Penerbit Akademia Baru

Journal of Advanced Research in Biofuel and Bioenergy

Journal homepage: www.akademiabaru.com/arbb.html
ISSN: 2600-8459

Feasibility Study and Kinetics of Biohydrogen Production by *Escherichia coli* from Hydrolyzed Sago Wastewater

Open Access

Noor Illi Mohamad Puad[1,*], Nur Syafiqah Yasmin Saipullah[1], Tami Astie Ulhiza[1]

[1] Department of Biotechnology Engineering, Kulliyyah of Engineering, International Islamic University. Malaysia (IIUM), P.O. Box 10, 50728 Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:
Received 11 June 2018
Received in revised form 14 August 2018
Accepted 19 August 2018
Available online 11 September 2018

ABSTRACT

The demand of hydrogen gas (H_2) has increased considerably in recent years since it is a clean energy source and used as feedstock for some industries. However, H2 is most commonly produced from non-renewable sources that is considered as a less eco-friendly process. To avoid any environmental issues, another alternative is to produce this renewable H2 biologically. In this study, sago wastewater (SWW) was utilized as a substrate for Escherichia coli (E. coli) as the H2-producing bacteria. The main aim is to compare the performance of E. coli in producing biohydrogen (bioH2) from acid-hydrolyzed SWW and pure glucose in terms of total cell number, glucose concentration and bioH2 production. Based on the results, E. coli was able to produce bioH2 from SWW with about 30% less than pure glucose for its cumulative bioH2 production. Several kinetic parameters for bioH2 production by E. coli using SWW were determined from Monod model which are Yxs (1.7128 g/g), Yps (0.175 mol/mol), Ypx (0.0001 g/g), μ 1/0.345 h and td (2.01 h). In addition, a cumulative H2 production curve fitted by the modified Gompertz equation suggested that Hmax, Rmax and λ from this study were 1 mL, 0.05 mL/h, 1.003 h, respectively. The findings from this study concluded the potential of using hydrolyzed SWW in producing bioH2.

Keywords:
Biohydrogen, Escherichia coli (E. coli), Sago wastewater, Monod equation, Gompertz model

1. Introduction

Hydrogen (H_2) does not only have the highest energy content per unit weight among the established gaseous fuels, but it also produces water vapour as the only by-product upon its combustion [1]. However, most of H_2 is commonly produced from non-renewable sources such as natural gas (50%), petroleum-derived naphthenes and distillates (30%), coal (18%), and electricity produced from variety of fuels (2%). Since this process leads to the reduction of non-renewable energy sources and is considered as a less eco-friendly, it is vital to discover a sustainable energy source [2]. Another method to produce renewable H_2 is by biological decomposition of water and

* *Corresponding author.*
E-mail address: illi@iium.edu.my (Noor Illi Mohamad Puad)

organic compounds derived from biomass which is known as biohydrogen (bioH_2) as is not an expensive process since waste can be used as the raw material or substrate for its production [3]. This study focused on the dark fermentation process in which H_2-producing bacteria are the vital constituent to yield bioH_2 and the most widely-studied are belong to genus *Clostridia, Enterobacter* and *Clostridium* [4]. Dark fermentation is a process where carbohydrate-rich substrate such as food waste or organic wastewater is fermented with selected microorganisms to produce bioH_2 [4,5]. In this study, the substrate used was SWW which is also known as sago processing effluent. In common practice, SWW is usually discharged to the nearby river and this might affect the ecosystem in the long term period. SWW is rich with carbohydrates and can achieve sustainable low cost of bio-related product such as bioethanol and bioH_2 [6,7]. In addition, the effluent from sago industries has high chemical oxygen demand (COD) and total solids indicating high organic contents. Both parameters are important and need to be exploited for bioH_2 production along with proper pH control on substrate selection composition [5].

There are many factors that can be improved to optimize bioH_2 production such as type of H_2-producing bacteria. *Escherichia coli* (*E. coli*) is a fast growing bacterium and also well studied in the biotechnological operations [8]. However, to date, reports specifically on producing bioH_2 from SWW by *E. coli* is still scarce. Besides, kinetics study enable researchers to observe and understand specific process or reactions and further used for scaling-up the process. It has been reported that *E. coli* produced 3.12 mol H_2/mol substrate from strap molasses in sugar industry [1]. Based on the notion, this study compares bioH_2 yield of *E. coli* from SWW and glucose to observe the feasibility of using SWW as a substrate. In addition, kinetic parameters of *E. coli* in terms of cell growth rate, substrate uptake and bioH_2 production were also determined.

2. Experimental Method

2.1 Collection and Preparation of SWW

SWW was collected from a sago processing factory in Batu Pahat, Johor, Malaysia in which it consists of sediment and wastewater. Characterization of SWW in terms of pH, COD, total dissolved solid (TDS), total suspended solid (TSS), volatile suspended solid (VSS) and total carbohydrates was performed according to Clesceri [9] (Data for characterization is not shown). The collected SWW was first autoclaved to avoid the unwanted bio-degradation by any microorganims. The sediment of SWW was filtered and then dried under sunlight before it was blended into the powder form. Then it was packed in air-tight bags and stored at the room temperature for further use.

2.2 Bacterial Culture

E. coli strain W1485 (ATCC 12435) was obtained from the Department of Biotechnology Engineering, Kulliyyah of Engineering, IIUM. *E. coli* was sub-cultured on petri dishes using agar Luria-Bertani (LB) medium. The plates were incubated at 37 °C, and after 24 hours of growth, the fresh colonies were used to prepare the inoculum. For inoculum preparation, a loopful of colonies was added into the sterilized conical flask containing 150 mL of LB medium and then the flask was placed in a shaking incubator at 250 rpm and 37 °C for 12 hours [8].

2.3 Acid Pre-treatment of SWW

About 2 g of sago sediment and 100 mL of raw SWW (without sediment) were mixed with 150 mL of 1 M of H_2SO_4 [10]. The solution was stirred for 1-2 minutes before placing it into a water bath

at the temperature of 100 °C for 60 minutes. The solution was then let to cool in the ice water. After few minutes, filtration was done by using Whatman filter paper (90 mm) to remove any solid particles in the pre-treated SWW solution. The hydrolysate was then neutralized with 2 M NaOH until its pH reached 6.5 [7]. Next, the solution was submerged again into ice water bath to absorb the heat of neutralization and to avoid further decomposition of reducing sugar. The total reducing sugar (glucose) in hydrolysate was then analyzed using 3, 5-Dinitrosalicylic acid (DNS) assay method [11].

2.4 Experimental Set-up for Fermentation Process

The set-up of the experiment is illustrated in Figure 1. The left-side syringe was used to collect about 3 mL of sample for cell growth and substrate uptake analysis. Meanwhile another tube was to direct the produced bioH_2 gas to the gas trap cylinder for H_2 volume measurement.

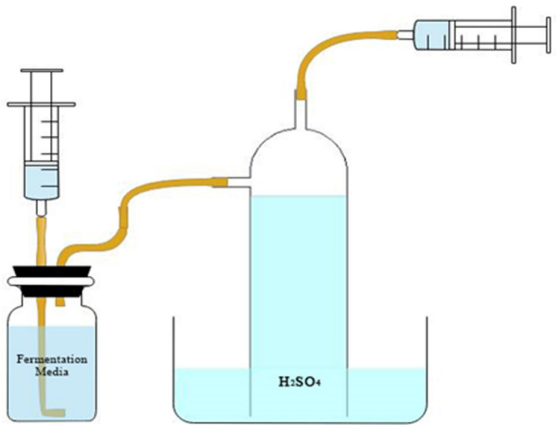

Fig. 1. Experimental set-up of fermentation process for bioH_2 production

A 500 mL Schott bottle with a working volume of 500 mL was used to carry out the fermentation process for bioH_2 production. About 150 mL of fresh inoculum was added into 350 mL of fermentation media (30% (v/v)). The concentration of each inoculum for every run was kept constant with OD_{600} equals to 1.90. The medium was stirred at 10 rpm and its temperature was controlled at 40 °C using a hot plate magnetic stirrer throughout the 24 hours of fermentation period [8] using hydrolyzed SWW and glucose, respectively. The sampling frequency was taken every two hours.

2.4 Analysis
2.4.1 Estimation of cell concentration

Initially 7 tubes containing 9 mL of LB broth medium were prepared for serial dilution. Then, 1 mL of fermented media (containing biomass) was diluted in the first tube and shaken well for uniform mixing. Another 1 mL of sample from the first tube was added to the second tube. The procedure was repeated until the seventh tube. From the seventh tube, 10 µL of sample was spread to the agar plate by using a hockey stick. After 24 hours of growth in an incubator, the colonies in the plate was ready for counting by using colony counting method [12].

2.4.2 Hydrogen gas production using water displacement method

The biogas produced was collected from the headspace of the gas trap cylinder. The volume of the gas is the volume of water displaced in the gas trap cylinder. The concentration of H_2 gas was measured using H_2 gas analyzer (GRI-8310 Portable Gas Detector). About 1 mL of gas was collected from the gas trap cylinder by a gas tight syringe.

2.5 Kinetics Study

In this study, several kinetic parameters including specific growth rate (μ), doubling time (t_d), and specific rate of product formation (q_p) were determined according to Monod model [12] as listed in Eq (1), (2), and (3). The specific growth rate of *E. coli* was determined from the slope of ln X *vs* time plot. The plot was taken at the exponential phase in which the μ is equal to μ_{max}.. From the same plot, doubling time and specific rate of product formation was determined accordingly.

$$\mu=\frac{lnX-lnX_0}{t} \quad (1)$$

$$t_d=\frac{ln\,2}{\mu} \quad (2)$$

$$q_p=Y_{px}\mu+m_p \quad (3)$$

In addition, Gompertz equation (Eq. 4) [13] was also applied to determine cumulative volume of hydrogen production, H_t (mL). By fitting the actual to the simulated curve, other parameters including maximum hydrogen production potential, H_{max} (mL); maximum rate of hydrogen production, R_{max} (mL/h), and lag phase, λ (h) were generated.

$$H(t)=H_{max}.exp\{-exp\left[\frac{R_{max,e}}{H_{max}}(\lambda-t)+1\right]\} \quad (4)$$

3. Result and Discussion

3.1 Effect of Acid Hydrolysis on SWW

Pretreatment of SWW is essential for enhancing the hydrolysis rate and increasing the amount of fermentable sugar from the lignocellulose wastes so that the sugars are readily available for *E. coli* to consume. Initially, glucose content in the raw SWW was 0.293 g/L. After acid hydrolysis using 1 M H_2SO_4 at the temperature of 100 °C for 60 minutes, the glucose content was increased to 2.095 g/L. It shows that acid hydrolysis can significantly breakdown the a lot of fermentable sugar into glucose [2]. This finding is in agreement with the previous one which reported that there was a dramatical increase of 86% glucose concentration after acid hydrolysis [8].

3.2 Comparison of E. coli Performance in Producing Biohydrogen from Hydrolyzed SWW and Glucose

The comparison of *E. coli* performance for bioH_2 production using hydrolyzed SWW and pure glucose, respectively, are interpreted in three different plots (Figure 2) which are in terms of total cell number (TCN) (cell/mL), glucose concentration (g/L) and cumulative of bioH_2 production (mL). All fermentation parameters were kept constant *i.e.* fermentation temperature of 40 °C with 10 rpm of agitation rate [8].

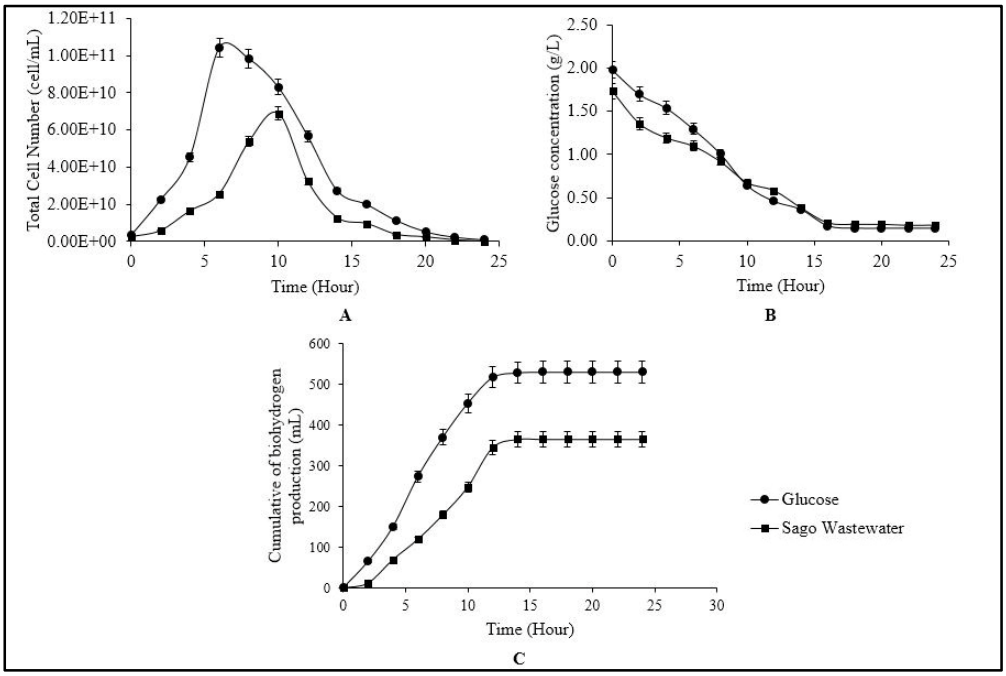

Fig. 2. Comparison of sago wastewater and glucose in terms of (A) *E. coli* total cell number (TCN); (B) glucose concentration; and (C) cumulative of biohydrogen

E. coli reached a higher maximum TCN in glucose (8th hour) than its counterpart in the hydrolyzed SWW medium (10th hour). *E. coli* entered its stationary phase after 10th hour while in glucose it was after 8th hour of inoculation. For *E. coli* substrate uptake, throughout the fermentation process, glucose concentration in glucose and hydrolyzed SWW medium were decreasing equally until the 14th hour of fermentation before both concentrations remained constant at 0.15 g/L and 0.18 g/L, respectively. During the fermentation process, 530 mL (117 564 ppm) of bioH_2 was accumulated in the glucose medium while in the hydrolyzed SWW, bioH_2 production was about 365 mL (92 520 ppm). Although *E. coli* growth and bioH_2 production using hydrolyzed SWW were lower than pure glucose, it shows that SWW has the potential to be utilized as a substrate for bioH_2 production by *E. coli*.

3.3 E. coli Cell Growth, Substrate Uptake and Biohydrogen Production using Hydrolyzed SWW

It was reported that in a bacterial growth phase, hydrogen starts to be produced in the log phase and reached its peak in the stationary phase [8]. In this study, bioH_2 production was generated simultaneously with *E. coli* cell division from the 1st to 14th hours after inoculation (Figure 3), showing that bioH_2 production is a growth-associated product [14]. As illustrated in Figure 3, glucose concentration in SWW medium showed the opposite trend than biomass and production of bioH_2. The initial glucose concentration was recorded to be 1.73 g/L at 0th hour. As the fermentation process proceed, glucose concentration declined till the 14th hour of fermentation (0.38 g/L).

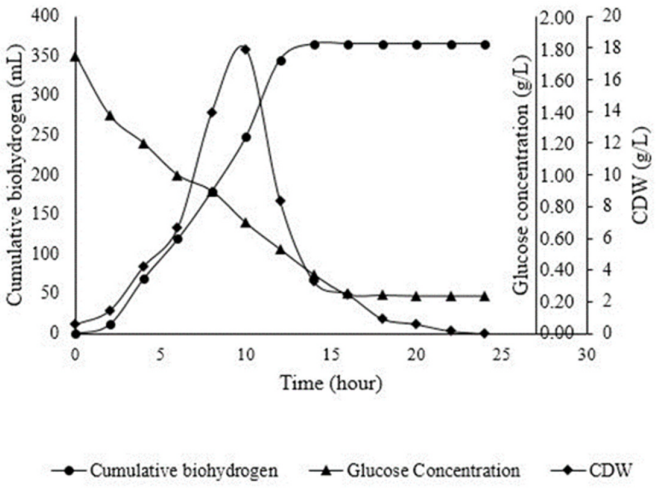

Fig. 3. The profile of *E. coli* in terms of biomass, substrate and product in the function of time using hydrolyzed SWW

3.4 Kinetic Parameters of Biohydrogen Production

3.4.1 Yield of biohydrogen production and bacterial growth

The yield of production, Y_{xs}, Y_{ps} and Y_{px} can be identified from the slope of the graph of biomass *vs* substrate, product *vs* substrate, and product *vs* biomass, respectively and the values are listed in Table 1. The calculated bioH_2 yield for both hydrolyzed SWW and glucose were comparatively lower than the theoretical yield (2 mol H_2/mol substrate) [15]. It was highlighted that the yield of bioH_2 produced by *E. coli* from SWW was 2.22 mol H_2/mol substrate [7]. The variation might be due to different SWW used that might have different characteristics thus affecting the performance of *E. coli* in producing bioH_2.

Table 1
Various yield values for *E. coli* fermentation using glucose and SWW

Yield	Glucose	SWW
Y_{xs} (g/g)	4.01	1.71
Y_{ps} (mol/mol)	0.82	0.18
Y_{px} (g/g)	0.00009	0.0001
Cumulative bioH_2 (mL)	530	365
BioH_2 yield (mol/mol)	0.25	0.16

E. coli exhibits mixed acid fermentation whereby alcohol and complex mixture of acid was produced as a by-product along with bioH_2 [16]. Based on the stochiometric equation (Eq. 5), 1 mol of glucose fermentation by *E. coli* produced maximum 2 moles of theoretical hydrogen yield with acetate and lactate as by-products. However, in this study, the true yield of product from substrate was lower than the theoretical yield. Therefore, optimization is required to increase the yield.

$$C_6H_{12}O_6 + H_2O \rightarrow 2H_2 + CH_3COOH + CH_3CH(OH)COOH + CO_2 \quad (5)$$

3.4.2 Specific rate of bacterial growth and product formation

The specific growth rate (μ), doubling time (t_d), and specific rate of product formation (q_p) were determined from Monod model as described in Section 2.5.. As illustrated in Figure 4, it was observed that the exponential growth occurred from the 1st to 10th hour and the μ value was estimated to be 0.345 h^{-1} while the doubling time, t_d was 2.01 h. The specific rate of product formation, q_p was found to be 3.45×10^{-5} h^{-1}.

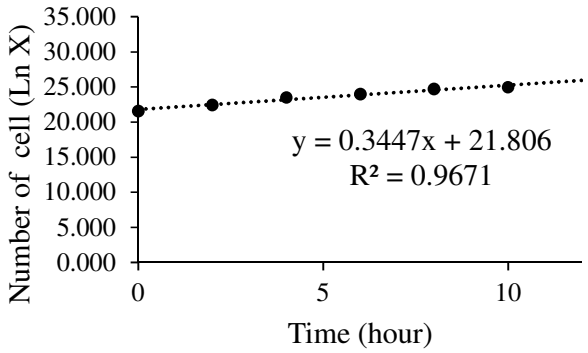

Fig. 4. Logarithmic of *E. coli* growth during $bioH_2$ production

3.5 Cumulative Biohydrogen Production

The Modified Gompertz model was used to fit the actual cumulative $bioH_2$ (Figure 5). From the model fitting curve, H_{max}, R_{max}, and λ were found to be 1 mL, 0.05 mL/h, 1.003 h, respectively. Apart from that, Table 2 shows the comparison of kinetic parameters obtained from the modified Gompertz equation using various substrates and bacteria. It was found that by using sodium formate, *E. coli* can produce maximum cumulative hydrogen of 740 mL [8], higher than the one achieved in this study which used SWW as substrate. On the other hand, *Enterobacter aerogenes (E. aerogenes)* strain performed better in producing $bioH_2$ from glucose (H_{max} 3285.03 mL) [17] as compared to *Enterobacter cloacae (E. cloacae)* (H_{max} 635.20 mL) [16]. It can be concluded that the simulated values of kinetic parameter obtained in this study were lower as compared to aforementioned studies, possibly due to different condition parameters used during the fermentation. For this study, modified Gompertz model was applied instead of other models such as Richards and Logistic because the kinetic parameters needed in this study were more suitable to find by using modified Gompertz and it is not a complex model compared to Richards. The best fit was observed with Richards model and modified Gompertz equation as it has flexible point of inflection while Logistic was more suitable for the cell growth rate [18].

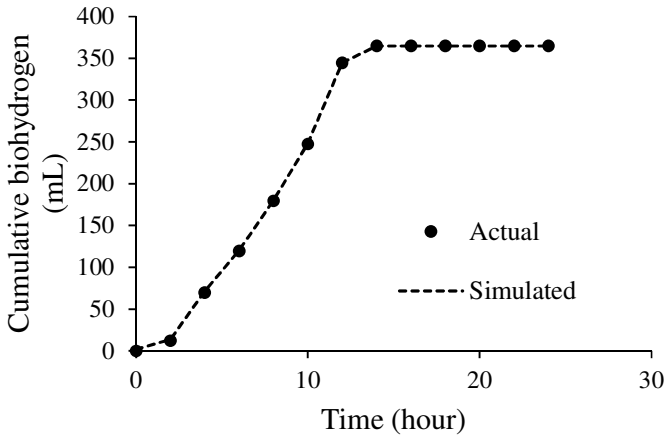

Fig. 5. Modified Gompertz equation fitted for biohydrogen production by *E. coli*

Table 3
Comparative review of kinetic parameters as obtained from modified Gompertz equation

Bacteria	Substrate	H_{max} (mL)	R_{max} (mL/h)	λ (h)	Reference
E. cloacae	Glucose	635.20	95.11	1.22	[16]
E. aerogenes	Glucose	3285.03	116.42	2.19	[17]
E. coli	Sodium-formate	740	35	2	[8]
E. coli	Sago wastewater	1	0.05	1.003	Recent study

4. Conclusions

Acid hydrolysis has increased glucose concentration in SWW by 86% which is from 0.293 g/L to 2.095 g/L. BioH_2 production of *E. coli* from the hydrolyzed SWW was compared with glucose medium in term of total cell number, substrate uptake and cumulative of bioH_2. All related fermentation parameters such as temperature and agitation rate were kept constant for all runs of experiment. The production of bioH_2 in SWW was lower compared to the glucose by 30%. Throughout the 24 hours of fermentation, the growth phases (lag phase, exponential phase, stationary phase and death phase) of *E. coli* were identified. The observed yield such as *Yxs*, *Yps*, and *Ypx* from this study were found to be 1.7128 g/g, 0.175 mol/mol, and 0.0001 g/g, respectively. Apart from that, μ and t_d of *E. coli* during its exponential growth were determined as 0.345 h^{-1} and 2.01 h, respectively. Lastly, the modified Gompertz model was used to fit the actual cumulative bioH_2 data from experiment to give the value of H_{max}, R_{max}, and λ as 1 mL, 0.05 mL/h, 1.003 h, respectively. From this study, it can be concluded that SWW can be utilized as a substrate in order to produce bioH_2 in the future and at the same time, will protect the environment. However, bioH_2 from this study is relatively low compared to other studies. Hence it needs further optimization in terms of process conditions or modification of strain's genes in order to make the production more feasible.

Acknowledgement
We would like to acknowledge the Ministry of Higher Education Malaysia (MOHE) for funding this project under the Fundamental Research Grant Scheme (FRGS 14-145-0386).

References

[1] Chittibabu, G., Kaushik Nath, and Debabrata Das. "Feasibility studies on the fermentative hydrogen production by recombinant Escherichia coli BL-21." *Process Biochemistry*41, no. 3 (2006): 682-688.

[2] Mullai, P., Eldon R. Rene, and K. Sridevi. "Biohydrogen production and kinetic modeling using sediment microorganisms of pichavaram mangroves, India." *BioMed research international* 2013 (2013).

[3] T. A. Ulhiza, N. I. M. Puad, and A. S. Azmi, "Review on biohydrogen production by dark fermentative bacteria using starch-containing waste as a substrate," *Journal of Advanced Researsch in Materials Science* 1, no. 1 (2017): 21–31.

[4] Kapdan, Ilgi Karapinar, and Fikret Kargi. "Bio-hydrogen production from waste materials." *Enzyme and microbial technology* 38, no. 5 (2006): 569-582.

[5] Sabariswaran, Kandasamy, Paramasamy Papitha, Ravichandran Indumathi, Sundararaj Selvakumar, and Samuel Paul Raj. "Biohydrogen production from sago effluent." *J Biodivers Environ Sci* 3 (2013): 17-23.

[6] Awg-Adeni, Dayang Salwani, K. B. Bujang, Mohd Ali Hassan, and Suraini Abd-Aziz. "Recovery of glucose from residual starch of sago hampas for bioethanol production." *BioMed research international* 2013 (2013).

[7] Puad, Mohamad, Noor Illi, Sarina Sulaiman, Azlin Suhaida Azmi, Yusra Shamsudin, and Maizirwan Mel. "Preliminary study on biohydrogen production by E. coli from sago waste." *Journal of Engineering Science and Technology* 10, no. 2 (2015): 11-21.

[8] Koók, László, Ákos Szabó, Péter Bakonyi, Gábor Tóth, Katalin Bélafiné Bakó, and Nándor Nemestóthy. "Process simulation of integrated biohydrogen production: hydrogen recovery by membrane separation." *AGRÁRINFORMATIKA/JOURNAL OF AGRICULTURAL INFORMATICS* 5, no. 2 (2014): 45-54.

[9] L. S. Clesceri, Standard methods for the examination of water and wastewater, 20th ed. American Public Healt Association, 1998.

[10] Puad, Noor Illi Mohamad, Nurainin Farhan Abd Rahim, and Azlin Suhaida Azmi. "Acid Pretreatment of Sago Wastewater for Biohydrogen Production." In *E3S Web of Conferences*, vol. 34, p. 02012. EDP Sciences, 2018.

[11] Miller, Gail Lorenz. "Use of dinitrosalicylic acid reagent for determination of reducing sugar." *Analytical chemistry* 31, no. 3 (1959): 426-428.

[12] J. Monod, "The Growth of Bacterial Cultures," Annu. Rev. Microbiol., vol. 3, no. 1, pp. 371–394, 1949.

[13] Zwietering, M. H., Il Jongenburger, F. M. Rombouts, and K. Van't Riet. "Modeling of the bacterial growth curve." *Applied and environmental microbiology* 56, no. 6 (1990): 1875-1881.

[14] Kumar, Narendra, and Debabrata Das. "Enhancement of hydrogen production by Enterobacter cloacae IIT-BT 08." *Process Biochemistry* 35, no. 6 (2000): 589-593.

[15] B. Ward, "Chapter 11 - Bacterial Energy Metabolism," in Molecular Medical Microbiology (Second Edition), Y.-W. Tang, M. Sussman, D. Liu, I. Poxton, and J. Schwartzman, Eds. Boston: Academic Press, 2015.

[16] Khanna, Namita, Kanhaiya Kumar, Sona Todi, and Debabrata Das. "Characteristics of cured and wild strains of Enterobacter cloacae IIT-BT 08 for the improvement of biohydrogen production." *International Journal of Hydrogen Energy* 37, no. 16 (2012): 11666-11676.

[17] P. Karthic, S. Joseph, and N. Arun, "Optimization of Process Variables for Biohydrogen Production from Glucose by Enterobacter aerogenes," Open Access Sci. Reports, vol. 1, no. 2, pp. 1–6, 2012.

[18] Ramprakash, Balasubramani, and Karuppan Muthukumar. "Influence of sulfuric acid concentration on biohydrogen production from rice mill wastewater using pure and coculture of Enterobacter aerogenes and Citrobacter freundii." *International Journal of Hydrogen Energy* 43, no. 19 (2018): 9254-9258.