Research Article

Effect of Pretreatment and pH on Biohydrogen Production from Sago Industrial Effluent Containing Mixed Microbes

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Abstract

In this study, starch rich sago industrial effluent was fed as substrate for biohydrogen production by a lab scale set up of batch anaerobic fermentation. The results showed that biohydrogen production was high in the reactor with acid treated effluent at pH 5, hydrogen production potential (P) was 83.65 mL, maximum production rate (R_m) was 29.45 mL/h and λ phase was 72.96 h. Maximum hydrogen production yield was 92.94 mL/g carbohydrate at the pH of 5 in ASIE. The biohydrogen production progress at the above mentioned the condition expressed the Gompertz model with R^2 0.9955. pH and pretreatment of inoculum was essential for the effective production of biohydrogen. Though the sago industrial effluent has deleterious properties, starchy content of the effluent was acts as feed stock for biohydrogen production.

Keywords: Biohydrogen; Energy, Sago Industry, Effluent, Pretreatment, Fermentation, Anaerobe, Water displacement, Gas Chromatography, Carbohydrate consumption

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Introduction

Today the global energy necessities are mainly depends on fossil fuels, but reservoirs of the primary energy will be depleted within a few decades. The wide use of fossil fuels cause major global issues such as increasing greenhouse emissions, accelerated climate change, depletion of the ozone layer and loss of biodiversity [1]. Hydrogen is one of the promising alternative energy sources, valuable gas, recyclable and has high conversion efficiency and produce water as end product when it combusted as fuel. Aerobic, anaerobic and photosynthetic microbes are used in the biological production of hydrogen. Organic wastes from various industries are considered as the source of biohydrogen. Sago industry produces different types of wastes such as bark, pith residue and waste water [2]. For the production of sago requires 20,000 – 30,000 L of water per ton of tapioca. After the production process, more or less equal volume of water will be released out from the industry. The water arising from washing of the tapioca roots and the supernatant from the starch settling tanks constitute the effluent, is highly organic, acidic and emits foul smell [3, 4].

Sago industrial effluent is the substrate for various aerobic and anaerobic bacteria, so it can be used as the inoculums for the biohydrogen production. The pretreatment of the inoculums such as acid, base and heat permit the selective enrichment of the specific group of bacteria by inhibiting the activity of hydrogen consuming methanogenic bacteria and other hydrogen consuming bacteria [5, 6]. Some researchers have succeeded in producing a continuous hydrogen production using the inocula without any pre-treatment [7, 8], and others have reported on various pretreatments which can be added to the inoculum or culture to enhance hydrogen production and its sustainability by harvesting the spore forming Clostridial bacteria [9, 5]. In this present study, the aim is to investigate the effects of pretreatment of SIE and initial pH on biohydrogen production and to optimize the reaction parameters for the enhancement of biohydrogen production.

Experimental

Collection and Characterization of Sago industrial effluent

The effluent was collected from the sago industry situated in Attur, Salem District, Tamilnadu, India. The effluent was characterized by their physicochemical parameters. pH and electric conductivity were measured using portable pH meter and digital conductivity meter respectively. All the other parameters were analyzed according to the Standard methods of the American Public Health Association [10].

Inoculum Pretreatment

SIE was used as the inoculums for the production of hydrogen with anaerobic fermentation. To inactivate the methanogenic and other hydrogen consuming bacteria, the acid and heat treatment were performed. The acid pretreatment was conducted by adjusting the pH of the effluent to 3.0 with 1 mol/l of HCl and maintaining it for 24 hrs. The heat pretreatment was performed by boiling the effluent at 80^{o}C for 2 hrs [11]. In order to enrich the mixed bacteria in the effluent, nutrient solution was prepared with 40g $NaHCO_3$, 5g NH_4Cl, 5g NaH_2PO_4. $2H_2O$, 5g K_2HPO_4. $3H_2O$, 15g $FeSO_4$. $7H_2O$, and 0.85g $MgCl_2$. $6H_2O$ and made up to one litre [11].

Biohydrogen Production Experiment

Batch experiments were conducted in 500 mL glass bottles with a working volume of 300 mL. 250 mL of effluent and 50 mL of nutrient solution was added to each glass bottles. Three sets of experiments were performed for acid treated sago industrial effluent (ASIE) and heat treated sago industrial effluent (HSIE) as well as untreated sago industrial effluent (USIE). USIE considered as control. Different pH values of the each set of experiment were adjusted to 4, 4.5, 5, 5.5, and 6 with 1mol/L HCl and 1mol/L NaOH. Each bottle was flushed with nitrogen gas for one min to create anaerobic environment. The initial concentration of total carbohydrate present in the SIE was 1.2 g/l. The reactions were conducted at room temperature and incubated for seven days (168 hrs). The gas production was recorded with the time intervals of 24 hrs. All the experiments were done in triplicates.

Analytical Methods

The total carbohydrate concentration was determined by Phenol – Sulphuric acid method, with D-glucose as the standard [12]. The amount of total biogas formed from batch experiment was measured by downward water displacement method. The data were averaged with standard error. The biogas was sampled from the head space of the bottles and was used to analyze its composition. The fraction of hydrogen in the collected gas was determined using a Gas Chromatograph (Chemito 7610 series) equipped with a thermal conductivity detector (GC - TCD) and Poropak Q column for this study. The temperature of the column, the injection port and the detector were 60^{o}C, 60^{o}C and 90^{o}C, respectively. Nitrogen was used as the carrier gas at a flow rate of 30 mL min^{-1}. Gas samples (500 µL) in the head space were collected using a pressure lock gas syringe and injected into the injection port. The results were interpreted using the software available with the GC (Iris 32 Lite).

Kinetic Modeling of Biohydrogen production

The Gompertz equation (Eq. (1)) was used to describe the progress of cumulative biogas and biohydrogen production [13].

$$H(t) = P.\exp\{-\exp[(Rm.e/P)(\lambda - t) + 1]\} \quad (1)$$

where H (mL) was the cumulative biogas and biohydrogen production at time t (h), P was the production potential (mL), Rm was the maximum production rate (mL/h) and λ was the lag time (h). Sigma plot software 13.0 was used to solve the eq. (1). The hydrogen production yield was calculated by dividing the hydrogen production potential by the amount of glucose consumed [14]. The substrate degradation efficiency was estimated by dividing the amount of carbohydrate consumed by the amount of initial carbohydrate [15].

Results and Discussion

Characterization of Sago industrial effluent

The physicochemical characters of sago industrial effluent were shown in **Table 1**. The effluent from the ago industries appeared to be pale white in color with pungent smell and has high amount of dissolved and suspended solids.

Effect of initial pH on biohydrogen production:

To study the effect of initial pH on biohydrogen production, the pH of the effluent sample (USIE, ASIE and HSIE) was adjusted from 4, 4.4, 5, 5.5 and 6 with 1mol/l HCL and 1 mol/l NaOH. The final pH of the effluent was measured in each treated sample after incubation period (168 hrs). In each experiment, the pH was decreased at the end of

reaction (**Table 2**). In the case of pH 5 in each experiment a high variation was observed when compared with the other pH. In ASIE, the initial pH of 5 was decreased to 3.6. Decreased pH also enables suppression of hydrogen consuming bacteria and lowering of retention time encourages hydrogen production. The highest variance in the final pH values was observed in sample from methane producing bioreactor depending on the pretreatment methods (pH values of 8 to 4.5 - 6.1) [16].

Table 1 Physicochemical characterization of SIE

Sl. No	Parameters	Observation
1	pH	4.2 ± 0.2
2	Electric Conductivity (µs/cm)	3600 ± 12
3	Total Dissolved Solids (TDS) (mg/l)	5500 ± 76
4	Total Suspended Solids (TSS) (mg/l)	2250 ± 54
5	Fixed Residue (mg/l)	2215 ± 93
6	Volatile Residue (Organic) (mg/l) @ 550^{o}C	677.1 ± 18
7	Dissolved Oxygen (mg/l)	10.3 ± 0.57
8	Biological Oxygen Demand (mg/l)	840 ± 22
9	Chemical Oxygen Demand (mg/l)	1600 ±41
10	Sulphates (mg/l)	36 ± 4.8
11	Chlorides (mg/l)	340 ± 17
12	Calcium (mg/l)	1.2 ± 0.21
13	Phosphates (mg/l)	93 ± 2.7
14	Residual Chlorine (mg/l)	0.1 ± 0
15	Total Carbohydrate (mg/l)	1200 ± 27

Table 2 The alteration in pH before and after biohydrogen production in USIE (Control), ASIE and HSIE

Initial pH	Final pH		
	Untreated (Control)	Acid Treated	Heat Treated
4	3.6 ± 0.03	2.7 ± 0.06	3.2 ± 0.05
4.5	4.2 ± 0.05	3.7 ± 0.03	4.1 ± 0.03
5	4.5 ± 0.03	3.6 ± 0.08	4.4 ± 0.05
5.5	4.9 ± 0.11	4.4 ± 0.08	4.6 ± 0.03
6	5.03 ± 0.05	5.4 ± 0.06	4.8 ± 0.03
Control	3.96 ± 0.15	3.4 ± 0.08	3.9 ± 0.08

The fraction of biohydrogen in the biogas was determined by Gas Chromatography (GC). The cumulative biohydrogen was maximum at the pH of 5 in USIE, ASIE and HTSE. The cumulative biogas and biohydrogen volume at the various pH were showed in **Figure 1**.

Effect of pretreatment on biohydrogen production

Two different inoculums pretreatment method were performed to hydrogen production and found that the acid pretreatment showed maximum hydrogen production of 38.17 % at the pH of 5. In previous study, the acid pretreated effluent with added starch showed the hydrogen yield of 255 mL/g starch at pH 7 [17]. The volume of biohydrogen produced when the mixed culture was pretreated using HCl at pH 2 was 3.2 times higher than that obtained without acid treatment [18]. Fermentation of acid-pretreated duckweed resulted in a biohydrogen production of up to 75 mL hydrogen per g dry duckweed in 7 days [19]. The cumulative biogas produced in the untreated, acid treated and heat treated SIE was 120, 208 and 176.3 mL/l respectively. In this study, ASIE showed higher production of biogas and biohydrogen at the pH of 5. **Figure 2** shows the cumulative biohydrogen production in USIE, ASIE and HSIE.

Gompertz non linear curve fitting equation was used to measure the hydrogen production potential (P), maximum production rate (R_m) and lag phase (λ). The hydrogen production potential, maximum production rate and lag phase were obtained from the Gompertz curve fitting equation given in **Table 3**. The hydrogen production potential was high in ASIE (83.65 mL) followed by HSIE (49.43 mL) at the lag phase of 72.96 and 67.24 hrs respectively. The production of hydrogen at untreated, acid treated and pretreated conditions with pH of 5 expressed the Gompertz model with R^2 0.9841, 0.9955 and 0.9826 respectively. These R^2 values indicated a strong correlation between the

experimental data and fit. Methane production was not noticed thorough out the incubation period of all sets of reactors. The maximum hydrogen production rate was also high in ASIE when compared with the USIE and HSIE.

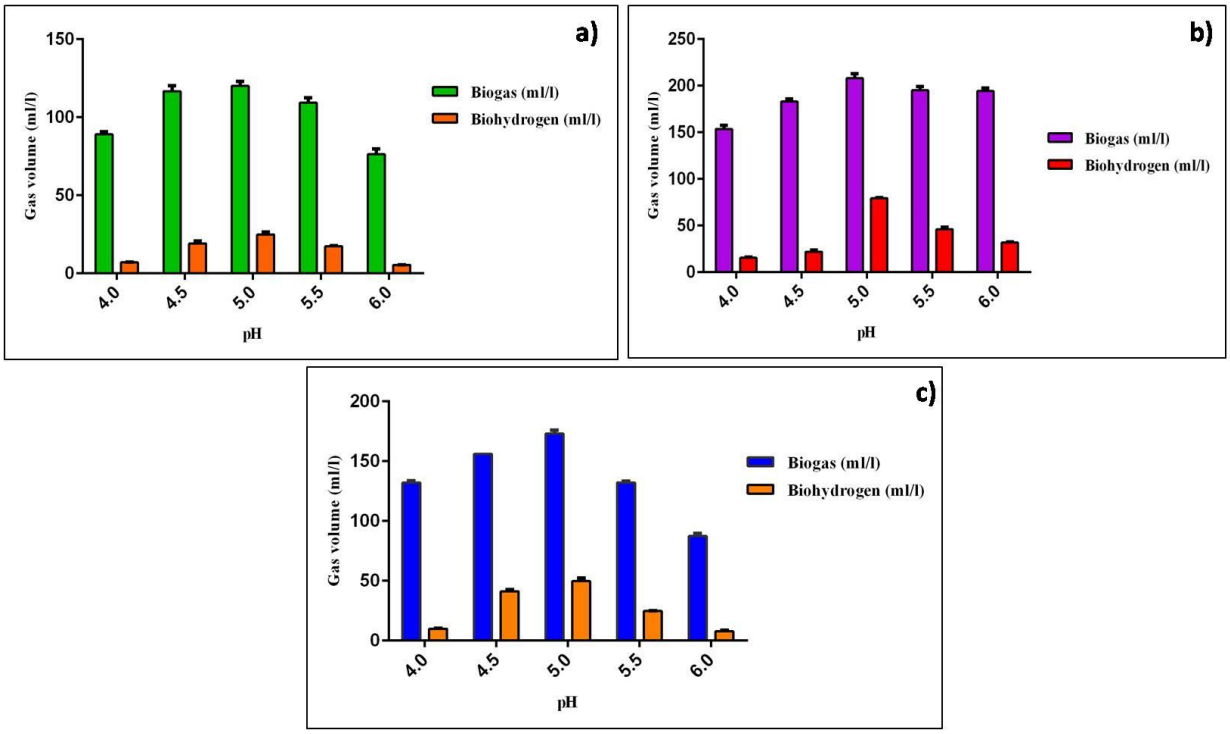

Figure 1 The cumulative biogas and biohydrogen production at various pH in. (a) USIE, (b) ASIE, (c) HSIE

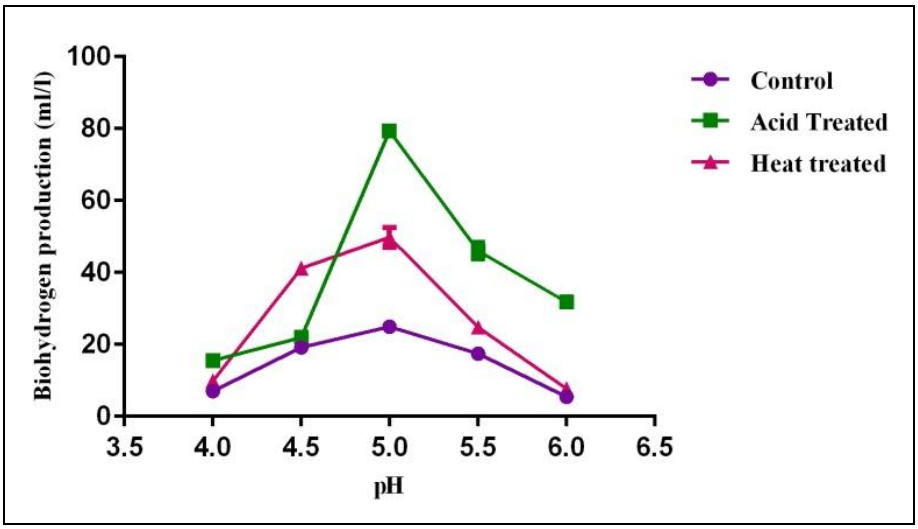

Figure 2 Cumulative biohydrogen production at different pH with untreated and pretreated effluent

Table 3 Calculated parameters using the modified Gompertz equation for biohydrogen production at pH 5

Treatment	Cumulative Biohydrogen (mL/l)	P (mL)	Rm (mL/h)	λ (h)	R^2	F value	P value
USIE	24.93	24.27	27.21	55.58	0.9841	124.14	0.0003
ASIE	79.04	83.65	29.45	72.96	0.9955	439.46	> 0.0001
HSIE	49.75	49.43	25.5	67.24	0.9826	113.1	0.0003

Effect of initial substrate concentration on biohydrogen production

Initial carbohydrate concentration of the SIE was 1.2 mg/l. The substrate degradation efficiency was determined by dividing the amount of carbohydrate consumed by the amount of initial carbohydrate. The biohydrogen yield was calculated by the amount of carbohydrate consumed and the biohydrogen production. **Figure 3** showed the effect of substrate degradation on cumulative biohydrogen production and biohydrogen yield. The concentration of the initial

carbohydrate was decreased after the incubation period and the maximum consumption was noticed in ASIE at the pH of 5. The data of carbohydrate consumption and biohydrogen yield were given in **Table 4**.

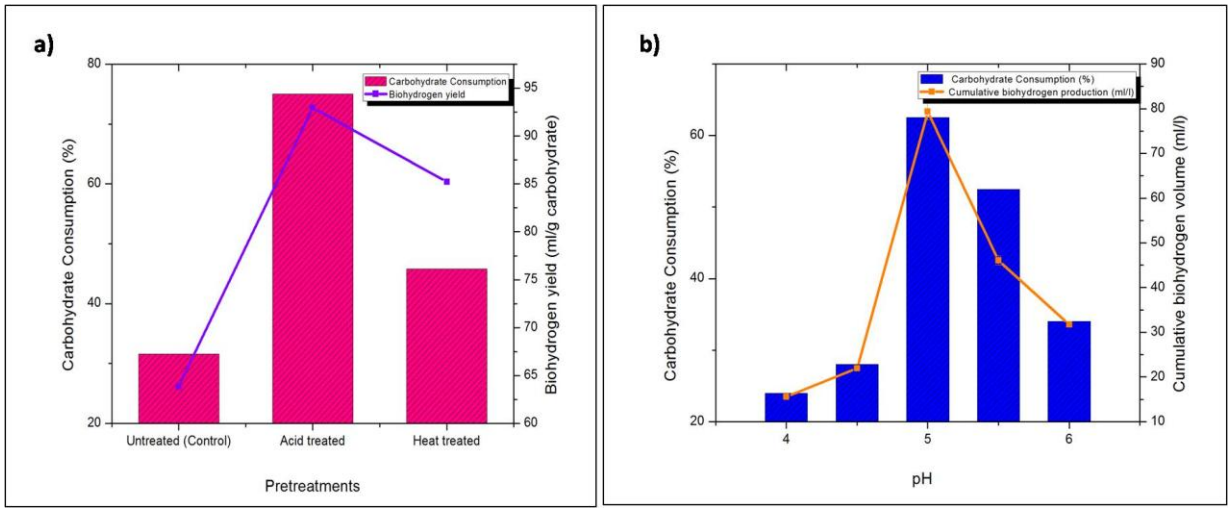

Figure 3 Carbohydrate consumption rate and (a) biohydrogen yield of USIE, ASIE and HSIE at pH 5 (b) cumulative biohydrogen production at various pH in ASIE

Table 4 Effect of carbohydrate concentration and the biohydrogen yield

Treatment	Initial Carbohydrate concentration (g/l)	Final Carbohydrate concentration (g/l)	Substrate Degradation Efficiency (SDE) (%)	biohydrogen yield (mL/ g carbohydrate)
USIE	1.2	0.82	31.6	63.86
ASIE	1.2	0.30	75	92.94
HSIE	1.2	0.65	45.8	85.22

The biohydrogen yield was based on carbohydrate consumption. Maximum biohydrogen yield of 92.94 mL/g carbohydrate was recorded at the pH of 5 in ASIE. The biohydrogen yield was increases when the substrate degradation efficiency was increased. In ASIE, the SDE was maximum (75%) when compared with HSIE (45.8%) and USIE (31.6 %).

Conclusion

This study was focused on the effects of pretreatment and pH on the biohydrogen production from sago industrial effluent. The effluent has the deleterious effects, but the starchy nature could be acts an effective feedstock for the biohydrogen production. In this, the modified Gompertz non-linear curve fitting equation gave the hydrogen production potential, rate and lag phase. The biohydrogen yield was directly depends on the hydrolysis and breakdown of the carbohydrate. 92.94 mL of biohydrogen yield was obtained from 1 g of carbohydrate already present in the effluent. The addition of carbohydrate to the media as the added substrate, it will also produce excessive biohydrogen.

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