

Potential of Producing Hydrogen Gas from Straw by Anaerobic Thermal Shocked Biological Sludge to Create Clean Energy

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Abstract

Hydrogen gas has been used widely in industrial aspect, especially as clean energy. On one hand, it reduces the dependency on fossil fuels; on the other hand it solves the environmental polluting issue and also reduces the greenhouse gases emitted to the environment. Producing hydrogen by chemical and physical methods require higher fee and investment. Producing hydrogen from renewable biomass, especially agricultural waste such as straws, can produce with low cost and decrease the amount of agricultural waste and pollutions. This article introduces the result of the beginning research to produce hydrogen gas from straw using biotechnology. Bacteria source to hydrolyze cellulose to median product such as glucose, lactate, acetate ethanol... and hydrogen gas is cultivated and enriched from biological sludge, taken from water spinach field and grow in medium 1658 MMS with glucose added straw (M2) and not added straw (M1). Hydrogen gas was introduced to growing environment to remove O_2 and create suitable environment for bacteria to grow. Thermal shock in 70 -80 °C condition in 72h, to exclude unbeneficial bacteria for hydrogen production and create suitable environment for bacterial benefit to fermentation to hydrogen to develop, then stabilize at 37 °C in 20 days. Microbes retrieved from M2 is used to hydrolyze straws by diluting it with nutrient solution at ratio 1: 4. Adding straw to the solution, proceed fermentation in 2 phases, phase 1 with straw content 33,3 g/L and phase 2 with straw content 130 g/L, in thermal shocked condition at between 55-60 °C. In fermentation, straw produce hydrogen above 50 days, dissolve organic content in bioreactor in phase 2 indicate that total amount of organic content is 6674.79 ± 1397.21 mg/L, methanol is 303.13 ± 108.95 mg/L. Hydrogen content in fermentation at phase 1 reached 6% and at phase 2 reached 17,82%. Growing thermophilic anaerobic bacteria from biological sludge thermal shocked for fermentation of straw to produce hydrogen, starting point shows that it is simple, economical comparing to other biotechnology of producing hydrogen and can be continued for industrial application

Keywords: Straw; Hydrogen; Bacteria; Thermal shock

Introduction

Hydrogen is a sustainable clean fuel source and considered to be the energy source of the future [1]. Around the globe, there are many hydrogen producing methods such as chemical method, converting hydrocarbon (fossil fuel, biomass) by heat method, electrolysis method, biological method, nano technology [2-5]. However, each method has different pros and cons. Chemical method is simple, easily performed; yet require high investment expense, suitable for producing a small amount in the laboratory. The chemical-physical method is highly economical efficient, using natural gases but require high investment, operation and maintenance. Electrolysis and using nano technology has the best advantage is utilizing the infinite energy of the sun, material of the inflow is water, high efficiency, however initial investment is quite large [6,7]. The biological method is relatively new, has many potential but still remain in experimental phase [7].

Vietnam is a manufacturing country and export rice at the top of the world, following by is a large amount of remaining straw, effecting the environment. Study to treat and utilize straw – agricultural waste, producing hydrogen by biological technology not only utilize straw as a clean energy source, but also has high application in industrial as well as daily lives; simultaneously reduce environmental pollution, green house effects...

Among all the producing H_2 method, anaerobic fermentation is available, hydrogen producing rate is high and capable of using many organic waste as substrate [8-10].

Materials and Methods

Experimental setup

The experimental set-up is shown in Figure 1. The laboratory bioreactor (liquid volume 10 L) was made of glass and was temperature controlled at 50-60 °C by running water through the outer mantle of the reactor. The biologically active reactor volume was 8 L, containing smoothly grinded straw and enriched bacteria as well as nutrient solution. Ar is continuously added to bioreactor as carrier to lead the air product out, produced gas is led to a saturated $\text{Ca}(\text{OH})_2$ bath to remove H_2S , CO_2 , the system is continuously operated in 60 days' time.

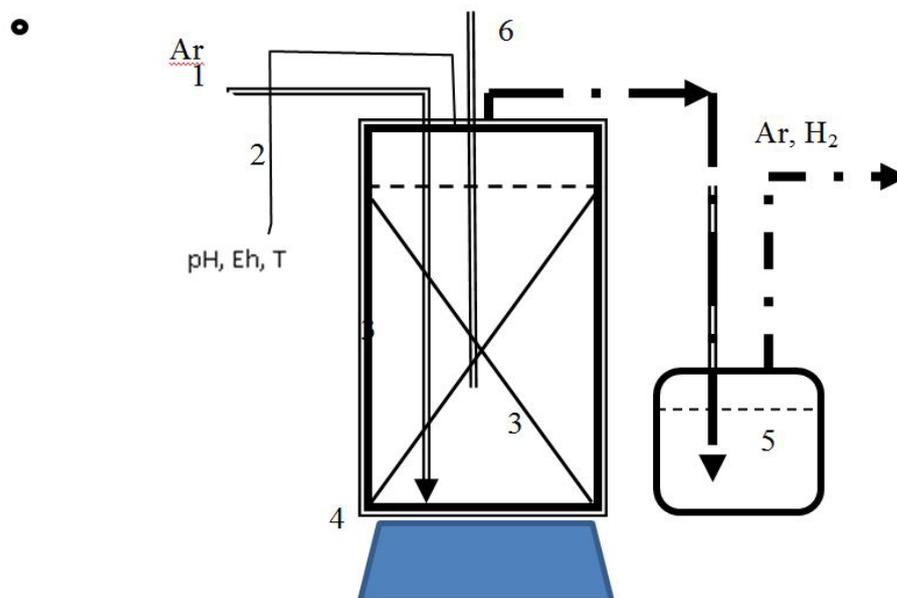


Figure 1: Layout of the biological hydrogen producing from straw reactor (1: Ar gas; 2: measurement of pH temperature, redox potential; 3: biological reactor; 4: heating magnetic stirrer; 5: lime cream tank; 6: Sample taken point)

The pH-electrode, redox-electrode, Temperature-electrode is inserted into the bioreactor and is connected to the meter online. The adjustment of the pH of the solution through the solution NaOH and H_2SO_4 in the input.

Medium: Enriching thermophilic anaerobic bacteria producing hydrogen gas as well as fermenting straws in bioreactor using nutrient solution with content indicated in Table 1 and 2. And isolation environment is shown in Table 3. All chemicals used were of extra pure quality and supplied by Merck (Darmstadt, Germany).

Nr.	Substance	Concentration
01	NaCl	6,93 (g/l)
02	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1,75 (g/l)
03	$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	1,38 (g/l)
04	KCl	0,16 (g/l)
05	NaBr :	25,0 (mg/l)
06	H_3BO_3	7,5 (mg/l)
07	$\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$	3,8 (mg/l)
08	KI	0,025 (mg/l)
09	CaCl_2	0,38 (g/l)
10	KH_2PO_4 :	0,5 (g/l)
11	$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$	0,5 (g/l)
12	$(\text{NH}_4)_2\text{Ni}(\text{SO}_4)_2$	2,0 (mg/l)
13	Resazurin	1,0 (mg/l)
14	Wolfe mineral solution	15,0 (ml/l)
15	Starch	5 (g/l)

Adjust final pH to 6.5 with H_2SO_4 . Prepare anaerobically under 80% N_2 , 20% CO_2 . Autoclave.

Table 1: ATCC medium 1658

Nr.	Substance	Concentration
01	$N(CH_2CO_2H)_3$	1,5 (g/l)
02	$MgSO_4 \cdot 7H_2O$	3.0 (g/l)
03	$MnSO_4 \cdot H_2O$	0.5 (g/l)
04	NaCl	1,0 (g/l)
05	$FeSO_4 \cdot 7H_2O$	0.1 (g/l)
06	$CoCl_2 \cdot 6H_2O$	0.1 (g/l)
07	$CaCl_2$	0.1 (g/l)
08	$ZnSO_4 \cdot 7H_2O$	0.1 (g/l)
09	$CuSO_4 \cdot 5H_2O$	0.01 (g/l)
10	$AlK(SO_4)_2 \cdot 12H_2O$	0.01 (g/l)
11	H_3BO_3	0.01 (g/l)
12	$Na_2MoO_4 \cdot 2H_2O$	0.01 (g/l)

Adjust to pH 6.5 with KOH to dissolve the compound.

Table 2: Wolfe's Mineral Solution: Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS.)

Nr.	Substance	Concentration
01	$C_6H_{12}O_6$	10.0 (g/l)
02	NaCl	5.0 (g/l)
03	Yeast	5.0 (g/l)
04	$FeSO_4 \cdot 7H_2O$	0.5 (g/l)
05	Agar	18.0 (g/l)

Table 3: Isolation media

Cultivation isolation of bacteria: Before experiment, Thermophilic anaerobic bacteria that produce hydrogen was taken from biological sludge in water morning glory field, centrifuged at rate 8500 rounds/min in 15 minutes, sludge afterward was thermal shock at 60 °C in 1 hour.

After pretreatment, bacteria were cultivated in the enriching environment: 200g centrifuged sludge + 600 ml nutrient solution (according to ATCC medium: 1658 MMS).

Solution was stirred and heated in range of 70 – 80 °C simultaneously add H_2 continuously in 72 hours aiming to create a suitable environment before put into stirrer at rate 170 round per min in moderation at 37 °C in 20 days.

Portion of cultivated and enriched samples:

- Sample 1 (M1): 200 g centrifuged, heat shocked sludge + 600 ml nutrient + 5 g glucose.
- Sample 2 (M2): 200 g centrifuged, heat shocked sludge + 600 ml nutrient + 5 g glucose + 5 g straw.

Dilute original sample by nutrient solution in ratio of 1:4, add 20 g dry minced straw into the sample, put into thermostats at 60 °C in 30 days. Bacteria sample after cultivated was mixed with 250 g straw and 5 L nutrient in bioreactor, kept in range 55 – 60 °C.

Sampling and analysis: Gas sample is taken once per day in the outflow of $Ca(OH)_2$ saturated bath. Organic compounds analysis sample is taken directly in reactor.

MeOH was determined with a gas chromatograph (Haedspace-Gaschromatographie) using a method adapted from (Uematsu *et al.* 2002). Soluble organic compounds such as Glucose, ethanol, acetate, lactate... in sample was analyzed with high performance liquid chromatography (HPLC 1100, Agilent 1200, using column 87H with pumping pressure 60 bar, temperature 60 °C with flowrate 0.6 ml/min, detector RID. Gas phase composition was determined with a gas chromatograph (Fisons Instruments GC 8000) equipped with two columns: 1.5 m X Y4' Teflon packed with Chromosorb 108 (60 to 80 mesh), and 1.2 m X Yirr stainless-steel packed with mol. sieve 5A (60 to 80 mesh). Hydrogen, methane, nitrogen were determined with a gas chromatograph equipped with detector (WLD), using a method adapted from Köhler 2000. Protein was determined with photometrically using a method adapter from Bradford 1976. Hydrogen retention time is measured with instruments for interpenetrated flow vessels.

Results and Discussions

Isolate anaerobic thermophilic hydrogen producing bacteria

To produce hydrogen from straw biologically, it require hydrogen producing bacteria. Therefore the first step is to isolate this bacteria specie [11]. Enrichment methods using acid, chemical, wet heat-, dry heat-shock, freezing and thawing was carried out for cattle manure sludge, and increased H_2 production using acid pretreatment was 1.9–9.8 times greater compared to control sludge [12]. A

comparative study was made across acid, heat and chloroform treatment on sewage sludge during immobilized H_2 production in a mixed-culture under anaerobic conditions. Chloroform treatment inhibited hydrogen consuming bacteria, avoided fast conversion of H_2 to acetic acid and repeated culture showed H_2 production till 15 days [13]. Heat pretreatment was the best among other pretreatments (acid and base) for enriching H_2 producing bacteria from anaerobic sludge [14]. The increased H_2 production depends on the source of the seed sludge and the pretreatment method. Heat treatment is a simple, effective and practical method in comparison to other methods for enriching H_2 -producing bacteria from different seed source [14-17]. In this study, hydrogen producing bacteria was not used from biological waste but isolated from active sludge from water morning glory field, where contain communities of bacteria.

Figure 2 and 3 indicate the result of enriching anaerobic thermophilic bacteria from natural biological sludge in condition of thermal shocked, both straw added and non-straw-added. In thermal shocked condition, aerobic bacteria and some anaerobic bacteria that cannot adapt to high temperature do not exist; combine with aerating hydrogen into the cultivating environment, forming a suitable environment for hydrogen producing bacteria.

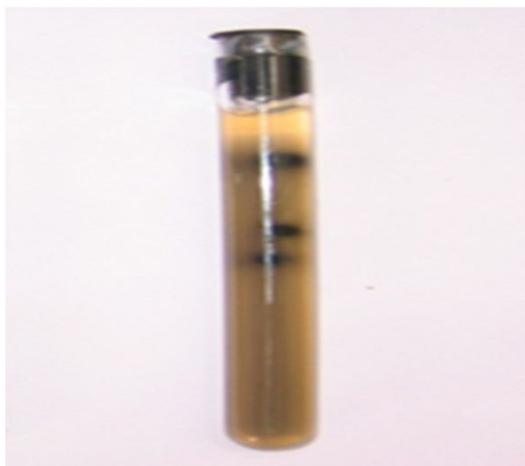


Figure 2: Sample of anaerobic thermophilic bacteria enriched in natural thermal shocked non adding straw environment (M1)



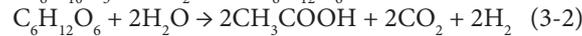
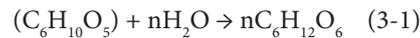
Figure 3: Sample of anaerobic thermophilic bacteria enriched in natural thermal shocked straw added environment (M2)

By adding straw to provide another carbon source, simultaneously create medium for bacteria activities, provide suitable condition for not only hydrogen producing bacteria but also cellulose hydrolysis bacteria and some other bacteria beneficial for producing hydrogen, therefore in the same cultivating condition, the amount of bacteria received in thermal shocked straw added sample (Figure 2) is higher than non-straw-added sample (Figure 3). Combine with the analysis result of hydrogen (Figure 4) produced in the cultivating process prove that in the sample exist hydrogen producing bacteria as stated in the aim, therefore, the product is continue to be used for the study process. As can be seen, thermal shocked used in this study is simply, using natural available bacteria, and using carbon source from agriculture waste to create low cost and industrial applicable comparing to using costly medium enrichment pretreatment method. This study utilize the natural bacteria source to clean the environment and providing clean energy source.

Content of dissolved organic compound

In the process of producing hydrogen from cellulose by biotechnology, the content of short chain organic compound such as glucose, lactate, acetate are the key of producing hydrogen, as well as methanol, ethanol can affect the process, thereby determine the

efficiency of the fermentation producing hydrogen [18-20]. The mechanism of producing hydrogen using fermentation of straw are as follow [18-22]:



Glucose created during the hydrolysis process of cellulose from straw, is an important organic source to provide for the hydrogen producing process biologically [21]. Lactate was produced from glucose hydrolysis, on one hand, it was consumed to enhance the growth of bacteria, on the other hand it is used as organic source to provide for bacteria to produce hydrogen [23].



Acetate is the co-product along with hydrogen in the fermentation process [12,24]. Methanol is formed through the reaction of bacteria with inflow material which are H_2 and CO_2 , reacted as follow:



The reaction create methanol by the activities of bacteria is the final reaction in the biological reaction chain in the anaerobic environment using hydrogen as reducer [22,24].

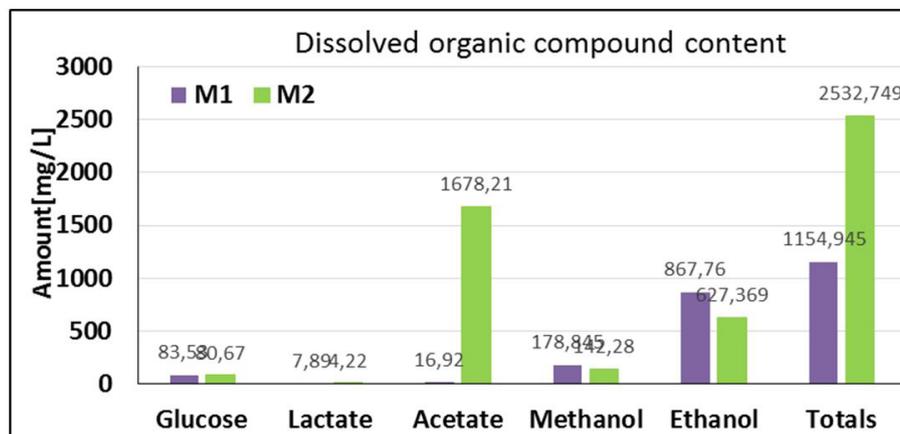


Figure 4: Amount of organic compound (sample 1) formed in condition of thermal shocked non-straw-added cultivation

The result of organic content analysis indicate that: in the environment of thermal shocked and non-straw-added sludge, after 20 days of cultivating bacteria, the total amount of dissolved organic compound in M2 is much higher than M1, proving the conversion of cellulose into dissolved organic compounds by bacteria occur well, especially converting into glucose, this product provides carbon source for bacteria producing hydrogen [12,19,24]. The glucose content in M1 is higher than M2 although M2 was added straw, the lactate content is also higher than M2, however, the amount of acetate is much lower than M2, this prove the hydrolysis of glucose to lactate and acetate is better, meaning producing more hydrogen.

The amount of methanol and ethanol is also higher than M2, caused by the created hydrogen reacting with CO_2 to form methanol and some bacteria consume hydrogen in M2 is limited [24]. Also, in both samples, the ethanol rate is high, bacteria hydrolyzing glucose to ethanol is growing and the bacteria converting ethanol to hydrogen in this environment is unsuitable, the ethanol amount afterward is still high [25,26].

It can be seen that the process of thermal shocking biological sludge in nature in condition of adding straw is a suitable environment, can enrich hydrogen producing bacteria from straw, thereby able to use them to ferment straw to produce hydrogen without consuming expensive hydrogen producing bacteria product.

Efficiency of producing hydrogen in cultivating process

The result of analyzing hydrogen received in different cultivating environments is shown in Figure 5.

The result indicates that, in the condition of cultivating without adding straw (M1), hydrogen does appear at first, even though with a small amount, its maximum rate reached 1,31%, after that the produced amount decrease and after 46 days hydrogen does not appear. This proves in the sample exist hydrogen producing bacteria, however due to not adding straw as carbon source, the amount of carbon in the cultivating process decreased as the original carbon provided is only sufficient for bacteria to grow, therefore carbon is insufficient for the process of producing hydrogen

In M2, the amount of H₂ received in average of 4,633%, much higher than M1 and it is released relatively equal during the whole process of cultivating. This proves in thermal shocked condition, not only removed bacteria harmful for producing hydrogen but also enriched aerobic producing hydrogen bacteria.

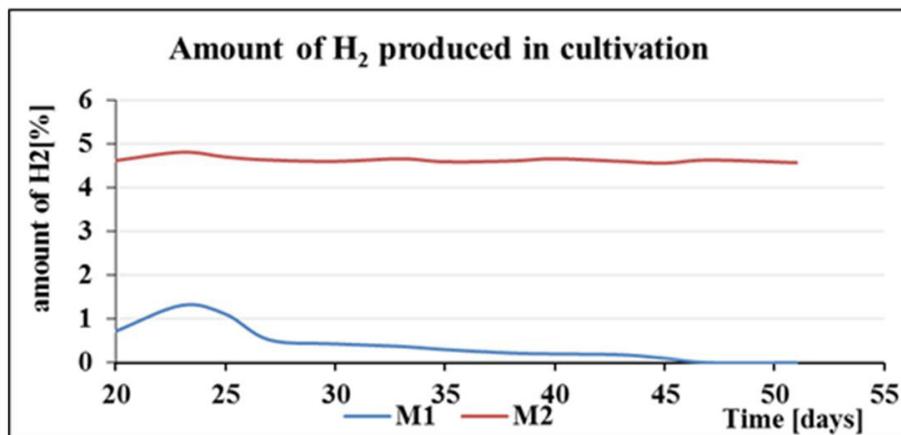


Figure 5: Ability to produce Hydro in different cultivating condition: thermal shocked without adding straw (M1) and thermal shocked straw added (M2), Duration 30 days (from 02-03 to 01-04 year 2017)

Adding straw to the environment not only provide carbon source for the process of fermenting straw producing hydrogen to increase the efficiency of producing hydrogen, but also create a suitable environment for enriching bacteria hydrolyzing cellulose into glucose, forming a medium for bacteria activities, especially favorable for hydrogen producing bacteria to grow. Therefore the condition of M2 can result in bacteria serve the process of producing hydrogen and produce hydrogen from straw. In the following research, experiments are conducted using bacteria cultivated in M2.

Amount of dissolved organic compound and efficiency of producing hydrogen in bioreactor

Bacteria were taken from M2, afterward diluted by nutrient solution, adding minced straw with original amount of 33,33g/l, temperature during the fermentation is kept at 55-60 °C. After 25 days of operating the bioreactor, continue adding straw into the reactor with total amount of 130 g/l, aiming to enhance the carbon source for the system.

The results of analyzing dissolved organic compound indicates that (Figure 6), when increase the amount of straw in the reactor, the total amount of organic compound in the bioreactor measured is very high, its value reached 6674,79 ± 1397.21 mg/l much higher than original (2532,75 mg/l), this shows the positive efficiency of adding a large amount of straw. Glucose content reached 4353,64 ± 1076.32 mg/l, this shows that the cellulose decomposing bacteria has adapted and operated productively, play an important role in creating carbon source for producing hydrogen by bacteria. However, glucose in this condition is much greater comparing to glucose content in the first phase, a part of glucose is transforms into lactate, a part transforms into acetate (1488.04 ± 487.64 mg/L) as well as ethanol to produce hydrogen. Result of lactate content indicates that its amount is higher than the first phase, but lower than acetate and ethanol, this shows the conversion of lactate into acetate and producing hydrogen is very good, combine with the content of ethanol shows that in this phase the ethanol content is lower than the first phase which indicate the process of producing hydrogen from ethanol occur very well.

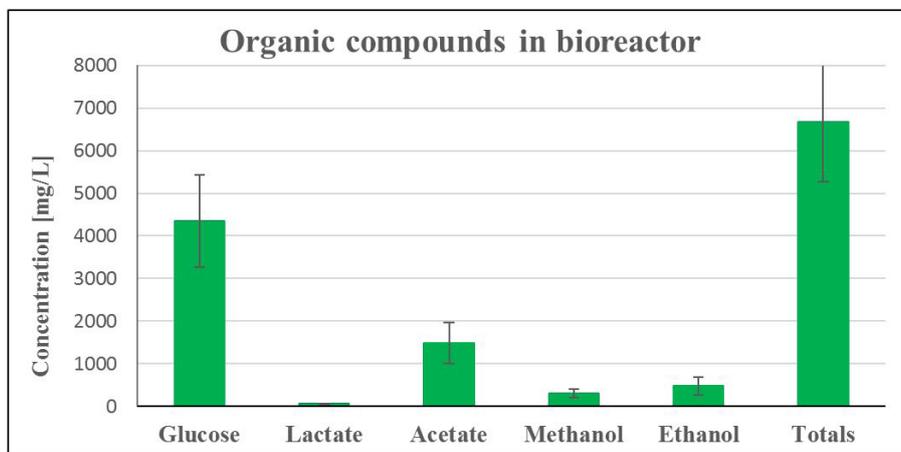


Figure 6: Content of organic compounds in bioreactor during fermentation producing Hydrogen

Based on the transformation of organic compound combine with analysis results of H_2 content (Figure 7) shows that, during the first period of the operating process in the first 5 days, hydrogen was not formed, in the next 20 days, hydrogen was produced in stability, cover approximate 5 to 6 %, this is strongly related to the content of dissolved organic compounds especially glucose (Zhang *et al.* 2006).

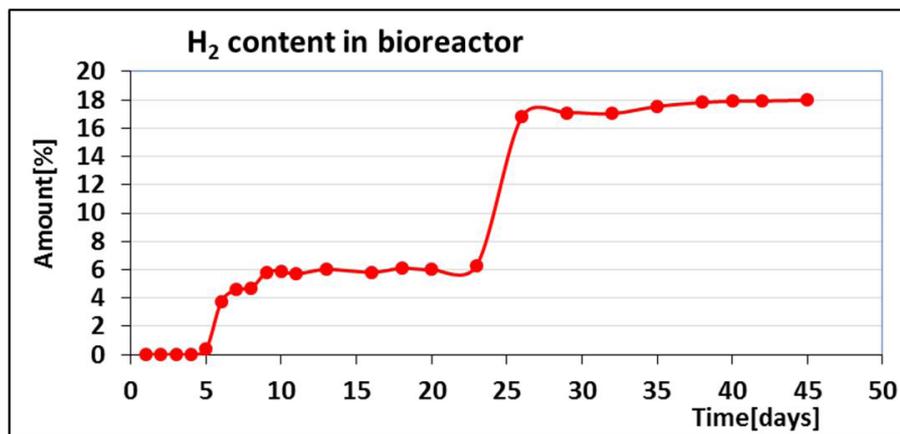


Figure 7: Result of Hydrogen content in bioreactor in fermentation

With the low amount of organic compound capable of producing hydrogen, lead to low amount of hydrogen produced due to equilibrium [19]. In the later phase, by adding straw, after a period to stabilize the environment, glucose as well as acetate, ethanol increased, the amount of H_2 increased and reached 17,82% and quite stabilized. This can prove the stabilization was established. Therefore, straw can have a great effect on the amount of organic compound, former compound of hydrogen producer and through that affect the efficiency of forming hydrogen.

Conclusions

Through the study, producing hydrogen from straw using biotechnology has proceeded enriching anaerobic bacteria producing hydrogen successfully from natural bacteria source in the condition of thermal shocked with straw added as a carbon source, this lessen the initial investment for the production and able to control the bacteria source.

Using straw, an available agricultural waste to produce hydrogen by fermentation method using natural bacteria source with relatively high efficiency, provide clean energy, simultaneously contribute to reduce polluting the environment, open a new path in producing clean energy.

This method could be applied to industrial production, however, further research is still required to find the optimum condition for producing hydrogen from straws.

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