SIMULTANEOUS AZO DYE DEGRADATION AND BIOHYDROGEN PRODUCTION BY STRAIN L17 FORM GASTROINTESTINAL ORIGIN

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I would like to dedicate this thesis to special people in my life:

To my mother who always gives me energy and without her encouragement and support I could not achieve this degree.

To my amazing father who always supports me during my difficult moments, for his patience and all the he sacrifices he has made during this difficult stage of my life.

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ABSTRACT

Nowadays, with the increase in the population of the world, energy consumption is one of the important issues. Environmental pollution is another issue related to the pollution rise. Hence, simultaneous treatment of the pollution with energy generation is very desirable. Azo dye from textile industry has the potential to be used as feedstock for energy generation, for example hydrogen production via biological treatment. In this study, strain L17 from *Enterobacteriaceae* family was used to degrade azo dye in microaerophilic condition in different minimal and rich media to select the best media for biohydrogen production via anaerobic fermentation. A minimal medium, MMP5, was chosen as the best media due to the absence of glucose and nutrient broth. L17 was inoculated into the anaerobic MMP5 medium and incubated to detect the hydrogen production, and simultaneous dye degradation. Residual Gas Analyzer was used to detect the production of hydrogen and other gases produced by L17 fermentation. It was found that L17 produced hydrogen as early as 24 hours after fermentation starts. In order to detect potential gene related to this hydrogen production, hydrogen 3 large subunit (hycE) was targeted for PCR amplification, sequencing and amino acids sequence prediction. Hydrogen 3 has been reported to play a role in hydrogen production in *Enterobacteriaceae*. This study supports the potential for simultaneous azo dye wastewater treatment and hydrogen production using L17 strain as the biological agent.

ABSTRAK

Pada masa kini, penggunaan tenaga adalah menjadi salah satu kepentingan disebabkan peningkatan populasi di dunia. Faktor lain yang menyebabkan peningkatan pencemaran adalah disebabkan oleh pencemaran alam sekitar. Oleh yang demikian, rawatan pencemaran dengan penggunaan tenaga adalah diperlukan. Azo pewarna dari industri tekstil, contohnya penghasilan hidrogen melalui rawatan biologi mempunyai potensi sebagai bahan mentah untuk penghasilan tenaga. Dalam kajian ini, strain L17 digunakan untuk mengurai pewarna azo dalam keadaan mikroaerofilik yang berbeza minimal dan media yang memenuhi kriteria dipilih untuk penghasilan biohidrogen melalui fermentasi dan anaerobik. Media minimal, MMP5 dipilih sebagai media kerana ketiadaan glukosa dan cecair media. L17 diinokulasi ke dalam media MMP5 anaerobik dan diinkubasi untuk mengesan penghasilan hidrogen dan dalam masa yang sama mengurai warna. Fermentasi L17 dengan menggunkan RGA untuk mengesan penghasilan hidrogen dan gas-gas lain. Didapati L17 menghasilkan hidrogen seawal 24 jam selepas fermentasi bermula 3 subunit besar hidrogen (hycE) disasarkan untuk memperbanyakkan PCR, turutan dan jangkaan turutan amino asid. Hidrogen 3 dalam *Enterobacteriaceae* memainkan peranan dalam penghasilan hidrogen. Kajian ini menyokong keupayaan strain L17 sebagai agen biologi untuk merawat sisa pewarna azo dan penghasilan hidrogen.

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LIST OF ABBREVIATIONS AND SYMBOLS

˚C	-	Degree Centigrade Celsius
µL	-	Microliter
CH4	-	Methane
CO	-	Carbon monoxide
CO2	-	Carbon dioxide
COD	-	Chemical Oxygen Demand
g	-	Gram
hr	-	Hours
H2/mol	-	Hydrogen/molarity
H2O	-	Dihydrogen oxide (Water)
Kg	-	Kilogram
mg	-	Milligram
mg/L	-	Milligram/Liter
min	-	Minute
mL	-	Milliliter
mM	-	Millimol
w/v	-	weight/volume
v/v	-	volume/volume
w/w	-	weight/weight
NA	-	Nutrient Agar
O2	-	Oxygen
PCR	-	Polymerase Chain Reaction
RGA	-	Residual Gas Analyzer

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Nowadays, with the increase in the population of the world, energy consumption is one of the important issues. Scientists are looking for alternative fuels that can reduce or overcome the problems that are caused by fossil fuels. The new alternative fuel is biofuel that can be used as transportation fuel. This type of fuel can decrease the greenhouse gas emission. For producing biofuel, organic materials such as, oilseed, cellulose, starch, industrial wastes and animal fats can be the sources (Carere *et al*., 2008).

Over the past few years, the interest in one type of the biofuel is more than other and it is hydrogen (bio-hydrogen). Hydrogen can be used for generating electricity and transportation fuel because it possesses high energy yield, renewable and the consumption does not emit greenhouse gases. There are many methods for hydrogen production, such as fermentation of biomass using microorganisms, coal gasification, reforming of hydrocarbons, photo fermentation, electrolysis, and other biological routes (Agrawal *et al*., 2008). There are many different pathways of hydrogen production in microorganisms, like dark fermentation of organic waste materials. This approach has advantages such as no light energy required, high rate of cell growth and no oxygen limitation problems (Hallenbeck *et al*., 2002; Nath *et al*., 2004).

To date, scientists are focusing on producing hydrogen from wastewater which has organic wastes to reduce the cost of hydrogen production (Yu *et al*., 2002). Hydrogen production from wastewater is a promising technology not only for treatment of wastes, but the waste can also be used for treatment and hydrogen production simultaneously. This method is cheaper, can be carried out using local feedstock and eco-friendly. One of the important sources is textile waste water, which has a good potential for producing hydrogen by azo-dye degradation and can be simultaneously treated. The discharge of textile wastewater to the environment causes the environment pollution. Thereby, the treatment of textile wastewater can help to reduce environment pollution and produce hydrogen (Lay *et al*., 2012).

The environmental problems created by the textile industry have received increased attention for decades, because this industry is one of the largest generators of contaminated effluents (Solís *et al*, 2012). The effluents mainly are from dyeing and finishing processes and are associated with the water pollution caused by the discharge of untreated or poorly treated effluents. Due to the concern about the potential hazard of textile wastewater, many techniques have recently been developed to find more efficient methodologies for the treatment of wastewater. Microorganisms have also been selected for the development of textile wastewater treatments (Solís *et al*., 2012).

Hydrogen can be generated from different supply (Figure 1.1). Hydrogen production from wastewater may be produced in two ways: 1- light driven process and 2- anaerobic fermentation. To date, dark fermentation or anaerobic fermentation is receiving more interests because of its rapid, simple, and easy operation (Liu *et al*., 2008). The production of hydrogen in large scale is important to reduce cost and to obtain higher efficiency. Scientists use the microorganisms and supply the optimum medium and environment for higher hydrogen production. Some parameters such as pH, temperature, metal ion concentration, inoculums size, and L-cysteine addition have been optimized (Chen *et al*., 2010).

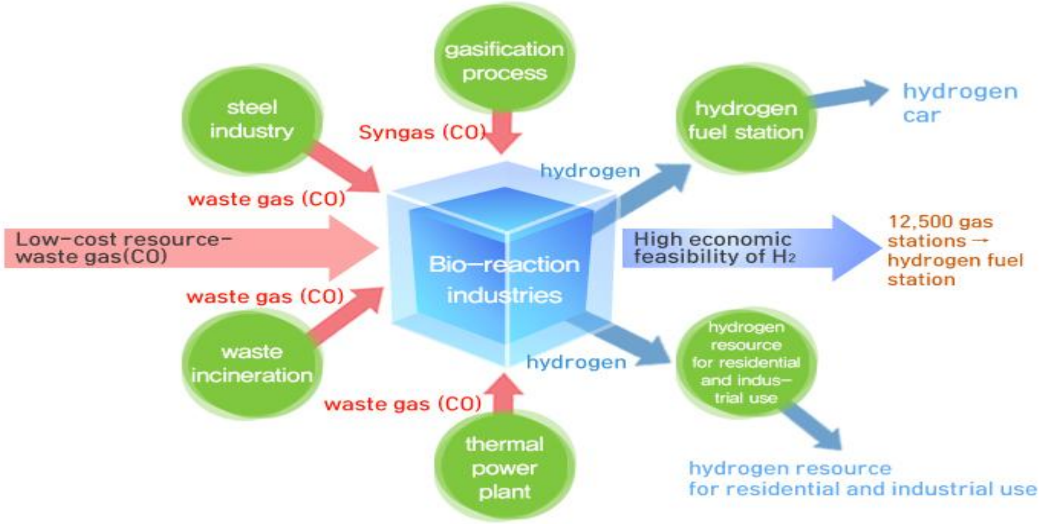

Figure 1. 1 Biohydrogen production

[http://www.ensoltek.com/?_p=rnd_3_1 (Accessed on 12.04.2013, 5:45 PM)]

1.2 Statement of Problem

The environmental problems created by the textile industry which mainly arises from dyeing and finishing processes and are associated with the water pollution caused by the discharge of untreated or poorly treated effluents. Wastewater resulting from these processes has Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), suspended solids, salinity, a wide range of pH (5–12) and the recalcitrance of organic compounds, such as azo dyes (Gavrilescu, 2008). Worldwide, 280,000 tons of textile dyes are discharged in industrial effluents every year (Jin *et al*., 2007), and the main concerns involve the adverse effects of azo dyes in the environment. If the dyes are broken down anaerobically, aromatic amines are generated, which are very toxic, carcinogenic and mutagenic. Azo-dyes are the most widely used dyes and represent over 60% of the total dyes (Yuzha Fu *et al*., 2001). Due to a general concern about the treatment of wastewater, a large number of investigations have been recently developed to find more efficient methodologies for the treatment of wastewater, and microorganisms have been selected for the development of textile wastewater treatments. The application of microorganisms for the biodegradation of azo-dyes is an attractive alternative to the development of bioremediation processes for the treatment of textile wastewater (Solís *et al*., 2012).

Global fossil fuel energy reserves are going to be exhausted in near future and the environmental pollution problems associated due to their usage compel scientists to search for alternate energy sources that can substitute the conventional ones. Hydrogen production using wastewater is a promising technology. It not only helps in the treatment of the waste but also in generation of the eco-friendly energy source. Organic wastewater is a potential bioenergy source by anaerobic fermentation technology. Cotton textile industries are characterized by high water consumption during the pre-treatment and dyeing processes. Thus, the wastewater contains a variety of polluting compounds the sources of which are the natural impurities extracted from the cotton fiber, the processing chemicals and the dyes. The discharge of textile wastewater to the environment causes environment pollution (Lay *et al*., 2012).

The textile industry employs one of the most complicated processes in the manufacturing sectors, which involves washing, dyeing and finishing processes. The processes produce a complex wastewater containing a type of dye, surfactants, as well as many textile additives. As a result, textile plants produce highly. Many recent studies focus on anaerobic hydrogen fermentations. The substrates for hydrogen dark fermentation typically use sucrose or starch, which are not economically feasible. Waste and/or wastewater containing high concentrations of carbohydrates, generated from textile industries, agricultural processes and food industries are preferred for economic reasons. Many researchers have studied the production of hydrogen via the anaerobic fermentation of waste and wastewater (Li *et al*., 2012). So this study was proposed as a proof of concept towards applications of dark fermentation for dye wastewater treatment and biohydrogen generation.

1.3 Significance of Study

Hydrogen is one of the most powerful and clean energy carriers (Thompson *et al*., 2008). Hydrogen can be converted to electricity by using a fuel cell also, which is common in developed countries (Nakada *et al*., 1999). The focus of this study is to investigate hydrogen production by bacteria of gastrointestinal origin using azo-dye as substrate. It will be used as a model for the application of the bacteria towards treatment of dye-containing wastewater with simultaneous hydrogen generation.

1.4 Objectives of Study

The objectives of this study are as follows:

1. To investigate the growth of gastrointestinal (GI) bacteria in minimal media with azo-dye as sole carbon source.

2. To investigate the hydrogen production ability of the bacteria using azo dye as carbon source in minimal medium.

3. To identify the potential gene(s) that could be responsible for hydrogen production during dye degradation.

1.5 Scope of Study

This study was investigated the hydrogen production ability by strain L17 from *Enterobacteriaceae* family, earlier identified as *Citrobacter freundii*. Azo-dye was used as a carbon source and dye degradation and hydrogen production was investigated. The hydrogen production was investigated in minimal medium, lets bacteria use azo dye as a sole carbon source. Finally, the putative gene responsible hydrogen production by strain L17 was proposed.

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