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Characteristics of Organic Wastewater Degradation on Hydrogen Fermentation

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수소발효의 유기성 폐수 분해 특성

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요 약

연속형 혐기성처리 반응조에서 배양된 수소발생 슬러지를 이용하여 중온 조건에서 회분식 혐기성 처리방법으로 유기성 폐수로부터 전환되는 수소가스 및 대사산물들에 대한 연구를 수행하였다. 수소발생에 대한 기질로는 sucrose를 이용하였다. 처리과정에서 발생된 누적수소가스, 휘발성지방산(VFAs) 및 solvents는 Gompertz equation을 이용한 비선형회귀분석을 통하여 계산하였다. 처리과정 중 수소가스는 반응초기에 발생하였고, 발생된 가스내 수소가스가 차지하는 비율은 약 20%이었다. 반응 전과정에서 메탄가스는 발생하지 않았다. 비수소가스발생율은 sucrose 농도가 40 g/l일 때 0.956 ml/g VSS/h이었으며, sucrose농도가 300 g/l의 경우는 0.011 ml/g VSS/h이었다. 수소가스 발생하는 기간 동안 VFAs의 생성은 acetate, butyrate의 순으로 높게 생성되었으나, propionate로의 전환은 발견되지 않았다. solvents의 경우 butanol이 가장 높게 발생하였다.

Keywords : Anaerobic treatment, Hydrogen fermentation, Sucrose, VFAs

I. Introduction

The limited supply and the probability of future increases in petroleum prices has initiated interest in chemical and fuel production from biomass fermentations.[1] Four basic processes are available for the production of hydrogen gas from nonfossil primary energy sources: water electrolysis, thermochemical, radiolytic and biological processes. Electrolytic hydrogen is presently produced competitively for industrial use, but only in areas where cheap electricity is readily available.[2] Moreover, on account of the increasing awareness of environmental problems, such as greenhouse effect, biological hydrogen production from renewable biomass represents an important development in the area of bioenergy production.

The hydrogen and solvent-forming clostridia share with many other fermentative organisms the ability to form a variety of metabolic end-products (butanol, ethanol, acetic and butyric acids, and gaseous hydrogen and carbon dioxide), which arise through the operation of different pathways for metabolism of carbohydrate. The variety of chemicals, both acids and neutral solvents, produced in clostridial fermentations is one of the factors that has brought them to prominence as bacteria with biotechnological potential. At the same time, the formation of multiple products in a single fermentation is a serious drawback when the intention is to obtain a particular metabolite. Optimization of culture conditions can go some way towards alleviating this limitation, and many reports have been obtained which show altered product profiles.[3,4] But those causal relationships between hydrogen and solvent production are incidental or secondary to some other basic studies such as investigation in the anaerobic digestion of sewage sludge,[5] or are only partly understood. Also the effects of substrate concentrations, nitrogen, acids and agitation, on the hydrogen and solvent production have been reported.[6,7]

This work was performed with batch reactors for hydrogen production using sucrose as a model substrate. The purpose of this study is to describe the metabolism under various sucrose concentrations, evaluate the characteristics of hydrogen production.

II. Materials and Methods

1. Seed Microorganism

The organism, which was isolated from soybean-meal obtained from a silo which exploded owing to the accumulation of biological produced hydrogen, was used for batch experiments. This microorganism was cultivated in a five-liter chemostat reactor with a working volume of 3 *l*, operated at a temperature of 37 ± 1°C and HRT (hydraulic retention time) of 10 hours. The chemostat reactor was continuously fed with a solution of sucrose (10 g/*l*) and maintained at pH 6.0 by a pH controller (Fine, FD-02).

2. Fermentations and Procedure

The experiments were performed in 120 m*l* glass bottles with 80 m*l* of assay medium containing 5, 10, 20, 30, 40, 50, 100, 200 and 300 g sucrose/*l* under a mesophilic condition of 37 ± 1°C. Each liter of media contained 200 g of NH_4HCO_3, 100 g of KH_2PO_4, 10 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of NaCl, 1.0 g of $Na_2MoO_4 \cdot 2H_2O$, 1.0 g of $CaCl_2 \cdot 2H_2O$, 1.5 g of $MnSO_4 \cdot 7H_2O$, and 0.278 g of $FeCl_2$. The initial pH of the fermentor was setted to 6.0 with a potassium hydroxide (1N KOH) and hydrochloric acid (1N HCl) solution. The fermentation stopped when hydrogen production reached zero. The biogas production was determined by a modified serum bottle technique.[8]

3. Analytical Methods

Gas Phase Analysis The sample was analyzed for its hydrogen content in a Shimadzu 8A gas chromatograph equipped with a thermal conductivity detector (TCD). The separation was packed with Porapak Q 50/80 mesh (GL Sciences). The column, injection port, and detector block were maintained at 100, 70 and 100°C, respectively. Methane and carbon dioxide were determined using a second GC-TCD of the same model with a 2 m stainless column packed with Porapak T (50/80 mesh). Linear calibration curves for both H_2, CH_4 and CO_2 were obtained by injecting samples of the purge gases (Standard gases, GL Sciences).

VFAs and Solvents Analyses The concentrations of the major acids produced by the fermentation were determined using a third GC of the same model with a flame ionization detector (FID) and a 2 m glass column packed with Unisole F-200 (30/60 mesh). The operational temperatures for the injection port, the oven and the FID were 170, 145 and 170°C, respectively. The solvents, including ethanol, propanol and butanol, were analyzed using a GC-FID of the same model with a 2 m glass column packed with Gaskuropack 54 (60/80 mesh). The operational temperatures for the injection port, the oven and the FID were 200, 185 and 200°C, respectively. Calibrations based on peak height were made using a standard solution containing all the components listed above.

pH, VSS and Carbohydrate Analyses The pHs of samples were determined by a TOA pH meter equipped with a GST-5425C probe. The concentrations of volatile suspended solids (VSS) were determined by the procedures described in Standard Methods.[9] Carbohydrate analysis was determined by the phenol-sulfate method,[10] and was measured spectrophotometrically at 490 nm (Hitachi, model 100-20).

III. Results and Discussions

1. Metabolic Features on Bioconversion of Sucrose into Hydrogen

In order to elucidate the relation between the formations of hydrogen, VFAs and alcohols during the conversion of the sucrose into hydrogen by mixed microorganism, the samples (i.e., 5, 10, 20, 30, 40, 50 100, 200 and 300 g sucrose/*l*) were cultured to monitor their metabolites including acetate, butyrate, ethanol and butanol. Fig. 1 shows the carbon and electron flow illustrating the metabolic pathway when microflora converted the sucrose into hydrogen gas. These curves reveal, as do all of the others, that there is a virtually negligible lag phase. Hydrogen production immediately increased to a sharp short-lived peak which was followed by a gradual decline until the end of the fermentation (Fig. 1a). Fig. 1a also shows the changes in pH that take place during one of the experiments. On the whole these values remain fairly constant, but there is a tendency for the pH to decrease through the experiment (data not shown). This decrease is at most by two unit and coincides with an appreciable decrease in the quantity of sucrose and a concomitant increase in the formation of bioproducts. Hydrogen composition in biogas was around 20% except for initial

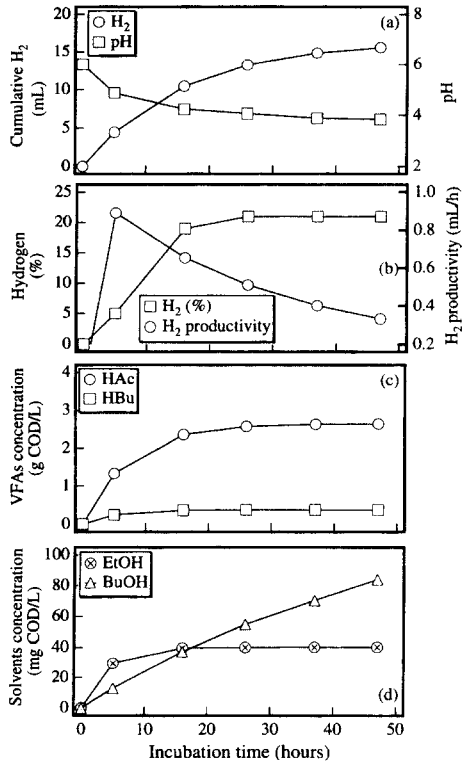

Fig. 1. Hydrogen production and metabolites in batch degradation of 50 g/*l* sucrose: (a) cumulative hydrogen production and pH variation, (b) composition of hydrogen in biogas and volumetric productivity, (c) production of volatile fatty acids and (d) solvents.

inoculation, and there was no methane found throughout this study. During the period of hydrogen production, the acetate concentrations increased to almost 2.0 g/*l*, however, butyrate was not eminently produced and also propionate was not found in the samples (Fig. 1c). This imply that cellular NADH is low and electrons released from acidogenesis may be metabolized to acetate with the benefit to the cell that additional ATP is generated by the terminal reaction catalysed by acetate kinase. However, when NADH concentration is elevated butyrate (or butanol) formation may be favored.[11] Apparently, the production of VFAs (e.g., acetate and butyrate) accompanied hydrogen production during the conversion of the sucrose by microflora. In Fig. 1d, the reason for the decrease of hydrogen production with the time courses is that the medium is supersaturated with hydrogen and butanol play a role as a sink of the reducing power (hydrogen).[12] Also, culture pH values decreased from around 6.0 to 4.0, and then the hydrogen production ceased. Lee *et al.*[13] found that hydrogen evolution maximally proceeds at alkaline initial pH in batch experiments. Hydrogen formation is inhibited because of a marked drop in intracellular pH after the external pH fell below 4.0.[14] As seen in Fig. 1, the maximum hydrogen productivity occurred during the acid phase of fermentation. These results were similar to those obtained with *Clostridium* sp. fermenting carbohydrates.[15]

2. Estimations of Specific Hydrogen Production Rate

To evaluate specific hydrogen production rate (m*l*/g VSS/h), each cumulative hydrogen production (H) data was fitted to a modified Gompertz equation,[16]

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

where P is hydrogen production (m*l*), Rm is maximum hydrogen production rate, (m*l*/h), λ is lag-phase time (hour) and e is 2.718281828, which are suitable models for describing the progress of cumulative biogas/hydrogen production in a batch experiment. Subsequently, the specific hydrogen production rate (m*l*/g VSS/h) was calculated by dividing Rm by the dry biomass weight (g VSS). In this study, specific hydrogen production rate represented hydrogen-producing bacterial activity on the sucrose.

3. Chracteristics of Specific Production Rate

Fig. 2 shows the relation between the estimated specific production rates of hydrogen and metabolites using Eq. 1. The data calculated by Eq. 1, Fig. 2a, reveal that specific hydrogen production rate increased from 0.314 to 0.956 m*l*/g VSS/h as to the sucrose concentrations changed from 5 to 40 g/*l*. However, it dropped to 0.1 m*l*/g VSS/h when the concentration exceeded 100 g/*l*. The production rates for VFAs and solvents were occurred similar to the results of hydrogen (Fig. 2b and 2c). It means that alteration of microbial activity on the VFAs formations occurred simultaneously with hydrogen production. Also, the decrease of specific hydrogen production rate (Table 1) might partly be influenced by metabolic products, such as VFAs and hydrogen. Hydrogen gas is a significant inhibitor of the growth of hydrogen-producing *Clostridium*

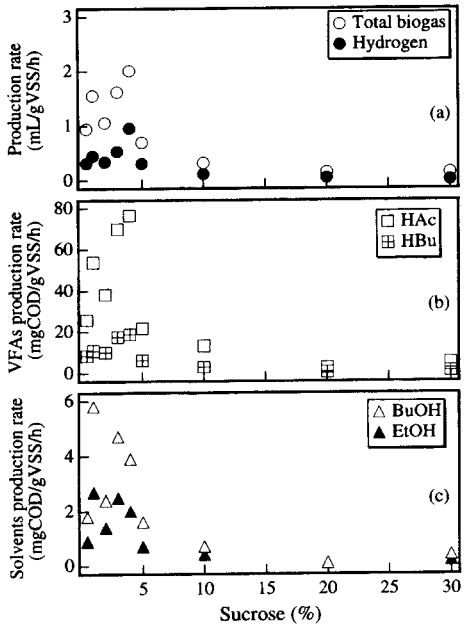

Fig. 2. Specific production rates of (a) biogas and hydrogen, (b) volatile fatty acids and (c) solvents.

Table 1. The calculated parameter values converting various amounts of sucrose into hydrogen

Sucrose (g/l)	H_2 (ml/g VSS/h)	Products Activity (mg COD/g VSS/h)			
		Acetate	Butyrate	Ethanol	Butanol
5	0.314	25.8	8.4	0.9	1.8
10	0.448	53.9	10.8	2.7	5.8
20	0.340	38.2	10.1	1.4	2.4
30	0.531	69.7	17.5	2.5	4.7
40	0.956	76.5	18.7	2.0	3.9
50	0.309	21.6	6.2	0.7	1.6
100	0.114	13.1	2.9	0.4	0.7
200	0.046	2.6	0.5	0.1	0.1
300	0.011	5.1	1.0	0.2	0.4

cellobioparum, thus it inhibits the metabolism shifting from soluble sugar to hydrogen/acids production. These metabolic properties also may limit the biological hydrogen production on a commercial scale[17] although hydrogen has been generated from sugarcane.[18]

IV. Conclusions

This work was performed with batch reactors for hydrogen production using sucrose as a model substrate under a mesophilic condition of 37 ± 1°C. Based on the results and discussion, the following conclusions were derived:

1. Hydrogen production immediately increased to a sharp short-lived peak which was followed by a gradual decline until the end of the fermentation. The content of hydrogen gas in biogas produced were around 20%, there was no significant methane found in the biogas.

2. The maximum hydrogen production ratewere 0.956 ml/g VSS/h for 40 g/l sucrose. The maximum substrate utilization ratio was 99% at initial sucrose 5 g/l.

3. The acetate was produced more higher than that of other VFAs during the period of hydrogen production. However, butyrate was not eminently produced and also propionate was not found in the samples. The production rate for VFAs and solvents were occurred similar to the results of hydrogen.

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