Biochemical Hydrogen Potential Assessment with Anaerobic Batch Biodegradation of Organic Leachate from Aerobic Bio-leaching Bed Feeding Kitchen Waste and Napier Grass

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1　Introduction

Napier Grass is an herbage which consists of abundant cellulose. Due to its advantages of easy planting, high-growing rate (it can be reaped every 8 or 10 weeks), high yield density per year (dry matter yield is about 60~80 ton/hectare/year), inexpensive and high electron density (COD/TOC = 3.02 gCOD/gTOC), we decided to choose Napier Grass as one of the substrates in this study. In Napier Grass, nevertheless, considering the abundant cellulose which can lead to the difficulties in hydrolysis, we added kitchen waste as another substrate in order to co-metabolize with Napier Grass. Kitchen waste is a mixture containing different kinds of organic compound such as starch, protein, cellulose, fat and so on. Therefore, we speculated that the cellulose-hydrolyzing microorganisms might exist in kitchen waste. In addition, kitchen waste contains not only high organic loading but also a great deal of nutrients and trace elements, so it can provide microorganisms with abundant nutrition. That is why we want to add kitchen waste to co-metabolize with Napier Grass in order to increase the efficiency of hydrolysis.

In the conventional anaerobic biohydrogen reactor, the efficiency of hydrolysis is limited by the anaerobic condition. In this study, therefore, the hydrolysis is conducted under aerobic condition. We divide the fermentative biohydrogen into two parts. The first part is to hydrolyze cellulose using aerobic leaching bed. The second part is to produce hydrogen with the leaching from the leaching bed. Because of the fine and long configuration of cellulose and its difficulty in hydrolysis, cellulose in high concentration can result in fouling easily on reactor such as fluidized bed, CSTR and so on. So this study exploited the aerobic leaching bed to solve the fouling problems. Dividing the fermentative biohydrogen into two parts, we can optimize the conditions of hydrolyzing cellulose and producing hydrogen respectively in order to increase the efficiency of hydrolysis and hydrogen production.

This study examined the feasibility of producing hydrogen by fermentation of the leaching from the ABB in batch culture. The seeding is from the anaerobic hydrogen fermentor fed with vegetable kitchen waste and we use the leaching as the substrate to carry out the biochemical Hydrogen Potential (BHP) test with different food to microorganism ratio (S0/X0). Discussing the pH variation, water quality variation, cumulative hydrogen yield, hydrogen production rate and so on, we hope to find out if the leaching is suitable for producing hydrogen or not and the optimistic condition for hydrogen production as well.

2   Materials and Methods

The biochemical hydrogen potential test was modified from biochemical methane potential test (Owen, Stuckey et al. 1979 [1]). The carbohydrate was determined by phenol-sulfuric method (Herbert, Philipps et al. 1971 [2]). Water quality analyses were conducted according to the procedures described in the Standard Method 19th edition (APHA 1995 [3]). Microbial diversity was monitored by Terminal-Restriction Fragment Length Ploymrphism (Duangmanee, Padmasiri et al. 2007 [4]) and primers used in this assay were EUB338-6FAM and 1392R-HEX and the restriction enzyme for digestion was MseI.

3   Results and Discuss

3.1   The water quality of the leaching

Table 2 shows that the leaching contains total COD (49 g/L), soluble COD (43 g/L) and the total COD contains up to 88% soluble COD. From this percentage, we can know that the solid content is not high and the predominant utilizable nitration is soluble. Soluble TOC is 17 g/L and soluble COD to soluble TOC ratio is 2.49 which is very close to the value of glucose which is 2.67. This ratio (2.49) revealed that reductive carbon in the leaching is very abundant and therefore it can provide sufficient electrons for the subsequent hydrogen production process. From the result of Figure 1, we can find that the majority is lactic acid containing about 49% of electron in the leaching. The secondary is organic nitrogen. The leaching contains 27% and 9% of electron as soluble and solid organic nitrogen respectively. The last one is carbohydrate. Therefore, we can know from the electron mass balance that there is plentiful nitrogen in the leaching and this is contributive to the growth of microorganisms in the subsequent BHP test. The extra 6% unknown can be ignored due to the operational errors.

Table 2:   The water quality analyses of aerobic leaching fed with Napier Grass and kitchen waste mixture.

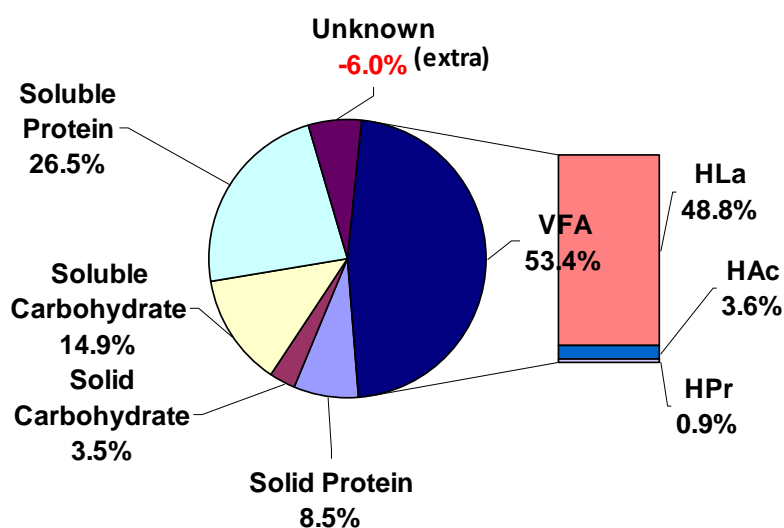| Characteristics		Unit	Mixed effluent of trickling bed
Solid	SS	g/L	4.71 ± 0.145
	VSS	g/L	4.34 ± 0.152
Carbo-hydrate	Total	g hexose/L	7.65 ± 0.351
	Soluble	g hexose/L	6.21 ± 0.491
	Reducing sugar	g hexose/L	1.06 ± 0.141
Protein	Org-N$_{total}$	g N/L	1.88 ± 0.012
	Org-N$_{soluble}$	g N/L	1.39 ± 0.12
	NH$_4^+$-N	g N/L	0.13 ± 0.007
COD	COD total	g/L	49.5 ± 0
	COD soluble	g/L	43.6 ± 0
VFA Conc.	Lactate	g/L	22.7 ± 0.82
	Acetate	g/L	1.7 ± 0.013
	Propionate	g/L	0.30 ± 0.003
Carbon	TOC solid	g C/g	0.20
	TOC liquid	g C/L	17.47
	TOC total	g C/g	0.11

Figure 1:   Electron distribution in the leaching from the leaching bed fed with kitchen waste and napiergrass.

3.2   The biochemical hydrogen potential tests (BHP)

The batch test was proceeded about 120 hours. The cumulative hydrogen and carbon dioxide production curve of different food to microorganism ratio (S_0/X_0) is showed in Figure 2. The feed blank (S_0/X_0=0 g COD/ g VSS) produced almost no hydrogen, so the high concentration of COD and carbohydrate would not disturb the experimental result.
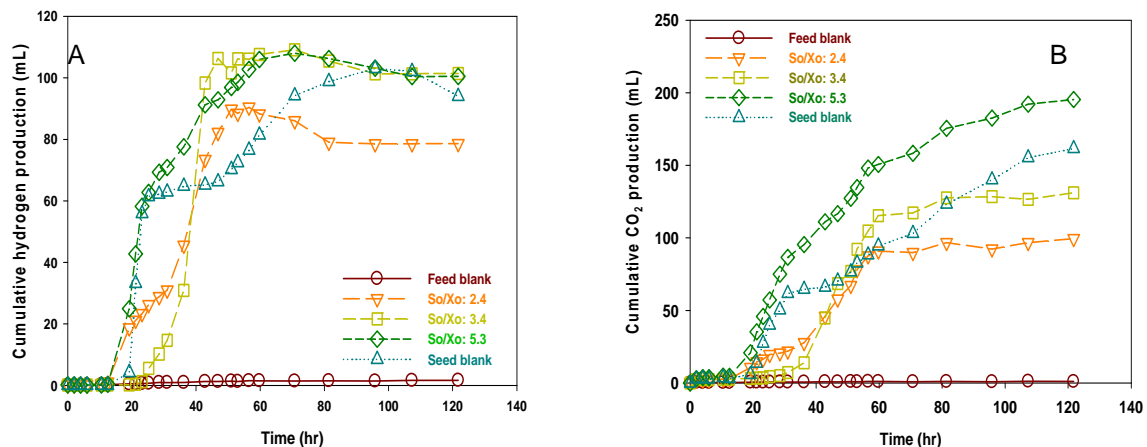

Figure 2:   Cumulative (A) Hydrogen (B) Carbon dioxide production curve of different substrate concentrations degraded by hydrogen producing bacterium.

Except the feed blank, others produced hydrogen obviously. When the batch test is carried on about 60 hours, the hydrogen production rate became much lower except the seed blank which still had the tendency to produce hydrogen. The higher the S_0/X_0 was, the more hydrogen yield would be produced and also the shorter the lag phase was. It is worth paying attention that the seed blank had quite good hydrogen yield about 94 ml and its maximum hydrogen production rate 21.2 ml/hr was even higher than that of S_0/X_0=3.4g COD/ g VSS and S_0/X_0=5.3 g COD/ g VSS. The hydrogen concentration in each group was over 45%. The group which S_0/X_0 is 3.4 mg COD/mg VSS possessed the highest hydrogen concentration about 62%. The cumulative carbon dioxide production curve was similar to that of hydrogen. According to the increase of the S_0/X_0, the carbon dioxide yield increase. On the sixtieth hour, the production of carbon dioxide in each group slowed down except for the seed blank. We speculate that it is because of the two-stage hydrogen production in the seed bland. In other words, microorganisms degrade organic matter in two different time. The first and the second one were about the twenty-first and sixtieth hour respectively. As a result, microorganisms were still carrying the biochemical reaction out at the sixtieth hour and this led to the increasing production of carbon dioxide.
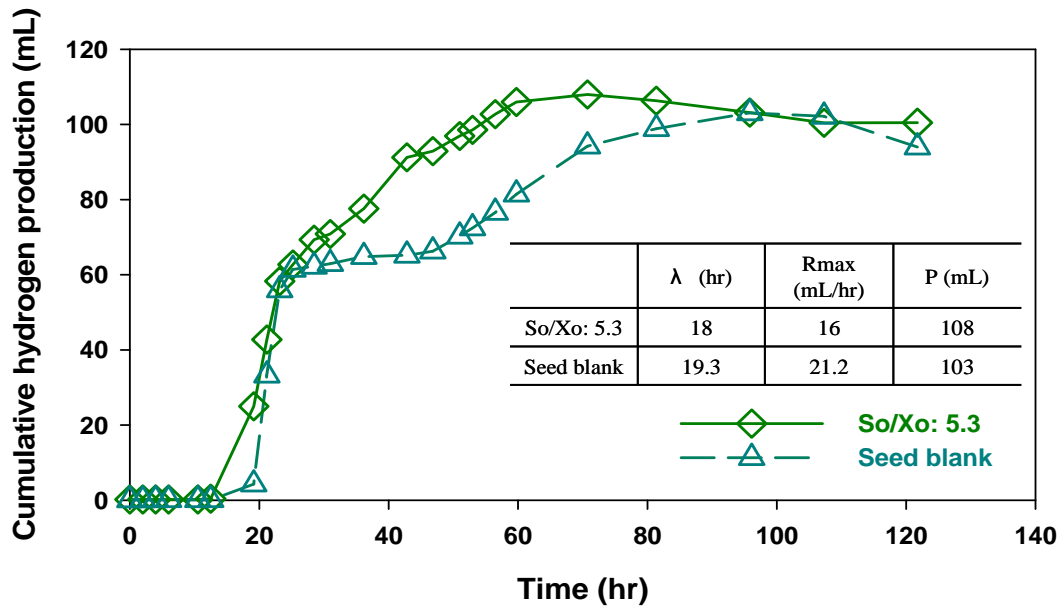
Figure 3:   **Cumulative hydrogen curve of So/Xo:5.3, Seed blank degraded by hydrogen producing bacterium**

Due to the quite good hydrogen production of the seed blank, we compared it with the highest S_0/X_0 group (S_0/X_0=5.3 g COD/ g VSS) intentionally. According to the regression of the improved Conpertz equation (2), the lag phases of the seed blank and the highest S_0/X_0 group (S_0/X_0=5.3 g COD/ g VSS) were very similar and the values of them were 19.3 and 18 hr respectively (Fig3). The hydrogen yields of these two groups were similar also. However, the R_{max} of the seed blank was 21.2 ml/hr and it is higher than that of the highest S_0/X_0 group which was 16 ml/hr. According to this comparison, hence, we can speculate that there was abundant hydrogen-producing organisms in the leaching and they were possible to be the contributors in this biochemical hydrogen potential test (BHP test).

$$y = P\exp\left\{-\exp\left[\frac{R_m e}{P}(\lambda - t) + 1\right]\right\}$$

(2)
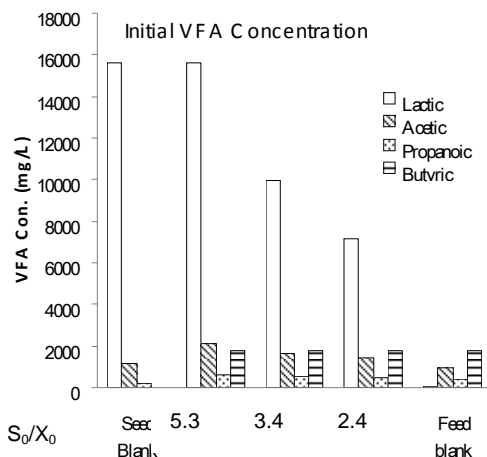
Figure 4: Initial VFA concentration at
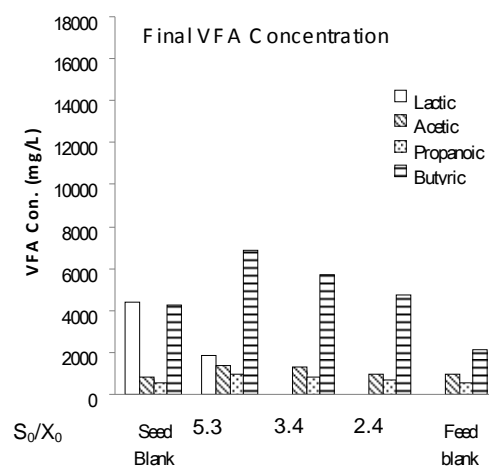   different conditions

Figure 5: Final VFA concentration at
           different conditions

Figure 4 and Figure 5 show the volatile fatty acids (VFAs) concentration before and after experiment respectively. Degraded after 120 hours, lactic acid in each group has quite good degradation rate. In the groups which S_0/X_0 are 2.4 and 3.4, there are approximate 100% degradation rate. Moreover, the degradation rates of the group which S_0/X_0 is 5.3 mg COD/ mg VSS and the seed blank are 88% and 72% respectively. The degradation rate of acetic acid in each group is about 30% except the 3.4 mg COD/mg VSS group which degradation rate is 21%. On the other hand, propionic and butyric acids have obvious production. The concentration of propionic acid increase according to the increase of the S_0/X_0. The highest production, which is 376 mg/L, appears in the highest S_0/X_0 group (S_0/X_0=5.3 g COD/ g VSS). The secondary is the seed blank and its propionic acid production is 354 mg/L. Butyric acid has a like increasing tendency with propionic acid. Its concentration also increase with the increase of the S_0/X_0, but the increasing extent is much more than that of propionic acid. The increasing production in each group is over 3,000 mg/L. The highest S_0/X_0 group (S_0/X_0=5.3 g COD/ g VSS) has the highest production (5,097 mg/L). The secondary is also the seed blank which production is 4,261 mg/L.

3.3 The result of T-RFLP

In this study, we exploited the Terminal Restriction Fragment Length Ploymrphism (T-RFLP) to observe the variety of the microorganism relationships. The advantage of this technique is that it can examine the microorganism diversity quickly, so it is a beneficial tool to the mixed culture system. The results from this assay show that the microorganism relationships between the feed and the seed blank vary obviously (Fig. 6). In the feed blank, the fragments of 497 bp (forward) and 318 bp (reverse) are predominant. The microorganism matched with these fragments is similar to *Thermoanaerobacterium thermosaccharolyticum*. This microorganism can utilize starch, cellodextrin, sucrose, xylose and other small molecular saccharides to produce hydrogen, acetic and butyric acid [O-Thong et al., 2007]. In addition, the forward fragments of 227 bp and 255 bp are predominant in the seed blank. The microorganism matched with 255 bp (forward) and 314 bp (reverse) is also similar to

Thermoanaerobacterium thermosaccharolyticum, which is reported in (Wang, Li et al. 2009). Comparing the analytic results of S_0/X_0 5.3 group with that of the seed blank, we can find that the position of both fragments has some difference. It infers that while the microorganisms in the seed and the feed are competing with each other, the former will be disappeared and finally the microorganisms in the feed will be the dominant. This phenomenon, hence, can illustrate that the microorganisms in the feed can utilize the feed more efficient than in the seed. Moreover, comparing the results of S_0/X_0 5.3 group with the others, we can know that the microorganisms cultured in the serums finally were dominated by the microorganisms came from the feed. We can verify, therefore, that there is abundant hydrogen-producing microorganisms in the leaching. As a result, we can infer that anaerobic hydrogen-producing microorganisms can still exist in the system of aerobic leaching bed. Being the seed directly, therefore, the microorganisms in the leaching can be exploited in the subsequent fermentative biohydrogen reactor.
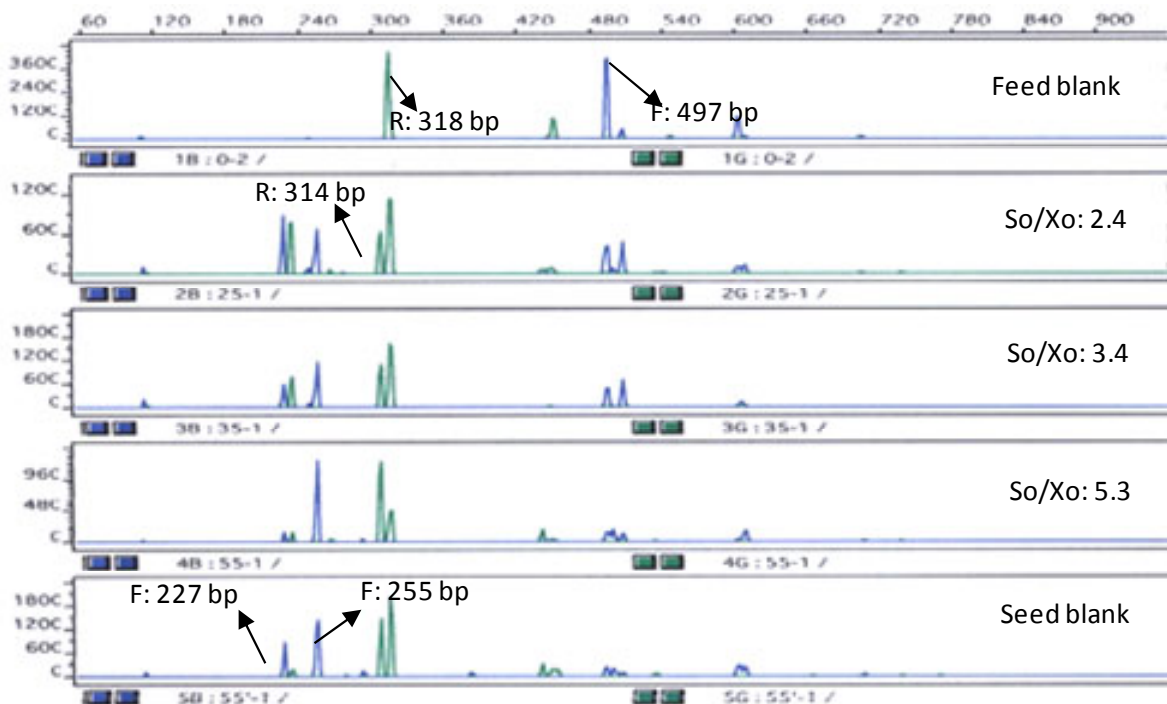

Figure 6: **T-RFLP analysis with MseI digested 16S-rDNA fragment. The total DNA was extracted from final liquid of BHP test fed with trickling bed effluent. (F: forward, R: reverse)**

4   Conclusions

1. In the leaching of aerobic leaching bed, the soluble COD, organic nitrogen and carbohydrate are 88%, 78% and 81% of the total respectively and this infers that the leaching bed has good hydrolyzing ability. Lactic acid (49% of the total COD) is maintained abundantly in the leaching and it can be utilized by the subsequent hydrogen-producing process.

2. Due to the similarity of hydrogen yield, hydrogen concentration, lag phase in the seed blank and the highest S_0/X_0 group (S_0/X_0=5.3 g COD/g VSS), and the higher hydrogen production rate (21.2 ml/hr) of the seed blank, we can infer that there are

abundant hydrogen-producing microorganisms in the feed and they are very possible to be the major contributors in this fermentative hydrogen experiment.

3. According to the results of T-RFLP, we can know that the leaching possesses abundant hydrogen-producing microorganisms which major species is Thermoanaerobacterium thermosaccharolyticum matched with the fragment length of 255 bp and this microorganism will grow better than those in the seed and ultimately become the predominant species.

4. In summary, we can utilize the microorganisms in the leaching as inoculums for the subsequent fermentative hydrogen production reactor.

Acknowledgements

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