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Review

Harnessing of biohydrogen from wastewater treatment using mixed fermentative consortia: Process evaluation towards optimization☆

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ABSTRACT

Utilizing wastewater as a potential source for renewable energy generation through biological routes has instigated considerable interest recently due to its sustainable nature. An attempt was made in this communication to review and summarize the work carried out in our laboratory on dark fermentation process of biohydrogen (H_2) production utilizing wastewater as primary substrate under acidogenic mixed microenvironment towards optimization of dynamic process. Process was evaluated based on the nature and composition of wastewater, substrate loading rates, reactor configuration, operation mode, pH microenvironment and pretreatment procedures adopted for mixed anaerobic culture to selectively enrich acidogenic H_2 producing consortia. The fermentative conversion of the substrate to H_2 is possible by a series of complex biochemical reactions manifested by selective bacterial groups. In spite of striking advantages, the main challenge of fermentative H_2 production is that, relatively low energy from the organic source was obtained in the form of H_2. Further utilization of unutilized carbon sources present in wastewater for additional H_2 production will sustain the practical applicability of the process. In this direction, enhancing H_2 production by adapting various strategies, viz., self-immobilization of mixed consortia (onto mesoporous material and activated carbon), integration with terminal methanogenic and photo-biological processes and bioaugmentation with selectively enriched acidogenic consortia were discussed. Application of acidogenic microenvironment for *in situ* production of bioelectricity through wastewater treatment employing microbial fuel cell (MFC) was also presented.

1. Introduction

More recently capturing of energy in the form of hydrogen (H_2) from fermentative wastewater treatment is assuming prominence. Biohydrogen is considered as sustainable and green alternative in the realm of fossil fuel depletion and environmental pollution. It is a carbon neutral energy source satisfying the criteria of an environmentally benign

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technology and thus believed that H_2 fuel-based economy would be less polluting than a fossil fuel-based economy. In this direction, considerable interest is observed on various biological routes of H_2 production using bio-photolysis, photo-fermentation and heterotrophic dark fermentation processes or by a combination of these processes. Among them, dark fermentation is considered as a viable method on practical front which is gaining importance and further leading to open a new avenue for the utilization of renewable energy sources [1–26]. Moreover, the process can be operated at ambient temperature (30–40 °C) and pressure [1–6].

Fermentative conversion of the substrate to H_2 is generally manifested by a diverse group of bacteria in a series of complex biochemical reactions similar to anaerobic conversion. Anaerobic degradation of complex organic material to methane (CH_4) requires four major steps and five physiologically distinct groups of microorganisms [26,27]. The process involves a series of biological processes manifested by multispecies reactions in which hydrocarbons are converted from complex to simple molecules and ultimately to carbon dioxide (CO_2) and CH_4 through a series of fermentative reactions mediated by a diverse group of microorganisms [4,26–28]. Fermentative/hydrolytic microorganisms hydrolyze (hydrolysis) complex organic polymers to monomers, which are further converted to a mixture of low-molecular-weight organic acids [volatile fatty acids (VFAs)] and alcohols (acidogenesis/acetogenesis) by obligatory H_2 producing bacteria. Obligatory H_2 producing acetogenic bacteria (AB) oxidize fermentative products to acid intermediates and H_2, which also include acetate production from H_2 and CO_2 by acetogens and homoacetogens. Finally acetoclastic methanogens convert organic acids to CH_4 and CO_2 (methanogenesis). Preliminary elements of methanogenic food web are expected to play a role in biological H_2 production which is considered to be dynamic in nature. Many thermophilic systems use syntrophic oxidation of acetate to CO_2 and H_2 by acetogenic or homoacetogenic bacteria coupled with H_2 consumption by hydrogenotrophic methanogens [29]. H_2 producing acetogenic bacteria grow in syntrophic associations with hydrogenotrophic methanogens, which keep the partial pressure of H_2 low enough to allow acetogenesis to become thermodynamically favorable by a process referred to as interspecies H_2 transfer [30]. The stepwise breakdown of the molecules is manifested by a specific group of microorganisms that thrive during the catabolic process and capture energy of the hydrocarbons. H_2 production from organic substrates is limited by the thermodynamics of the hydrogenase reaction, which involves the enzyme-catalyzed transfer of electrons from an intracellular electron carrier molecule to protons where, most of the observed H_2 can be attributed to electrons derived from a single reaction by oxidative decarboxylation of pyruvate catalyzed by pyruvate:ferredoxin oxidoreductase (PFOR) [26]. PFOR are intracellular enzymes which catalyze the transfer of electron to proton especially in *Clostridial* sp. and this pathway may vary from species to species. Hydrogenase, the last rate-limiting enzyme of H_2 release during the process of energy metabolism, plays a key role in H_2 producing metabolism [25,31]. The metabolic interaction between various groups of organisms is essential and the operation of dynamic H_2 producing system requires a solid understanding of the important factors involved. Optimization of process parameters for improving the overall microbial process is one of the important ways to enhance H_2 yield as well as substrate degradation and assumes significance prior to up-scale the process.

Reducing the cost of wastewater treatment and finding ways to produce useful products from wastewater has been gaining importance in view of environmental sustainability. One way to reduce the cost of wastewater treatment is to generate bio-energy, such as H_2 gas from the organic matter present in wastewater, at the same time accomplishing treatment. Wastewater generated from various industrial processes are considered to be the ideal substrates because they contain high levels of easily degradable organic material, which results in a net positive energy or economic balance. H_2 production by using wastewater as fermentative substrate with simultaneous treatment of wastewater is assuming importance and is an effective way of tapping clean energy from renewable resource in a sustainable approach [2,4–26]. Major advantages of energy produced from wastewater are the absence of environmental emissions, simultaneous recovery of energy and wastewater treatment. Technical feasibility, simplicity, economics, societal needs and political priorities are the key factors to choose any bioprocesses that will be used to treat wastewater in future [26]. Therefore, in this communication, the work carried out in our laboratory for the past six years on H_2 production by fermentation (acidogenic/acetogenesis) utilizing wastewater as primary substrate employing mixed consortia is reviewed and discussed in the direction of optimizing the process.

2. Process optimization

Origin and nature of mixed culture used as parent inoculum and application of pretreatment methodology for selective enrichment of required microflora, pH microenvironment, nature of substrate and its composition, co-substrate addition, fermentation period, reactor configuration and mode of reactor operation are some of the critical factors governing the fermentative H_2 production process and requires optimization prior to up-scaling.

2.1. *Pretreatment of mixed culture*

Employing mixed anaerobic culture is extremely important and practical approach to achieve H_2 production on a large scale. Using mixed microbial community needs selection of culture according to requisite function and should be well suited to the non-sterile, ever-changing and complex nature of substrate/wastewater. Biological H_2 production (acidogenic/acetogenesis) by dark fermentation (chemo-organotrophic) process shares many common features with methanogenic anaerobic digestion, [4,26]. The mixed communities involved in both bioprocesses share some common elements but with one important difference: successful H_2 production requires inhibition of H_2 consuming microorganisms, such as homoacetogens and methanogens. Typical anaerobic mixed cultures could not produce H_2 as it is an intermediate for CH_4 formation, and was rapidly consumed