Sociae Polites

EISSN 2620-4975
ISSN 1410-3745

INCREASED LOW-COST BIOHYDROGEN PRODUCTION USING CO-CULTURE FERMENTATION OF DAIRY WASTEWATER

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Abstract

Biohydrogen is one of the most desired clean source energy due to the multi-purpose function and its production can be achieved via dark and photofermentation. However, the challenge comes from the expense of utilized fermentation substrate. Hence, utilizing low-cost and abundance material is needed. Dairy wastewater is considered to be a desirable alternative substrate due to high organic content. Co-culture fermentation of Clostridium butyricum and Rhodopseudomonas palustris with sucrose-containing substrate increased hydrogen production yield better than using separated batches of both cultures. Volatile fatty acids (VFA) as the end-product of dark fermentation by Clostridium butyricum could be utilized by R. palustris. This literature research is addressing the potential of dairy wastewater as a co-culture fermentation substrate to increase biohydrogen yield in low-cost. The research method is a literature review using secondary data carried out by identifying, assessing, and interpreting findings on relevant research topics. The result of this research is dairy wastewater used as a co-culture fermentation substrate can enhance biohydrogen production by promoting growth rate of both bacteria and by increasing the production of VFA. The theoretical yield of biohydrogen produced from co-culture fermentation of dairy wastewater is 8,44 gram higher than the theoretical yield of biohydrogen produced from singular fermentation of non-wastewater substrate.

Keywords: biohydrogen, co-culture fermentation, dairy wastewater

DOI: 10.33541/sp.v22i1.3302
Sociae Polites : Majalah Ilmiah Sosial Politik
Faculty of Social and Political Science, Universitas Kristen Indonesia
ISSN 1410-3745 print/ ISSN 2620-4975 online
Volume 22, Number 1 (Indonesia Clean Energy Conference)
Pages 1-14

1. Introduction

1.1 Background

Energy demand continues in line with economic growth and population explosion. The increasing use of fossil fuels as energy sources can have negative impacts which are inevitable. The environtmentally friendly energy source can be the best option to substitute fossil fuels usage. Nowadays, global concern for energy is not only focused on obtaining an environmentally friendly energy source, yet concern on obtaining an economical energy source. In achieving these goals, United Nations declared Global Goals 7 “Affordable and Clean Energy” into Sustainable Development Goals (SDGs) programme. Thus, innovation in obtaining an affordable energy source is one of the actions to achieve that goal.

Biohydrogen is one of renewable alternative energy sources which are multipurpose and environmentally friendly. Biohydrogen production needs few energy, organic raw material, and does not produce pollution during the production (Liu et al., 2012). Biohydrogen production can be obtained by fermentation process with microorganisms such as bacteria (Osman et al., 2020). The bacteria that can be used in this process are *Clostridium butyricum* and *Rhodopseudomonas palustris*. *C. butyricum* is Anaerobic gram positive bacteria that produce biohydrogen through dark fermentation (Kao et al., 2016; Cassir et al., 2016). While, *R. palustris* is purple nonsulfur photosynthetic bacteria (PNBS) that produced biohydrogen by photo fermentation (Kao et al., 2016).

Biohydrogen production can be increased by combining both bacteria in the co-culture system. Co-culture is the system of fermentation that involves two or more microorganisms (Tesfaw and Assefa, 2014). This system involves dark and photo fermentation. Dark fermentation is done by *C. butyricum*, while photo fermentation is done by *R. palustris*. Both process will proceed simultaneously and produce biohydrogen separately

The expensive medium raw material cost is one of the challenges in producing biohydrogen (Osman et al., 2020). That challenge can be solved by using potential wastewater as a co-culture fermentation substrate. This will give economical value in producing biohydrogen and be the solution in solving wastewater problems in the environment (Otoo and Drechsel, 2018). Potential wastewater as a co-culture fermentation substrate is the wastewater that has high carbohydrate content. The carbon content in carbohydrates serves as a source of energy for microbes which can affect growth rates and the production of primary and secondary metabolites (Singh et al., 2017). One of the wastewater that has rich carbohydrates is dairy wastewater (Ataso, 2020).

The production of dairy wastewater in Indonesia is increasing in line with the increasing dairy product production in Indonesia. Dairy wastewater production in a dairy factory can produce almost 1000 m^3/ day wastewater (Handayani et al., 2020). Dairy wastewater contains organic compounds such as carbohydrates, amino acids, and lipids which are converted into sugars, acids, and fatty acids after undergoing the hydrolysis process (Demirel et al., 2005). Organic compounds content in that wastewater can be used as a substrate for microbes. This is evidenced by the use of dairy wastewater as a substrate in MCF (Microbial Fuel Cell) application (Mathuriya and Sharma, 2010).

Based on the description above, this literature study is conducted to examine the potency of using dairy wastewater as a co-culture substrate in producing biohydrogen. This literature study is expected to provide an overview of increasing environmentally friendly biohydrogen with low cost production so that alternative energy sources will be obtained to support the achievement of SDGs Goals 7: Affordable and Clean Energy.

1.2 Research Question

The research questions in this study are as follows:

1. How many is the theoretical yield of biohydrogen produced from co-culture fermentation of dairy wastewater.
2. How much is the cost of biohydrogen production saved from using co-culture fermentation of dairy wastewater.

1.3 Purpose and Objectives

The objectives of this study are as follows:

1. To acknowledge how many theoretical yields of biohydrogen is produced using co-culture fermentation of dairy wastewater.
2. To acknowledge how much cost of biohydrogen production is saved from using co-culture fermentation of dairy wastewater.

2. Literature Review

2.1 Biohydrogen

Biohydrogen can be defined as hydrogen which produced through biological processes (usually by bacteria) as bioenergy which comes from organic waste (Demirbas, 2009). Biohydrogen has a great potential for renewable energy in the future. Biohydrogen production will produce H_2 in sustainability, producing greenhouse gases (environmentally friendly) and can be easily converted into electrical energy (Rathore et al., 2018). In addition to producing energy that does not have a negative impact on the environment, hydrogen as an alternative fuel also has the highest energy content, which is around 141 MJ/kg (Singh and Das, 2019). Biohydrogen in general can be produced in three ways, fermentation, biophotolysis, and bioelectrochemical system. The fermentation process is very dependent on the characteristics of the substrate which carbohydrates are the most suitable type of substrate for fermentation (de Vrije and Classen, 2003). The fermentation process consists of two types, dark fermentation and photofermentation. The dark fermentation process can produce H_2 without requiring light whereas photofermentation requires light in the production of H_2. According to Elsharnuborry et al. (2013) the dark fermentation process will produce a higher H_2 yield than the photofermentation process.

Biophotolysis is a way of producing hydrogen from water with energy from sunlight using a biological system. In this process, O_2 and H_2 molecules are produced, with light as the energy source (Oh et al., 2013). Biophotolysis can occur directly and indirectly. In a direct biophotolysis, water splits by light energy with a wavelength of 680 nm to produce protons, electrons and oxygen. The electrons then transferred through PS II and PS I to an amount that is potentially sufficient to reduce ferredoxin (Fd) until the hydrogenase enzyme changes from $NADP^+$ to NADPH to produce H_2 (Osman et al., 2020). Meanwhile, the indirect biophotolysis process requires two stages of photosynthesis from light energy to carbohydrates as a form of chemical energy. In this process, H_2 is produced with CO_2 under anaerobic conditions supported by light. Bioelectrochemical system is a system which converts chemical energy into electrical energy (and vice versa) while using microbes as catalysts (Bajracharya et al., 2016). This system integrates microorganisms or other plant-based catalysts with electrochemical methods to enhance reducing or oxidation metabolism. In general, this system shows the process of generating electricity or achieving a reduction reaction regulated by the transfer of electrons between an electron acceptor and an electron donor (Fernandez et al., 2015).

2.2 Dairy Wastewater

Dairy industry is one of the types of food industry that contributes to pollution with pollutants being organic and normally consist of ⅓ dissolved, ⅓ colloid, and ⅓ suspended substances, while inorganic materials are usually present mainly in solution (Pathak et al., 2016). Dairy industry generates 0,2-10 liters of effluent per liter of processed milk with an average generation of about 2,5 liters wastewater per liter of milk (Shete, 2013). Dairy wastewaters are characterized by high BOD (biological oxygen demand) and COD (chemical oxygen demand) concentration, generally containing fat, nutrients, lactose, detergent, and cleansing agent (Singh et al., 2014).

Dairy industry processes involving receiving and storing of raw materials, processing of raw material into processed products, packing, storing processed products, and several additional processes are examples of a variety of processes or operations that are performed in dairy industries. The initial process such as homogenization, standardization, and separating are common to most plants and products. In the dairy industry, the amount of wastewaters generated during the initial process, balancing (equilibrating), stopping, and cleansing (Shete, 2013).

In dairy industries, the common strategies for wastewater treatment are grease trap, oil-water separator to remove floatable solids, flow equalization, and clearers to isolate suspended oil (Dongre et al., 2020). Technologies such as coagulation or flocculation and oxidation process have been developed several years to remove the organic matter from dairy wastewaters. These methods are effective for wastewater treatment but are expensive, large power demands, more chemical consumptions, and large area availability (Pathak et al., 2016).

Dairy wastewaters are known to be used as substrates for microorganism growth. It proved by the use of dairy wastewaters as substrates for mixed anaerobic inoculum to generate hydrogen with different treatment methods (Mohan et al., 2007). Dairy wastewaters are also known to be used as substrate for Microbial Fuel Cell application (Dongre et al., 2020). Dairy wastewaters contain various organic compounds such as carbohydrate, amino acid, and lipid that are converted into glucose, acid, and fatty acid after the hydrolysis process, therefore dairy wastewaters can be used as substrate for microorganism (Demirel et al., 2005).

2.3 Biohydrogen Production via Co-Culture Fermentation

Co-culture is a fermentation process to obtain biohydrogen using a batch reactor containing two different bacterial cultures. The production of biohydrogen through the fermentation of two bacterial cultures in a co-culture system provides efficiency in the time, place, and quantity of the fermentation substrate. However, the use of co-culture must examine several parameters to be optimized so that biohydrogen production can run optimally. These parameters are pH, temperature, inoculum ratio, medium component, and control anaerobic conditions (Du et al., 2020).

Two bacteria that can be used in the co-culture system are *Clostridium butyricum* and *Rhodopseudomonas palustris*. Lo et al., (2010) proved that the two bacteria could associate to produce biohydrogen production using sequential dark-photo fermentation. C. *butyricum* will produce biohydrogen and COD (Chemical Oxygen Demand) in dark fermentation. The COD from dark fermentation will be used by R. *palustris* to produce biohydrogen through a photofermentation process. Co-culture fermentation system with disaccharide sugar substrates such as sucrose and lactose will produce biohydrogen of 278.6 mL/L/h.

a) *Clostridium butyricum*
   C. butyricum is a gram-positive, anaerobic, bacillus-shaped bacterium, has spores and can produce high amounts of butyric acid. This bacterium was first isolated from the intestines of pigs by Prazmowski in 1880 (Cassir et al, 2015). Since then, research on these bacteria has been going on massively from various types of samples in the environment. A recent study found *C. butyricum* strains in 302 of the 978 samples tested (31%) (Ghoddusi and Sherburn, 2010). The highest percentage of isolates were found in soil, vegetables, soured milk, and cheese (Mountzouris et al., 2010). This

data shows that the bacteria are adaptive to milk waste. As a fermentative bacteria, clostridia produces short chain fatty acids (SCFAs), especially butyrate and acetate (Hamer et al., 2008). *C. butyricum* is strictly an anaerobic oxidizer of glucose to lactate, acetate, butyrate, ethanol and gases (H_2 and CO_2). This bacterium is the optimal H_2 producer because it is able to produce 4 mol-H_2 / mol-glucose (Seppala et al., 2011).

b) *Rhodopseudomonas palustris*

Rhodopseudomonas genus belongs to the Alphaproteobacteria class and has red to reddish brown pigments, rod-shaped, phototrophic, and motile. These bacteria can be isolated from soil and freshwater sediments. All strains contain bacteriochlorophyll a and carotenoids from the spirilloxanthin series (Ramana et al., 2012). *Rhodopseudomonas palustris* is used to phototrophically produce hydrogen from acetate and butyrate, which are the main soluble products of acidogenic dark fermentation (Carlozzi, 2012).

3. Results and Discussions

This study uses a literature review method to obtain information related to the research. Information gathered is obtained from scientific articles, scientific websites, books, and news pages published over the past 20 years. The keywords searched from any source covers the words biohydrogen, co-culture fermentation, dark fermentation, photofermentation, *Clostridium butyricum*, *Rhodopseudomonas palustris*, dairy wastewater, cheese whey waste. The results of this literature review are then used as a basic consideration in a research study that will provide scientific information regarding economical and efficient biohydrogen production. The views and results discussed in this study are the development of ideas from theory and experimental results from previous studies.

4. Result and Discussion

4.1 Biohydrogen Production using Co-culture Fermentation of Dairy Wastewater

Biohydrogen production using co-culture fermentation enables the efficient use of fermentation substrate. Utilizing obligate and facultative anaerobes could promote biohydrogen production with carbon source as the main substrate. The cultures of *C. butyricum* and *R. palustris* are chosen to be the inoculants in co-culture fermentation to achieve efficient biohydrogen production through dark and photo-fermentation. The hydrogen produced is the accumulation of biohydrogen resulting from both fermentation. In co-culture fermentation, carbon source from organic substrate is utilized by *C. butyricum* and resulting biohydrogen and volatile fatty acids (VFAs) as the secondary metabolite products. Still in the same batch, VFA produced from dark fermentation is utilized by *R. palustris* as their sole photofermentation substrate to produce more biohydrogen.

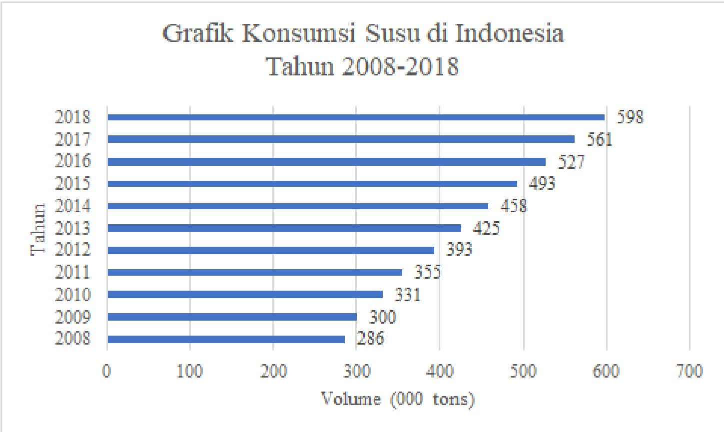
Abundance and cost of the fermentation substrate is one of the challenging issues in biohydrogen production using co-culture fermentation. To overcome such issues, utilizing carbon rich wastewater as the fermentation substrate will be a strategy to attain economical worth biohydrogen production. Dairy wastewater is one of the carbon-rich wastewater which contains 430-18,045 mg/L of chemical oxygen demand (COD), 40-8,240 mg/L of biological oxygen demand (BOD), and lactose as the main biodegradable carbohydrate (Ashekuzzam et al., 2019; Atasoy, 2020). Dairy wastewater abundance could be represented by the high rate of dairy production. Based on the Indonesian Central Bureau of Statistics (2019), dairy products availability is increasing every year in Indonesia. The increasing dairy products production is equivalent to increased demand.

Table 1. Fresh Milk Production in Indonesia year 2013-2018

Year	Offer (Tons)	Growth
2013	786.849	-
2014	800.749	1,76%
2015	835.124	4,29%
2016	912.735	9,29%
2017	928.108	1,68%
2018	951.003	2,46%

(Source: Badan Pusat Statistik, 2019)

Figure 1. Chart of Milk Consumption in Indonesia



(Source: weeklyindoperspective.org, 2018)

The volume of milk consumption from the chart above shows that milk consumption rose by 8,3% in 2008-2013 and continuously rose by 7% in 2014-2018. The increasing consumption demand is equivalent to production rate and waste production. One of the dairy products is cheese. Normally, a kilogram of cheese production process produces 9 liters of whey (Okamoto et al, 2019). From 25 kg of fresh milk with 0,12% citric acid addition in a cheese production process produces 23,88 kg of whey and 3,98 kg of curd (Komar et al., 2009). Whey is a byproduct produced during the cheese production process. It represents 85% - 90% of the volume of original milk and retains 55% of nutrients. Of the total whey solids, 75% is lactose (Slavov, 2017).

Dairy wastewater is used as a co-culture fermentation substrate because it has high organic content. Utilization of carbon-rich wastewater as a substrate for anaerobic fermentation is an effective method for producing biogas (Dębowski et al., 2020). *C. butyricum* can utilize dairy wastewater through dark fermentation. Utilization of dairy wastewater as dark fermentation substrate produces secondary metabolites such as higher VFA (acetic acid 886 ± 465 mgCOD/L) than VFA results in control fermentation medium (acetic acid 168 ± 103 mgCOD/L) (Atasoy, 2020). In co-culture fermentation, *R. palustris* bacteria will immediately utilize VFA from dark fermentation as an energy source to produce biohydrogen production through photofermentation. The availability of VFA, especially acetic acid, can support the growth of *R. palustris* because acetic acid is the best substrate for growth compared to pyruvate, ethanol, and lactate (Jiao et al., 2012).

The presence of *R. palustris* in co-culture fermentation could act as a buffer to maintain constant pH in the medium. A medium with a constant pH promotes optimum growth for *C. butyricum*. For instance, in a co-culture fermentation of cellulose with *C. cellulolyticum* and *R. palustris*, bacteria *R. palustris* act as a buffer that will affect metabolism of *C. cellulolyticum* to only use less ATP to maintain membrane potential. Such impact will contribute to better developed metabolism of *C. cellulolyticum* and eventually will achieve higher growth rate of the bacteria and higher consumption of cellulose (Jiao et al., 2012).

Hydrogen is a growth rate-associated product (Mullai et al., 2013). Luedeking-Piret Model elaborates the association noting that growing cells will produce products in a constant proportion with their growth (Mu et al., 2006). Therefore, optimum growth rate that can be achieved by *C. butryricum* and *R. palustris* can contribute to biohydrogen production rate.

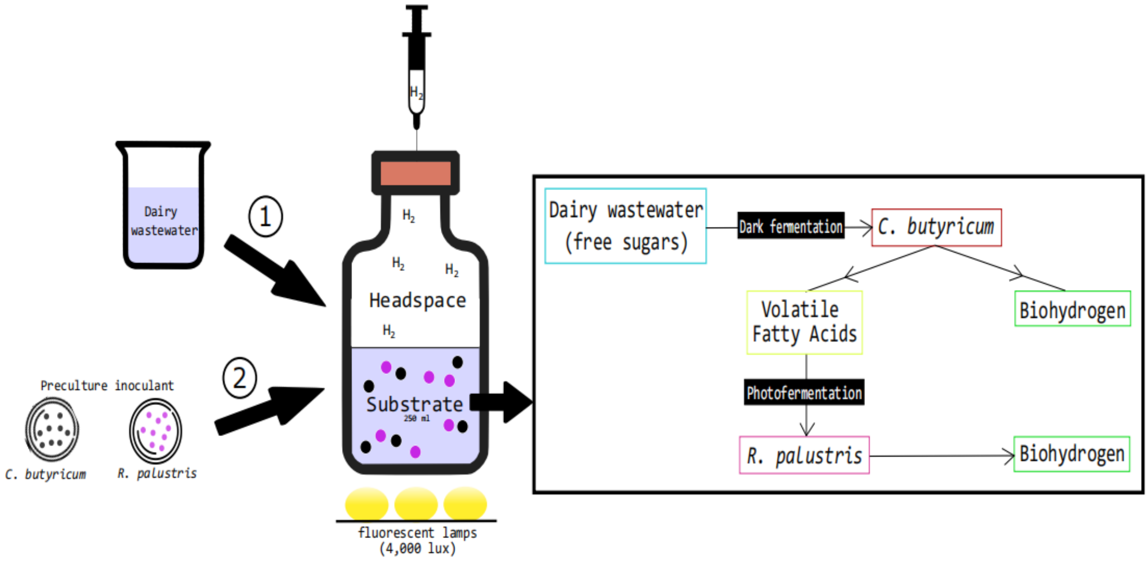
4.1.1. Co-culture Fermentation Method

In a co-culture fermentation, different inoculants and a carbon rich substrate are needed. The inoculants used are an obligate anaerobes preculture, *Clostridium butyricum*, and a facultative anaerobes preculture, *Rhodopseudomonas palustris*. Dairy wastewater, especially cheese whey with 75% lactose contained, is used as co-culture fermentation substrate.

Co-culture fermentation method is utilizing both dark and photofermentation to produce accumulated biohydrogen in a single bioreactor or fermentor. Serum bottle with 500 ml volume is used as a fermentor for a laboratory-scale fermentation. A fermentor is filled with 250 ml of cheese whey as a co-culture fermentation substrate and 25 ml of each *C. butyricum* and *R. palustris* inoculants. The serum bottle is sealed with butyl rubber stopper and aluminum crimp-seal then the condition is made anaerobic by oxygen-free argon for 15 minutes. Both cultures in the serum bottle are cultivated anaerobically for 5 days with initial pH of 7.0 under mesophilic conditions (35-37°C). The bottle serum is illuminated with 4.000 lux to promote the growth of *R. palustris* (Abo-Hasheh et al., 2011; Jiao et al., 2012; Kao et al., 2016).

The inoculants for co-culture fermentation will immediately enter the exponential growth phase. Maximum biohydrogen production achieved from co-culture fermentation could be harvested in 71 hours after inoculation. Biohydrogen produced can be indicated by decreasing pH to 5,2 (Kao et al., 2016). Accumulation of biohydrogen produced then disperse in the headspace section of the bottle to furthermore be collected through a gas tight syringe. The collected biohydrogen sample is stored in a bottle with high purity argon to be evaluated for its maximum cumulative value of production and biohydrogen yield (Lo et al., 2010; Jiao et al., 2012).

Figure 2. Co-culture Fermentation Method Scheme



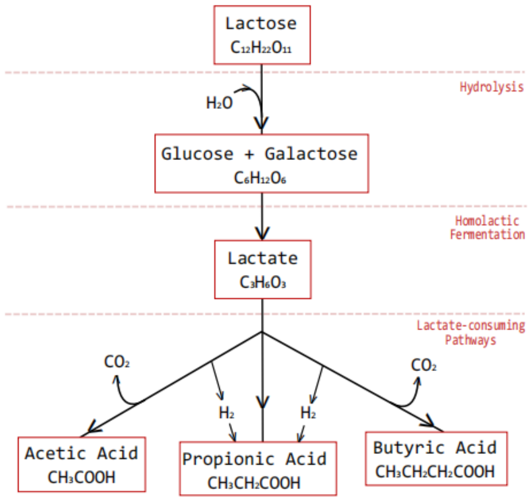
(Source: Author's Design, 2021)

4.2. Theoretical Yield of Biohydrogen from Dairy Wastewater

Biohydrogen production yield from co-culture fermentation of dairy wastewater can be predicted by calculating the theoretical yield. The maximum yield can be estimated from dark and photofermentation stoichiometry. Theoretical yield is used to estimate the maximum yield that could be obtained from a certain value of reactant (The LibreText Libraries, 2019).

In dark fermentation, the lactose content from dairy wastewater, especially from cheese whey, will be hydrolyzed into glucose and galactose which then undergo homolactic fermentation and produce lactic acid. Lactic acid will produce end products ie. acetic acid, propionic acid, butyric acid, and hydrogen through lactate-consuming pathway (Asunis et al., 2020).

Figure 3. Lactose Fermentation Pathway



Source: Author's Design, 2021

a) Mol of Hydrogen Calculation based on Stoichiometry

The reaction equation of lactose-dark fermentation is shown below (Sikora et al., 2013):

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2\ C_6H_{12}O_6$$
$$2C_6H_{12}O_6 + 4\ H_2O \rightarrow 8\ H_2 + 4\ CO_2 + 4\ CH_3COOH$$

Based on stoichiometry, hydrogen that will be produced from converting 1 mole of lactose into acetic acid is as much as 8 moles of hydrogen. In addition, lactic acid contained in cheese whey also could be utilized to produce biohydrogen for 6 moles of hydrogen per 1 mole of lactic acid. Hydrogen is obtained from lactic acid through homolactic fermentation with such reaction equation (Sikore et al., 2013):

$$C_3H_6O_3 + 3H_2O \rightarrow 6H_2 + 3CO_2$$

Other products from dark fermentation, the organic acid or VFA, include acetic acid, propionic acid, and butyric acid then furthermore utilized by *R. palustris* through photofermentation in co-culture fermentation. The reaction equation of organic acid photofermentation is shown below (Reungsang et al., 2018) :

Acetic acid $CH_3COOH \rightarrow 2H_2O + 4H_2 + 2CO_2$
Propionic acid $CH_3CH_2COOH \rightarrow 4H_2O + 7H_2 + 3CO_2$
Butyric acid $CH_3CH_2CH_2COOH \rightarrow 6H_2O + 10H_2 + 4CO_2$

Every 1 moles of acetic acid will produce 4 moles of hydrogen, 1 moles of propionic acid will produce 7 moles of hydrogen, and 1 mole of butyric acid will produce 10 moles of hydrogen.

b) Gram of Hydrogen Calculation

Cheese whey waste contains 154 g/L of lactose and 3,1 g/L of lactic acid (Moreno et al., 2015). Dark fermentation of cheese whey by *C. butyricum* produce 886±465 mgCOD/L of acetic acid, 1390±498 mgCOD/L of propionic acid, and 1610±697 mgCOD/L of butyric acid (Atasoy, 2020). Below is the *theoretical yield* (TY) calculation of hydrogen produce from cheese whey using co-culture fermentation:

Dark Fermentation:

1) Lactose:

TY of H_2 = 154 g $C_{12}H_{22}O_{11}$ x 1 mol $C_{12}H_{22}O_1$ /180 g $C_{12}H_{22}O_{11}$ x 8 mol H_2/2 mol $C_{12}H_{22}O_{11}$ x 16 g H_2 = 54,75 g H_2

2) Lactic Acid:

TY of H_2 = 3,1 g $C_3H_6O_3$ x 1 mol $C_3H_6O_3$/ 360 g $C_3H_6O_3$ x 24 mol H_2/ 4 mol $C_3H_6O_3$ x 48 g H_2/ 1 mol H_2 = 3,571/1,44 = 2,48 g H_2

Photofermentation:

1) Acetic Acid:

TY of H_2 = 0,886 g CH_3COOH x 1 mol CH_3COOH/ 60 g CH_3COOH x 4 mol H_2/ 1 mol CH_3COOH x 8 g H_2/ 1 mol H_2 = 0,47 g H_2

2) Propionic Acid:

TY of H_2 = 1,39 g CH_3CH_2COOH x 1 mol CH_3CH_2COOH/ 74 g CH_3CH_2COOHx 7 mol H_2/ 1 mol CH_3CH_2COOH x 14 g H_2/ 1 mol H_2 = 1,84 g H_2

3) Butyric Acid:

TY of H_2 = 1,61 g $CH_3CH_2CH_2COOH$ x 1 mol $CH_3CH_2CH_2COOH$ / 88 g $CH_3CH_2CH_2COOH$ x 10 mol H_2/ 1 mol $CH_3CH_2CH_2COOH$ x 20 g H_2/ 1 mol H_2 = 3,65 g H_2

Accumulation of theoretical yield obtained from 1 liter of cheese whey wastewater using co-culture fermentation is 63, 19 grams of hydrogen.

The theoretical yield of hydrogen obtained from co-culture fermentation with whey cheese waste produced 8,44 gram of hydrogen higher than the yield of biohydrogen produces using only dark fermentation of lactose with the artificial fermentation substrate.

4.3. Biohydrogen Production Cost

Production costs are costs that must be incurred by a company in producing a product. One of the production costs in producing this biohydrogen are determined based on the materials used. There is indeed a different cost generated from using wastewater as the main fermentation substrate. Therefore, to acknowledge how much biohydrogen production cost is saved from using dairy wastewater as the fermentation substrate, we need to elaborate on the non-wastewater substrate used to produce the biohydrogen.

The table below shows how much biohydrogen production cost is generated when using the non-wastewater as the main substrate.

Table 2. Fermentation Substrate Cost

Artificial Fermentation Substrate Composition adapted from Cardoso et al. (2014)	KH_2PO4	21 grams	Rp94.500
	K_2HPO_4	49 grams	Rp67.032
	$MgSO_4$	7 grams	Rp84
	Yeast extract	21 grams	Rp126.000
	Meat extract	3,5 grams	Rp7.700
	$(NH_4)_2SO_4$	7 grams	Rp9.100
	Laktosa	140 grams	Rp11.200
TOTAL			Rp315.616

When compared with the design cost of using the artificial fermentation substrate adapted from Cardoso et al. (2014), the use of cheese whey waste as a co-culture fermentation substrate to produce biohydrogen will save IDR 315.616.

5. Conclusions and Recommendations

5.1 Conclusions

Biohydrogen production using co-culture fermentation of dairy wastewater could be used to offer efficient biohydrogen production with economical value. Dairy wastewater used as a co-culture fermentation substrate promotes higher production of volatile fatty acids from dark fermentation which then contributes to the growth rate of *R. palustris*. The presence of *R. palustris* in co-culture fermentation also helps promote the growth of *C. butyricum* by acting out as a buffer in the medium to keep the pH balance. By promoting the growth rate of both inoculants, it contributes to a higher rate of hydrogen production.

The abundance and lower cost of dairy wastewater is likely to enable the notion of increased biohydrogen production in low-cost. From theoretical yield calculation, it can be concluded that the theoretical yield of biohydrogen produces using co-culture fermentation of dairy wastewater is 8,44 gram higher than the yield of biohydrogen produces using only dark fermentation of lactose with the artificial fermentation substrate. Biohydrogen production cost using dairy wastewater also saved IDR 315.616-, compared to biohydrogen production cost using non-wastewater.

5.2 Recommendations

The idea addressed in this study needs more advance consideration regarding the actual yield of biohydrogen produced and the entire cost of production needed besides the replacement of substrate fermentation cost. It is hoped that this recommendation will allow the development of co-culture fermentation of dairy wastewater on an industrial scale.

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