A REVIEW ON INFLUENCE OF PRETREATMENT AND SYSTEM OPERATING CONDITION FOR HYDROGEN PRODUCTION FROM WASTEWATER
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Abstract: H₂ is a sustainable and viable form of green/alternative energy source. This study reviews the work on H₂ production from wastewater treatment relating to inoculum pretreatment and system working conditions. Process was assessed and examined taking into account pretreatment methodology procedures applied on mixed parent anaerobic culture to selectively enrich acidogenic culture, operating pH and retention time in concurrence with wastewater type as substrate.

Keywords: Biohydrogen, pH, Chemical Oxygen Demand, Wastewater treatment.

1. INTRODUCTION
Capturing of energy as molecular biohydrogen (H₂) especially from wastewater treatment process is gaining prominence. Various biological routes of H₂ production include biophotolysis, photo-fermentation and dark-fermentation processes or by a combination of these processes1-4. Fermentative conversion of substrate to H₂ is complex biochemical process manifested by diverse group of bacteria by a series of biochemical reactions similar to anaerobic conversion⁵.
Anaerobic conversion requires four major steps and five physiologically distinct groups of microorganisms where hydrocarbons are converted from complex to simple molecules through H₂ and acid intermediates (Fig. 1). Hydrocarbons are ultimately converted to carbon dioxide (CO₂) and methane (CH₄). Fermentative/ hydrolytic microorganisms hydrolyze complex organic polymers to monomers, and then ferment monomers to a mixture of low-molecular-weight organic acids and alcohols.
Obligatory H₂ producing acetogenic bacteria (AB) oxidize fermentation products to acid intermediates and H₂, which also include acetate production from H₂ and CO₂ by acetogens and homoacetogens and finally acetoclastic methanogens convert organic acids to CH₄ and CO₂⁵-⁷. Many thermophilic systems use syntrophic oxidation of acetate to CO₂ and H₂ by acetogenic or homoacetogenic bacteria coupled to H₂ consumption by hydrogenotrophic methanogens⁵. H₂-producing AB grow in syntrophic associations with hydrogenotrophic methanogens (H₂ consuming), which keep H₂ partial pressure low enough to allow acetogenesis to become thermodynamically favorable by interspecies H₂ transfer⁵. Using mixed consortia as biocatalyst for H₂ production can be a practical and
promising option for scaling up of technology especially when wastewater is used as substrate.

This review summarizes the work carried out on H₂ production pertaining to inoculum pretreatment and system operating conditions during wastewater treatment. Process was evaluated and discussed based on pretreatment procedures applied on mixed anaerobic culture to selectively enrich acidogenic H₂ producing culture, operating pH, hydraulic retention time (HRT) and substrate loading rate in concurrence with wastewater used as fermentable substrate.

II. PRETREATMENT METHOD

Pretreatment of parent inoculum/culture used in fermentative H₂ production process permits selective enrichment of specific group of bacteria. H₂ production with dark fermentation process shows many common features with methanogenic anaerobic digestion. Typical anaerobic mixed cultures cannot produce H₂ as it is rapidly consumed by methane-producing bacteria. Most effective way to enhance H₂ production from anaerobic culture is to restrict or terminate methanogenesis process by allowing H₂ to become an end product in metabolic flow. Induction of H₂ accumulation in fermentative consortia is related with inhibition of H₂ consumers (especially methanogens), which is essential for its further scale-up and industrial application. It is important for acidogenic H₂ producing process and also inhibits bacteria that are not required especially when mixed cultures are used as parent inoculum. Physiological differences between H₂ producing bacteria and H₂ uptake bacteria form fundamental basis for methods used for preparation of H₂ producing seeds.

H₂ producing inoculum. Seed preparation affects both start up and overall efficiency of H₂ production.

Figure 1: Anaerobic process of wastewater treatment depicting both acetogenesis/acidogenic and methanogenic process during conversion of complex organic material to methane and carbon dioxide.
Heat-shock Treatment (HST)

HST facilitates suppression of non-spore forming bacteria and allows growth of spore forming bacteria, which are important for H₂ production. HST relies on thermal suppression of methanogenic Archaea and non-sporulating bacteria, thereby enriching culture with sporulating H₂ producing bacteria. It also facilitates suppression of methanogenic activity, which leads to H₂ production associated with concomitant volatile fatty acid (VFA) generation. HST parameters vary with temperatures (80-104°C) and exposure times (15-120 min). It is efficient to remove H₂ consuming bacteria while protecting spore forming bacteria and reported to repress methanogenic activity completely. HST kills vegetative cells of non-spore-forming microorganisms, some of which consume H₂ such as methanogens but in this process it could also kill H₂-producing microorganisms (Enterobacter sp), which are unable to sporulate. On the contrary, HST may activate dormant spores of strains able to germinate and produce H₂ when conditions become optimal, for example, spores of Clostridial species.

Acid Treatment

Most methanogens are limited to a relatively narrow pH range (6.8-7.2), while most H₂ producing bacteria can grow over a broader pH range. Methanogens are repressed by controlling cultivation conditions at low pH (5.5). Acid treatment is efficient in removing H₂ consuming bacteria and also protects spore-forming bacteria by repressing methanogenic activity. Acidic pH (5.0-5.5) was considered to be ideal for effective H₂ production due to its influence on repression of methanogenic activity thus indirectly promoting H₂ producers within the system.

Load-shock Treatment (LST)

LST is reported more effective than HST due to the presence of higher diversity of microbes, as no physical or chemical treatment was applied. On the other hand, overloading or shock-load treated sludge led to accumulation of organic acids, resulting in decrease of pH from 5.5 to 4.6; hence, not suitable for growth of methanogens. LST facilitates preparation of H₂ producing by physical pretreatment by means of direct cultivation of digested sludge without any chemical pretreatment. LST is reported to be effective compared to base, acid, chemical (BESA) and HST methods for enriching thermophilic H₂ producing seeds, as it completely repressed methanogenic activity along with good H₂ production.

Chemical Treatment

H₂ consuming methanogens in mixed culture could be eliminated by applying inhibiting chemicals [iodopropane, acetylene and 2-bromoethanesulfonic acid (BESA)]. BESA is a structural analog of co-enzyme-M specifically found in methanogens only. Chemical treatment by BESA facilitates selective inhibition of methanogenic activity without disturbing H₂ production where co-enzyme M reductase complex, a chief component for methanogenesis, inhibits. In addition, BESA could inhibit acetate producing process and long-term operation with BESA addition were not found sustainable and likely to have side effects on H₂ producing bacteria as well as occurrence of BESA-resistant mutants in anaerobic fermentation. Chemical pretreatment method has an advantage of being readily applied as and when required.
III. FACTORS INFLUENCING BIOHYDROGEN PRODUCTION

System operating conditions significantly influence overall performance of H2 production process in association with wastewater treatment. Data enveloping analysis (DEA) performed on H2 production process illustrated the importance of pH microenvironment, nature of mixed consortia, composition, nature and complexity of fermentative substrate used.

**pH**

Bacteria respond to changes in internal and external pH by adjusting their activity and synthesis of proteins associated with many different processes, including proton translocation, amino acid degradation, adaptation to acidic or basic conditions and virulence. Depending on organism and growth conditions, changes in external pH can bring about subsequent alterations in several primary physiological parameters, including internal pH, concentration of other ions, membrane potential and proton-motive force. This is especially important for fermentative H2 production where activity of acidogenic group of bacteria is considered to be crucial and rate limiting. pH plays a critical role in governing metabolic pathways of organism where activity of AB is considered to be crucial. pH are integral expressions of acid-base conditions of any anaerobic process and also an intrinsic index of balance between two of the most important microbial groups. Production of acids gradually reduces buffering capacity of system, which, in turn, resulted in a concomitant decline in the system pH in all experimental variations studied, due to accumulation of organic acids leading to process inhibition. If pH is not maintained in optimum range, cessation of H2 production will result along with marked shift in microbial population.

**Hydraulic Retention Time (HRT)**

HRT also influences H2 generation process. Fermentation time is considered as an important operational parameter to restrict the process of methanogenesis during acidogenic H2 production. It facilitates metabolic shift in concurrence with extended fermentation time, nature of inoculum, nature of substrate, applied loading rate and fermentation pH. A number of workers have used short HRT and acidophilic pH. Optimum HRT between 8.0 and 14 h has been reported to yield maximum H2 with inhibition of methanogenesis. Continuous H2 production from a mixed culture was observed at long HRT of 3 days (pH 6.4) without encountering problems with methanogenesis. Provision of short HRT’s during reactor operation may wash out slow growing methanogens and can reduce reactor size and capital cost.

**Substrate Loading Rate**

Apart from wastewater characteristics, substrate/ organic loading rate (OLR) of wastewater had marked influence on H2 production. H2 yields were inversely related to glucose feeding rate, while highest H2 yields were observed at lowest glucose loading rate. Glucose concentration exceeding 2 g/l (as co-substrate) showed suppression in H2 production. Feed consisting of only glucose as substrate showed low H2 yield, while feed with chemical
wastewater admixed either with glucose or with sewage wastewater as co-substrates demonstrated relatively high H₂ yield. Adding glucose and sewage wastewater as co-substrates along with chemical wastewater showed positive influence on H₂ generation rate. H₂ production was studied using chemical wastewater as substrate in which a marked reduction in H₂ production rate was observed with increase in OLR [specific hydrogen yield - 13.44 mol H₂/kg COD·day (6.3 kg COD/m³·day), 8.23 mol H₂/kg COD·day (7.1 kg COD/m³·day) and 6.064 mol H₂/kg COD·day (7.9 kg COD/m³·day)]⁴,10,17. Increase in H₂ yield may be due to end product inhibition by over-accumulated (supersaturated) H₂ in liquid at high COD. H₂ production rate increased with increase in initial glucose concentration from 0.5-2.0% but dropped at 2.5% indicating substrate inhibition¹⁷.

However, for effective H₂ yield and substrate degradation, significantly diverse system operational conditions are required individually under anaerobic microenvironment¹⁷. Balancing conditions for combined effective performance and process optimization in this direction are especially important to sustain economic viability¹⁷,18. In this direction, a study was performed to evaluate the system performance by combining both output parameters using two diverse mathematical approaches [data enveloping analysis (DEA) and Taguchi design of experimental (DOE) methodology]⁴, 19. Feed composition showed stronger influence followed by pH and pretreatment with respect to H₂ production. Acidophilic pH responded favorably to H₂ generation. Feed composition showed significant influence on H₂ production and substrate degradation¹⁹.

Concluding Remarks
Pretreatment applied to parent inoculum, operation conditions such as feeding and operating pH, HRT, etc. significantly contribute to H₂ production and substrate degradation apart from nature and characteristics of wastewater. Amount of substrate degradation is important when process efficiency is considered when dealing with wastewater as fermentative substrate for H₂ production. Balancing the conditions for combined effective performance are especially important for up-scaling the process and to sustain its economic viability. One of the vital aspects to be paid significant attention is non-utilized organic fraction, which remains as a soluble fermentation product from acidogenic H₂ production process.

REFERENCES
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