Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 99 (2008) 6941–6948
BIORESOURCE TECHNOLOGY

Effect of substrate loading rate of chemical wastewater on fermentative biohydrogen production in biofilm configured sequencing batch reactor

Y. Vijaya Bhaskar, S. Venkata Mohan *, P.N. Sarma

Bioengineering and Environmental Centre, Indian Institute of Chemical Technology, Hyderabad 500 007, India

Received 13 November 2007; received in revised form 9 January 2008; accepted 11 January 2008
Available online 4 March 2008

Abstract

The influence of substrate loading rate on fermentative hydrogen (H_2) production was studied in biofilm configured sequencing batch reactor using chemical wastewater as substrate. Reactor was operated with selectively enriched anaerobic mixed microflora at different organic loading rates (OLRs; 6.3, 7.1 and 7.9 kg COD/m^3 day) after adjusting the feed to a pH of 6.0 (acidophilic) to provide suitable environment for acidogenic bacterial function. Variation in H_2 production rate was observed with change in OLR [specific hydrogen yield – 13.44 mol H_2/kg COD_R day (6.3 kg COD/m^3 day), 8.23 mol H_2/kg COD_R day (7.1 kg COD/m^3 day) and 6.064 mol H_2/kg COD_R day (7.9 kg COD/m^3 day)]. H_2 yield showed reasonably good correlation with pH drop [6.3 kg COD/m^3 day (R^2 – 0.9796), 7.1 kg COD/m^3 day (R^2 – 0.9973), 7.9 kg COD/m^3 day (R^2 – 0.9908)]. Increase in OLR showed marked reduction in COD removal efficiency [22.6% – 6.3 kg COD/m^3 day; 19.8% – 7.1 kg COD/m^3 day and 17.2% – 7.9 kg COD/m^3 day].

Keywords: Mixed microflora; Wastewater treatment; Periodic discontinuous batch reactor; Acidophilic; Acidogenic

1. Introduction

Even though the world's energy requirement is being replenished by fossil fuels that serve as a primary energy source, the unfettered use of fossil fuels is causing potential disastrous effects on the world climate. The release of green house gases from fossil fuels leads to global warming and acid rain (Vijayaraghavan et al., 2006). Among many alternative pathways, ''hydrogen (H_2) energy'' has been receiving special attention as a potential and sustainable replacement for fossil fuels as it has low emission and is environmentally benign and cleaner. H_2 can be produced through different routes and from various substrates. Biological H_2 production from a renewable biomass such as the organic fraction of waste or wastewater has considerable potential with respect to solving global environmental issues (Levin et al., 2004). Biological H_2 production using fermentative process, photosynthetic bacteria or algae is environmental friendly and the reactors are mostly operated at ambient temperatures and pressures (Fascetti et al., 1998; Das and Verziroglu, 2001). Studies on H_2 production by anaerobic fermentation were carried out using pure cultures of bacteria such as *Enterobacter* (Kumar and Das, 2000), *Rhodopseudomonas* and *Citrobacter* (Oh et al., 2003), *Bacillus* (Lin and Chang, 2004), *Escherichia* (Chittibabu et al., 2006), *Bacillus coagulans* IIT-BT S1 (Kotay and Das, 2007) and *Clostridium* (Wang et al., 2003) or combined bacteria, i.e., *Clostridium* and *Enterobacter* (Yokoi et al., 2002) to degrade monosaccharides and disaccharides, cellulose and starch in the laboratory-scale studies. Production of H_2 by mixed cultures is generally preferred from an engineering point of view because of low cost, ease of control,

* Corresponding author.
E-mail address: vmohan_s@yahoo.com (S. Venkata Mohan).

0960-8524/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2008.01.029

and possible use of organic wastes as substrates (Fang et al., 2006). More recently researchers have also been studying the use of anaerobic mixed microflora enriched from a natural population of bacteria for the production of H_2 (Ueno et al., 2001, 2007; Mu et al., 2006; Cheong and Conly, 2007; Cooney et al., 2007; Venkata Mohan et al., 2007a,b,c,d). Various attempts were made to generate H_2 from wastes like paper mill wastes (Idania et al., 2005), starch effluent (Zhang et al., 2003), food processing wastewater (Shin et al., 2004; Ginkel et al., 2005), domestic wastewaters (Ginkel et al., 2005), rice winery wastewater (Yu et al., 2002), dairy wastewater (Venkata Mohan et al., 2007a), chemical wastewater (Venkata Mohan et al., 2007b,c,d), distillery and molasses based wastewater (Ren et al., 2007; Venkata Mohan et al., in press), wheat straw wastes (Fan et al., 2006) and palm oil mill effluent (Atif et al., 2005; Vijayaraghavan and Ahmad, 2006). In this communication, experimental data pertaining to studies carried out on fermentative H_2 production at various organic loading rates utilizing chemical wastewater as substrate in biofilm configured reactor was presented and discussed.

2. Experimental

2.1. Wastewater

Designed synthetic wastewater (SW) [(g/L) glucose – 3.0, NH_4Cl – 0.5, KH_2PO_4 – 0.25, K_2HPO_4 – 0.25, $MgCl_2{\cdot}6H_2O$ – 0.3, $FeCl_3$ – 0.025, $NiSO_4$ – 0.016, $CoCl_2$ – 0.025, $ZnCl_2$ – 0.0115, $CuCl_2$ – 0.0105, $CaCl_2$ – 0.005 and $MnCl_2$ – 0.015] and composite chemical wastewater (CW) were used as substrates for H_2 production. The characteristics of SW are pH 7.64, total dissolved solids (TDS) – 1350 mg/L, chemical oxygen demand (COD) – 5840 mg/L, biochemical oxygen demand (BOD_5) – 3910 mg/L, chlorides – 184 mg/L, sulfates – 12 mg/L, phosphates – 230 mg/L and total nitrogen – 140 mg/L. The CW was composite/combined chemical wastewater collected from a common effluent treatment plant in Hyderabad, India where wastewater received from about 100 chemical processing industries is being treated. The characteristics of CW are pH 7.80, TDS – 11,000 mg/L, suspended solids – 920 mg/L, oil and grease – 14 mg/L, COD – 9840 mg/L, BOD_5 – 2950 mg/L, chlorides – 5096 mg/L, sulfates – 1750 mg/L, phosphates – 360 mg/L and total nitrogen – 125 mg/L. Characteristically, the wastewater is complex in nature due to its composite nature and low-biodegradability (BOD_5/COD – 0.3).

2.2. Mixed microflora

Anaerobic mixed microflora acquired from an operating laboratory-scale upflow anaerobic sludge blanket (UASB) reactor treating chemical wastewater for the past three years was used as parent inoculum. The inoculum was used after cyclic pretreatment sequences (four times) changing between heat-shock (100 °C; 2 h) and acid (pH 3 adjusted with orthophosphoric acid (88%); 24 h) treatment. Orthophosphoric acid treatment was given to inhibit the growth of methanogenic bacteria (MB), at the same time to selectively enrich the H_2 producing acidogenic bacteria (AB) (Venkata Mohan et al., 2007b). Resulting selective enriched acidogenic mixed consortia (pH (1:10), 7.7 ± 0.2; total solids, 18.53 ± 0.44 g/L, suspended solids (SS), 13.54 ± 0.32 g/L; volatile suspended solids (VSS), 7.62 ± 0.16 g/L)was used as parent inoculum for reactor startup.

2.3. Bioreactor design and operation

Biofilm configured anaerobic reactor (AnSBBR; working volume – 4 L, liquid volume – 2 L; gas holding capacity – 0.35 L) was fabricated using 'perplex' material using leak proof sealing. Inert stone chips (0.02–0.03 m; void ratio – 0.54) were used as packing material to support the growth of H_2 producing acidogenic mixed microflora. The bioreactor was designed for operation in upflow mode and was operated in periodic discontinuous batch (PDBR)/sequencing batch (SBR) mode with a total cycle period (hydraulic retention time) of 24 h consisting of 15 min of FILL, 23 h of REACT (anaerobic), 30 min of SETTLE and 15 min of DECANT phases. The reactor after inoculation with mixed culture was initially operated with synthetic feed (SW) as substrate to support the biofilm formation on the packing medium and to facilitate adaptation at low OLRs (OLR – 2.4 kg COD/m^3 day) by adjusting the pH of the feed to 6 (Venkata Mohan et al., 2007b). Fermentative H_2 production was evaluated by operating the reactor at variable OLRs of 6.3 kg COD/m^3 day [CW (50%) and SW (50%); COD – 7.84 g/L], 7.1 kg COD/m^3 day [CW (75%) and SW (25%); COD – 8.84 g/L] and 7.9 kg COD/m^3 day [CW (100%); COD – 9.84 g/L]. Constant COD removal and gas production were considered as indicators for the successful formation of biofilm and then the reactor was operated at higher OLR. At the beginning of each cycle, immediately after withdrawal (earlier sequence), a pre-defined volume (1.5 L) of wastewater was fed to the reactor during FILL phase. The contents of the reactor were circulated with reactor outlet in a closed loop at recirculation rate (recirculation volume to feed volume ratio) of 3 during the REACT phase to achieve a homogeneous distribution of the substrate as well as uniform distribution of requisite consortia along the reactor depth. The influent pH was adjusted to 6.0 using concentrated orthophosphoric acid (88%) before the reactor was fed with wastewater. Peristaltic pump controlled by preprogrammed electronic timer (ETTS, Germany) was used to regulate the FEED, recirculation, and DECANT operations. The controller was programmed to operate on a repeatable 24 h cycle. All experiments were performed at a mesophilic (room) temperature of 28 ± 2 °C.

2.4. Biochemical analysis

Biogas (H_2) collected in the headspace of bioreactor was estimated regularly using electrochemical H_2 sensor [microprocessor based pre-calibrated system, FMK satel-