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Microbiome involved in anaerobic hydrogen producing granules: A mini review

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ABSTRACT

This mini review overviewed the latest updates on the anaerobic hydrogen fermentation using the granulation technology and the microbiome involved in the process. Additionally, the implication of various reactor design and their microbial changes were compared and provided the new insights on the role of microbiomes for rapid granules formation and long term stable operation in a continuous mode operation. The information provided in this communication would help to understand the key role of microbiomes and their importance in anaerobic hydrogen producing granular systems.

1. Introduction

Growing concern over fuel crisis and development of industries has constantly increased the requirement of energy and environmental restraints. Apart from liquid and solid fuel sources, gas based fuels are becoming a natural, easily and largely available clean form of energy [1,2]. Hydrogen is known as an eco-friendly, economical and 2.75 times energy efficient than other fuels [3,4]. The combustion of hydrogen liberates water molecules and energy instead of greenhouse gases [1,4]. Even though hydrogen fuel is beneficial but still is produced via high energy intensive conventional methods like water electrolysis and steam reforming of fossil fuels [5]. Alternating the hydrogen production source and method will improvise the quality and cost effectivity of hydrogen fuel [5]. Therefore researchers have started utilizing fermentation and photosynthesis for the production of bio hydrogen. Substrates like organic waste, microorganism, wastewater has grabbed attention towards bio hydrogen production [5]. Biological methods of hydrogen production possess significant importance like controlled operation environment and substrate conversion specificity [6]. It is a new trend for producing hydrogen with zero pollution and low energy utility for achieving both wastewater treatment and generation of clean energy [7,8].

Biohydrogen production can be done via two system namely dark and photo fermentation [9]. Hydrogen production by photo fermentation is a promising technology, which occurs in the availability of light using photosynthetic bacteria (PSB) [10,11]. This method can be coupled with wastewater treatment under controlled condition (ambient light and temperature). Researchers have always preferred dark fermentation under anaerobic condition in which the loss of energy is lesser in comparison with photo fermentation [12]. Production of hydrogen can be done using facultative anaerobes, anaerobes, photosynthetic microbes and methylotrophs. Different microbes like *Clostridium butyricum*, *E.coli*, *Ruminococcus albus*, *Rhodospirillum rubrum*, *Rhodospirillum capsulate* are some examples of hydrogen producing bacteria using glucose as sole carbon source [13]. The major requirements for biohydrogen production includes the choice of efficient microbes,

Abbreviations: CSTR, continuous stirred tank reactor; I-CSTR, immobilized continuous stirred tank reactor; UASB, upflow anaerobic sludge blanket reactor; EPS, extra polysaccharides; FBR, fixed bed reactor; HRT, hydraulic retention time; HPG, hydrogen producing granules; HPR, hydrogen production rate; HY, hydrogen yield.

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selection of highly productive bioreactor and suitable raw material for biohydrogen conversion [14].

The fermentation process or production of biohydrogen can be performed using different specialized anerobic fermenters like packed bed fermenter, upflow sludge blanket fermentor, upflow filter and fluidized bed fermentor. Among them upflow sludge blanket fermenter are effective in producing biohydrogen [15]. Instead of using sludge, granulation of microbes increases the reaction efficiency of a fermenter for biohydrogen production [16].

Granulation has been used for biohydrogen production; it is a complex route which involves physico-chemical, mechanical, hydrodynamics and microbial interaction for producing biohydrogen [17]. Microbial community of inter and intra species clump or attaches together using the extracellular polymeric substance and vanderwaals force to form a granule like structure. The granular form of microbes is more influential than free floating planktonic cells [17]. Till date the exact mechanism behind formation of granules is unclear. In this form the microorganisms effectively treats wastewater and the conventional anaerobic methanogenic and acidogenesis process also gets enhanced [18,19]. The hydrogen producing granular (HPG) can be formed in four ways they are adsorption, entrapment, encapsulation and polymer based granulation [20]. HPG is the attachment of microbes with different supporting material into a granular complex structure [21]. The granulation process increases maintains higher concentration of microbial biomass which entraps more organic materials and lesser reactor size [19]. Granulation also prevents or resists the toxicity of organic and inorganic pollutants [22]. Under favorable condition HPGs are formed using activated charcoal, clay and polymers as supporting material [21]. The microbes present in the granules secrete adhesive extracellular polymeric substances (EPS) which supports granulation of the microbes. It prevents the microbes from external harsh conditions [23,24]. The three layer of EPS includes first layer as tightly packed stable bound EPS, second layer is the loosely stick EPS and the final layer is dispersible slime EPS [25]. Immobilization of microbial consortium of *Rhodopseudomonas palustris* have higher rate of hydrogen productivity Guevara-Lopez and Buitron [26]. Production of biohydrogen using conventional suspended bioreactors is widely performed but the production rate is not sufficient in comparison to immobilized bioreactors [26]. In conventional bioreactors higher concentration of biomass leads to operation failure. Therefore integrating immobilization techniques with modern bioreactors will increase the biohydrogen production efficiency and cost [26].

Apart from these factors, the substrate used for biohydrogen production plays a major role. The substrates selected for biohydrogen production should produce high yield, must cheaper and easily available [27,28]. The abundance of cellulosic materials makes them a suitable substrate for biohydrogen production. Nowadays researchers are focusing on using wastewater rich with organic material for the production of hydrogen [28]. The combination of all this factor will give an effective and stable way for biohydrogen production. Recently, Banu et al. [17] overviewed the HPG formation mechanism and compared various hydrogen production ability of the system. Moreover, the development of granular reactor system for hydrogen production has been practiced in recent times, the understanding of microbial dynamics populations is essential for process optimization and possible scale up of the system. Thus, the present review highlights the importance of key microbial populations involved in the hydrogen production system and their roles were discussed.

2. Microbiome involved in granulation process

In general, granulation system has been observed in both aerobic and anaerobic sludge [29,30]. The granulation formed by an interaction between the active microbial groups and extracellular polymeric substances released by the microbes [31]. The microbial aggregates tend to form granules by the action of key microbes during the hydrogen fermentation process. Fang et al. [32] reported that methanogenic granules contains the active carbohydrate degrading fermentative acidogenic microorganisms. For instance Liang et al. [33] stated that initial acid shock pretreatment of the seed sludge alters the charge of the bacterial cells, resulting in lower repulsive vanderwaals force between the microbial consortia (enriched with *Clostridium* sp.) aggregates to form mature granules. Hung et al. [34] identified the presence of *Streptococcus* sp. and *Clostridium* sp. community forming a mesh like structure in the granular biomass. The presence of *Streptococcus* sp. provides the stability to the hydrogen producing consortia (*Clostridium* sp.) by secreting the extra polysaccharides (EPS) and maintaining the granular structure at low HRT. The biofilm forming species of *Ruminococcaceae* and *Clostridiaceae* has enhanced hydrogen production by forming fine granules [35]. The hydrogen producing granules (HPG) was accelerated by the EPS synthesis activity, whereas the biofilm developed on the surface of the carbon nanotubes which retain the active biomass inside the upflow anaerobic sludge bed reactor (UASB) and provided a stable hydrogen yield of 2.45 mol/mol glucose. In an investigation by Rafrafi et al. [36], mixed community of bacteria were influenced by sub-dominat groups during hydrogen production. The presence of sub dominant species *Bacilli* sp. with a self flocculation property, increases the abundace of dominant hydrogen producing bacteria *Clostridium* sp. thereby providing a stable granular property of the biomass and which can withstand the harsh conditions such as high organic loading rates and low HRT.

Sivagurunathan et al. [37] noticed that, granulation process improved a hydrogen production rate in the I-CSTR system. At the hydraulic retention time (HRT) of 3 h, the biomass concentration was noted as 6 g VSS/L with the presence of active groups of *Selenomonas sp, Klebsiella oxytoca* and *Clostridium* sp., while at a short HRT of 1.5, the biomass concentration surged to a low value of 3.5 g VSS/L, due to the wash out of *Selenomonas* sp. in the I-CSTR system. The outcome demonstrated that the biomass concentration of the granular system related to the presence of active groups of hydrogen producing bacteria and granular producing bacteria. Barca et al. [38] showed that the addition of *Clostridium acetobutylicum* and *Desulfovibrio vulgaris* provided the stable hydrogen production and mechanical stability to the biofilm in an UASB reactor. The introduction of sulfate reducing bacteria along with the hydrogen producing bacteria aided in exchange of the nutrients/electrons, formation of the cell aggregates and cell adhesion properties between them and resulted in a stable hydrogen productivity of 2.3 L H_2/L-d with a co-culture [34].

2.1. Granulation in suspended cell system

Park et al. [39] demonstrated that self-aggregated granular biomass consisted of a bacterial populations of Clostridia, Bacilli, and Proteobacteria. The microbial aggregates formed by the presence of hybrid immobilized catalyst in a short span of less than 20 days facilitates the stable hydrogen production during various process disturbances in a continuous stirred tank reactor (CSTR) operation. The sub-dominant group of *Bacilli* population increased during disturbance phase, while *Clostridium* population was increased during the recovery phase of the process. Due to the robustness of the granular biomass, the self-aggregated microbial granules provide the mechanical stability to the CSTR. The process upsets were recovered in 4–7 days period. In another study Kumar et al. [40] identified the presence of diverse functional consortia of *B*acilli, Clostridia, Gammaproteobacteria, and Bacteroidia populations providing the stability of the microbial granular system at various HRT in a CSTR. The presence of biofilm forming

Sporolactobacillus and *Enterobacteriaceae facilitates the formation of hydrogen, lactate, acetate and butyrate as metabolic end products. The low proportion of* Gammaproteobacteria and *Bacteroidia* at a HRT of 2 h resulted in the washout of the active biomass causing change in the granular structure which affected the hydrogen production rate.

Sivagurunathan et al. [25] observed a rapid granules formation in a USAB reactor fed with galactose after transferring the CSTR grown self- flocculated mixed consortia. The microbial biomass grown in a CSTR had a self-flocculated property due to the highest shear forces via agitation conditions. The self-flocculated biomass rapidly formed a granular biomass in a UASB and matured in the system. The presence of major self-flocculating populations of *Sporolactobacillaceae* and *Prevotellaceae* groups along with the active hydrogen-producing *Clostridium* sp were involved in the overall reaction. The presence of biofilm forming bacteria and hydrogen producers maintains a syntrophic relation between them and aided in a better biomass holding ability under short HRT operation.

2.2. Granulation in attached cell system

Muri et al. [41] indentified that the bacterial adhsesion is the key step for microbial granulation process in a anaerbic packed bed reactor. The support materials with postive charge attracts and adsorbs the bacteria facilating granular biofilm formation. The microbial populations present in the granular biofilm actively belongs to the group of *Clostridia*, *Eneterobacter* and *Bacilli* sp. They are involved in the biochemical conversion of glucose to hydrogen and organic acids. Jamali et al. [42] investiagted the impact of granular activated carbon (GAC) as a support matrix for biofilm development in an immobilized cell system. In their report, biofilm formation was noticed on the surface of the GAC with the diversifed group of *Bacillus* sp. This bacterial populations enables a stable hydrogen production with a higher cell retention in the system. Dessi et al. [43] assesed the hydrogen producing biofilms in a FBR system with mesophilic and thermophilic conditions. Their outcomes showed that the biofilm community in the FBR system varied between the operational temperature conditions. The mesophilic reactor dominated with *Clostridium* and *Ruminiclostridium* community, whereas the thermophilic reactor dominated with *Thermoanaerobacterium* sp.

The reactor design plays a significant roles in the granulations process, as it affects the stability of the microbial granules, better mass transfer between the substrates and microbes and improved cell retention. In recent years, Kim et al. and his co workers extensively studied the performances of the HPG systems in various reactor configurations such as CSTR, UASB, FBR and dynamic memmbrane module reactor. The hydrogen production performances and the role of key microbes involved in the granulation process with various reactor systems are summarized in Table 1. As seen in the Table, the HPR and microbial community tends to vary with the type of the reactors used. For instance, the CSTR reactor (Park et al. [39]) fed with galactose was dominated with the microbial commmunity structure of *Sporolactobacillaceae, Bacillaceae, Enterobacter, Clostridium, and Prevotella* sp. whereas, the CSTR reactor fed with algal hydrolysate Kumar et al. [44], showed the dominance of the microbial community with *Enterococcus, Lactobacillus and Clostridium* sp. The *Prevotella* sp. was observed in both CSTR and UASB reactor. The FBR reactor was dominated with *Enterococcus, Lactobacillus and Clostridium* sp. The results showed that the diverse microbial community is essential for stable and efficient hydrogen production in a granule forming hydrogen production system.

2.3. Microbial changes in the granulation system

Hernańdez-Mendoza, et al. [45], demonstrated that adaption of anaerobic granules with different feeding regimes affected the microbial community dynamics and hydrogen production performances. Despite using the same inoculum seed source the operational strategy of continuous and discontinuous mode adoption leads to the enrichment of various microbial community structures in the system. The continuous mode adoption is a good strategy for achieving a HPR of 1.7 L/L-d with an enrichment of *Clostridium* sp. and *Escherichia* coli. In case of discontinuous mode operation the non-hydrogen producers (*Desulfobacca acetoxidans, Desulfobulbus propionicus* and *Burkholderia* sp.) were retained in the anaerobic granules, which affected the HPR with a value of 0.8 L/L-d. Recently, Konjan et al. [46] assesed the adaptation of the granular seed sludge in both UASB and biofilm forming reactor for hydrogen production from xylose. The adapted granular biomass in UASB reactor and biofilm reactor showed a similar populations of *Thermoanaerobacterium* sp. and *Clostridium* sp. and responsible for hydrogen production from xylose, however the hydrogen production performances varied between the reactor operation, UASB showed a peak HPR of 15.1 L/L-d at 4 h HRT, while biofilm reactor showed a peak HPR of 19.4 L/L-d at 2 h HRT. The differences in HPR showed that biofilm reactor is a feasible reactor for stabilizing the microbial granules at low HRT operation and provided a maximum hydrogen production from xylose substrate.

Ning et al. [47] observed the formation of HPG in an UASB reactor using continuous mode operation with a fixed HRT of 10 h. By adjusting the substrate concentration (1–10 g COD/L), the HPG formed gradually in the UASB reactor from a suspension to a granular biomass. The granules observed by SEM analysis showed a distinct pattern of microbial morphology with a rod, cocci, and filamentous indicates that the HPG formation and maturation requires an interaction between the different groups of microbial populations. Further, the characterization of the HPG communities by DGGE analysis revealed that during the initial phase with low organic loading rate (OLR) the microbial community dominated with *Janthinobacterium* sp., Uncultured beta *proteobacterium, Janthinobacterium* sp., *Variovorax paradoxus, Variovorax sp*., further increasing OLR, the microbial dynamics varied with the enrichment of *Oxalobacteraceae* bacterium, *Janthinobacterium* sp.,

Table 1
HPG performances in various reactor configurations and their microbial community structure.

Microorganism	Substrate	Reactor	HRT (h)	HPR (L/L-d)	Reference
Gammaproteobacteria, Clostridia and *Bacilli*	Galactose	CSTR	8	10.8	[39]
Sporolactobacillaceae, Bacillaceae, Enterobacter, Clostridium, and Prevotella sp.	Galactose	CSTR	3	25.9	[40]
Enterococcus, Lactobacillus and Clostridium sp.	Algal hydrolysate	CSTR	24	2.7	[44]
Klebsiella, Prevotella, Clostridium, and Sporolactobacillaceae sp	Galactose	UASB	3	32.7	[25]
Klebsiella, Enterobacter, Clostridium, and Sporolactobacillaceae sp	Galactose	UASB	2	56.5	[50]
Enterobacter sp., Enterococcus sp., Lactobacillus sp., and Clostridium sp.	Galactose	FBR	2	65.5	[51]
C.butyricum and *Lactobacillus* sp.	Glucose with 5-HMF	FBR	6	20.0	[52]
Enterobacter, Lactobacillus and Clostridium sp.	Galactose with 5-HMF	FBR	6	26.6	[53]
C.butyricum and *Lactobacillus* sp.	Galactose	MBR	3	51.38	[54]

Clostridium sp., *V. paradoxus*, *Variovorax* sp. and Uncultured bacterium clone C2. In another study, Zinatizadeh et al. [48] reported that initial pretreatment of the granular sludge affected the structural stability of the HPG. The chemical treatment aided by 0.1% chloroform supplementation provided less structural damage to the granule, whereas thermal treatment (90 C for 60 min) showed disintegration of the granular structure. Jung et al. [49] observed a rapid formation of HPG in an UASB reactor with a fixed HRT of 5 h and high-recirculation rate. The HPG granules developed with increased self-flocculating particle size and rapidly formed HPG granules in a short time of 30 days and further matured at 60 days of operation. The matured HPG contains a dominant microbial group of *Clostridium* sp., *Anaerobacter* sp. *and Acetanaerobacterium* sp.

3. Conclusions

The HPG formation is a unique process combined with many physio-chemical factors and biological process. In this review, the key role of active microbial populations was overviewed and their information has been provided. Monitoring the key dominant and sub-dominant microbial species is crucial for easier kinetic control and development of stable bioprocess system. In majority of the HPG system the *Clostridium* sp. are the key hydrogen producing bacteria along with the non-hydrogen producing bacteria such as *Lactobacilli, Prevotella, Selenomonas sp, and Devibrio* sp. Understanding the interaction between the biofilm forming bacterial community and hydrogen producing community is paramount importance for developing a stable microbial consortium and it could be explored further for sustainable hydrogen production.

Conflict of interest

Authors declare no conflict of Interest.

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