradiation, and ozonation (Selcuk, 2005). Nevertheless, many of these techniques are expensive and time-consuming hence cannot be regarded as practical alternative for treating huge waste streams.

Treating dyes with the adoption of biological methods making use of microorganisms to decolourise and biodegrade azo dyes in anaerobic, aerobic or joint anaerobic-aerobic preparation mechanism have proven to be productive. Among the most reliant means which own the ability to decolourise synthetic dyes is the adoption of microorganisms containing bacterial strains, fungi and yeast (Ferreira *et al.*, 2000; Saratale *et al.*, 2011). Several authors have described proper decolourising potential by microbial blend culture compared to pure culture (Chan *et al.*, 2011; Chan *et al.*, 2012; Joshi *et al.*, 2008; Khehra *et al.*, 2005).

Earlier authors have indicated that azo dyes experience two chronological processes; anaerobic that generates amines and a successive step of aerobic conduct (Hong *et al.*, 2007). Amines are considered as more poisonous and recalcitrant when compared to the family compound. These can be eliminated under aerobic condition with the use of custom made microorganisms. Hence, it is of great relevance to prepare dye-comprising waste before disposal and discharge.

1.2 Statement of Problem

Unethical release of textile dye waste matter containing azo dyes and their metabolites in aqueous environment is aesthetically obnoxious and result to a decrease in sunlight infiltration, which in turn reduces photosynthetic processes, dissolved oxygen absorption, and quality of water, and had acute poisonous impact on aquatic flora and fauna, causing severe ecological damages globally (Vandevivere *et al*., 1998).

Numerous synthetic azo dyes and their metabolites are lethal, carcinogenic, and mutagenic (Saratale *et al.,* 2011; Singh and Arora, 2011). Furthermore, plentiful information point to the fact that textile dyes and emissions have poisonous effects on the germination rates and biomass of many plant species which have essential environmental roles, such as been a source of livelihood to living things, soil erosion prevention and organic material provision that is so important to soil fertility (Ghodake *et al*., 2009). In biological treatment under anaerobic circumstances, bacteria can diminish azo bonds by azoreductase enzyme and discharge ingredient amines that are susceptible to aerobic biodegradation. Acclimatised ecologically friendly and economical decolourising bacteria generate more efficient methods of lowering dye pollution. For that reason, handling of manufacturing effluents containing azo dyes and their metabolites is essential prior to their final release to the environment.

1.3 Objectives of the Study

The general objectives of this research were:

1. To utilize bacteria *Citrobacter* sp.A1, *Citrobacter* sp.L17, and *Enterococcus* sp.C1 in consortium to degrade Remazol Black B in batch effectively.

2. To modify surface of clinoptilolite with surfactant HDTMA-Br for immobilisation of bacterial consortium for continuous decolourisation study.

3. To assess capacity of decolourization by the selected consortium in a continuous up flow packed bed column and followed by aerobic degradation.

1.4 Scope of Study

In this research, the aim is to use a bacterial consortium capable of decolourising model azo dye Remazol Black B (RBB) in batch and in continous system. The capacity of decolourisation of chosen consortium designated as NAR-2 were evaluated both in batch and continous systems. The degree of decolourisation and percentage for the two systems were examined and compared. As was earlier highlighted, the decrease of azo dyes yielded aromatic amines which are more dangerous than the parent compound. Hence, the concentration of amines following decolourisation and degradation were examined with the use of ammoniacal nitrogen test analysis (TAA). The concentration of amines should be lowered after aerobic degradation of decolourised RBB, indicating NAR-2 consortium's capability to degrade or even mineralise amines.

1.5 Significance of Study

This study focus on biological treatment in batch with suspended mix culture and as continuous system utilizing biofilm formation of NAR-2 on cheap and robust support material, clinoptilolite in the degradation of textile dye, RBB under sequential microaerophilic-aerobic environment. It is hoped that from this study, the efficiency of this system in treating real textile wastewater can be improved. The use of support material such as clinoptilolite has several advantages over suspended culture, because the support can be reused over many cycles and provide a less toxic environment to the bacteria. Consequently, environmental pollution can be reduced with an ecofriendly system as such this. It is hoped that this study can offer an economic wastewater treatment alternative to the textile industry.

REFERENCES

Ab.llah, N. (2012). SURFACTANT MODIFIED CLINOPTILOLITE IMMOBILISED WITH BACTERIAL CONSORTIUM FOR EFFICIENT AND ECOFRIENDLY DECOLOURISATION OF ACID RED 27. Master Thesis. Universiti Teknologi Malaysia, Skudai.

Abrahart, E.N. (1977). Dyes and their intermediates. 2nd edition. London: Edward Arnold.

Ahmad, S. A. I., Nayeemul Bari, S. M. and Mohiuddin, M. (2012). Biofilm: multicellular living of the unicellular bacteria *Int. J. Biosci*. **2** (6):59-71.

Akay, G., Erhan and E., Keskinler, B. (2005). Bioprocess intensification in flow-through monolithic microbioreactors with immobilized bacteria. *Biotechnol. and Bioeng*. **90**: 180-190

Aksu, Z. and Donmez, G. (2005). Combined effects of molasses sucrose and reactive dye on the growth and dye bioaccumulation properties of Candida tropicalis. *Process Biochem*. **40**: 2443–2454.

Al-Bastaki, N. (2004). Removal of methyl orange dye and Na2SO4salt from synthetic waste water using reverse osmosis. Chemical Engineering and Processing: Process Intensification. **43**(12): 1561-1567.

Allen, R. L. M. (1971). Colour Chemistry. Great Britain: Thomas Nelson Ltd.

Allison, D. G. and Sutherland, I. W. (1987). The role of exo-polysaccharides in adhesion of freshwater bacteria. *J. Gen. Microbiol.* **133**: 1319-1327

Allison, D. G., Ruiz, B., SanJose, C., Jaspe, A. and Gilbert, P. (1998). Extracellular products as mediators of the formation and detachment of Pseudomonas fluorescens biofilms. FEMS Microbiol. Lett. **167**: 179-184.

Anal, A. K. and Stevens, W. F. (2005). Chitosan–alginate multilayer beads for controlled release of ampicillin. *Int. J. Pharm*. **290**: 45–54.

Asgher, M., Kausar, S., Bhatti, H. N., Hassan Shah, S. A., & Ali, M. (2008). Optimization of medium for decolorization of Solar golden yellow R direct textile dye by Schizophyllumcommune IBL-06. *International Biodeterioration and iodegradation*. **61**(2): 189-193.

Bakhshi Z., Najafpour, G., Neya, B. N., Kariminezhad, E., R. Pishgar and Moosavi, N.(2011). Recovery of upflow anaerobic Packed bed reactor from high organic load during startup for phenolic wastewater treatment. *Chem. Ind. Chem. Eng. Q*.**17** (4): 517−524.

Banat, I.M., Nigam, P., Singh, D., Marchant, R. (1996). Microbial dcolourisation of textile dye containing effluents: A review. *Bioresource Technology*. **58**: 217-227.

Barragán, B. E., Costa, C., and Carmen Marquez., M. (2007). Biodegradation of azo dyes by bacteria inoculated on solid media. *Dyes and pigments* **75**(1): 73-81.

Benkli, Y. E., Can, M. F., Turan, M. and Celik, M. S. (2005). Modification of organo-zeolite surface for the removal of reactive azo dyes in fixed-bed reactors. *Water Research*. **39**(2-3): 487-493.

.Bowman, R. S. (2003). Applications of surfactant-modified zeolites to environmental remediation. *Microporous and Mesoporous Materials.* 61(1-3): 43-56.

Brown, D. and. Laboureur, P. (1983a). The Degradation of Dyestuffs: Part I.

Primary Biodegradation under Anaerobic Conditions. *Chemosphere*. 12(3): 397-404.

Brown, D. and Laboureur, P. (1983b). The Aerobic Biodegradability of Primary Aromatic Amines. *Chemosphere*. 12(3): 405-414.

Brown, D. and. Hamburger, B. (1987). The degradation of dyestuffs: Part III – Investigations of their ultimate degradability. *Chemosphere.* **16**(7): 1539-1553.

Buitron, G, Quezada, M. and Moreno, G. (2004). Aerobic Degradation of the Azo DyeAcid Red 151 in a Sequencing Batch Biofilter. Bioresour. Technol. **92**:143-149.

Bryers, J. D. (Ed.). (2000). Biofilms II: process analysis and applications. Wiley-Liss.

Byberg, R. (2012). Photocatalytic Degradation of a Series of Direct Azo Dyes Using Immobilized TiO2. Doctoral dissertation. Worcester Polytechnic Institute.

Carliell, C.M., Barclay, S.J., Naidoo, N., Buckley, C.A., Mulholland, D.A. and Senior, E. (1995). Microbial Decolourisation Of A Reactive Azo Dye Under Anaerobic Conditions. Water SA. **21**: 61-69.

Castillo-Carvajal, L., Ortega-González, K., Barragán-Huerta, B. E., & Pedroza-Rodríguez, A. M. (2012). Evaluation of three immobilization supports and two nutritional conditions for reactive black 5 removal with Trametes versicolor in air bubble reactor. *African Journal of Biotechnology.***11**(14): 3310-3320.

Chan, G.F., Rashid, N.A.A., Koay, L.L., Chang, S.Y., Tan, W.L. (2011)." Identification and optimization of novel NAR-1 bacterial consortium for the biodegradation of Orange II". *Insight Biotechnol*. **1**: 7–16.

Chan, G. F., Rashid, N. A. A., Chua, L. S., Nasiri, R., and Ikubar, M. R. M. (2012). Communal microaerophilic–aerobic biodegradation of Amaranth by novel NAR-2 bacterial consortium. *Bioresource Technology* **105**: 48-59

.Chang, Y. C., and Chou, C. C. (2002). Growth and production of cholesterol oxidase by alginate-immobilized cells of Rhodococcus equi No. 23.*Biotechnology and applied biochemistry*. **35**(2): 69-74.

Chacko, J. T., and Subramaniam, K. (2011). Enzymatic degradation of azo dyes—a review. *Int J Environ Sci.* **1**(6): 1250-1260.

Chakrabarti, T., Subrahmanyam, P., and Sundarsan B. (1988). Biodegradation of recalcitrant industrial wastes. Biotreatment Systems-Vol. II. Wise D., editor. CRC Press, Inc., Boca Raton, FL.

Chen, K. C., Wu, J. Y., Liou, D. J., and Hwang, S. C. J. (2003). Decolorization of the textile dyes by newly isolated bacterial strains. *Journal of Biotechnology*.**101**(1): 57-68.

Chen, B. Y. and Chang, J.-S. (2007). Assessment upon species evolution of mixed consortia for azo dye decolorization. *J. Chin Inst. Chem. Eng*. **38**: 259-266.

Chin Wang, C., Mei Lee, C., Jen Lu, C., Shang Chuang, M., and Zong Huang, C. (2000). Biodegradation of 2, 4, 6-trichlorophenol in the presence of primary substrate by immobilized pure culture bacteria. *Chemosphere*. **41**(12): 1873-1879.

Chinwetkitvanich S., Tuntoolvest M., and Panswad T. 2000. Anaerobic Decolorization of Reactive Dyebath Effluents by a Two -Stage UASB System with Tapioca as a Co-substrate. *Water Research*. **34**(8): 2223-2232

Costerton, J. W., Stewart, P. S. and Greenberg, E. P., (1999). Bacterial biofilms: a common cause of persistent infections. *Sci*. **284**: 1318–1322

Davis, M. E. (1991). Zeolites and molecular sieves: not just ordinary catalysts. *Industrial Engineering Chemical Resource*. 30: 1675-1683.

Deive, F. J., Domínguez, A., Barrio, T., Moscoso, F., Morán, P., Longo, M. A., and Sanromán, M. A. (2010). Decolorization of dye Reactive Black 5 by newly isolated thermophilic microorganisms from geothermal sites in Galicia (Spain).*Journal of hazardous materials*. **182**(1): 735-742.

Dı´az , M. P., Boyd, K. G, Grigson, S. J. W. and Burgess, J. G. (2002). Biodegradation of crude oil across a wide range of salinities by an extremely halotolerant bacterial consortium MPD-M, immobilized onto polypropylene fibers.Biotechnol. *Bioeng*. **79**:145–153.

Donlan, R. M. (2002). *Biofilm: Microbial life on surfaces*. USA: Medscape Publisher.

Ersoy, B. and Çelik, M. S. (2003). Effect of Hydrocarbon Chain Length On Adsorption of Cationic Surfactants Onto Clinoptilolite. *Clays Clay Min*. **51** (2): 173-181.

Ferreira, V. S., Magalhaes, D. B., Kling, S. H., Da Silva, J. G., & Bon, E. P. S. (2000). N- Demethylation of methylene blue by lignin peroxidases from Phanerochaete chrysosporium. *Applied Biochemistry and Biotechnology*. **84**: 255–265.

Firmino, P. I. M., da Silva, M. E. R, Mota, F. S. B. and dos Santos, A. B. (2011). Applicability of anthraquinone-2,6-disulfonate (AQDS) to enhance colour removal in mesophilic UASB reactors treating textile wastewater. *Braz. J. Chem. Eng*. **28**(4):617-624.

Fitzgerald, S. W., and Bishop, P. L. (1995). Two Stage Aerobic/Aerobic Treatment of Sulfonated Azo Dyes. *J. Environ. Sci. Health*. **30**(6): 1251-1276.

Forgacs, E., Cserháti, T., Oros, G. (2004). Removal of synthetic dyes from wastewaters: a review. *Environ Int*. 30: 953-971.

Ganesh R. (1992). Fate of Azo Dyes in Sludges. Masters Thesis, Virginia Polytechnic Institute and State University. 193

Ganesh, R., Boardman, G. D., and Michelsen, D., (1994), Fate of azo dyes in sludges. *Wat Res*. **28**(6): 1367-1376.

Georgiou, D., Hatiras, J., and Aivasidis, A. (2005). Microbial immobilization in a two-stage fixed-bed-reactor pilot plant for on-site anaerobic decolorization of textile wastewater. *Enzyme and Microbial Technology*. **37**(6), 597-605.

Ghodake, G. S., Talke, A. A., Jadhav, J. P., & Govindwar, S. P. (2009). Potenntial of Brissica juncea in order to Treat Textile Effluent Contaminated Sites. *International Journal of Phytoremediation.* **11**(4) 297-312.

Gingell, R., Walker, R. (1971). Mechanism of azo reduction by Streptococcus faecalis II, the role of soluble flavins. *Xenobiotics*. **1**(3): 231-239.

Glaudio, P.D., Colombo, P., Colombo, G., Russo, P. and Sonvico, F. (2005). *Internat. J..Pharmaceutics*. **302**(1–2): 1–9.

Goksungur, Y., Gunduz, M. and Harsa, S. (2005). Optimization of lactic acid production from whey by *L. casei* NRRL B-441 immobilized in chitosan stabilized Ca-alginate beads. *J. Chem. Technol. Biotechnol*. **80**:1282–1290

Hamedaani, H. R., Sakurai, A., & Sakakibara, M. (2007).Decolorization of synthetic dyes by a new manganese peroxidase producing white rot fungus. *Dyes and Pigments*. **72**: 157–162.

Hentzer, M., Teitzel, G.M., Balzer, G.J., Heydorn, A., Molin, S., Givskov, M. and Parsek, M.R. (2001). Alginate overproduction *affects Pseudomonas aeruginosa* biofilm structure and function. *J. Bacteriol*. **183**:5395-5401.

Heydorn, A., Ersboll, B., Kato, J., Hentzer, M., Parsek, M.R., Tolker-Nielsen, T.,Givskov, M., Molin, S. (2002). Statistical analysis of Pseudomonas

aeruginosa biofilm development: impact of mutations in genes involved in twitching motility, cell-to-cell signaling, and stationary-phase sigma factor expression. *Appl. Environ. Microbiol*. **68**:2008-2017.

Hildenbrand, S., Schmahl, F.W., Wodarz, R. Kimmel, R., and Dartsch, P. C. (1999) Azo dyes and carcinogenic aromatic amines in cell culture. *International Archives of Occupational and Environmental Health*. **72**(3): 52-56.

Hong, Y., Guo, J., Xu, M., and Sun, G. (2007). Reduction and partial degradation mechanisms of naphthylaminesulfonicazo dye Amaranth by Shewanelladecolorationis S12. *Applied Microbiology Biotechnology*. **75**(3): 647-654

Hrenovic, J., Kovacevic, D., Ivankovic, T. and Tibljas, D. (2011). Selective immobilization of Acinetobacter junii on the natural zeolitized tuff in municipal wastewater. *J. Colloids and Surfaces B: Biointerfaces*. **88**: 208–214.

Hrenovic, J. , Rozic, M., Ivankovic, T. and Farkas, A. (2009). Biosorption of phosphate from synthetic wastewater by biosolids. *Cent. Eur. J. Biol*. **4**(3): 397–403.

Hrenovic, J., Rozic, M., Sekovanic, L. and Anic-Vucinic, A. (2008). Interaction of surfactant-modified zeolites and phosphate accumulating bacteria. *J. Hazard.Mater*. 156: 576–582.

Hrenovic, J., Tibljas, D., Ivankovic, T. , Kovacevic, D. and Sekovanic, L. (2010). Sepiolite as carrier of the phosphate-accumulating bacteria *Acinetobacter junii*. *Appl. Clay Sci*. **50**:582–587.

Hogan, D. and Kolter, R. (2002). Why are bacteria refractory to antimicrobials *Curr.Opin. Microbiol*. **5**:472–477.

Ikubar, M. R. B. M. (2010). Decolourisation of amaranth by bacterial consortium immobilised onto modified zeolite-NaX, Universiti Teknologi Malaysia, Skudai. Bachelor Degree

Isaka, K., Yoshie, S., Sumino, T., Inamori, Y., and Tsuneda, S. (2007). Nitrification of landfill leachate using immobilized nitrifying bacteria at low temperatures. *Biochemical Engineering Journal.* **37**(1): 49-55.

Işik, M. and Sponza D. T. (2003). Effect of oxygen on decolorization of azo dyes by Escherichia coli and Pseudomonas sp. and fate of aromatic amines. *Process Biochemistry.* **38**(8): 1183-1192.

Işık, M. and Sponza D. T. (2004). A batch kinetic study on decolorization and inhibition of Reactive Black 5 and Direct Brown 2 in an anaerobic mixed culture. *Chemosphere.* **55**(1): 119-128.

Iskakov, R. M., Kikuchi, A. and Okano, T. (2002). Time-programmed pulsatile release of dextran from calcium-alginate gel beads coated with carboxy-npropylacrylamide copolymers. *J. Controlled Release*. **80**(1-3): 57-58.

James, G. A., Beaudette, L. and Costerton, J. W. (1995). Interspecies bacterial interactions in biofilms. *J. Ind. Microbiol*. **15**: 257-262.

Joshi, T., Iyengar, L., Singh, K., Garg, S. (2008). Isolation, identification and application of novel bacterial consortium TJ-1 for the decolorization of structurally different azo dyes. *Bioresour. Technol*. **99**: 7115–7121.

Kalyani, D. C., Telke, A. A., Dhanve, R. S., & Jadhav, J. P. (2009). Ecofriendly biodegradation and detoxification of Reactive Red 2 textile dye by newly isolated *Pseudomonas sp. SUK1*. *Journal of Hazardous Materials.* **163**(2): 735-742.

Karatas, M., and Dursun, S. (2007). Bio-decolourization of azo-dye under anaerobic batch conditions. *J. Int. Environmental Application & Science.* **2**(1-2): 20-25.

Khan, R., and Banerjee, U. C. (2010). Decolorization of azo dyes by immobilized bacteria. *Biodegradation of Azo Dyes Springer*. 73-84.

Khehra, M.S., Saini, H.S., Sharma, D.K., Chadha, B.S., Chimni, S.S. (2005). Comparative studies on potential of consortium and constituent pure bacterial isolates to decolorize azo dyes. *Water Res*. **39**: 5135–5141.

Khodakov, A. Y., Kustov, L. M., Kazansky, V. B., and Williams, C. (1993). Infrared spectroscopic study of the interaction of cations in zeolites with simple molecular probes. Part 3.- Adsorption and polarization of methane and ethane on cationic forms of high-silica zeolites. *J. Chem. Soc., Faraday Trans.* **89**(9): 1393-1395.

Kim, S. Y., An, J. Y., and Kim, B. W. (2008). The effects of reductant and carbon source on the microbial decolorization of azo dyes in an anaerobic sludge process. *Dyes and pigments*. **76**(1): 256-263.

Kudlich, M.,. Hetheridge, M. J., Knackmuss, H. J., and Stolz, A. (1999). *Environ. Sci. Technol*. **33**:. 836.

Klepacz-smółka, A., Paździor, K., Ledakowicz,S., Sójka-ledakowicz, J, Mrozińska, Z.and Żyłła, R. (2009). Kinetic studies of decolourisation of concentratesFromnanofiltration treatment of real textile effluents in anaerobic/aerobic sequencingbatch reactors. *Environ. Protect. Eng*. **35** (3): 1-11.

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

Li, Z. (1999). Sorption kinetics of hexadecyltrimethylammonium on natural clinoptilolite. *Am. Chem. Soc.* (1999), 15, 6438-6445.

Li, Z., Alessi, D. and Allen, L. (2002). Influence of quaternary ammonium on sorption of selected metal cations onto clinoptilolite zeolite. *Journal of Environmental Quality*. **31**(4): 1106-1114.

Libra, J. A., Borchert, M., Vigelahn, L., and Storm, T. (2004). Two stage biological treatment of a diazo reactive textile dye and the fate of the dye Metabolites. *Chemosphere* **56**(2): 167-180.

Lilan Sollan, K. (2008). *SEQUENTIAL ANAEROBIC-AEROBICBIODEGRADTION OF REMAZOL BLACK B USING BACTERIAL CONSORTIUM IN A STIRRED TANK REACTOR. Master Thesis. Universiti* Teknologi Malaysia, Skudai.

Loyd, Chapman K. (1992). Anaerobic/Aerobic Degradation of a Textile Dye Wastewater. Masters Thesis, Virginia Polytechnic. Institute and State University 184 pp.

Margeta, K., Logar, N. Z., Šiljeg, M., and Farkaš, A. (2013). Natural Zeolites in Water Treatment–How Effective is Their Use.

Mbuligwe, S., E. (2005). Comparative treatment of dye-rich wastewater in engineered wetland systems (EWSs) vegetated with different plants. *Water research.* **39**(2): 271-280.

Mi-Na, Z., Xue-Pin, L. and Bi, S. (2006). Adsorption of Surfactants on Chromium Leather Waste. *J. Soc. Leather Technol*. *Chem*. **90**: 1.

Méndez-Paz, D., Omil, F. and Lema, J. M. (2005). Anaerobic treatment of azo dye Acid Orange 7 under batch conditions. *Enzyme and Microbial Technology*. **36**(2-3): 264-272.

Mezohegyi, G., Kolodkin, A., Castro, U.I., Bengoa, C., Stuber, F., Font, J., Fabregat, A.and Fortuny, A. (2007). Effective Anaerobic Decolorization of Azo Dye Acid Orange 7 in Continuous Upflow Packed-Bed Reactor Using Biological Activated Carbon System. *Indust. Eng. Chem. Res*. **46**: 6788-6792

Mohanty, S., S., Dafale, N., and Rao, N. N. (2006). Microbial decolorization of reactive black-5 in a two-stage anaerobic–aerobic reactor using acclimatized activated textile sludge. *Biodegradation* **17**(5): 403-413.

Moosvi, S., Keharia, H., and Madamwar, D. (2005). Decolourization of textile dye Reactive Violet 5 by a newly isolated bacterial consortium RVM 11.1. *World Journal of Microbiology and Biotechnology.* **21**(5): 667-672.

Mumpton, F. A. and Fishman, P. H. (1977). The application of natural zeolites in animal science and aquaculture. *Journal of Animal Science*. 45(5): 1188-1203.

Nessler, J. (1868). Ueber Bestimmung des Ammoniaks und der Salpetersäure in sehr verdünnten Lösungen. *Fresenius' Journal of Analytical Chemistry*. **7**(1): 415-416.

Naimabadi, A., Attar, H. M., and Shahsavani, A. (2009). Decolorization and biological degradation of azo dye reactive Red2 by anaerobic/aerobic sequential process. *Iranian Journal of Environmental Health Science & Engineering*. ***6***(2): 67-72.

Ogugbue, C. J., Morad, N., Sawidis, T. and Oranusi, N. A. (2012). Decolorization and partial mineralisation of a polyazo dye by Bacillus firmus immobilized within tubular polymeric gel. *3 Biotech*. **2**(1): 67-78.

O'Neil C., Lopez A., Estevez S., Hawkes F.R., Hawkes D.L., and Wilcox S. (2000). Azo- dye degradation in an anaerobic-aerobic treatment system operating on simulated textile effluent. *Appl. Microbiol. Biotechno*. **53**:249-254.

Pagga U. and. Brown, D. (1986). The Degradation of Dyestuffs: Part II. Behaviour of Dyestuffs in Aerobic Biodegradation Tests. *Chemosphere*. **15**(4): 479-491

Pazarlioglu,N. K., Urek, R.O. and Ergun, F. (2005)..Biodecolourization of Direct Blue 15 by immobilized Phanerochaete chrysosporium. *Proc. Biochem*. **40** (5):1923-1929.

Pearce, C.I., Guthhrie, J.T., and Lloyd, J.R. (2003). The Removal of Colour From Textile Wastewater Using Whole Bacterial Cells : A Review. *Dyes and Pigments*. **58:** 179-196.

Perei, K., Rakhely, G., Kiss, I., and Kovacs, K.L. (2001). Biodegradation of sulfanilic acid by Pseudomonas paucimobilis. *Appl. Microbiol. Biotechnol.* **55**: 101-107.

Plum, A., Braun, G., and Rehorek, A. (2003). Process monitoring of anaerobic azo dye degradation by high-performance liquid chromatography–diode array detection continuously coupled to membrane filtration sampling modules.*Journal of Chromatography A*. **987**(1): 395-402.

Razo-Flores E., Luijten M., Donlon B., Lettinga G., Field J. (1997). Biodegradation of Selected Azo Dyes Under Methanogenic Conditions. *Water Science Technology*. **36**(6-7): 65-72.

Rahman, R. N.Z.., Farinazleen, M. G., Abu Bakar, S. and Mahiran, B. (2006). Biodegradation of hydrocarbon contamination by immobilized bacterial cells. *J.Microbiol.* **44** (3):354–359.

Rehorek, A., and Plum, A. (2006). Online LC-MS-MS process monitoring for optimization of biological treatment of wastewater containing azo dye concentrates. *Analytical and bioanalytical chemistry*. **384**(5): 1123-1128.

Sandhya, S. (2010). Biodegradation of azo dyes under anaerobic condition: role of azoreductase. *Biodegradation of Azo Dyes, Springer*: 39-57.

Sanjeev Kumar, S., Kumar, M. S., Siddavattam, D., and Karegoudar, T. B. (2012). Generation of continuous packed bed reactor with PVA–alginate blend immobilized *Ochrobactrum sp.* DGVK1 cells for effective removal of N,N-dimethylformamide from industrial effluents. *Journal of hazardous materials.* **199**: 58-63.

Saratale, R.G., Saratale, G.D., Chang, J.S., Govindvar, S.P. (2011). Bacterial decolorization and degradation of azo dyes: a review. J. *Taiwan Inst. Chem. E*. **42**: 138–157.

Scott, G.V. (1968). Spectrophotometric determination of cationic surfactants with orange II. *Anal. Chem*. **40**(4): 768-773.

Selcuk, H. (2005). Decolourization and detoxification of textile wastewater by ozonation and coagulation processes. *Dyes and Pigments*. **64**: 217-222.

Sen, S., & Demirer, G. N. (2003). Anaerobic treatment of real textile wastewater with a fluidized bed reactor. *Water Research*. **37**(8): 1868-1878.

Senan, R. C., Shaffiqu, T. S., Roy, J. J. and Abraham, T. E. (2003). Aerobic Degradation of a Mixture of Azo Dyes in a Packed Bed Reactor Having Bacteria-Coated Laterite Pebbles. *Biotechnol. Prog*. **19**: 647-651.

Seshadri, S., Bishop, P. L., and Agha, A. M. (1994). Anaerobic/Aerobic Treatment of Selected Azo Dyes in Wastewater. *Waste Management*. **14**(2): 127-137.

Sharma, D.K., Saini, H.S., Singh, M., Chimni ,S.S. and Chadha, B.S. (2004). Biological treatment of textile dye Acid violet-17 by bacterial consortium in an up-flow immobilized cell bioreactor. *Lett. Appl. Microbiol*. **38**: 345–350.

Shieh, W. and Hsu, Y. (1996). Biomass loss from an anaerobic fluidized bed reactor.*Water Res*. **30**:1253–1257.

Singh, K., and Arora, S. (2011). Removal of synthetic textile dyes from wastewaters: a critical review on present treatment technologies. Critical Reviews in *Environmental Science and Technology*. **41**(9), 807-878.

Simose, M., Bennett, R. N., and Rosa, E. A. (2009). Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. *Natural product reports.* **26**(6): 746-757.

Stolz, A. (2001). Basic and applied aspects in the microbial degradation of azo dyes. *Appl Microbiol Biotechnol*. **56**: 69-80.

Tan, N. C. G., Prenafeta-Boldu, F. X., Opsteeg, J. L., (1999). Biodegradation of azo dyes in cocultures of anaerobic granular sludge with aerobic aromatic amine degrading enrichment cultures. *Applied Microbiology Biotechnology*. **51**: 865-871.

Tan, N. C. G. (2001). Integrated and Sequential Anaerobic/Aerobic Biodegradation of Azo Dyes. Environmental Technologies to Treat Sulfur Pollution – Principles and Engineering. 377-392.

Tavaker, M., Svobodova, K., Kuplenk, J., Novotny, C., & Pavko, C. (2006). Biodegradation of Azo Dye RO16 in different reactors by immobilized Irpex lacteus. Acta Chimica Slovenica. **53:** 338–343.

Tony, B.D., Goyal, D. and Khanna, S. (2009). Decolorization of Direct Red 28 by mixed bacterial culture in an up-flow immobilized bioreactor. *J. Ind .Microbiol.Biotechnol*. **36**:955–960.

Tope, A. M., Jamil, K., and Baggi, T.R. (1999). Transformation of 2,4, 6-trinitrotoluene (TNT) by immobilized and resting cells of Arthrobacter sp. *J. Hazard Subst.Res*. **2**(3):1–8.

Vaidya, A. A. and Datye, K. V. (1982). Environmental pollution during chemical processing of synthetic fiber. *Colorate* **14**: 3–10.

Van Der Zee, F.P. (2002). Anaerobic azo dye reduction. Doctoral Thesis. Wageningen University.

Van der Zee, F. P. and Villaverde S. (2005). Combined anaerobic–aerobic treatment of azo dyes—a short review of bioreactor studies. *Water Research* **39**(8): 1425-1440

Vandevivere, P.C., Bianchi, R. and Verstraete, W. (1998). Review: Treatment and reuse of wastewater from the textile wet-processing industry: Review of emerging technologies. *J. Chem Technol. Biotechnol*. **72**: 289-302.

Verma, P. and. Madamwar, D (2003). Decolourization of synthetic dyes by a newly isolated strain of Serratia marcescens. *World Journal of Microbiology and Biotechnology*. **19**(6): 615-618.

Wallace, T.H. (2001). "Biological treatment of a synthetic dye water and an industrial textile wastewater containing azo dye compounds". Master thesis.Virginia Polytechnic Institute.

Wang, L. and Langley, D. (1975). Determining Cationic Surfactant Concentration. *Prod. R and D*. **14**: 210-212.

Wang, L., Sheltona, R.M., Cooper, P.R., Lawson, M., Triffitt,J.T. and Barralet, J.E.(2003). Evaluation of sodium alginate for bone marrow cell tissue engineering. *Biomater*. **24**: 3475–3481

Wang, H., Zheng, X. W., Su, J. Q., Tian, Y., Xiong, X. J., and Zheng, T. L. (2009). Biological decolorization of the reactive dyes Reactive Black 5 by a novel isolated bacterial strain *Enterobacter* sp. EC3. *Journal of hazardous materials* **171**(1): 654-659.

Warchoł, J., Misaelides, P., Petrus, R. and Zamboulis, D. (2006). Preparation and application of organo-modified zeolitic material in the removal of chromates and iodides. *J. Hazard. Mater*. **137**: 1410-1416.

Webb, J.S., Givskovy, M. and Kjelleberg, S. (2003). Bacterial biofilms: prokaryotic adventures in multicellularity. *Curr. Opin. Microbiol*. **6**(6):578-85.

West, T. P. and Strohfus, B. (2001). Polysaccharide production by immobilized Aureobasidium pullulans cells in batch bioreactor. *Microbiol Res*. **156**:285–288

Wong, P.K., and Yuen, P.Y. (1996). Decolourisation and biodegradation of methyl red by Klebsiella pneumoniae R-13. *Water Res*. **30**:1736–1744.

Wuhrmann, K., Mechsner, K. and Kappeler, T. (1980). Investigation On Rate-Determining Factors In The Microbial Reduction Of Azo Dyes. *Eur. J. Applied Microbiology Biotechnology*. **9**: 325-338

Yongjie, M. (2004). Biological remediation of dyes in textile effluent: A review on current technologies. *Biotechnology Advanced.* **11**: 52-85.

Zollinger, H. (2003). Color chemistry: syntheses, properties, and applications of organic dyes and pigments.3rd edition Wiley-Vch.

Zouari-Mechichi, H., Mechichi, T., Dhoui, A., Sayad, S., Martínez, A. T., and Martínez, M. J. (2006). Laccase purification and characterization from Trametes trogiiisolated in Tunisia: decolorization of textile dyes by the purified enzyme. *Enzyme and Microbial Technology* **39**: 141–148.