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

NADHIRAH AMINAH BT AZIZAN

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Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

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To my beloved parents and family members:

Azizan Yahya
Rohanah Baharom
Ahmad Faiz Azizan
Roziyati Abdullah
Nadiah Aminah Azizan
Ahmad Zharif Azizan
Norhayati Baharom
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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. Penyajikan 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.
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LIST OF SYMBOLS

% - Percentage
°C - Degree Celsius
g - Gram
kg - Kilo gram
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
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<td>BSA</td>
<td>Bovine Serum Albumin</td>
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<td>CFE</td>
<td>Cell free extract</td>
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<td>COD</td>
<td>Chemical Oxygen Demand</td>
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<td>Culture Supernatant</td>
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<tr>
<td>df</td>
<td>Dilution factor</td>
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<td>FADH</td>
<td>Flavin adenine dinucleotide (reduced)</td>
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<td>NADH</td>
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<td>OD</td>
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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.
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