

# Heat Pre-Treatment of Beverages Wastewater on Hydrogen Production

**S Z Uyub\*, N S Mohd<sup>1</sup> and S Ibrahim**

Department of Civil Engineering, Faculty of Engineering, University of Malaya,  
Kuala Lumpur, Malaysia.

E-mail: sitizulaihayub@gamil.com

**Abstract.** At present, a large variety of alternative fuels have been investigated and hydrogen gas is considered as the possible solution for the future due to its unique characteristics. Through dark fermentation process, several factors were found to have significant impact on the hydrogen production either through process enhancement or inhibition and degradation rates or influencing parameters. This work was initiated to investigate the optimum conditions for heat pre-treatment and initial pH for the dark fermentative process under mesophilic condition using a central composite design and response surface methodology (RSM). Different heat treatment conditions and pH were performed on the seed sludge collected from the anaerobic digester of beverage wastewater treatment plant. Heat treatment of inoculum was optimized at different exposure times (30, 90, 120 min), temperatures (80, 90 and 100°C) and pH (4.5, 5.5, 6.5) in order to maximize the biohydrogen production and methanogens activity inhibition. It was found that the optimum heat pre-treatment condition and pH occurred at 100°C for 50 min and the pH of 6.00. At this optimum condition the hydrogen yield was 63.0476 ml H<sub>2</sub>/ mol glucose (H<sub>2</sub> Yield) and the COD removal efficiency was 90.87%. In conclusion, it can be hypothesized that different heat treatment conditions led to differences in the initial microbial communities (hydrogen producing bacteria) which resulted in the different hydrogen yields.

## 1. Introduction

Due to the climate change issues and concerns regarding the limited non-renewable energy systems (fossil-based), many researchers have been working on new sustainable energy sources that could substitute fossil fuels. At present, a large variety of alternative fuels have been investigated and explored and hydrogen gas is considered as one of possible solution for future due to its unique characteristics.

Hydrogen can be produced through various methods such as reforming of hydrocarbons, coal gasification, electrolysis, photochemical process and biological hydrogen production. Among the different approaches to generate hydrogen, the dark fermentation is the simplest process in obtaining biohydrogen, which can be done through anaerobic digestion of organic matter [1].

In the recent biohydrogen studies, many of the studies used seed sludge from wastewater treatment plants (WWTPs) or pure culture as the inoculum [2-8]. However, very limited studies have used seed sludge from the industrial wastewater. Other than the different type of wastewater used as inoculum, environmental and operation factors play important roles in hydrogen production efficiency during dark fermentation process. The factors are initial pH, substrate and inoculum concentration, temperature and



nutrient were discussed in several review articles [9-13]. Although, many studies have demonstrated the role of variables on fermentative hydrogen production, discrepancies are still exists with regard to optimum of initial pH, pre-treatment of inoculum, temperature and inoculum size.

With regard to pre-treatment of the inoculum, among the commonly studied technologies are heat pre-treatment, repeated aeration, chloroform, acid as well as alkali pre-treatment. Among all, heat pre-treatment is the most commonly used pre-treatment of inoculum as it is proven to be able to suppress non-sporulating bacteria such as methanogens. Moreover, under appropriate conditions,  $H_2$  producing endospore becoming active and multiplying which then resulted in increase the biohydrogen production.

Therefore, due to the disagreement arises from this issues this study was initiated in order to find the optimum conditions for heat pre-treatment that will maximize biohydrogen production, hence making anaerobic digestion feasible alternative in generating biohydrogen.

## 2. Materials and Methods

### 2.1 Substrate and seed sludge

The sludge used as the inoculum was obtained from an anaerobic digester of beverage factory located at Bangi, Selangor. The collected sludge is stored in a cold room at 4°C prior to use for experiment. This is to inhibit the degradation of the effluent from microbial activities.

The physical and chemical characteristics of the wastewater, which were pH, total suspended solid (TSS), volatile suspended solid (VSS), chemical oxygen demand (COD) and Total Kjeldahl Nitrogen (TKN) were analyzed according to APHA Standard Method [14]. Table 1 showed the physical and chemical characteristics of the inoculum.

In order to find the optimum heat pre-treatment condition of the sludge and initial pH, temperature with the exposure time and pH were chosen as variables. The temperature was varied from 80°C to 100°C and exposure time was chosen at 30, 90, and 120 min. The pH was varied as 4.5, 5.5 and 6.5.

**Table 1.** Chemical Characterization of Beverage Sludge.

Parameter	Beverage Sludge
Total suspended solids	14996.67 ± 128.97 mg/L
Volatile suspended solids	10497.67 ± 90.28 mg/L
Chemical Oxygen Demand	3625.67 ± 60.68 mg/L
TKN	296.17± 2.80 mg/L
pH	6.58± 0.01

### 2.2 Experimental design

The 158ml serum bottles with working volume of 100ml were used as batch reactor. For each experiment, the sludge was prepared according to experimental design. The inoculum was added, and followed by the addition 1g of glucose and pH adjustment solution (1 N HCl or 1 N NaOH). The bottles were sealed with rubber stopper and flushed with  $N_2$  gas (99.99%) for 5 minutes at 10 ml / min in order to provide anaerobic condition. All experiments were conducted in an incubator shaker at 150 rpm for 24 hours in anaerobic mesophilic conditions (35°C).

Full factorial design allows for simultaneous study of several factors and the interaction between factors on response. A full factorial experimental design was employed in running experiments to optimize selected parameters for efficient  $H_2$  production. Design Expert version 7.0 was designated for this study to

generate statistical analysis, experimental designs, and optimization of the variables. The variables are coded according to equation (1).

$$x_i = \frac{X_i - X_i^*}{\Delta X_i} \quad (1)$$

Where  $x_i$  is the coded value of  $i^{\text{th}}$  test variable,  $X_i$  is the real value of  $i^{\text{th}}$  test variable,  $X_i^*$  is the value of  $X_i$  at the center point and  $\Delta X_i$  is the step size.

**Table 2.** Levels and variables for full factorial design.

Symbols	Variables	Levels		
A	Temperature	80°C	90°C	100°C
B	Time	30 min	90 min	120 min
C	pH	4.5	5.5	6.5

### 2.3 Analytical Method

Biogas volume was determined using gas tight syringe at room temperature and pressure (760 mm Hg). The biogas composition was measured using gas chromatograph (Perkin Elmer, AutoSystem GC) equipped with a thermal conductivity detector and data acquisition system. The temperature of the oven, injector and detector were 80°C, 200°C, and 200°C respectively with Argon as a carrier gas. The analytical procedure of APHA Standard Methods was used to determine COD, TSS, VSS and pH of the sample [14].

## 3. Result and Discussion

### 3.1 The optimization for maximum hydrogen yield.

The optimal values of the studied factors, i.e., heat treatment conditions (exposure time and temperature); pH and their interaction on hydrogen production were further analyzed using RSM. 20 experiments were carried out according to the schedule in Table 3 designated using Design Expert 7.0. to find the optimum heat treatment conditions and pH.

**Table 3.** Central composite design.

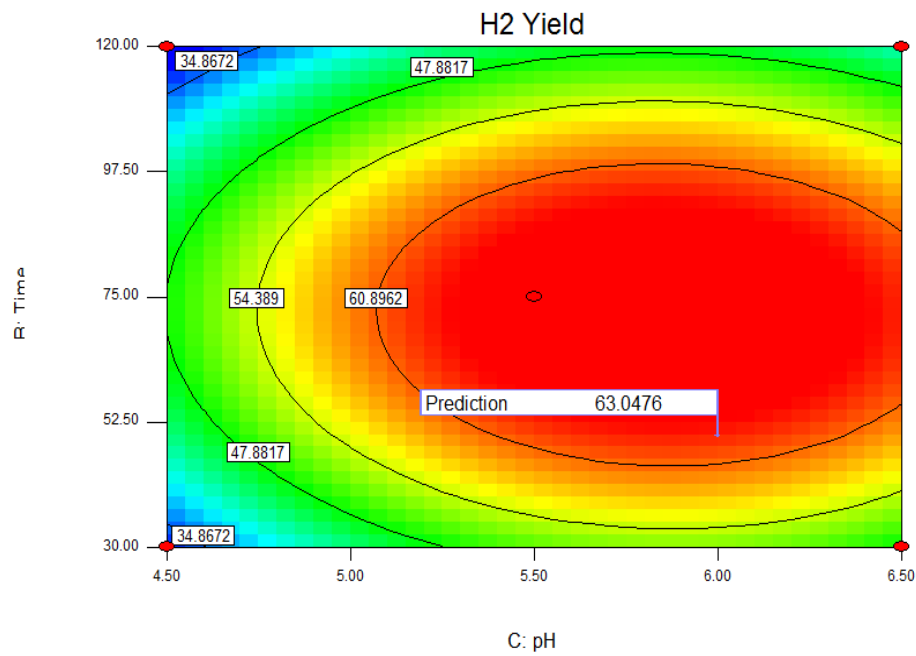
<b>Std</b>	<b>Run</b>	<b>Temp (°C)</b>	<b>Exposure Time (min)</b>	<b>pH</b>
<b>13</b>	1	90	75	4.5
<b>14</b>	2	90	75	6.5
<b>7</b>	3	80	120	6.5
<b>8</b>	4	100	120	6.5
<b>6</b>	5	100	30	6.5
<b>18</b>	6	90	75	5.5
<b>9</b>	7	80	75	5.5
<b>12</b>	8	90	120	5.5
<b>16</b>	9	90	75	5.5
<b>1</b>	10	80	30	4.5
<b>2</b>	11	100	30	4.5
<b>15</b>	12	90	75	5.5
<b>5</b>	13	80	30	6.5
<b>20</b>	14	90	75	5.5
<b>11</b>	15	90	30	5.5
<b>17</b>	16	90	75	5.5
<b>19</b>	17	90	75	5.5
<b>10</b>	18	100	75	5.5
<b>4</b>	19	100	120	4.5
<b>3</b>	20	80	120	4.5

For the beverage sludge, multiple regression analysis using Design Expert 7.0 software showed the importance of main effects and interaction of the three variables based on the response which was H<sub>2</sub> yield.

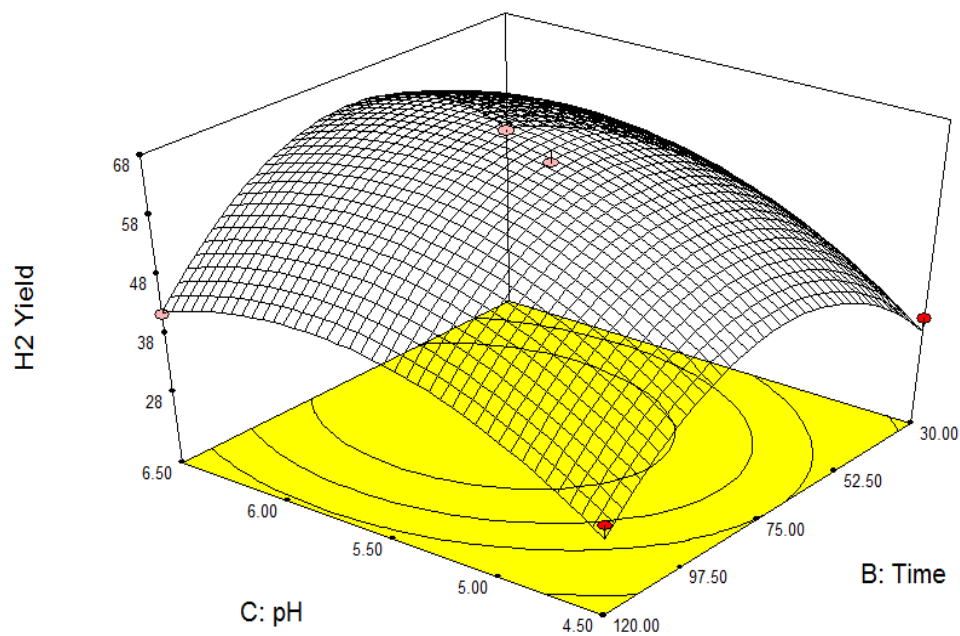
Equation (2) described the quadratic regression model in terms of actual factors based on analyses of variance (ANOVA) of the model.

$$\begin{aligned}
 H_2 \text{ Yield} = & +56.48 + 9.53A + 10.63B + 5.46C + 1.04AB + 2.00AC - 8.35 B^2 - 10.58C^2 \\
 & - 14.00A^2 B - 9.49 A B^2
 \end{aligned}
 \tag{2}$$

A, B and C are the coded values of temperature of heat treatment, exposure time and pH respectively. The ANOVA of quadratic regression model demonstrated that the model was statistically significant. The coefficient of multiple determinations, R<sup>2</sup> of the model was 0.834 which means that the model could explain 82.34% of the total variation in the system.



**Figure 1.** Contour lines.



**Figure 2.** Response surface plot.

To generate three-dimensional response surface plots, one variable was kept as the central level and the other two variables were varied within the experimental range. Hydrogen yield over pH and exposure time were estimated using two-dimensional contour lines and three-dimensional response surface. The heat treatment conditions chosen for this study that ranged from 80°C to 100°C, did not show any significance effect, probably due to its small range.

Data analyses using RSM showed that maximum hydrogen yield and COD removal efficiency was achieved when heat pre-treatment was done at 100°C for 50 min at pH 6.00. At optimum condition, the predicted hydrogen yield was 63.0476 ml H<sub>2</sub>/ mol glucose and the COD removal efficiency was 90.87%. The composite desirability was 0.904 which means that the probability to get predicted response was 90 times out of 100. However, in comparison to the other studies they were differences in the optimum heat treatment conditions and optimal pH for biohydrogen production and the possible reason behind the disagreement was the differences in term of source, temperature and other environmental factors [15].

Figure 1 showed that at 100°C, hydrogen yield increase with the increases of pH and exposure time. However, as the exposure time get longer, the hydrogen yield started to decrease. This was probably because high temperature and longer exposure time may have suppressed hydrogen producing bacteria.

Apparently, pre-treatment had a great influence on the biohydrogen production. Among all pre-treatment technologies, heat pre-treatment is the most commonly used pre-treatment of inoculum and it was proven to be able to suppress hydrogen consuming bacteria and at the same time enriching the hydrogen producing bacteria, thus maximize the hydrogen yield [16-17]. In agreement with other studies, the heat treatment applied in this study had successfully suppressed the HCB based on the absence of methane gas detected in the GC analysis [18].

In this study, it was found that at pH 6.00 is the maximum hydrogen production and COD removal efficiency was at the maximum. This was in agreement with a study done by Ren et al, indicated that pH 6 and 6.5 is the optimum operating pH that can improve acidogenesis and to avoid excessive propionate production [19]. Additionally, a study by Manyi-Loh et al. reported that lower pH slowed down the growth rate of methanogens and methanogenic biomass activities [20].

### 3.2 Verification of optimum conditions

The triplicate batch tests were conducted to verify the optimum condition analyzed by RSM. At given optimum heat treatment conditions and pH; 100°C for 50 min at pH 6.00, the hydrogen yield and COD removal efficiency slightly lower than the expected value (88.76% and 60.1766 ml H<sub>2</sub>/ mol glucose).

## 4. Conclusion

This study proved that heat treatment of sludge can significantly increase biohydrogen production as well as suppressing the methanogenic bacteria efficiently. It can also be concluded that the different heat treatment conditions led to differences in the microbial communities i.e. hydrogen producing bacteria which resulted in the different hydrogen yields. Data analysis from RSM showed that the optimum heat pre-treatment condition and pH was 100°C for 50 min at pH 6.00. In future, further tests will be conducted to identify the different combinations of different environmental and operational parameters in order to determine the most ideal conditions of biohydrogen production.

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