Bio-hydrogen Production from Tempeh and Tofu Processing Wastes via Fermentation Process using Microbial Consortium

by Wara Dyah Pita Rengga

Bio-hydrogen Production from *Tempeh* and *Tofu* Processing Wastes via Fermentation Process using Microbial Consortium: a Mini-Review

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Abstract. One of alternative energies that can replace fossil fuels is hydrogen. Hydrogen can be used to generate electricity and to power combustion engines for transportation. Bio-hydrogen produced from tempeh and tofu processing waste can be considered as a renewable energy. Bio-hydrogen produced from tempeh and tofu processing waste is beneficial because the waste of soybean straw and tofu processing waste is plentiful, cheap, renewable and biodegradable. Specification of tempeh and tofu processing waste were soybean straw and sludge of tofu processing. They contain carbohydrates (cellulose, hemicellulose, and lignin) and methane. This paper reviews the optimal condition to produce bio-hydrogen from tempeh and tofu processing waste. The production of bio-hydrogen used microbial consortium which were enriched from cracked cereals and mainly dominated by *Clostridium butyricum* and *Clostridium roseum*. The production process of bio-hydrogen from tempeh and tofu processing waste used acid pre-treatment with acid catalyzed hydrolysis to cleave the bond of hemicellulose and cellulose chains contained in biomass. The optimal production of bio-hydrogen has a yield of 6-6.8 mL/g at 35-60 °C, pH 5.5-7 in hydraulic retention time (HRT) less than 16 h. The production used a continuous system in an anaerobic digester. This condition can be used as a reference for the future research.

INTRODUCTION

Energy is one of primary needs in human daily life besides water and food. The problem is that the demand of energy keep rising and multiplying while the global petroleum reserves are decreasing and will cease to dominate, which surely will create an global energy crisis[1]. Therefore, it is needed to search for alternative energy sources. In the last decades, research efforts have focused mainly on the production of bioethanol and biodiesel as liquid fuels. However, the problem is that the use of biofuels, e.g. bioethanol and biodiesel have produced carbon monoxide and carbon dioxide as emissions, which can be considered as hazardous air pollutants[2]. The problem of air pollutants can be solved by using more clean energies. Hydrogen can be considered as one of alternative clean energies with a great potential to replace fossil fuels, which produces zero pollutants (i.e. *green energy*). This hydrogen can be produced by utilizing biomass as a main source, thus it can be called as "bio-hydrogen".

In Indonesia, the citizens are very fond with two traditional foods, called *tempeh* and *tahu* or *tofu*. The wastes from tempeh and tofu processing can be utilized as a potential source for bioenergy (i.e. bio-hydrogen). These

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wastes can be utilized via a fermentation process to produce bio-hydrogen. The fermentation process involved microorganism activity under anaerobic conditions[3]. The main components of sludge tofu waste processing were reductive sugar, sucrose, starch and volatile acids[4]. Whereas, the waste product of tempeh processing was soybean straw that contains about 44-48 % of cellulose, 12-15 % of hemicelluloses, 5-14 % of lignin and only 2-5 % of ash[5]. Soybean straw is a biodegradable cellulose resource, but it has never been fully utilized. Normally, the waste of the soybean straw is combusted, which then resulted in air pollution problem, due to CO_2 emission[6]. Therefore, the utilization of soybean straw to produce bio-hydrogen is a promising alternative path to solve energy problem and environmental problem simultaneously.

The process of making tempeh from soybean includes dehulling, soaking, cooking, drying, incubation and refrigeration[5]. Whereas, the process of making tofu includes grinding, cooking (boiling), filtration, protein coagulation, preservation, and packaging[7]. During the tofu production process in tofu industry, almost 30 % of soybean turned into waste, whereas in the tempeh industry, about 220 million tons of soybeans and an equivalent amount of byproducts (straw) are produced in the world every year[8]. All of these tofu and tempeh wastes are potential resources for an alternative renewable energy. Therefore, in this case, the problem of fuels scarcity and wastes can be solved by a fermentation process. The bio-hydrogen production is a form of renewable sources or organic waste, generating hydrogen on top of an additional benefit of waste tempeh and tofu reduction[9]. Tempeh and tofu waste processing are potential to be converted into bio-hydrogenbio-hydrogen by using fermentation process.

Hydrogen is used in the gas phase so that there is no pollutants produced during the process. Bio-hydrogen can be used directly (or after purification) in combustion engines for transportation while in fuel cells it can be used for producing electricity. It has high energy content per unit of weight (142 kJ/g) and since water is the only by-product generated by oxidative combustion, this makes hydrogen the ideal and most environmentally friendly alternative to fossil fuels[10]. Hydrogen is recognized as the most promising energy carrier since it is clean, pollution-free, sustainable, and efficient[11]. Molecular hydrogen has the highest energy content per unit weight among the known gaseous fuels (143 GJ ton^{-1})[12] and is the only carbon-free fuel which ultimately oxidizes to water as its combustion product. The objective of this paper is to review the optimal conditions to produce maximum yield of bio-hydrogen from tempeh and tofu processing waste via fermentation process using microbial consortium.

METHODS

Feedstock Pretreatment

There are some pretreatment methods for the mixed cultures for enriching hydrogen-producing, mainly include heat-stock, acid-base, aeration, freezing and thawing, chloroform, sodium 2-bromoethanesulfonate or 2-moethanesulfonic acid and iodopropane, and every pretreatment methods have different property[13]. Different pretreatments of tempeh and tofu processing waste are summarized in Table 1. to obtain a better pretreatment method for a given fermentative hydrogen production process.

TABLE 1. Feedstock pretreatment of wastes

Waste stock	Substrate	Fermentation system	Reactor type	Maximum hydrogen yield (mL/g)	Optimal pretreatment method	Ref. No.
Soybean straw of tempe	Cellulose	Anaerobic mixed bacteria	Batch	6	Acid	14
Sludge of tofu	Sucrose	Anaerobic digester sludge	CSTR	6.8	Acid	15
Bean curd	Cellulose	Anaerobic microflora	Batch	2.54 mL/g	Acid	16
Dietary fibers of tofu residue	Sucrose	Anaerobic thermophilic mixed culture	Batch	1.48 mL/g	Acid	17

CSTR: Continuous stirred tank reactor

As shown in Table 1, the highest bio-hydrogen production/yield from tempeh and tofu processing waste was reached by acid pretreatment and continuous system in an anaerobic digester.

Inoculum and H_2 Fermentation

The mixed cultures are simpler to operate and easier to control and may have a broader source of feedstock than the pure cultures. Because of that, fermentative hydrogen production processes using mixed cultures are more practical than those using pure cultures[18]. Pure cultures may be difficult to use for hydrogen production from organic wastes (food waste) because pure culture is easily out-competed by various non-hydrogen producers[19]. Inoculum and H_2 fermentation of tempeh and tofu processing waste are summarized and compared in Table 2.

TABLE 2. Inoculum and H_2 fermentation

Waste stock	Inoculum	H_2 fermentation				Ref. No.
		pH	Temperature (°C)	Hydraulic Retention Time (h)	Reactor type	
Soybean straw of tempeh	Mixed cultured were dominated by Clostridium Butyricum	7.0	35	10.56	Batch	14
Sludge of tofu	Microbial consortium were dominated by Clostridium Butyricum	5.5	60	6	Continous fermentation digester sludge	15
Bean curd	Clostridium sp	7.5	35 ^{0}C	12 h	Batch	16
Dietary fibers of tofu residue	Clostridium sp	5.5	60 ^{0}C	15 h	Batch	17

As shown in Table 2, the optimal condition to produce bio-hydrogen is strongly influenced by hydraulic retention time (HRT). The hydraulic retention time (HRT), also known as hydraulic residence time is a measure of the average length of time that a soluble compound remains in a bioreactor. HRT is an important controlling factor in the production of biohidrogen because it affects the contact time between the different reactants in the reactor. An HRT should be carefully selected to optimize process performance. The optimal conditions to produce bio-hydrogen from tempeh and tofu processing waste are when pH 5.5-7.0, temperature 35-60 °C, hydraulic retention time (HRT) of 6-16 h. The effect of HRT on bio-hydrogen production was investigated in a mixing reactor. The mixed culture was established by washing out propionate producing bacteria to show the value of HRT in bio-hydrogen production[20].

As mentioned previously, the bio-hydrogen production of tempeh and tofu processing waste involving Clostridium bacteria were carried out in two stage processes. The first stage was done at pH 7.0 and temperature of 35 °C with HRT of 10.56, which obtained hydrogen yield of 6 ml/g. Soybean straw was the first waste stock to be feed in this process, because it produced by-product in acid condition such lactate acid. The second stage was done at pH 5.5 and temperature 60 °C with HRT 6 h, which obtained maximum hydrogen yield of 6.8 mL/g waste stock. The feed for the second stage was the mixture of treated soybean straw and tofu sludge. The purpose of this process is to produce nio-hydrogen with maximal yield and short HRT. Figure 1 shows the schematic diagram of the bio-hydrogen production from tempeh and tofu processing waste via fermentation process.

Many factors may influence the bio-hydrogen production from tempeh and tofu processing waste using microbial consortia in an anaerobic digester, which include: inoculum, substrate, reactor type, hydraulic retention time (HRT), pH and temperature[13,20]. Inoculum with mixed bacteria consumes hydrogen produced by hydrogen-producing bacteria. When mixed cultures are treated under harsh conditions, hydrogen-producing bacteria would have a better chance than some hydrogen-consuming bacteria to survive. The inoculum are microbial consortia that was enriched by *Clostridium butyricum* and *Clostridium roseum*.

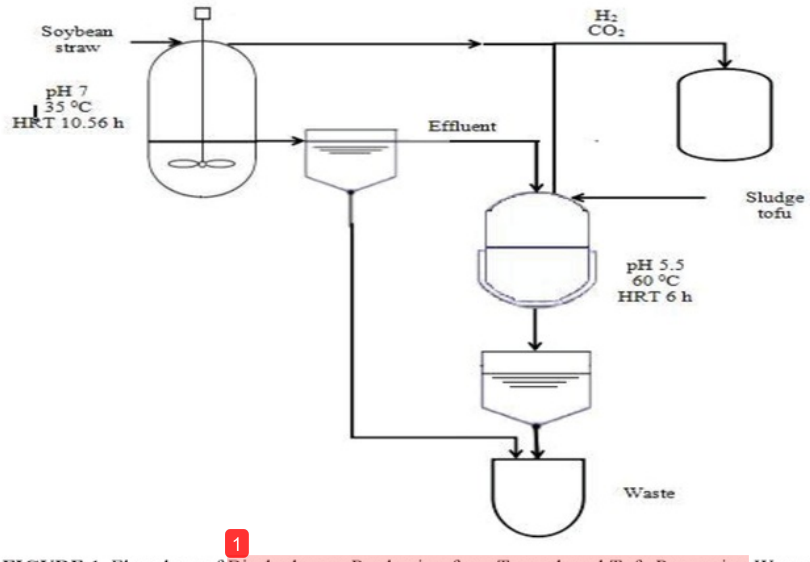

FIGURE 1. Flowsheet of Bio-hydrogen Production from Tempeh and Tofu Processing Waste

The substrates used are soybean straw of tempeh and sludge of tofu from their respective processing waste. Some complex substrates are not ideal for fermentative bio-hydrogen production because they will need some methods to pretreatment feedstock[21]. Tempeh and tofu waste processing contain substrates with simple structures that suitable for fermentative bio-hydrogen with acid pretreatment for feedstock pretreatment. Fermentative hydrogen production using continuous processes is more practical than batch process for large-scale operations. Continuous stirred tank reactor (CSTR) was widely used for continuous fermentative hydrogen production[21]. As mentioned previously, bio-hydrogen production is strongly influenced by the HRT. Increasing HRT can increase the bio-hydrogen production, however HRT at much higher levels could decrease the production of bio-hydrogen[22]. Optimal bio-hydrogen production by tempeh and tofu waste processing reached at HRT of 6 h. Steady microbial population and hydrogen yield observed as the HRT increased from 6 h to 12 h with a sampling day at 34 days (run 2-5) indicate that hydrogen yield representing the capability of microorganisms to convert carbohydrate into hydrogen gas is independent of the HRT.

The Ph is one factor that influences the activities of bio-hydrogen production, it affects the hydrogenase activity and the metabolism pathway. Inappropriate range, increasing pH could increase the ability of bio-hydrogen production during fermentation process[23]. Optimal pH in bio-hydrogen production from tempeh and tofu waste processing is approximately 5.5. This condition compatible with other research studies for bio-hydrogen of food waste, where optimal hydrogen production appears to take place at pH of 5.0 - 6.0 for food wastes[24,25]. The pH at much higher levels could decrease the yield of bio-hydrogen[23]. Another factor that influence bio-hydrogen production are temperature. Increasing temperature could increase the ability of bio-hydrogen production, but the temperature at much higher levels could decrease bio-hydrogen production[13]. The optimal temperature for fermentative bio-hydrogen production is not always same, it fall into the mesophilic range (around 37 °C) and thermophilic range (approx. 55 °C), respectively[18]. In case of bio-hydrogen production from tempeh and tofu processing waste, the optimal temperature was 60 °C.

CONCLUSIONS

Bio-hydrogen production via fermentation process using a mixture of tempeh and tofu processing waste in anaerobic digester can be considered to be one of promising processes of continuous hydrogen production. The bacteria used in bio-hydrogen production is microbial consortium which were dominated by *Clostridium butyricum* and *Clostridium roseum*. Based on the reviewed references, there are several variables that are used to produce bio-

hydrogen from tempeh and tofu processing waste. Bio-hydrogen optimal production has a yield of 6 - 12,8 mL/g with conditions at 60 °C, pH 5.5-7, in HRT less than 16 h, for a continuous system in an anaerobic digester.

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