INDUCED ENZYME ACTIVITIES BY ACCLIMATISED BAC-ZS MIXED CULTURE DURING THE TREATMENT OF ACID ORANGE 7

NADHIRAH AMINAH BT AZIZAN

A dissertation submitted in partial fulfillment of the
requirements for the award of the degree of
Master of Science (Biotechnology)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

JULY 2014

To my beloved parents and family members:

Azizan Yahya

Rohanah Baharom

Ahmad Faiz Azizan

Roziyati Abdullah

Nadiah Aminah Azizan

Ahmad Zharif Azizan

Norhayati Baharom

ACKNOWLEDGEMENT

I would like to express my deepest appreciation to my supervisor, Assoc. Prof. Dr Zaharah Ibrahim for the valuable comments, remarks, guidance and advices whilst giving me the leeway to work on my own way in accomplishing this project. Without her supervision and constant help, the accomplishment of this dissertation would not have been possible.

My sincerest thanks also to the laboratory staffs and fellow friends especially Environmental Biotechnology Laboratory 1 members (Kak Nida, Hanif, Fahmi, Wong, Ivy, Lam, Lim, Neoh, Kak Fareh, Kak Nad, Ahmad Idi) and my other colleagues (Suparman Mohd Said, Jiha, Zara, Lin, Kak Dalila, Soraya) who are always keen to lend a helping hand throughout my difficulties in completing this project. I am so grateful to know all of you and really indebted for the wonderful memories that we shared throughout the past years.

My special thanks are extended to my beloved parents (Azizan B.Yahya and Rohanah Bt Baharom), family and Mr S for their infinite support, concern and patience throughout my difficulties in completing my dissertation. I warmly appreciate their endless encouragement and understanding.

Last but not least, I would like to thank to those who are involved directly or indirectly during the accomplishment of this project. Thank you very much.

ABSTRACT

Azo dyes are the most common group of synthetic colourants released into the environment. Improper discharge of effluents containing azo dyes and their metabolites into the water bodies are detrimental as it generates highly coloured wastewater, and releases compounds that can be toxic, carcinogenic or mutagenic to living organisms. The acclimatised BAC-ZS mixed culture was able to decolourise azo dye, Acid Orange 7 (AO7) in a sequential facultative anaerobic-aerobic condition. The whole genome sequencing showed two possible enzymes are associated with decolourisation and degradation of AO7 which are azoreductase and NADH peroxidase. Both azoreductase and NADH peroxidase were produced intracellularly during treatment of AO7 using the sequential facultative anaerobic-aerobic condition. The maximum activity of azoreductase was obtained during the facultative anaerobic condition while the maximum activity of NADH peroxidase was obtained during the aerobic condition. During the facultative anaerobic condition (2 hours of static condition), azoreductase activity was about 2 fold (0.013U/mg) higher than aerobic conditions which only produced specific activity of 0.006 U/mg. These indicated that azoreductase was induced during the facultative anaerobic condition while NADH peroxidase was mainly induced during the aerobic condition. Its highest activity was obtained during the exponential phase under aerobic condition (48 hours agitation) with specific activity of 4.63 U/mg.

ABSTRAK

Pewarna azo adalah kumpulan pewarna sintetik yang sering dibebaskan ke alam sekitar. . Pembuangan kumbahan yang mengandungi pewarna azo dan metabolitnya secara tidak teratur ke dalam kawasan pengairan adalah memudaratkan kerana ia menghasilkan sisa air berwarna serta melepaskan sebatian yang bersifat toksik, karsinogenik atau mutagen terhadap organisma hidup. Kultur campuran teradaptasi BAC-ZS berkebolehan untuk menyahwarna pewarna azo melalui proses berturutan anaerob fakultatif-aerob. Penjujukan keseluruhan genom menunjukkan dua enzim yang mungkin terlibat dalam proses penyahwarnaan dan degradasi pewarna azo, Acid Orange 7 (AO7), iaitu enzim azoreductase dan NADH peroxidase. Kedua-dua enzim azoreductase dan NADH peroxidase menghasilkan enzim secara intrasel dalam keadaan anaerob fakultatif dan aerob. Aktiviti maksimum bagi azoreductase telah diperolehi ketika dalam keadaan fakultatif anaerob manakala aktiviti maksimum bagi NADH peroxidase diperolehi dalam keadaan aerob. Azoreductase menunjukkan aktiviti enzim 2 kali lebih tinggi (0.013U/mg) ketika dalam keadaan anaerob fakultatif (keadaan statik selama 2 jam) berbanding keadaan aerob yang hanya menghasilkan aktiviti spesifik sebanyak 0.006 U/mg. Penghasilan azoreductase telah dirangsang dalam keadaan anaerob fakultatif manakala NADH peroxidase penghasilannya dirangsang dalam keadaan aerob. Aktiviti tertinggi bagi NADH peroxidase telah diperolehi pada fasa eksponen dalam keadaan aerob (penggoncangan selama 48 jam) dengan spesifik aktiviti sebanyak 4.63 U/mg.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

%	-	Percentage
°C	-	Degree Celsius
g	-	Gram
kg	-	Kilogram
L	-	Liter
M	-	molarity
mg	-	Miligram
min	-	Minute
mL	-	Milliliter
mm	-	Millimeter
nm	-	Nanometer
ppm	-	Part per million
rpm	-	Rotation per minute
U	-	µmol per minute
w/v	-	Weight/volume
µL	-	Micro Liter
µmol	-	Micromol

LIST OF ABBREVIATIONS

AO7	-	Acid Orange 7
BAC-ZS	-	*Brevibacillus panacihumi*, *Lysinibacillus fusiformis* and *Enterococcus faecilis*.
BSA	-	Bovine Serum Albumin
CFE	-	Cell free extract
COD	-	Chemical Oxygen Demand
CS	-	Culture Supernatant
df	-	Dilution factor
FADH	-	Flavin adenine dinucleotide (reduced)
NADH	-	Nicotineamide-adenine-dinucleotide (reduced)
OD	-	Optical Density

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

Textile industry is one of the main sources of severe pollution problems worldwide due to the high concentration of dyes in the wastewaters. There are different textile dyes being produced and commercially available in the whole world. According to Zaharia *et al.* (2012), about 10,000 different textile dyes are produced annual production of approximately 7.105 metric tonnes. The highest production of textile dyes and improper discharged of the effluent lead to serious water pollution. Approximately, 2-20% of the textile dyes are directly discharged as aqueous effluents into the water bodies (Zaharia *et al.,* 2012). The discharge of dye-containing effluents into the water bodies is detrimental because it produces wastewater which is highly coloured and contains products of incomplete breakdown of azo dyes that can be toxic, carcinogenic or mutagenic to living organisms. Examples of carcinogens are benzidine, naphthalene and other aromatic compounds which may remain in the environment for a long period of time if the wastewater or effluents are not treated properly (Zaharia *et al.,* 2009).

In general, azo dyes are widely used in various industries such as textile, printing application, cosmetics and food. About 3000 different azo dyes are used to

satisfy the consumers' demands for colors appeal in food, textile and printing industries (Coughlin *et al.,* 2002). Besides, azo dyes also have interesting characteristics such as low cost, heat stable and do not fade when exposed to light or oxygen and definitely have color varieties which are favorable to be applied to the industries.

Textile industries produce wastewater that is coloured and affects the aesthetic value of water bodies with high content of toxic chemicals. Reactive azo dyes are known to be stable (in terms of wide range of pH, heat stable and insensitive to light and oxygen) and xenobiotic which make them recalcitrant. Thus, they cannot be fully degraded by conventional wastewater treatment processes that involve light, chemicals or activated sludge (Chung *et al.,* 1992; Chacko *et al.,* 2010).

Various azo dyes, mainly aromatic compounds, show both acute and chronic toxicity. This is because azo dyes and their breakdown products (toxic amines) can be adsorbed via the gastrointestinal tract, skin, lungs, and damage of DNA that can lead to the genesis of malignant tumors (Zaharia *et al.,* 2009). In addition, it may also give negative impact to the aquatic life. For example, high concentrations of textile dyes in water bodies will prevent the penetration of sunlight, thereby upsetting biological activity in aquatic life and also the photosynthesis process of aquatic plants or algae (Wang *et al.,* 2011; Zaharia *et al.,* 2009). In order to overcome the problem, several treatment methods had been applied such as physico-chemical methods and biological treatment methods to treat dye wastewater to meet the discharge level according to the Environmental Quality Act (Zhao *et al*., 2010).

Both physical and chemical treatment methods give negative impacts and not practical to be applied as they commonly result in the accumulation of hazardous sludge, secondary pollution due to the formation of hazardous byproducts, high chemical consumption and high maintenance costs. The presences of sludge or secondary wastes in the physical treatment poses difficulties in disposal, high cost

sludge management and limit the reusability of these methods. The chemical treatment process also requires high consumption of reagent, high power consumption for certain chemicals which lead to high maintenance cost (Anbalagan, 2012).

Alternatively, biological treatment method is more reliable as it is more environmentally friendly and cost effective. It may involves variety of bacteria which is able to decolorise dye-containing wastewater and also capable of completely mineralise many reactive dyes under specific optimum conditions (Van der Zee and Villaverde, 2005; Lim *et al.,* 2013). This approach consists of sequential facultative anaerobic-aerobic phase along its treatment process.

During the anaerobic process, the azo bonds undergo reductive cleavage of azo bonds and contribute to the decolourisation of the dye. However, it produces aromatic amines that are carcinogenic which will be further oxidized into less harmful products during the aerobic phase treatment (Erkurt *et al.,* 2010). Hence, the combination of the two phases helps in the treatment of azo dye by decolourization and removal of toxic metabolites. (Anbalagan, 2012).

Acid Orange 7 (AO7) is one of azo dyes present in textile wastewater and is difficult to treat because of the presence of sulfonic acid groups in its chemical structure . Extensive studies had been carried out to achieve effective degradation processes of AO7 (Liu *et al.,* 2013*;* Bay *et al.,* 2014). Because of this, several predictions of AO7 degradation pathway had been proposed in order to understand, investigate and analyse the intermediates or by products formed during the treatment process.

1.2 Statement of Problems

There are a lot of treatment processes that had been proposed in order to increase the effectiveness such as physical, chemical and biological treatment process. One of the physical methods is based on coagulation-flocculation of dyes but this method is restricted to certain types of dyes. Chemical treatment process involves the use of oxidizing agent such as ozone, hydrogen peroxide and permanganate. However, it is less practical for treating dyes that are insoluble in water, has low COD removal capacity as well as the high cost of the oxidizing agents (Saratale *et al.,* 2011). One of the most widely used treatment processes is the biological treatment process as it owns several advantages over others such as cost-effectiveness and environmental friendly.

The mechanisms of microbial degradation are by enzymatic degradation. This involves both reductases and oxidases in order to achieve the standard of water quality in decolourization and detoxification of dye-contaminated effluent before being released into the environment (Solis *et al.,* 2012). However, not all of the enzymes can be induced or activated during the degradation of azo dyes. A complete genome sequence analysis is capable of detecting the functional gene via that gives an overview to the possible expression of the gene within the cellular compartments and the behavior of the whole organisms. The results of the whole genome sequencing can be used to detect the presence of genes encoding for enzymes in a genome (Claudel-Renard *et al.,* 2003). The genes, however, may not be expressed under certain experimental conditions. Based on the whole genome sequencing, enzymes that are related to decolourisation and degradation of azo dyes have been detected which are NADH peroxidase and azo reductase. The presence of the enzymes for decolorization and degradation of azo dyes were tested experimentally.

1.3 Objectives

The primary aim of this study was to detect the presence of several enzymes based on the full genome sequencing of each microbe in a mix bacterial culture (Bay *et al.*, 2014). There are two (2) enzymes which are nadh dependent azoreductase and NADH peroxidase that might involve in decolourisation and degradation of AO7 by the mix culture in a sequential anaerobic/aerobic treatment process. Thus, the main objectives were:

a) To apply the acclimatised mixed bacterial culture for the decolourisation and degradation of AO7 under sequential facultative anaerobic-aerobic condition by looking into decolourisation of AO7, growth profile of the mixed culture and COD removal of the culture.
b) To determine the localization of azoreductase and NADH peroxidase produced by the mix culture
c) To determine enzyme activities of azoreductase and NADH peroxidase during treatment of AO7

d) Scope of study

In this research study, bacterial consortium or also known as *MicroClear* would be used for treating colored wastewater taken from a local textile industry. However, instead of using the real textile wastewater, mono azo dye, Acid Orange 7 (AO7) was used as the model dye to study the biodegradation process. The experiments were initiated by decolorisation of AO7 by the mix culture in the sequential facultative anaerobic-aerobic conditions based on its optimum conditions (Bay *et al.*, 2014). The two (2) enzymes, azoreductase and NADH peroxidase that are mainly related to

decolourisation and degradation respectively, were assayed during the two different phases of the treatment process.

1.4 Significance of study

Detection and quantification of azoreductase and NADH peroxidase that involve in decolorization and degradation of AO7 are important to provide strong evidence in determining the degradation pathway of AO7 based on the full genome sequencing of the bacterial mixed culture. Modifications or alterations for better performance of the potential microbes can be done if a clear degradation pathway is obtained. Successful quantification of the enzyme production during two different conditions will give important results and thus, can be applied to improve the effectiveness of azo dyes degradation particularly AO7 in the future.

REFERENCES

Allaby M. (2008). A Dictionary of Earth Sciences 3rd Edition. Oxford University Press, United Kingdom, London.

Anbalagan A. (2012). Combination of biological and photochemical treatment for degradation of azo dyes. *Degree project in applied biotechnology, Master of Science.* Biology Education Centre, Uppsala University, and Department of biotechnology, Lund University, Sweden.

Baban A., Yediler A. and Ciliz N.K. (2010). Integrated water management and CP implementation for wool and textile blend processes. *Clean.* **38**: 84-90.

Bafana A., Chakrabarti T., Muthal P. and Kanade G. (2009). Detoxification of Benzidine-Based Azo Dye by *Enterococcus gallinarum*: Time-Course Study. *Ecotoxicology Environment Safety*. **72(3)**: 960-964.

Banat I. M., Nigam P., Singh D. and Marchant R. (1996). Microbial Decolorization of Textile-Dye-Containting Effluents: A Review. *Bioresource Technology* **58**: 217-227.

Bae W. C., Lee H., Choe Y., Jahng D. and Lee S. *et al*. (2005). Purification and Characterization of NADPH-Dependent Cr (VI) Reductase from *Escherichia coli* ATCC 3345. *The Journal of Microbiology.* **43**: 21-27.

Bay H. H., Lim C.K., Kee T.C., Ware I., Chan G.K., Shahir S. and Ibrahim Z. (2014). Decolourisation of Acid Orange 7 Recalcitrant Auto-Oxidation Coloured By- Products using an Acclimatised Mixed Bacterial Culture. *Environment Science Pollution Research.* **21**: 3891–3906.

Bay, H.H. (2014). Decolourisation and degradation of Acid Orange 7 using an acclimatised BAC-ZS mixed bacteria culture. Doctor Philosophy, Universiti Teknologi Malaysia, Skudai.

Barros V. P. and Assis M. D. (2013). Iron phorphyrins as biomimetical models for disperse azo dye oxidation. *Journal of Brazil Chemistry Society.* **24**: 830-836.

Bragger J.L., Lloyd A.W., Soozandehfar S.H., Bloomfield S. F., Marriott C., Martin G. P., (1997). Investigations into the Azo Reducing Activity of a Common Colonic Microorganism. *International Journal of Pharmaceutics.* **157**: 61–71.

Bromley-Challenor K. C. A., Knapp J.S., Zhang Z., Gray N.C.C., Hetheridge M.J. and Evans M.R. (2000). Decolorization of an Azo Dye by Unacclimated Activated Sludge under Anaerobic Conditions. *Water Research***. 34**: 4410-4418.

Chang, J. S. and T. S. Kuo (2000). Kinetics of Bacterial Decolorization of Azo Dye with *Escherichia coli* NO3. *Journal of Bioresource Technology*. **75**: 107-111.

Chang J. and Lin C. (2001). Decolorization Kinetics of a Recombinant *Escherichia Coli* Strain Harboring Azo-Dye-Decolorizing Determinants From *Rhodococcus* sp. *Biotechnology Letters.* **8**: 631-636.

Chung K. T. and Cerniglia C.E. (1992). Mutagenicity of Azo Dyes: Structure Activity Relationship. *Mutation Research.* **277**: 201–220.

Carbona S. L., Sauvageut N., Glard J., Benachour A., Posteraro B., Auffray Y., Sangulnettl M. and Hartke A. (2007). Comparative Study of the Physiological Roles of Three Peroxidases (NADH Peroxidase, Alkyl Hydroperoxide Reductase And Thiol Peroxidase) in Oxidative Stress Response, Survival Inside Macrophages and Virulence of *Enterococcus faecalis. Molecular Microbiology.* **66:**1148-1163.

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

Cervantes F.J., van der Velde S., Letinga G. and Field J.A. (2000). Quinones as Terminal Electron Acceptors for Anaerobic Microbial Oxidation of Phenolic Compounds. *Biodegradation.* **11**: 313-321.

Chacko J.T. and Subramaniam K. (2010). Enzymatic Degradation of Azo Dyes- A Review. *International Journal of Environmental Sciences.* **1**: 2011-2021.

Chen B. Y. and Chang J.S. (2007). Assessment Upon Species Evolution of Mixed Consortia for Azo Dye Decolorization. *Journal of the Chinese Institute of Chemical Engineers.* **38**: 259-266.

Dos Santos A. B., Cervantes F. J. and van Lier J. B. (2007). Review Paper on Current Technologies for Decolourisation of Textile Wastewater:

Perspectives for Anaerobic Biotechnology. *Bioresource Technology*. **98**: 2369-2385.

Coughlin MF, Kinkle B.K. and Bishop P.L. (2002). Degradation of Acid Orange 7 in an Aerobic Biofilm. *Chemosphere.* **46**:11–19.

Chequer F. M., Dorta D. J. and de Oliveira D. P. and Hauser P. (2011). Azo Dyes and Their Metabolites: Does the Discharge of the Azo Dye into Water Bodies Represent Human and Ecological Risks? (Advances in Treating Textile Effluent). Rijeka, Croatia : InTech.

Cirik K., Kitis M. and Cinar O. (2013). The Effect of Biological Sulfate Reduction on Anaerobic Color Removal in Anaerobic-Aerobic Sequencing Batch Reactors. *Bioprocess Biosystem Engineering*. **36**: 579-589.

Claiborne A., Ross R.P. and Parsonage D. (1992). Flavinlinked Peroxide Reductases: Protein-Sulfenic Acids and The Oxidative Stress Response. *Trends Biochemistry Science.* **17:** 183–186.

Claudel-Renard C., Chevalet C., Faraut T. and Kahn D. (2003). Enzyme-specific profiles for genome annotation: PRIAM. *Nucleic Acids Research.* **31**: 6633-6639.

dos Santos A. B., Cervantes F.J. and van Lier J.B. (2007). Review Paper on Current Technologies for Decolourisation of Textile Wastewaters: Perspectives for Anaerobic Biotechnology. *Bioresource Technology*. **98**: 2369-2385.

Erkurt H. A., Arshad M., Banerjee U.C., Bardi L., Bazerra R. M. F. and Cinar Ö *et al*. (2010). Biodegradation of Azo Dyes. *Handbook of Environmental Chemistry*. **9**: 1-37.

Fernandez R.F. and Kunz D.A. (2005). Bacterial Cyanide Oxygenase is a Suite of Enzymes Catalyzing the Scavenging and Adventitious Utilization of Cyanide as a Nitrogenous Growth Substrate. *Journal of Bacteriology*. **187**: 6396-6402.

Field J.A., Stams A.J.M., Kato M., Schraa G. (1995). Enhanced Biodegradation of Aromatic Pollutants in Cocultures of Anaerobic and Aerobic Bacterial Consortia. *Antonie Van Leeuwenhoek.* **67**:47–77.

Fitzgerald S. W. and Bishop P.L. (1995). Two Stage Anaerobic/aerobic Treatment of Sulfonated Azo Dyes. *Journal of Environmental Science Health*. **30**: 1251-1276.

Forgacs, E., T. Cserhati, and G. Oros (2004). Removal of Synthetic Dyes from Wastewaters: A Review. *Journal of Environment International*. **30**: 953-971.

Fraser C.M., Eisen J.A., Nelson K.E., Pualsen I.T. and Salzberg S.L. (2002). Sequencing (You Get What You Pay For) The Value of Complete Microbial Genome. *Journal of Bacteriology.* **23**:6403-6405.

Glenn J.K., Akileswaran L.L. and Gold M.H. (1986). Mn(II) oxidation is the principal function of the extracellular Mn-peroxidase from *Phanerochaete chrysosporium*. *Journal of Biochemistry and Biophysics.* **251(2):**688-696.

Gomare S. S., Tamboli D.P., Kagalkar A.N. and Govindwar S.P. (2009). Eco-friendly Biodegradation of a Reactive Textile Dye Golden Yellow HER by *Brevibacillus laterosporus* MTCC 2298. *International Biodeterioration and Biodegradation*. **63**: 582-586.

Gonzales C. F., Ackerley D. F., Lynch S. V. and Matin A. (2005). ChrR, a Soluble Quinone Reductase of *Pseudomonas putida* that Defends Against H_20_2. *The Journal of Biological Chemistry.* **280**: 22590-22595.

Higuchi M., Yamamoto Y. and Kamio Y. (2000). Molecular Biology of Oxygen Tolerance in Lactic Acid Bacteria: Functions of NADH Oxidases and Dpr in Oxidative Stress. *Journal of Biosciences and Bioengineering.* **90(5)**:484–493.

Jadhav S. U., Jadhav U. U., Dawkar V. V., and Govindwar S.P. (2008). Biodegradation of Disperse Dye Brown 3REL by Microbial Consortium of *Galactomyces geotrichum* MTCC 1360 and *Bacillus* sp. VUS. *Biotechnology Bioprocess Engeneering*. **13**: 232-239.

Joshi, T., L. Iyengar, K. Singh, and S. Garg (2008). Isolation, Identification and Application of Novel Bacterial Consortium TJ-1 for the Decolourization of Structurally Different Azo Dyes. *Journal of Bioresource Technology* **99**: 7115-7121.

Kang T.S., Korber D. R. and Tanaka T. (2013). Influence of oxygen on NADH recycling and oxidative stress resistance systems in *Lactobacillus panis* PM1. *AMB Express.* **3**: 10-18.

Khadijah O., Lee K. K. and Mohd Faiz F. A. (2009). Isolation, screening and development of local bacterial consortia with azo dyes decolourising capability. *Malaysian Journal of Microbiology*. **5**: 25-32.

Khalid A., Arshad M., Crowley D., Erkurt H. A., Arshad M. *et al.* (2010). Biodegradation of Azo Dyes. *Handbook of Environmental Chemistry*. **9**: 1-37.

Khehra, M. S., Saini H.S., Sharma D.K., Chadha B.S. and Chimni S.S. (2005). Comparative Studies on Potential of Consortium and Constituent Pure Bacterial Isolates to Decolorize Azo Dyes. *Water Research.* **39**: 5135-5141.

Kirby, N., Marchant, R. and McMullan, G. (2000). Decolourization of Synthetic Textile Dyes by *Phlebia tremellosa. FEMS Microbiology Letters*. **188**: 93-96.

Kumar K., Devi S.S. and Krishnamurthi K. (2006) Decolorisation, Biodegradation and Detoxification of Benzidine Based Azo Dye. *Bioresource Technology*. **97**:407–413.

Lim C.K., Bay H.H., Aris A., Majid Z.A. and Ibrahim Z. (2013). Biosorption and Biodegradation of Acid Orange 7 by *Enterococcus Faecalis* Strain ZL: Optimization by Response Surface Methodological Approach. *Environment Science Pollution Research.* **20**:5056-5066.

Liu G., Zhou J., Wang J., Zhou M, Lu H. and Jin R. (2009). Acceleration of Azo Dye Decolorization by using Quinone Reductase Activity of Azoreductase and Quinone Redox Mediator. *Bioresource Technology.* **100**: 2791-2795.

Liu G., Zhou J., Wang J., Zhou M, Lu H. Jin R. and Qu Y. (2008). Enhancing Survival of *Escherichia Coli* by Expression of Azoreductase AZR Possessing Quinone Reductase Activity. *Applied Microbial Technology*. **80**: 409-416.

Leelakriangsak M. (2013). Molecular Approaches for Bacterial Azoreductases. *Songklanakarin Journal of Science and Technology*. **35**: 647-657.

Maier J., Kandelbauer A., Erlacher A., Cavaco-Paulo A., and Gubitz G.M. (2004). A New Alkalithermostable Azoreductase from *Bacillus* sp. Strain SF. *Applied Environmental Microbiology.* **70 (2)**: 837-844.

Mendez-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**: 264-272.

Miyoshi A, Rochat T, Gratadoux JJ, Le Loir Y, Oliveira SC, Langella P, Azevedo V (2003) Oxidative stress in *Lactococcus lactis*. *Genetic Molecular Research.* **2(4)**: 348–359.

Mohd Ramlan M. A., Azizan N.A., Bay H.H., Lim C.K., Mohamad S. E. and Ibrahim Z. (2012) Decolourisation of Reactive Black 5 by Azoreductase Produced by *Brevibacillus panacihumi* ZBI. *Jurnal Teknologi*. **59**: (11-16).

Moosvi S., Kher X. and Madamwar D. (2007). Isolation, Characterization and Decolorization of Textile Dyes by A Mixed Bacterial Consortium JW-2. *Dyes Pigmentation*. **74**:723–729.

Moutaouakkil A., Zeroual Y., Dzayri F.Z., Talbi M., Lee K and Blaghen M. (2003). Purification and Partial Characterization of Azoreductase from *Enterobacter agglomerans. Biochemistry and Biophysics.* **413**: 139-146.

Myers C. R. and Myers J.M. (1992). Localization of Cytochromes to the Outer Membrane of Anaerobically Grown *Shewanella Putrefaciens* MR-1. *Journal of Bacteriology*. **174**: 3429–3438.

Nachiyar C. V. and Rajakumar G. S. (2005). Purification and Characterization of an Oxygen Insensitive Azoreductase from *Pseudomonas Aeruginosa. Enzyme and Microbial Technology*. **36**: 503-509.

Ooi T., Shibata T. Sato R., Ohno H., Kinoshita S., Thuoc T.L. and Taguchi S. (2007). An Azoreductase, Aerobic NADH-Dependent Flavoprotein Discovered from *Bacillus* sp.: Functional Expression and Enzymatic Characterization. *Applied Microbiology Biotechnology*. 75: 377-386.

Panswad T., Iamsamer K. and Anotai J. (2001). Decolorization of Azo Reactive Dye by Polyphosphate and Glycogen Accumulating Organisms in An Anaerobic Aerobic Sequencing Batch Reactor. *Bioresource Technology*.**76**:151–159.

Pourbabaee A.A., Malekzadeh F., Sarbolouki M.N., Najafi F. (2006). Aerobic Decolorization and Detoxification of a Disperse Dye in Textile Effluent by A New Isolate Of *Bacillus* sp. *Biotechnology Bioengineering.* **93**: 631–635.

Ramalho P. A., Cardoso M.H., Cavaco-Paulo A. and Ramalho M.T. (2004). Characterization of Azo Reduction Activity in a Novel *Ascomycete* Yeast Strain. *Applied Environmental Microbiology*. **70 (4)**: 2279-2288.

Rai H., Bhattacharya M., Singh J., Bansal T.K.,Vats P. and Banerjee U.C. (2005). Removal of Dyes from the Effluent of Textile and Dyestuff Manufacturing Industry: A Review of Emerging Techniques with Reference to Biological Treatment. *Critical Reviews in Environmental Science and Technology* **35**: 219 – 238.

Rau J., Knackmuss H. and Stolz A. (2002). Effects of Different Quinoid Redox Mediators on the Anaerobic Reduction of Azo Dyes by Bacteria. *Environment Science Technology*. **36**: 1497-1504.

Robinson T., McMullan G., Marchant R., Nigam P. (2001). Remediation of Dyes in Textile Effluent: A Critical Review on Current Treatment Technologies with a Proposed Alternative. *Bioresource Technology*. **77**: 247–55.

Ronaghi M. (2001). Pyrosequencing Sheds Light on DNA Sequencing. *Genome Reasearch.* **11**: 3-11.

Sakamoto M and Komagata K. (1996). Aerobic Growth of and Activities of NADH Oxidase and NADH Peroxidase in Lactic Acid Bacteria. *Journal of Fermentation and Bioengineering*. **3**: 210-216.

Sanchez L. B., Elmendorf H., Nash T. E. and Muller M. (2001). NAD(P)H: Menadione Oxidoreductase of the Amitochondriate Eukaryote Giardia Lamblia: A Simpler Homologue of the Vertebrate Enzyme. *Microbiology*. **147**:561-570.

Saraswathi K., Balakumar S. (2009). Biodecolourization of Azo Dye (Pigmented Red 208) using *Bacillus firmus* and *Bacillus laterosporus*. *Journal of Bioscience Technology*. **1**:1–7.

Saratale R. G., Saratale G.D, Kalyani D.C, Chang J.S, and Govindwar S.P. (2009). Enhanced Decolorization and Biodegradation of Textile Azo Dye Scarlet R by using Developed Microbial Consortium-GR. *Bioresource Technology*. **100**: 2493-2500.

Saratale R. G., Saratale G. D., Chang J. S. and Govindwar S.P. (2011). Bacterial Decolorization and Degradation of Azo Dyes: A review. *Journal of the Taiwan Institute of Chemical Engineers*. **42**: 138-157.

Sarayu, K. and S. Sandhya (2010). Aerobic Biodegradation Pathway for Remazol Orange by *Pseudomonas aeruginosa*. *Applied Biochemistry Biotechnology*. **160 (4)**: 1241-1253.

Seesuriyachan P., Takenaka s., Kuntiya A., Klayraung S., Murakami S. and Aoki K. (2007) Metabolism of Azo Dyes by *Lactobacillus Casei* TISTR 1500 and Effects of Various Factors on Decolorization. *Water Research***. 41**: 985-992.

Solis M., Solis A., Perez H. I., Manjarrez N. and Flores M. (2012). Microbial Decolourisation of Azo Dyes. *Process Biochemistry*. **47**: 1723-1748.

Soloman, P.A., Basha, C.A., Ramamurthi, V., Koteeswaran, K. and Balasubramanian, N. (2009). Electrochemical Degradation of Remazol Black B Dye Effluent. *Clean.* **37**: 889-900.

Stolz A. (2001). Basic and applied aspects in the microbial degradation of azo dyes. *Applied Microbiol Biotechnology*. **56**: 69–80.

Supaka N. J., Somsak Delia K.D. and Pierre M.L.S. (2004). Microbial Decolorization of Reactive Azo Dyes in a Sequential Anaerobic– Aerobic System. *Chemical Engeneering Journal*. **99**: 169-176.

Telke A. A., Kalyani D.C., Dawkar V.V. and Govindwar S.P. (2009). Influence of Organic and Inorganic Compounds on Oxidoreductive Decolorization of Sulfonated Azo Dye C.I. Reactive Orange 16. *Journal of Hazardous Materials*. **172**: 298-309.

Vandevivere, P. C., R. Bianchi, and W. Verstraete (1998). Treatment and Reuse of Wastewater from the Textile Wet-processing Industry: Review of Emerging Technologies. *Journal of Chemical Technology and Biotechnology*. **72**: 289-302.

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**: 1425-1440.

Wang G. and Maier R. J. (2004). An NADPH Quinone Reductase of *Helicobacter pylori* Plays an Important Role in Oxidative Stress Resistance and Host Colonization. *Infection and Immunity*. **72**: 1391-1396.

Wang C. J., Hagemeier C., Rahman N., Lowe E., Noble M., Coughtrie M., Sim E. and Westwood I. (2007). Molecular Cloning, Characterisation and Ligand-Bound Structure of an Azoreductase from *Pseudomonas aeruginosa*. *Journal of Molecular Biology*. **373(5)**: 1213-1228.

Wang Z., Xue M., Huang K., Liu Z. and Hauser P (2011). Textile Dyeing Wastewater Treatment (Advances in Treating Textile Effluent). Rijeka, Croatia: In Tech.

Yoo E.S., Libra J. and Adrian L. (2001). Mechanism of Decolourization of Azo Dyes in Anaerobic Mixed Culture. *Journal of Environmental Engineering and Science*. **127**: 844–849.

Zaharia, C., Suteu D., Muresan A., Muresan R. and Popescu A. (2009). Textile wastewater treatment by homogenous oxidation with hydrogen peroxide. *Environmental Engineering and Management Journal*. **8**: 1359-1369.

Zaharia C., Daniela S. and Puzyn T. (2012). Textile Organic Dyes – Characteristics, Polluting Effects and Separation/Elimination Procedures from Industrial Effluents – A Critical Overview (Organic Pollutants Ten Years After the Stockholm Convention - Environmental and Analytical). Rijeka, Croatia : InTech.

Zhao H., Sun Y., Xu L. and Ni J. (2010). Removal of Acid Orange 7 in Simulated Wastewater using A Three-Dimensional Electrode Reactor: Removal Mechanisms And Dye Degradation Pathway. *Chemosphere.* **78**: 46-51.

Zhao. X and Hardin I.R. (2007). HPLC and Spectrophotometric Analysis of Biodegradation of Azo Dyes by Pleurotus ostreatus. *Dyes and Pigments.* **73**: 322-325.

Zimmermann T., Kulla H.G., Leisinger T. (1982) Properties of Purified Orange I1 Azoreductase, the Enzyme Initiating Azo Dye Degradation by *Pseudomonas* KF46. *European Journal Biochemistry.* **129**: 197-203.