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Bio-hydrogen Production by Hyperthermophilic Archeon, *Thermococcus onnurineus* NA1, Using Starch Containing Food Wastes

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Abstract

In this paper an attempt has been made to study the possibility of using food waste as a carbon source for growing Thermococcus onnurineus NA1 which produces H_2 as a byproduct of its metabolism. After varying the type of carbon sources - flour, corn powder, potato powder - added to the MYS medium in which the Thermococcus onnurineus NA1 is grown for 24 hours in a 80 degree oven, the content of H_2 was measured and compared using gas chromatography and the rate of growth was also compared using the change in pH. The concentration of each carbon source was also varied and analyzed using a gas chromatographer and pH meter. The possibility of recycling food waste to create H_2, a type of alternative energy, has great importance in suggesting an environmentally friendly means of creating energy, thus solving the problems of environmental pollution and energy crisis.

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1. Introduction

H_2 is being highlighted as an efficient and environmentally friendly energy source [1]. However, H_2 is currently produced from mining fossil fuels using steam reformation processes in the production of natural gas and naphtha.[2]. Recently, sustainable and environmentally friendly methods of producing H_2 have been developed, including microbial H_2 production by dark fermentation.

Hyperthrmophilic microorganism such as *Thermotoga*, *Caldicellulosiruptor saccharolyticus* [3], *Pyrococcus furiosus* [4], and *Thermococcus kodakaraensis* [5] have been turned out that produce between 2 and 4 mol of H_2 by dark fermentation. Recently, a new hyperthermophilic archaeon *Thermococcus onnurineus* NA1 has been isolated from deep-sea hydrothermal vent and it has been turned out that NA1 was able to produce H_2 from various carbon sources including carbon monoxide (CO), formate or starch [6, 7] Because *T. onnurineus* NA1 is effective in producing H_2 on starch, it is theoretically possible of NA1 to use food waste to increase cell density and H_2 production.

Recycling food waste as a carbon source would not only lessen food waste but also create useful clean energy in the form of H_2. Here, we report the cell growth and H_2 production by *T. onnurineus* NA1 from various carbon substrates such as potato, corn, flour, and a mixture of the three samples which are regarded as one of main components in food waste

2. Method and materials

2.1. Strain, media and culture condition

Thermococcus onnurineus NA1, which is used in this study isolated from a deep-sea hydrothermal vent area in the Manus Basin near Papua New Guinea[6]. This strain was obtained from marine biotechnology research department (courtesy of Dr. J. H. Lee) in KIOST (Korea Institute of Ocean Science & Technology).

T. *onnurineus* NA1 was pre-cultured in MYS (Mineral -Yeast extract- Starch) medium by using 20 ml-serum bottle sealed with aluminium cap and butyl rubber stopper at 80 ºC for 24 h. The MYS medium was composed of (g/L); yeast extract (3), soluble starch (5), NaCl (35), KCl (0.7), $MgSO_4$ (3.9), $CaCl_2 \cdot 2H_2O$ (0.4), NH_4Cl (0.3), Na_2HPO_4 (0.15), $NaSiO_3$ (0.03), $NaHCO_3$ (0.5), cysteine-HCl (0.5), resazurin (0.001). The media was supplemented with filter-sterilised 100X trace mineral solution [8] at a final concentration of 1X. After the media was autoclaved, the media was transferred into an anaerobic chamber (Coy, USA) to maintain the anaerobic condition. the medium was reduced by adding 0.005% (v/v) of 5% (w/v) $Na_2SO_4 \cdot 7H_2O$ and 1 ml/l of a filter-sterilised vitamin solution [9] was added. The initial pH of the media was adjusted to 6.5 by using 2 M HCl .

In order to test the potentials of hydrogen production on the utilization of low materials, 5 g/l of soluble starch (sigma) was replaced by each wheat, corn, and potato meals as the same concentration in the MYS medium. The raw materials were obtained form a grocery store. To observed the effect of initial concentration of raw materials on H_2 production, the concentrations of wheat, corn and potato meal were increased from 5 to 20 g/l. the culture was carried out as the same condition as described above.

2.2. Analytical and other methods

Accumulated gas production in the headspace of the serum bottles was periodically sampled by using gas-tight glass syringe. The gas composition was determined by using gas chromatography (YL 6100) (Yong lin, Korea) equipped with a thermal conductivity detector (TCD) and a flame ionisation detector (FID). The gas chromatography column used a packed column (3FT 1/8 IN Molsieve 5A Column, 10FT 1/8 IN Porapak N Column, Supelco, USA). The temperature of the injector, thermal conductivity detector, flame ionisation detector and oven was maintained at 120, 150, 250 and 40 °C, respectively. Argon was used as the carrier gas at a flow rate of 30 ml/min. The measurement of the detected gases was calculated by comparing the peak area to calibration curves by regression analysis using a standard gas.

The change of pH in the culture broth was measured by pH meter (Orion Star A211,Thermo scientific)

3. Result

It is well established that hyperthermophilic archaeon, *T. onnurineus* NA1, is one of the best hydrogen producing microorganism with eight hydrogenase clusters, which are two times that of other known bio-hydrogen producing microorganism. Furthermore, its use of starch as carbon source for cell growth has potential for food waste management as well as sustainable production of hydrogen for alternative green energy. It is known that this NA1 strain, which has been isolated from Papua New Guinea-Australia-Canada-Manus (PACMANUS) field, has optimal hydrogen production with 5 g/l of starch, 3 g/l of yeast extract at pH 6.5 [7].

Three raw materials- wheat, corn and potato- were tested for their ability to produce hydrogen under the optimized conditions. Each three raw materials were then tested with varying concentrations to compare the hydrogen producing efficiency with that of pure soluble starch. Mixed raw material was also tested to obtain further insight of the possibility of utilizing food waste for bio-hydrogen production by *T. onnurineus* NA1.

Starch in raw food material is not readily accessible for the *T. onnurineus* NA1 to utilize as a carbon source for metabolism that produces hydrogen as a by-product. Furthermore, processed raw food materials could contain detrimental substances that can inhibit the cell metabolism. Therefore, a screening test was first conducted to determine the possibility of bio-hydrogen production using starch containing food sources.

3.1 Possibility of bio-hydrogen production using starch containing food sources

Three starch containing substrates – wheat, corn and potato – was used as an alternative carbon source of pure starch. Each set was conditioned with 5 g/l of respective carbon source and 3 g/l of yeast extract, inoculated with 2% seed culture and incubated for 24hours in 80℃, pH 6.5. Double replicas were sampled for each carbon source with the pure starch set as a positive control and the blank samples for each set as a negative control. Gas composition in the head space was measured using gas chromatography, and the pH measured using the pH meter.

As shown in Fig.1, biological hydrogen production was positive using flour, corn powder, potato powder and mixed material. Thus, it was concluded that starch-containing food powder can be used as a feedstock for *T. onnurineus* NA1 and to produce hydrogen gas. However, on all three carbon sources and their mixture, NA1 produced about much less of hydrogen than that produced from pure starch. The mean concentration of hydrogen gas produced from starch was 24.3%. Compared to starch, NA1 produced 3.49%, 4.59%, 6.04%, and 2.85% of H_2 from flour, corn, potato, and mixed material, respectively. Therefore, of all the raw materials, it was confirmed that hydrogen gas was most actively produced by *T. onnurineus* NA1 from potato.

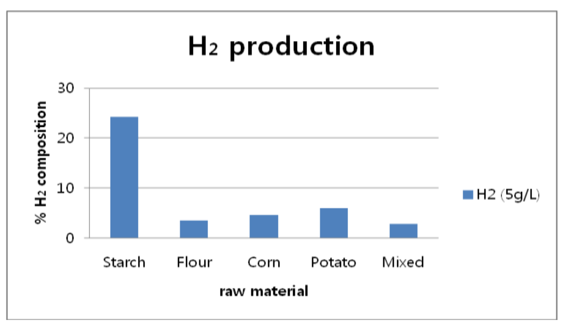

Fig1. H_2 production using 5g/L of each raw materials and mixed material by *T. onnurineus* NA1

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3.2 H_2 production according to substrate concentrations

When possibility of biological H_2 production from each carbon raw materials was confirmed, we carried out additional culture of *T. onnurineus* NA1 on each raw material to investigate the effect of substrate concentration on H_2 production with the concentration of raw materials except the mixed material. The result is shown in Fig.2. Even though the absolute level of H_2 production was much lower than that from starch, *T. onnurineus* NA1 could produce hydrogen gas in all concentrations of each raw material. As substrate concentration was increased from 5 to 20 g/L, the H_2 production from corn reached its maximum output at 10g/L with 5.82%, indicating the optimal substrate concentration. In the case of other raw materials, H_2 production steadily decreased, and it was only able to determine the pattern of H_2 production but not the optimal substrate concentration where H_2 production shows the highest value. In terms of H_2 production level, potato showed higher H_2 production compared to other raw materials for all three different concentrations, implying the most favorable substrate for NA1.

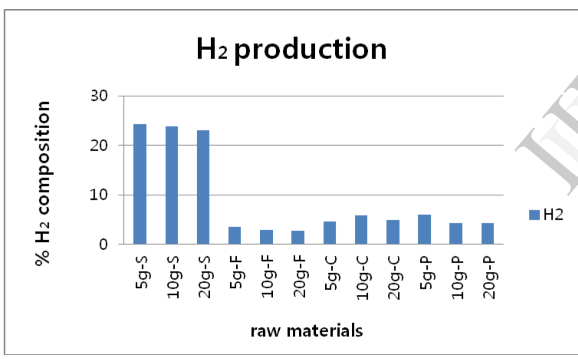

Fig2. H_2 production from starch or starch-containing raw materials with various concentrations by *T. onnurineus* NA1

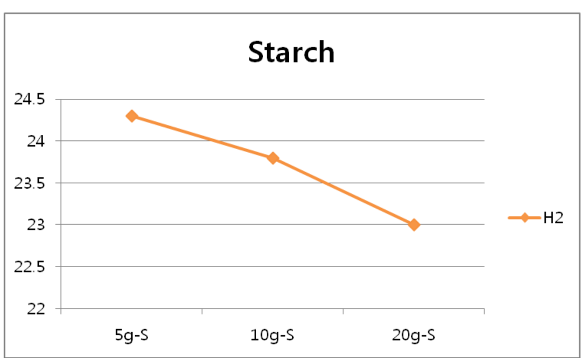

Fig3. H_2 production from starch with various concentrations

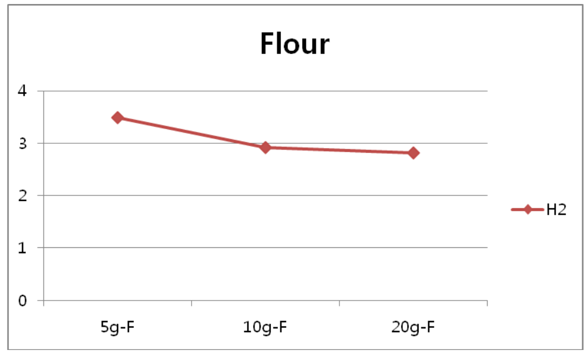

Fig4. H_2 production from flour with various concentrations

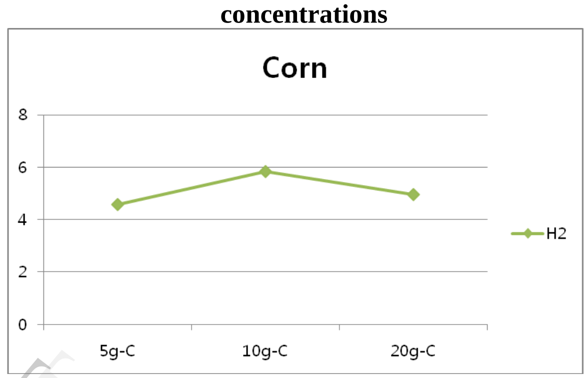

Fig5. H_2 production from corn with various concentrations

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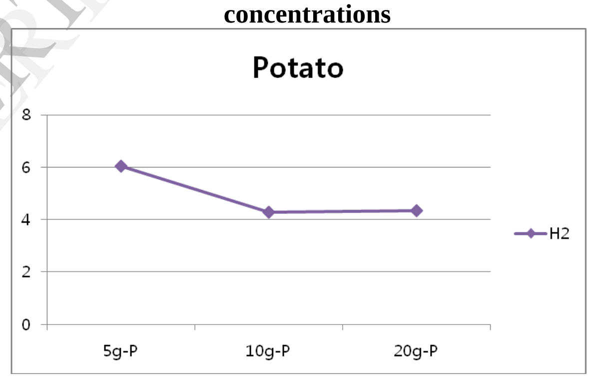

Fig6. H_2 production from potato with various concentrations

3.3 Summary of H_2 production

H_2 production from each raw material as feed stock of *T. onnurineus* NA1 was compared to that of starch. H_2 production from starch for all three concentrations were assigned 100%, and H_2 production from other raw materials for each different concentrations were calculated as the percentage of the H_2 produced when using starch as feedstock (Table 1). For 5g/l, potato had the highest H_2 production with 24.86%. Corn was second with 18.87%, flour was third with 14.36% and mixed sample was last with only 11.71%. For 10g/L, corn had the highest H_2 production with 24.45%, and potato (18.03%), flour (12.25%) following. For 20g/l, corn still had the highest H_2 production with potato and flour following. Overall, as the substrate concentration was increased, corn was the only raw material with an increase in H_2 production, while H_2 production steadily decreased on flour and potato.

Table1. Summary of H_2 production from raw materials compared to that of starch (%)

	5g/L	10g/L	20g/L
Starch	100	100	100
Flour	14.36	12.25	12.22
Corn	18.87	24.45	21.59
Potato	24.86	18.03	18.91
Mixed	11.71	.	.

4. Discussion

The intensity of air pollution and its social impact worldwide urges for more environmentally friendly technology to produce energy, for CO_2 from the burning of fossil fuel is understood to be the main cause of air pollution. The process of producing hydrogen for the use as hydrogen energy through *T. onnurineus* NA1 is thus one of the solutions to environmental problems because it reuses food waste, another source of pollution, and produces energy without any known harmful effects to the environment.

The first set of experiments was aimed to observe the difference in H_2 production by *T. onnurineus* NA1 depending on carbon sources. The three carbon sources - potato, flour, and corn - were all processed food products, thus possibly containing ingredients that could inhibit the cell growth. Seeing that all three carbon sources allowed the cells to grow and produce H_2, *T. onnurineus* NA1 can use processed food products, which are the majority of food waste, as sources of energy. Although the designated carbon sources allowed the cells to produce H_2 thus showing the possibility of using process food or food waste as a carbon source for *T. onnurineus* NA1, the amount of H_2 produced was significantly lower than that produced when soluble starch was used as a carbon source. The resulting amount of H_2 was lower than the theoretical value calculated by the percentage of starch contained in the three carbon sources. Although further research is needed, it seems possible that this is because three carbon sources probably have many unknown additives during process, thus it seems more difficult for NA1 to break down carbohydrate bonding in three carbon sources compared with pure starch probably due to the inhibition of amylase activity by unknown additives. Even after 48 hours, the amount of H_2 production was the same as that measured after 24 hours of incubation. This shows that the digestion of carbon sources is not a matter of time but a matter of the inhibition of amylolytic enzymes to break down the three carbon sources.

Among the three carbon sources, the sample using potato as its carbon source produced H_2 at the highest rate. Mixed starch samples also allowed *T. onnurineus* NA1 to produce H_2 although at a lower rate than other carbon sources. This implicates that waste products, which are essentially a mixture of carbon sources and other nutrients, can provide energy for *T. onnurineus* NA1 to grow and produce hydrogen. Although further research is necessary, it seems that the interaction between the diverse carbon sources caused a different type of molecule indigestible by *T. onnurineus* NA1.

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