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Improving photofermentative biohydrogen production by using intermittent ultrasonication and combined industrial effluents from palm oil, pulp and paper mills

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ABSTRACT

An ultrasonication technique was applied intermittently onto photofermentation broth during the first six hours of photofermentation to improve biohydrogen production by using *Rhodobacter sphaeroides* NCIMB8253. In this research, photofermentation broth consisted of a combination of palm oil (25%, v/v), pulp and paper (75%, v/v) mill effluents as well as liquid inoculum. The effects of amplitude (10, 20 and 30%, A) and ultrasonication duration (5, 10 and 15 min, T) were investigated in terms of their influences on photofermentative biohydrogen yield and total chemical oxygen demand (COD_{total}) removal. The recommended ultrasonication parameters were found at the middle range of amplitude and duration (A20T10). Using A20T10 intermittent treatment, the production of biohydrogen could be maximized up to 14.438 mL H_2/mL_{medium} with a COD_{total} removal and light efficiency of 52.2% and 7.412%, respectively. By comparing the treatment without intermittent ultrasonication, an increase of biohydrogen yield by 44.6% was achieved in A20T10 treatment. A total energy input of 306.1 J/mL (A20T10 treatment) was supplied to improve substrate consumption and light distribution during the photofermentation, which led to the increase of biohydrogen yield.

1. Introduction

Hydrogen has been widely regarded as a potential energy source in the future. The release of water as the only combustion product and high heating value are the two main benefits of hydrogen energy [1]. At present, most of the hydrogen is produced from natural gas, heavy oil, and naphtha as well as coal [2]. Together with the potential of using biological production method, hydrogen energy could be produced in a sustainable manner by reusing biomass or waste materials at ambient temperature and pressure [3,4]. Nonetheless, the practical feasibility of biohydrogen production is relatively limited by the low substrate conversion efficiency and biohydrogen yield.

Wang et al. [5] highlighted the importance of substrate transport and bacterial activities in the synthesis of photopigments, generation of ATP and activities of intracellular enzymes, as the dominating factors that influenced photofermentative biohydrogen production. Enhancement of both substrate transport and bacterial activities could be achieved by reducing the resistance of mass transfer from substrate solution into the cell via ultrasonication [5]. Ultrasonication is an irradiation of ultrasound waves with a frequency of higher than 20 kHz. In recent years, ultrasonication has gained many interests as a green technology which is used in environmental remediation for degrading pollutants in the wastewater [6,7]. Meanwhile, controlled ultrasound at low power can be applied as an innovative technology to stimulate various bioprocesses which led to an enhanced microbial productivity [8]. For example, Wang et al. [9] developed an efficient intermittent ultrasonication strategy, which consisted of a 5 min ultrasonic treatment (40 kHz) of cultures for two times with a 12 h interval, to enhance laccase production by *Trametes versicolor*. Laccase activity was found to be increased by 1.8 times as compared to the control due to the improvement of nutrients and metabolites transfer during ultrasonication [9]. Also, Wang et al. [5] found that the production of biohydrogen by *Rhodopseudomonas palustris* CQK01 was nearly doubled when an intermittent ultrasonication was used during photofermentation due to the faster release of hydrogen gas and the reduction of hydrogen partial pressure in the broth.

Zhu et al. [10] found that ultrasonication modified membrane morphology and broke up part of the cells, resulting in improvement of membrane permeability and bacterial activities. Although

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Wu et al. [6] described the advantages of using ultrasound technology as an environmentally friendly technology, the studies about the uses of ultrasonication onto the fermentation broth for improving photofermentative biohydrogen production are still very limited. Thus, intermittent ultrasonication technique was introduced in the present study to improve biohydrogen production and wastewater treatment, mainly by enhancing substrate uptake of the cells from the wastewater during photofermentation process.

In this study, combined-industrial effluents (25%, v/v palm oil mill effluent (POME) and 75%, v/v pulp and paper mill effluent (PPME)) were reused as a photofermentation substrate to produce biohydrogen by using purple non-sulfur bacteria, namely *R. sphaeroides* NCIMB 8253. The appropriate combination between POME and PPME as a single substrate was found to encourage both bacterial growth [11] and photofermentative biohydrogen production [12]. Intermittent ultrasonication was investigated as an improvement step of the photofermentative biohydrogen production. Ultrasound irradiation was applied intermittently every hour onto the photofermentation broth, consisted of combined effluents and liquid inoculum, during the first six hours of photofermentation. Two parameters, namely amplitude (10–30%) and ultrasonication duration (5–15 min), were investigated to obtain the recommended ultrasonication scheme to further enhance the biohydrogen production. To evaluate the efficiency of intermittent ultrasonication treatment, the values of biohydrogen yield, and the removals of the total chemical oxygen demand (COD_{total}) and soluble chemical oxygen demand ($COD_{soluble}$) were also determined. Additionally, a preliminary economic assessment of the proposed photofermentative biohydrogen production was performed to provide an initial estimation on the economic feasibility of intermittent ultrasonication as an improvement step in photofermentation.

2. Materials and method

2.1. Bacteria

The strain of photosynthetic bacteria *R. sphaeroides* NCIMB8253 was used in this study. The cultivation of bacteria was done on agar slants and incubated for 24 h under 4000 lx illumination (WalkLAB Digital Lux meter, Trans Instrument Pte Ltd, Singapore) and kept at 30 °C. After cultivation, the culture was maintained and preserved at 4 °C.

2.2. Collection of raw effluents and preparation of combined effluents

POME was collected from Seri Ulu Langat Palm Oil Mill Sdn. Bhd, while PPME was obtained from Muda Paper Mills Sdn. Bhd. The physical and chemical characteristics of these two effluents are shown in Table 1.

Before photofermentation, photofermentation substrates were prepared according to Budiman et al. [12]. A production medium contained 25 and 75% (v/v) of POME and PPME, respectively gave the best results for biohydrogen yield (4.67 mL H_2/mL_{medium}) [12]. Subsequently, this production medium was further pretreated to increase the bioavailability of nutrients based on the method described by Budiman and Wu [13]. Then, pH of photofermentation substrate was adjusted to 7.0 (Mettler Toledo FE20) and sterilized at 121 °C for 15 min (Hirayama Clave HV-85, Japan) before undergoing photofermentation experiment.

2.3. Experimental design

2.3.1. Inoculum preparation

Pre-activation of bacteria was conducted following the method described by Budiman et al. [11]. Two loops of bacteria were inoculated into a liquid inoculum under 4000 lx illumination and anaerobic condition for 24 h (30 °C) before undergoing photofermentation. The compositions of liquid inoculum (1 L) were: KH_2PO_4, 0.5 g; K_2HPO_4,1 g; $MgSO_4{\cdot}7H_2O$, 0.5 g; NaCl, 0.4 g; DL-malic acid, 1.0 g; sodium glutamate, 1.8 g; $CaCl_2{\cdot}2H_2O$, 0.05 g; yeast extract, 10 g; ferric citrate (0.1 w/v%), 5 ml; trace elements solution, 1 ml; vitamins solution, 1 ml; and HCl (37%), 0.68 ml. Trace elements solution (100 ml) contained: H_3BO_3, 0.06 g; $CoCl_2{\cdot}2H_2O$, 0.2 g; $ZnCl_2$, 0.07 g; $Na_2MoO_4{\cdot}2H_2O$, 0.04 g; $MnCl_2{\cdot}4H_2O$, 0.1 g; $NiCl_2{\cdot}6H_2O$, 0.02 g; and $CuCl_2{\cdot}2H_2O$, 0.02 g. Vitamin solutions (1 L) contained: thiamin, 500 μg; niacin, 500 μg; and biotin, 15 μg.

Further pre-activation of bacteria was performed according to the method proposed by Budiman et al. [11]. In a 100 mL Schott bottle, 10 mL of liquid inoculum was transferred into a growth medium (consisted of 25%, v/v POME and 75%, v/v PPME) and cultivated for 48 h, under 4000 lx, 30 °C, and anaerobic condition. pH of the medium was adjusted to 7.0 (Mettler Toledo FE20) and sterilization was done at 121 °C for 15 min (Hirayama Clave HV-85, Japan). Before pre-activated bacteria were transferred into photofermentation substrate, pretreatment of bacteria was done using ultrasonication at 256 J/mL (amplitude 30% for 10 min). Ultrasound waves were transmitted aseptically using QSonica Q700 Sonicator (20 kHz with a probe diameter of 12.7 mm) inside an ice bath. The probe and surrounding area of sonicator were sterilized with 70% ethanol solution and inserted from the top into the growth medium about 2 cm depth.

Ultrasonicated bacteria ($\sim 82.9 \times 10^8$ CFU/mL) from growth medium (10 mL) were then transferred into Schott bottle containing ultrasonicated photofermentation substrate (90 mL) inside a

Table 1
Raw effluents and control characteristics.

Parameter	Unit	POME	PPME	Combined effluents[a]
pH	–	4.3 ± 0.3	6.15 ± 1.3	7.00 ± 0.25
Turbidity	NTU	67,500 ± 1,910	4,700 ± 141	17,200 ± 100
COD_{total}	mg/L	84,450 ± 19,500	2,716 ± 125	25,200 ± 283
Total suspended solids	mg/L	19,610 ± 7,900	841 ± 878	6,520 ± 141
Total organic carbon	mg/L	4,251 ± 112.70	473.3 ± 18.56	1,433.5 ± 6.36
Total nitrogen	mg/L	650 ± 300.00	3.70 ± 3.65	170.5 ± 2.12
C/N ratio	–	6.54	128	8.41
Heavy metal content				
– Fe	mg/L	70.7 ± 1.65	0.50 ± 0.01	18.35 ± 0.64
– Zn	mg/L	7.53 ± 1.07	0.12 ± 0.01	2.01 ± 0.21
– Mn	mg/L	6.47 ± 1.43	0.09 ± 0.01	1.72 ± 0.09
– Mg	mg/L	1,144 ± 7.00	3.28 ± 1.08	289.5 ± 2.12
– Al	mg/L	334 ± 22.65	33.43 ± 1.10	109 ± 4.24

[a] Combined effluents: A combination of POME (25%, v/v) and PPME (75%, v/v) after initial pH adjustment.