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Ipomoea aquatica as a new substrate for enhanced biohydrogen production by using digested sludge as inoculum

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ABSTRACT

Ipomoea aquatica, a tropical plant was used as a new substrate, and the digested sludge (DS) was used as inoculum for biohydrogen production. In order to inhibit the hydrogen consuming bacteria (HCB), the DS was subjected to thermal and acid pretreatment to identify the optimum method. The results showed that thermal pretreatment was better than acid pretreatment. To further investigate the best thermal pretreatment condition of DS, response surface methodology (RSM) was employed. Consecutively, thermal pretreatment at 90 °C for 60 min was identified as the optimum pretreatment condition for inoculum. Further, *Ipomoea aquatica* used as substrate was also optimized under conditions like freezing, boiling, and alkali pretreatment to attain high hydrogen yield (HY). Frozen and dried *I. aquatica* demonstrated the highest HY of 217.16 mL/g-VS, which was manifold higher than control and other treatment conditions. The energy consumed in the fermentation process was evaluated which was lesser than energy produced in the process. Furthermore, a practical process was proposed. To the best of our knowledge, it's the first time that *I. aquatica* was used as substrate to produce hydrogen through an attractive process that could not only benefit the environment by water purification but also contributes to clean energy production.

1. Introduction

Currently global consumption of fossil fuels is equivalent to more than 11 billion tonnes of oil. Crude oil reserves are vanishing at the rate of 4 billion tonnes a year, and at this rate all known accessible conventional oil deposits will be gone by 2042 [1]. Hydrogen can provide an alternative source of energy to meet this rising global demand. It is a clean fuel with no combustion by-products other than water. The high energy density (122 kJ/g) makes it especially attractive as a mobility fuel to replace gasoline [2,3].

Conventional methods of hydrogen production like steam reforming, electrolysis and thermolysis have major drawbacks due to their hazardous nature and high energy consumption [4]. One option to overcome these problems would be to use biological means such as waste biomass to produce hydrogen through fermentation. The annual global yield of biomass residue exceeds 220 billion tonnes which potentially equals the energy of 60–80 billion tonnes of crude oil [5].

Ipomoea aquatica, commonly known as 'water spinach' a free floating semi-aquatic plant plays an important role in pollutant removal from lakes and ponds in order to overcome eutrophication [6]. However, the rapidly growing *I. aquatica* needs to be harvested as a resource to restrain secondary pollution in aquatic environments. Its high carbohydrate content of 54.2% and presence of mineral elements like K, Na, Ca, Mg, Fe and Mn could be a viable source for biohydrogen production [6]. Although there are many reports on hydrogen production from plants, there are no reports showing *I. aquatica* as a suitable substrate for biohydrogen production. In this research, for the first time *Ipomoea aquatica* was used to produce biohydrogen. Furthermore, for the plant substrate to be readily hydrolysed by bacteria, pretreatment is necessary [7–11]. In the present research, commonly used pretreatment methods for substrates with easy operation such as freezing, boiling and alkali pretreatment were examined. On the other hand, digested sludge (DS) is present in abundance all over the world and it's also an economical source of microorganisms. DS obtained from waste water treatment plant has been an efficient source of hydrogen producing bacteria (HPB), such as *Clostridium* and *Enterobacter* for biohydrogen production from various biomass like

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agricultural waste, food residues, plant residues, waste water and so on [8–12]. However, in a mixed culture system, under anaerobic condition some hydrogen consuming bacteria (HCB) existing in the DS, such as methanogens, homoacetogens and Archaea [13–16], often readily consume the hydrogen produced by HPB. Therefore, in order to harness hydrogen from a mixed culture system the HCB have to be inhibited by pretreatment [17]. Acid pretreatment using HCl [18–22] and thermal pretreatment [7,12,23,24] are the most widely accepted pretreatment methods for inoculum. However, the most efficient pretreatment for higher hydrogen production is still subject to much debate.

A wide range of temperature and residence time have been reported as optimum conditions for thermal pretreatment in different literature, as the best pretreatment differs according to the inoculum source [7,12–14]. Response surface methodology (RSM) is a statistical method useful for evaluating the significance of several explanatory variables, understanding the interactions of the various parameters affecting the process, and hence determining optimal conditions for desirable responses [25]. In this research the parameters (temperature and time) of thermal pretreatment of DS was optimized using RSM to enhance hydrogen production.

The objective of this research was to identify the capability of biohydrogen production from *I. aquatica* and optimize the process in order to obtain a practical fermentation system for higher biohydrogen production from water purification plants. Additionally, an overall energy balance of the entire process was calculated and comparison of the hydrogen yield with other researches was carried out.

2. Materials and methods

2.1. Inoculum preparation from digested sludge

Digested sludge obtained from a waste water treatment plant in Japan, was acclimatized for a week at 35 °C using a trace mineral solution and then used as inoculum. The inoculum preparation is explained in detail in our previous work [26]. For acid pretreatment the DS was adjusted to pH 3 using 2 M HCl. In case of thermal pretreatment, the DS was subjected to heating using a hot air oven (SHIMAZU, Japan) at the corresponding temperature and residence time. Initially to identify the best pretreatment method among acid and thermal pretreatment, 100 °C for 30 min was used as thermal pretreatment condition. Later the DS was pretreated thermally according to the temperature and corresponding residence time as designed by RSM software.

RSM, including two factors and a central composite design (CCD) was used in this research to study the effect of independent variables on dependent variables. The maximum H_2 concentration, accumulated H_2 and CH_4 concentration were chosen as response or dependent variables, while temperature (Factor A: 90 °C - 100 °C) and pretreatment time (Factor B: 15–60 min) were chosen as independent variables. Design expert version 6.0.6 was used as the software. Based on the response variables the best thermal pretreatment condition was identified and reported in the form of 2-D contour plots and 3-D response surface models. The thermal pretreatment conditions at 114.14 °C for 37.5 min and 85.85 °C for 37.5 min were used to calculate standard error and deviation by the program.

2.2. Substrate preparation from Ipomoea aquatica

I. aquatica was obtained from a supermarket in Ibaraki prefecture, Japan. It was divided into two categories-one category of the plant was pulverized and packed in air-tight bags and frozen (Frozen substrate). The other category of the plant was boiled for 1 min, pulverized and frozen in air-tight bags (Boiled substrate). This was done in order to prevent decomposition and also to study the effects of freezing and boiling as a pretreatment method. In the experiments to identify the optimum inoculum pretreatment condition only frozen *I. aquatica* was used as substrate.

In the further batches of fermentation to identify the optimum substrate pretreatment condition; the frozen and boiled substrates were dried using a hot air oven (SHIMAZU, Japan) at 105 °C for 24 h and used as substrate.

For alkali pretreatment method of substrate, the dried substrate was treated using 1% NaOH for 24 h followed by microwave heating for 1 min [7].

Raw *I. aquatica* was used as control.

2.3. Hydrogen fermentation experiment

To identify the best pretreatment method for inoculum, 50 mL serum bottles were used as bioreactors. Each bioreactor contained 2 g of frozen *I. aquatica* and 10 mL of thermal or acid pretreated inoculum and the control bioreactor contained untreated inoculum for the fermentation experiments. The pH in the bioreactors was adjusted to 5 using HCl (2 M) and NaOH (2 M) and the incubation temperature was set to 35 °C. The bioreactors were then sparged with Nitrogen gas (SHIMAZU, Japan) to create anaerobic conditions and the reactors were tightly sealed with rubber caps. The experiment was carried out in triplicate.

Further to identify the optimum thermal pretreatment temperature and time, RSM was used to design the experiments. For the RSM experiments, D-glucose (0.5 g) (WAKO, Japan) was used as substrate to identify the best thermal pretreatment condition, in order to maintain uniformity among the different runs of RSM. 25 mL of thermal pretreated inoculum was used and the other conditions were similar to the above experiments. The experiments were carried out in triplicate.

Using the optimum inoculum condition, the fermentation experiments to identify the best pretreatment method for *I. aquatica* was carried out. 2 g of frozen dried, boiled dried, alkali pretreated and unfrozen *I. aquatica* as control and 25 mL of optimized inoculum were used for fermentation respectively. The other experimental conditions were similar to the previous experiments.

2.4. Analytical methods

The biogas yield and composition was measured every day. Biogas was collected using 20 mL plastic syringes which were connected to the bioreactor using plastic tubes as connectors. The volume of the biogas was read directly using the scale on the syringe. The gas composition was detected via gas chromatography (GC-8A, SHIMAZU, Japan) using a machine equipped with a thermal conductivity detector (80 °C) and a Porapak Q column (60 °C). Nitrogen was used as the carrier gas. Dissolved Organic Carbon (DOC), Volatile Solids (VS) and hydrogen yield (HY) were determined in accordance with standard methods, and pH was detected using a pH meter. Also, the activity of the microorganisms which is indicated by the adenosine triphosphate (ATP) concentration [26] was evaluated on Day 2 using a Bac Titer-Glo™ Micro-bial Cell Viability Assay (Promega, USA).

2.5. Energy balance calculation

In order to demonstrate the efficiency of biohydrogen production from *I. aquatica,* in terms of overall energy obtained at the end of the process, the energy balance was evaluated. The energy consumed and produced for the overall process was calculated