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# Bio-electrohydrolysis as a pretreatment strategy to catabolize complex food waste in closed circuitry: Function of electron flux to enhance acidogenic biohydrogen production

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## ABSTRACT

A novel bio-electrohydrolysis system (BEH) based on self-inducing electrogenic activity was designed as a pretreatment device to enhance biohydrogen ( $H_2$ ) production efficiency of food waste. Experimental strategy involved two-stage integrated/hybrid operation with hydrolysis in initial stage followed by acidogenic fermentation for  $H_2$  production in second stage. After pre-treatment, catabolized food waste from control (anaerobic) and BEH (closed circuit mode of operation) system was used as substrate in a separate bioreactor to evaluate  $H_2$  production in dark-fermentation process. Pretreated-waste from BEH showed higher  $H_2$  production (29.12 ml/h; 24th h) than control (26.75 ml/h; 16th h). Higher cumulative  $H_2$  production and maximum substrate degradation were also noticed with BEH-pretreated substrate (CHP, 0.91 l; COD, 52.42%) than control (CHP, 0.68 l; COD, 43.68%). Under closed circuitry, anode served as an alternative electron acceptor promoting biotransformation of complex organics to simpler molecules through catabolism.

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## Introduction

Biohydrogen ( $H_2$ ) production using dark-fermentation process from renewable sources such as biomass and wastewater using mixed biocatalyst is generally preferred because of lower cost of raw material, ease of control and the possibility of using a wide range of organic waste as feedstock [1–13]. Exploitation of wastewater as substrate for  $H_2$  production with

concurrent wastewater treatment is an attractive and effective way of tapping clean energy from renewable resources in a sustainable approach [14–16]. The process yields dual benefit to the environment in terms of renewable energy generation with simultaneous waste stabilization. One of the major challenges that persist with the dark-fermentation process is the rate and yield of  $H_2$  production. Substrate/feed-stock and its composition are one of the rate limiting factors that persist with the dark-fermentative  $H_2$  production

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process. Carbohydrates are the main source of  $H_2$  during fermentative processes and therefore wastes/wastewater rich in carbohydrates can be considered as potential sources of  $H_2$  [17]. Food waste due to its high organic fraction can be considered as a viable substrate for  $H_2$  production, along with treatment which makes the process economically and environmentally feasible. This high organic fraction (carbohydrate) can be catabolized to simple sugars like hexoses and pentoses by hydrolysis. So that it can be easily available for the microbes.

In recent years, microbial fuel cell (MFC) has emerged as a promising yet challenging technology for the recovery of energy from waste besides its treatment and is gaining importance due to its sustainable nature. This process provides dual benefits of wastewater treatment and provides access to cheap and environmentally friendly energy. Wastewater contains complex organics, such as polysaccharides which undergo catabolism to form the corresponding monomer sugars [18,19]. Pre-treatment of waste/wastewater otherwise called as hydrolysis can enhance the bio-digestibility and increase the accessibility of enzymes to the materials, thus improving biogas yield from wastes [20]. Pre-fermentation as a pretreatment of composite canteen food waste and its subsequent evaluation as substrate for electricity production in MFC were reported earlier [21]. Pre-fermentation of food waste showed positive influence on energy recovery. MFC as hybrid bio-electrochemical system (BES) is receiving much attention due to its inherent advantage to degrade toxic and complex substrate. Anodic bacteria transfer electrons from a reduced electron donor (substrate) to the electrode and then transfer through an external circuit to the cathode, where the electrons get reduced in the presence of an electron acceptor [19]. Released electrons not only generate electricity but also involve in the removal of pollutants (substrate catabolism) due to the associated secondary reactions [18].

In this direction, pre-hydrolysis of food waste was performed in a novel bio-electrohydrolysis system (BEH) which was designed to take advantage of the *in situ* biopotential (self-inducing bioelectrogenic activity) developed during the operation. The main aim of BEH is to hydrolyze food waste prior to acidogenic fermentation towards enhancing the  $H_2$  production efficiency. Hydrolysis was carried out as a pretreatment step to simplify the composite food waste. The resulting hydrolysate was subsequently used as substrate in the second phase for  $H_2$  production. Comparative performance of the anaerobic process (control system without electrode assembly) and BEH (with electrode assembly) was evaluated separately using the same biocatalyst under identical operating conditions. BEH operation facilitates integration of the anaerobic process with self-induced bio-electrochemical hydrolysis in a single system. In the previous study [22], hydrolysis was carried out as a pretreatment step by applying variable external resistance ( $R_E$ ) such as 10  $\Omega$ , 100  $\Omega$ , 1000  $\Omega$  and compared with closed circuit mode. Considering the outputs from this study, detailed experiments were performed to evaluate the influence of closed (short) circuit mode of operation on electron flux, metabolic functions and electrochemical behaviour of the biocatalyst in comparison with anaerobic process (control without electrode assembly). Electrode assembly present in the BEH system functions as an

alternative electron acceptor, which promotes the biotransformation of complex organics to simpler ones [18, 22]. Experiments were carried out in two phases by coupling BEH with terminal dark fermentation process (acidogenic batch reactor, ABR). During the second stage of operation, hydrolysate collected from the pretreatment reactors was used as substrate for  $H_2$  production to evaluate the pretreatment efficiency so as to enhance the  $H_2$  production rate. The performance of the bioreactors was evaluated with respect to the chemical oxygen demand (COD), carbohydrate profile and bioelectrochemical behavior along with  $H_2$ .

## Materials and methods

### Bioreactors

The experimental methodology included two stage integrated (hybrid) operation. The initial system was designed to bring about hydrolysis by BEH through *in situ* bio-potential generated in the system itself followed by acidogenic fermentation for  $H_2$  production (ABR). In the first phase, bio-hydrolysis was carried out as a pretreatment step to simplify/catabolize the composite food waste and the hydrolysate obtained was used as substrate in the second phase to enhance  $H_2$  production.

### Bioelectrogenic hydrolysis system (BEH)

BEH resembled a single chambered fuel cell with an open air-cathode having a total/working volume of 0.55/0.50 l. BEH was fabricated using 'perspex' material in the laboratory (Fig 1). Non-catalyzed plain graphite plates (7.0  $\times$  3.5 cm; 0.5 cm thick; surface area 59.5 cm<sup>2</sup>) were used as anode and cathode. Copper wires were employed for connections after sealing with an epoxy sealant. The cathode was arranged on the top surface of BEH such that its bottom portion submerged in the anolyte leaving the top portion exposed to air. Anode was completely submerged in the anolyte. BEH was operated under closed circuit (CC) mode and without membrane. Provision was made to include sampling ports and wire input in the design. Proper care was taken during the operation, sampling, feeding and measurements to maintain anaerobic conditions. A control system dimensionally similar to BEH was operated parallel without electrode assembly to enumerate the performance of a conventional anaerobic hydrolysis in comparison to self-induced potential mediated bio-hydrolysis.

### Acidogenic batch reactor (ABR)

$H_2$  production was evaluated in two batch reactors operated in a suspend growth mode under acidogenic conditions in anaerobic microenvironment. The reactors were fabricated with glass with a total total/working volume of 0.30/0.25 l employing leak proof sealing with proper inlet and outlet arrangements. Pre-hydrolyzed substrate in both BEH and control reactors was evaluated in two separate reactors.

### Anaerobic consortia

Anaerobic consortium acquired from wastewater treatment plant was used as biocatalyst in both control and BEH reactors. Prior to inoculation, the culture was washed twice in saline

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