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# Improved biohydrogen production and treatment of pulp and paper mill effluent through ultrasonication pretreatment of wastewater

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ABSTRACT

Pulp and paper mill effluent (PPME), a rich cellulosic material, was found to have great potential for biohydrogen production through a photofermentation process. However, pretreatments were needed for degrading the complex structure of PPME before biohydrogen production. The aim of this study was to gain further insight into the effect of an ultrasonication process on PPME as a pretreatment method and on photofermentative biohydrogen production using *Rhodobacter sphaeroides* NCIMB. The ultrasonication amplitudes and times were varied between 30–90% and 15–60 min, respectively, and no dilution or nutrient supplementation was introduced during the biohydrogen production process. A higher biohydrogen yield, rate, light efficiency and COD removal efficiency were attained in conditions using ultrasonicated PPME. Among these different pretreatment conditions, PPME with ultrasonication pretreatment employing an amplitude of 60% and time of 45 min (A60:T45) gave the highest yield and rate of 5.77 mL $H_2$/mL medium and 0.077 mL $H_2$/mL h, respectively, while the raw PPME without ultrasonication showed a significantly lower yield and rate of 1.10 mL $H_2$/mL medium and 0.015 mL $H_2$/mL h, respectively. The results of this study demonstrated the potential of using ultrasonication as a pretreatment for PPME because the yield and rate of biohydrogen production were highly enhanced compared to the raw PPME. Economic analysis was also performed in this study, and in comparison with raw PPME, the highest net saving was $0.2132 for A60:T45.



## 1. Introduction

Worldwide industrial development during the past century has led to the depletion of fossil fuel, as global energy requirements are mostly dependent on them. Hydrogen gas has been identified as the fuel of the future due to its non-polluting nature. It produces water only upon combustion without emitting greenhouse gases into the environment. In accordance with sustainable development and waste minimization issues, biological hydrogen production from renewable sources with microorganisms has received considerable attention in recent years. Photofermentation by photosynthetic bacteria is one of the most promising biohydrogen production methods due to its high theoretical conversion yield and its capability to be used in a wide variety of substrates, including industrial wastes [1–3]. Using industrial waste as a feedstock for a photofermentative process is a desirable strategy to reduce the production costs of clean energy (biohydrogen) [4]. This photofermentative biohydrogen production process is strongly coupled with the photosynthesis electron transport system, through which the photosynthetic bacteria obtain energy [5]. Among all photosynthetic bacteria, *Rhodobacter sphaeroides* has been studied intensively due to its ability to grow under a variety of environmental conditions and produce enormous amounts of biohydrogen under light conditions [6].

According to Elbeshbishy et al. [7], any organic substrate rich in carbohydrates, fats and proteins is a viable substrate for promoting biohydrogen production. Thus, some industrial wastewaters may possess the potential to be directly reused as substrates in biohydrogen production [1,8,9]. However, a lignocellulosic type of wastewater may cause the photosynthetic bacteria to digest the substrate less effectively. For example, pulp and paper mill effluent

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(PPME) contains high lignocellulosic materials not readily biodegraded by microorganisms because of their complex structure, which is resistant to enzymatic hydrolytic attacks [10]. Therefore, pretreatment is suggested to remove or weaken the structure of lignin, reduce the crystallinity of cellulose and increase the surface area of the material so that the biohydrogen production efficiency could be enhanced through photofermentation.

Ultrasonication has been proven to be an effective method for degrading organic effluents into less toxic compounds, and it is able to mineralize the compounds completely in certain cases [11]. An extensive literature research indicated the lack of using ultrasonication on wastewater as a mechanical disintegration pretreatment method for biohydrogen production [7]. The findings obtained by Elbeshbishy et al. [7] showed that ultrasonication pretreatment exhibited a positive effect on both the biohydrogen production rate and yield, as both increased by approximately 93% and 83%, respectively.

In this present study, PPME without any additional nutrients or enhancers was reused as a substrate in a photofermentation process to produce biohydrogen. In addition, the novelty and main objective of this study was primarily the application of ultrasonication on PPME as a pretreatment step, as well as to explore the effect of ultrasonication on single stage biohydrogen production using pretreated PPME as a substrate. Understanding energy consumption is essential in ultrasonic-assisted pretreatment. Thus, the common effects of power and ultrasonication time were investigated further by varying the amplitude (30–90%) and ultrasonication time (15–60 min).

## 2. Materials and methods

### 2.1. Pulp and paper mill effluent (PPME)

PPME was collected from Muda Paper Mills Sdn Bhd in Kajang, Selangor, Malaysia. The wastewater was stored in a cold room at 4 °C to reduce the wastewater from undergoing biodegradation due to microbial action. Table 1 shows the PPME characteristics used in this study.

### 2.2. Preparations of activation and growth media

*R. sphaeroides* NCIMB 8253 (provided by the Department of Chemical and Process Engineering, Universiti Kebangsaan Malaysia) was used in this study. Re-modified Biebl and Pfennig activation medium was used for cell activation up to 24 h before transferring to growth medium, with 7.5 mM malic acid serving as the organic carbon substrate and 10 mM sodium glutamate as a nitrogen source. Re-modified Biebl and Pfennig medium [12] contained (g/L): $K_2HPO_4$, 1.0; $KH_2PO_4$, 0.5; $MgSO_4.7H_2O$, 0.5; NaCl, 0.4; $CaCl_2$, 0.05; and yeast extract, 10.0 and 5.0 mL ferric citrate solution (0.1% wt/vol), 1.0 mL trace elements solution and 1.0 mL vitamin solution. The trace elements solution contained (g/L): $H_3BO_3$, 0.06; $CoCl_2.2H_2O$, 0.2; $ZnCl_2$, 0.07; $Na_2MoO_4{\cdot}2H_2O$, 0.04; $MnCl_2{\cdot}4H_2O$, 0.1; $NiCl_2{\cdot}6H_2O$, 0.02; and $CuCl_2{\cdot}2H_2O$, 0.02. The vitamin solution (mg/L) contained thiamine, 0.5; nicotinic acid, 0.5; and biotin, 0.015.

Growth medium (100 mL) contained PPME with supplementation of 0.75 g/L and 1.32 g/L of malic acid and sodium glutamate, respectively. The initial pH of the growth medium was adjusted to 7 by using 1 mol/L HCl or NaOH solution. The cultures were incubated at 30 °C anaerobically with 7 k lux light intensity supplied by a fluorescent lamp for 72 h before *R. sphaeroides* were transferred into the biohydrogen production medium. Argon gas was used to prepare the anaerobic environment in an anaerobic chamber (Model 855-AC/EXP, Plas Labs, USA) by purging the medium for 10 min. Activation, growth and production media were autoclaved at 121 °C for 15 min before use.

**Table 1**
Physico-chemical characteristics of raw and unsonicated PPME.

| Parameter | Unit | Value |
|---|---|---|
| pH | | 6.9 ± 1.2 |
| C/N | | 128 ± 22 |
| Total COD (TCOD) | mg/L | 1441 ± 109 |
| Soluble COD (SCOD) | mg/L | 318 ± 22 |
| Lignin | mg/L tannins | 21 ± 4 |
| Cellulose | mg/L | 76.4 ± 31.3 |
| Soluble carbohydrate | mg/L | 170 ± 9 |
| Total phenol | mg/L | 5.25 ± 0.35 |
| Fe | mg/L | 0.50 ± 0.01 |
| Zn | mg/L | 0.12 ± 0.01 |
| Mn | mg/L | 0.09 ± 0.01 |
| Mg | mg/L | 3.28 ± 1.08 |
| Al | mg/L | 33.43 ± 1.10 |

### 2.3. Preparation of biohydrogen production medium via ultrasonication pretreatment

Biohydrogen production medium (67.5 mL) was prepared via ultrasonication pretreatment of raw PPME. No nutrients were added into biohydrogen production medium. A horn-type ultrasonicator (Qsonica Sonicator Q700) was used for ultrasonic irradiation, which was operated at 700 W and 20 kHz. The tip of the ultrasonicator horn (0.5 in. diameter) was placed in the center of the production medium. The amplitude (intensity) was adjustable from 1% to 99%. The PPME was pretreated at various amplitudes (30%, 60% and 90%) and ultrasonication times (15 min, 30 min, 45 min and 60 min).

### 2.4. Biohydrogen production using pretreated PPME

Biohydrogen production experiments were conducted in a series of 100 mL Schott bottles with a working volume of 75 mL. After inoculation of each bottle with 10% (v/v) inoculum, the sterilized medium was purged with oxygen-free argon gas for 10 min to create the anaerobic condition and was then sealed tightly with GL 45 multiport-system screw caps with 2 ports (GL 14 thread). Photofermentation experiments were performed in a water bath with a constant temperature of 30 °C and with light intensity up to 7 k lux supplied by a fluorescent lamp. The amount of gas that evolved was measured with a water displacement method using graduated cylinders filled with water that were partially submerged in a tub of water connected to the Schott bottles by rubber tubing. Each biohydrogen production experiment was performed in triplicate and the averages of the repeated experimental results were calculated.

### 2.5. Analysis

The composition of produced biogas was analyzed using a gas chromatography (Model 7890A, Agilent) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Molesieve 5A, Agilent). Helium was used as the carrier gas. The temperature of the injector port, the detector and the column were 240 °C, 240 °C and 140 °C, respectively. The soluble carbohydrate concentration was determined spectrophotometrically at 490 nm with a phenol sulfuric acid method using glucose as the standard Ref. [13]. The cellulose concentration was determined by the colorimetric method with a UV wavelength of 620 nm [14]. Total and soluble COD (TCOD and SCOD, respectively) were analyzed by the reactor digestion method using a COD analyzer (Hach Method 8000). The lignin concentration was analyzed using a DR