i n t e r n a t i onal j o u r n a l o f hydrogen energy 3 4 ( 2 0 0 9 ) 7 5 0 2 – 7 5 1 2

# Optimization of phototrophic hydrogen production by Rhodopseudomonas palustris PBUM001 via statistical experimental design

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## Introduction

Hydrogen ($H_2$) is considered as a promising and ideal source of energy for the future. This is due to the factor that it is clean and it produces an amount of energy that is higher than the one produced by other fuel on a mass basis (122 kJ g_1) [1]. In addition, its low heating value (LHV) is 2.4, 2.8 and 4 times higher than methane, gasoline and coal respectively [2]. For these reason, the amount of energy produced during $H_2$ combustion is higher than that released by any other fuel on a mass basis. Besides, significant use of $H_2$ will contribute to the reduction of energy-linked environmental impacts, including global warming due to anthropogenic carbon emissions, mobile source emissions such as CO, $NO_x$, $SO_x$, non-methane hydrocarbons, and particulates [3].

Current utilization of $H_2$ is equivalent to 3% of the energy consumption. It is used as a reactant in hydrogenation processes in ammonia and methanol production, an oxygen ($O_2$) scavenger, a fuel in rocket engines and a coolant in electrical generators [4]. In accordance with sustainable development and waste minimization issues, biological $H_2$ production from renewable sources, also known as ‘‘green technology’’ has received considerable attention in recent years [5].

Photosynthetic bacteria have been studied extensively, for their capacity to produce significant amounts of $H_2$. Hydrogen production by photosynthetic bacteria is mediated by nitrogenase activity particularly in the absence of molecular nitrogen and oxygen (i.e. in the presence of argon) [6–8]. However,$H_2$ production is not advantageous if argon is used as

sparging gas and this is a potential obstacle to an eventual efficient and economical commercial process. A study has discovered four mutant strains of the photosynthetic bacterium Rhodopseudomonas palustris that produced $H_2$ constitutively, even in the presence of ammonium, a condition where wild-type cells do not accumulate detectable amounts of$H_2$ [9].

Photosynthetic bacteria are found to be the most promising as compared to other microbial system due to its high substrate to product conversion yield, lack of oxygenevolving activity (which is desirable for biohydrogen production), ability to use a wide wavelength of light, and capability to consume organic substrates (derived from wastes) for $H_2$ production that also helps in bioremediation process (wastewater treatment) [10]. Photosynthetic bacteria carry out anoxygenic photosynthesis using organic compounds and reduced sulfur compounds electron donors, which are categorized as non-sulfur and sulfur photosynthetic bacteria respectively [11]. Some purple non-sulfur bacteria (PNS) are strong $H_2$ producers utilizing organic acids that are produced through the breakdown process of organic substrate, such as lactic, succinic and butyric acids or alcohol [12]. They constitute a non-taxonomic group of versatile organisms which can grow as photoheterotrophs, photoautotrophs or chemoheterotrophs-switching from one mode to another depending on available conditions such as degree of anaerobiosis, availability of carbon source ($CO_2$ for autotrophic growth, organic compounds for heterotrophic growth) and availability of a light source (needed for phototrophic growth) [6].

Palm oil mill effluent (POME) generated from palm oil milling processes to produce crude palm oil (CPO) is the most polluting agro-industrial effluent. It is estimated that 0.5–0.75 tonne of raw POME were produced for every tonne of fresh fruit bunches (FFB) processed [13]. POME is rich in organic carbon with a chemical oxygen demand (COD) of 50,000 mg/l, biological oxygen demand (BOD), 30,000 mg/l, oil and grease, 6000 mg/l, suspended solids, 59,350 mg/l and 750 mg/l of total nitrogen [14]. Several studies on biohydrogen production from POME have been carried out by using anaerobic microflora [15–17]. However, the photofermentation of $H_2$ production from POME by using photosynthetic bacteria has barely been explored.

In this study, $H_2$ production from POME by using phototrophic bacteria R. palustris strain PBUM001 was investigated. Response surface methodology (RSM) using Box-Behnken

design was employed in planning the experiments for evaluating the individual and interactive effects of process parameters namely, POME concentration, light intensity, inoculums size, pH and agitation rate on biohydrogen production and COD reduction simultaneously.

## 2. Materials and methods

### 2.1. Microorganism and medium

R. palustris PBUM001 was isolated from rice noodle processing wastewater [18] and used throughout this study. The PBUM001 strain was grown and maintained in Glutamate malate medium (GMM) [18]. The initial pH value of the medium was adjusted to 7.0 by 0.1 N NaOH. The cells were grown anaerobically at 30 _ 2 _C for 48 h under continuous illumination by tungsten light bulbs at 2.5 klux.

### 2.2. Sample collection and preparation

Palm oil mill effluent (POME) was collected from a local palm oil mill in Dengkil (Selangor, Malaysia). The collected POME was initially pre-settled for 24 h in 4 _C cold room and centrifuged at 7000 rpm for 15 min. The supernatant was stored in plastic bags in a freezer at _20 _C in order to prevent the wastewater from undergoing biodegradation due to microbial action [18]. The POME was thawed at 4 _C and the remaining solids in the POME were filtered with Whatman (No. 1) filter paper. The pH of filtered wastewater was adjusted to required pH (i.e. 6, 7, 8) and autoclaved at 121 _C for 15 min prior to its use as a culture media. The characteristics of the raw and pre-treated POME were analyzed [20].

### 2.3. Seed culture

Seed culture was prepared by adjusting the bacterial culture to 0.2 g drywt/L at $OD_{660}$ of 0.3. R. palustris PBUM001, at 10% (v/v) was acclimatized in sterilized 25% (v/v) diluted POME in serum bottles and were flushed with nitrogen ($N_2$) gas for 4 min and sealed with butyl rubber stoppers and aluminium caps. Then the cultures were grown under the same condition as growing in GMM. The serum bottle was agitated continuously at 150 rpm for 48 h. The acclimatized PBUM001 strain (seed culture) was used for further studies.

### 2.4. Experimental design and procedure

Response surface methodology (RSM) has been widely used in the optimization of biohydrogen production process [21–24]. The RSM used in this study investigates the effects of process parameters namely POME concentration, light intensity, inoculum size, agitation rate and pH on $H_2$ production and COD reduction by PBUM001 strain. Box-Behnken design was used which comprised of three levels, low (_1), central (0), and high (þ1). It was chosen because it required fewer experiments, was more efficient and easier to arrange and interpret in comparison to others. The level and code of variables considered in this study are shown in Table 2a. Accordingly, a total of 138 runs involving the replication of the central points for each six blocks, were used to optimize the process parameters (Table 2b). Two responses were predicted from RSM, that is, cumulative $H_2$ production (ml) and COD reduction (%). The Minitab_ 14 software was used in the experimental design, data analysis, quadratic model buildings, and graph (three-dimensional response surface and contour) plotting.

The experiments were conducted in serum bottles (total volume of 121 ml) containing sterilized POME with 50 ml working volume. The bottles were flushed with $N_2$ gas for 4 min and sealed with butyl rubber stoppers and aluminium caps. The samples were seeded and incubated for 66 h under various conditions of pH, light intensity (klux), inoculum size (% v/v), agitation rate (rpm) and POME concentration (% v/v). The composition of gas products was measured after 66 h. The biogas produced was collected using air-tight syringe (Terumo, Japan) and stored in bottles containing acidic water (<pH 3) using water displacement method in order to prevent dissolution of the gas components [15]. The gas composition was determined by gas chromatography (Shimadzu Corp., Japan, GC-8A), equipped with thermal conductivity detector (TCD), Porapak Q column, $N_2$ as carrier gas, detector/injector temperature at 100 _C, column temperature at 50 _C. 1 ml of the gas sample was injected in replicate.