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Systematic approach to assess biohydrogen potential of anaerobic sludge and soil rhizobia as biocatalysts: Influence of crucial factors affecting acidogenic fermentation

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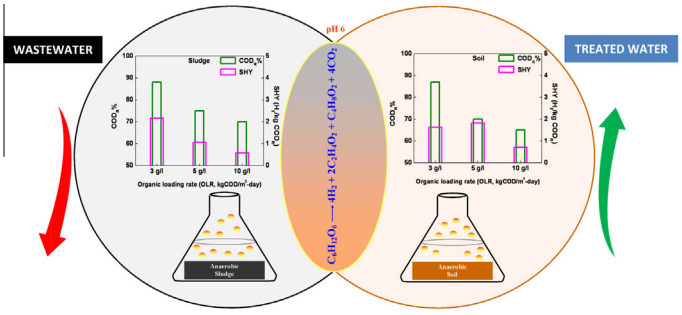
HIGHLIGHTS

- Biohydrogen potential of anaerobic sludge and soil were evaluated for H_2 production.
- Kinetics and Modeling studies for H_2 production along with statistical validation.
- Critical evaluation of both biocatalysts using voltammetric analysis.
- Pretreated agricultural soil as a biocatalyst showed efficient H_2 production at pH 6.

GRAPHICAL ABSTRACT



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ABSTRACT

A systematic protocol was designed to enumerate the variation in biohydrogen production with two different biocatalysts (sludge and soil) under different pH and organic loads. Both the biocatalysts showed cumulatively higher H_2 production under acidogenic condition (pH 6) than at neutral pH condition. The cumulative hydrogen production was non-linearly fitted with modified Gompertz model and statistically validated. Pretreated soil biocatalyst showed relatively higher H_2 production (OLR II, 142 ± 5 ml) than pretreated sludge (OLR I, 123 ± 5 ml); which was evidenced by substrate linked dehydrogenase activity and bio-electrochemical analysis. Experimental results revealed agricultural soil as a better biocatalyst than anaerobic sludge for all the operated process conditions. The voltammogram profiles and Tafel slopes revealed dominance of reductive catalytic activity of the pretreated inoculums substantiating dark-fermentation. Soil consortia showed low polarization resistance (2.24 kΩ) and high reductive electron transfer efficiency (1.17 $Vdec^{-1}$) at a high organic load; thus, rebating high H_2 production.

1. Introduction

In developing countries like India, the existing economic status depends majorly on the usage of fossil fuels and the need has been increasing exponentially every year (Hallenbeck et al., 2012; Venkata Mohan et al., 2009). The emissions emanated from fossil fuels are detrimental, and causes serious negative impact on living environment and climate change (Swamy et al., 2012, 2013). Therefore, the modern outlook of scientific community is to produce value-added products using bacterial biomass from negative valued waste (Mohanakrishna and Venkata Mohan, 2013; Venkateswar Reddy et al., 2012). Harvesting of bioH_2 as a renewable energy from wastewater addresses today's two most imperative concerns: energy security and global climate change; which have strong impact on our lives (Won and Lau, 2011). Dark fermentation is an eco-friendly biological process which has received significant interest

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in the recent decade owing to the fact that H_2 can be generated continuously (Venkata Mohan, 2009; Rittmann and Herwig, 2012). In this way, production costs of biologically mediated H_2 can compete cost-effectively with other conventional methods. Nevertheless, augmenting rate and yield are the two critical confronts for sustainable H_2 production (Hafez et al., 2010). Disparity in yields documented in the literature reflect the underlying influence of several process parameters, including substrate composition, type of inoculum, pre-treatment, reactor type, mode of reactor operation, operating variables such as temperature, hydraulic retention time (HRT), organic loading rate (OLR) and pH (Venkata Mohan et al., 2012).

Anaerobic bacteria are competent bio-catalysts in producing H_2 from organic waste. The potential anaerobic inoculums are mainly found in the natural environments such as compost, sewage sludge, cow dung, soil, etc. (Bansal et al., 2013). Mixed microflora can be directly used for continuous H_2 production, which is practically robust and more productive than single strains (Niessen et al., 2006). Yet, nature of microbial diversity of the parent inoculum can show a discrepancy in the H_2 production efficiency due to the coexistence of H_2-producing and consuming bacteria (Sarkar et al., 2013). Under such circumstances, pre-treatment of inoculum by selective or combined strategies is often practiced to enrich H_2 producers due to their adaptability in adverse conditions (Venkata Mohan et al., 2012). Besides, nature of biocatalyst, H^+ fluxes in/out bacteria (i.e. measure of pH values) during process operation is considered as the most pivotal parameter, due to its effects on hydrogenase activity, metabolic pathways and substrate hydrolysis. The H^+ ion concentration in the system is also critical for maintaining adequate ATP levels in the system, since in the presence of an H^+ ions excess ATP is used to ensure cell neutrality rather than to produce H_2 (Nazlina et al., 2011). The initial pH is also known to affect H_2 production through its influence on lag phase duration, spore germination (in those cases where a pre-treatment is preliminary applied to the inoculum) and the synthesis of enzymes (Kim et al., 2011). The OLR of a system may affect a number of operating issues, including volatile fatty acids (VFA) accumulation and pH changes, as well as variations in the composition of the active biomass, with consequent modifications of the associated metabolic pathways (Vijaya Bhaskar et al., 2008). These pathways can either be promoted or inhibited, depending on the adopted operating conditions.

The literature review gave a fair notion that there is a need for suitable microbial consortia with selective enrichment of H_2 producers and optimization of the crucial influential factors for better H_2 yield. Several types of inoculums have been used for anaerobic H_2 production in previous studies, such as compost, anaerobic sludge and isolated cultures; however, only a few have worked with soil/sediment. Therefore, in this study an attempt was made to compare and evaluate the H_2 production efficiencies of anaerobic sludge and agricultural soil inoculums with optimization of process parameters viz., type and nature of inoculum, initial pH and OLR. Bio-kinetic and statistical analyses were also performed to validate the obtained observations.

2. Methods

2.1. Selection and enrichment of the biocatalyst

Two different types of anaerobic mixed consortia (sludge and soil) were employed in this study. Anaerobic sludge was acquired from an existing commercial full scale wastewater treatment plant and anaerobic soil was taken from an agricultural field where groundnuts (*Arachis hypogaea*) were cultivated. The soil was sampled in an assorted pattern and a homogenized portion of the sample was taken by cone-quarter method for further studies. Both the inoculums were enriched with designed synthetic wastewater (DSW; glucose – 3 g/l, NH_4Cl – 0.5 g/l, KH_2PO_4 – 0.25 g/l, K_2HPO_4 – 0.25 g/l, $MgCl_2$ – 0.3 g/l, $CoCl_2$ – 25 mg/l, $ZnCl_2$ – 11.5 mg/l, $CuCl_2$ – 10.5 mg/l, $CaCl_2$ – 5 mg/l, $MnCl_2$ – 15 mg/l, $NiSO_4$ – 16 mg/l, $FeCl_3$ – 25 mg/l) for five days to activate the parent bacterial culture. Thereafter, the parent culture was pretreated with heat (100 °C, 2 h), acid-shock (pH 3 adjusted with orthophosphoric acid (88%, 24 h) and chemical treatment (2-bromoethane sulphonic acid sodium salt solution (BESA), 0.2 g/l, 24 h). For experimentation, an inoculum of about 20% (v/v) i.e. with volatile suspended solids (VSS) concentration of 4 g/l was added and then the initial pH was adjusted.

2.2. Experimental methodology

The experimental approach was designed keeping in view of the rapid protocol introduced by Venkata Mohan et al. (2012). This methodology was based on applied environmental conditions viz., inoculum pretreatment to enrich the growth of H_2 producers, operation with initial pH and appropriate OLR to maintain suitable pH levels for significant and stable H_2 production, fixed HRTs causing the wash-out of H_2 consumers. In this regard, a systematic approach was employed by considering crucial factors that significantly influence the overall H_2 process (Fig. 1). Experiments with sludge and soil were carried out, separately in two phases to optimize three main parameters viz., condition of inoculum/biocatalyst, initial pH and organic substrate load. The best resultant parameter was considered to the next level of optimization by assessing the bioH_2 potential (BHP) along with substrate degradation efficiency (ξ_{COD}). In phase I, four experiments at OLR I [5 g/l (3.20 kg COD/m^3-day)] was performed with different combinations of selected factors viz., untreated inoculum (UTI) and pretreated inoculum (PTI) with feeding pH of 6 and 7. The outcome from phase I yielded optimized conditions for two operational parameters viz., condition of biocatalyst and initial pH. While, phase II aims to establish optimum substrate organic load. Experiments were carried out at two OLRs one at lower [OLR II, 3 g/l (1.9 kg COD/m^3-day)] and the other at higher [OLR III, 10 g/l (6.23 kg COD/m^3-day)] compared to first

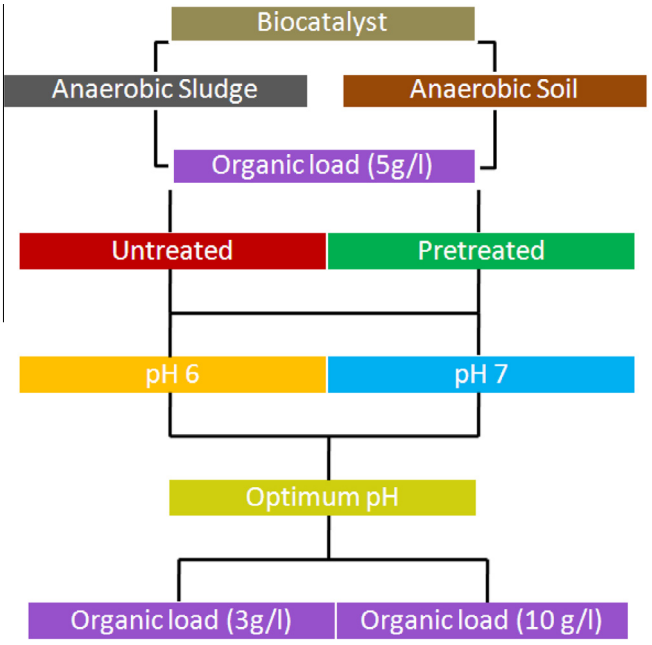

Fig. 1. Sequential flowchart illustrating the experimental methodology employed to enrich H_2 producers and to evaluate the bio-hydrogen potential of anaerobic sludge and soil inoculums.

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