Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/he

Effect of system optimizing conditions on biohydrogen production from herbal wastewater by slaughterhouse sludge

D. Sivaramakrishna [a], D. Sreekanth [a], M. Sivaramakrishnan [a], B. Sathish Kumar [a], V. Himabindu [a,*], M. Lakshmi Narasu [b]

[a] Centre for Environment, Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad 500 085, (A.P.) India

[b] Centre for Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad 500 085, (A.P.) India

ARTICLE INFO

Article history:
Received 21 April 2013
Received in revised form
3 February 2014
Accepted 5 February 2014
Available online 28 February 2014

Keywords:
Biohydrogen
Fermentation
Wastewater
pH control
Temperature

ABSTRACT

The study evaluates the biohydrogen production from herbal wastewater as the substrate by the enriched mixed slaughterhouse sludge as the seed source. In the following experiments, batch-fermentations are carried out with the optimum substrate concentrations, fermentation pH and fermentation temperature to observe the effects of H_2 production, hydrogen yield and other fermentation end products at different conditions. The hydrogen production is increased as substrate concentration increased up to 8 g COD/L WW, but drastically decreased at 10 g COD/L WW. When the pH of fermentation is controlled to 6.5, a maximum amount of hydrogen yield could be obtained. The hydrogen production is maximum at 50 °C (930 ± 30 mL/L WW) compared to 30 °C (436 ± 16 mL/L WW). Acid-forming pathway with butyric acid as a major metabolite dominated the metabolic flow during the hydrogen production. The experimental results indicated that effective hydrogen production from the herbal wastewater could be obtained by thermophilic acidogenesis at proper operational conditions.

1. Introduction

Due to abrupt climate change and global warming problems arising from excessive uses of fossil fuels, much attention goes to using non-carbonaceous fuels such as hydrogen (H_2) produced from renewable feedstock to reduce greenhouse gas emissions [1]. Hydrogen is a clean and renewable energy carrier with a high energy yield of 122 kJ/kg, thereby being considered as available alternative energy carrier of future [2]. Hydrogen can be produced by physical and chemical methods such as partial oxidation of fuel oil, steam reforming and gasification of coal. However, hydrogen production through biological method has attracted worldwide attention, due to its potential as an low-cost, inexhaustible and renewable source of clean energy [3].

The majority of fermentative hydrogen-producing micro-organisms belong to Clostridium [4] species of anaerobes,

* *Corresponding author*. Centre for Environment, Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad 85, Andhra Pradesh, India. Tel./fax: +91 040 23156133.
E-mail addresses: drvhimabindu@gmail.com, sivamicro@gmail.com (V. Himabindu).

0360-3199/$ – see front matter
http://dx.doi.org/10.1016/j.ijhydene.2014.02.026

Enterobacter [5], *Escherichia coli* of facultative anaerobes [6], Bacillus species of aerobes [7] and mixed anaerobic microbial populations from different sources (soil, sediment, compost, aerobic and anaerobic sludges) have been studied as microflora for H_2 production by dark fermentation [8]. From an engineering point of view, producing hydrogen by mixed cultures are generally preferred because of lower cost, ease of control and the possible use of organic waste as feedstock [9].

Recently, some studies on biohydrogen production from wastewaters and agricultural waste such as tapioca wastewater or cellulosic materials have been carried out [10,11]. Herbal wastewater (HWW) is an important renewable biomass resource. Herbal medicines are being used by about 80% of the world population primarily in the developing countries. Herbal medicines include herbal materials, herbs, herbal preparations and finished herbal products that contain active ingredients from plants or other plant materials. Of all the sciences, India possesses the longest and most glorious tradition in the field of herbal medicine. Herbal pharmaceutical wastewater is strong with chemical oxygen demand (COD) and biological oxygen demand (BOD) concentrations in the range of 21,960–26,000 mg l^{-1} and 1200–15,660 mg l^{-1} respectively and an equally high concentration of suspended solids (SS) of 5460–7370 mg l^{-1}. It cannot be directly discharged into surface water bodies, due to its highly biodegradable nature [12]. By applying suitable conditions, HWW can be converted into biohydrogen by fermentation that would be a dual environmental benefit in the direction of wastewater treatment along with sustainable bioenergy (H_2) generation [13].

Taking the factors above into account, HWW is selected as model compound of organic-rich wastewater in this study, and the key process factors of biohydrogen production, such as substrate concentration and characteristics, the type of inoculant used, fermentation pH and temperature are optimized by slaughterhouse sludge (SHS).

2. Materials and methods

2.1. Herbal wastewater

HWW used in this study is collected from the local herbal pharmaceutical manufacturing company in Vijayawada, India. It is collected from the outlet of the production process line. To make the feedstock for the experiments, raw herbal wastewater is diluted with tap water and frozen at −20 °C until being used. The characteristics of wastewater are shown in Table 1.

Table 1 – Composition of herbal wastewater.

Parameters	Unit	Content
pH	–	5.8 ± 0.45
Chemical oxygen demand (COD)	mg/L	16,162 ± 480
Biological oxygen demand (BOD)	mg/L	6852 ± 185
Total solids (TS)	mg/L	13,156 ± 896
Total suspended solids (TSS)	mg/L	1856 ± 562
Volatile suspended solids (VSS)	mg/L	1386 ± 563

2.2. Seed microflora

The sludge sample collected from the full scale anaerobic digester of up flow anaerobic sludge blanket reactor (UASB) of the local slaughterhouse industry in Hyderabad, India. The UASB is used to produce methane from the slaughterhouse wastewater. Prior to use, raw seed sludge is filtered through a screen (pore size, 2 mm) to remove any fiber-like, undigested materials before use. The anaerobic sludge is heated at 105 °C for 1 h to select for spore-forming hydrogen producers and then cooled at room temperature. Thereafter, pre-incubated with basal medium in an sealed container (1000 mL) at 36 ± 1 °C for about 24 h, the basal medium contains: glucose, 4 g/l; KH_2PO_4, 0.2 g/l, NH_4HCO_3, 1 g/l and 10 mL mineral salt solution (0.1 g/l $MgSO_4 \cdot 7H_2O$, 0.01 g/l NaCl, 0.01 g/l $Na_2MoO_4 \cdot 2H_2O$, 0.015 g/l $MnSO_4 \cdot 7H_2O$, 0.01 g/l $CaCl_2 \cdot 2H_2O$ and 0.0278 g/l $FeCl_2$).

2.3. Experimental apparatus

To start a batch reactor, inoculum (125 mL) is placed into 3.2 L batch reactors at a loading of 1 gVs L^{-1} working volume. The reactor is loaded with HWW (625 mL) at 4 gVS L^{-1} to achieve a food-to-microorganism ratio (F/M) of 4 and tap water is added to bring the working volume of the reactor to 1.4 L. The initial pH values of the medium are adjusted to 6.7 with a dilute acid/alkali solution. These bottles are purged with nitrogen gas to remove oxygen to keep the anaerobic environment and then closed with butyl rubber stoppers and connected to a control with plastic tubing. The reactor is placed on magnetic stirrer provided with heating mantle for continuously mixing with rotation speed of 150 rpm to provide better contact among substrates under mesophilic conditions (36 ± 1 °C). At each time interval, the total biogas volume is measured by releasing the pressure in the bottles using a gas-collecting vessel of displacement method with saturated brine. Each series is repeated three times for reproduction of values. The effluent biogas compositions are simultaneously monitored along with operational parameters and steady-state conditions are reported. The photograph representation of the reactor is provided in Fig. 1.

2.4. Analytical methods

The hydrogen gas percentage is calculated by comparing the sample biogas with a standard of pure hydrogen using a gas

Fig. 1 – Photograph of a batch reactor for Hydrogen production.