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Biohythane: An emerging future fuel

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Abstract
An industrialized country exceedingly depends on fossil fuels. These non-renewable resources are threatened by extinction at any time. The abundance of reliance on them led to a drastic increase in pollution destroying the environment. During combustion, hydrogen does not emit carbon dioxide and has a very high calorific value, hence it can be a significant alternative. The biohythane process includes biohydrogen production followed by biomethanation. Biohythane is a blend of hydrogen with methane, where hydrogen concentration is between 10 to 30% v/v. Its production can occur with various organic substrates by two consecutive anaerobic steps; the dark fermentation step is followed by the anaerobic digestion step, producing hydrogen and methane respectively. This paper initially presents the applications and advantages of this mixture, compared to hydrogen or methane, as different biofuels. The focus is on biohythane production from household waste, the most ample organic feedstocks accessible for anaerobic digestion, the major milestones and the future outcomes are revealed. The likelihood to co-digest sewage sludge and food wastes to enhance the yield is mainly looked into. Furthermore, improvements in the biohythane application in the automotive sector, as well as the reduction in environmental load is illustrated. The current study also explores a complete understanding of the different microorganisms involved in both the production of biohydrogen and biomethane.

Keywords: Biohythane, Biohydrogen, Biomethanation, Dark fermentation, Anaerobic Digestion, Organic wastes, Household Food Wastes

1. INTRODUCTION

Demands satisfied by fossil fuels pleased the country while earth tragically started to collapse due to degradation and climate instability by greenhouse emissions. The range of consumption of petroleum and coal currently is leading to exhaustion. An alternative for a source of energy is to be thought off. As a result of constant research for renewable and greener technologies, hydrogen is found to be a clean and carbon-free source of energy. With the highest energy density (143 GJ ton−1) hydrogen gives only water as a by-product upon combustion. Currently, with the help of operations such as oil/naphtha reforming, steam reforming of chemical/refinery industrial off-gases, water electrolysis and coal gasification [15], approximately 368,000 trillion cubic litres of hydrogen is commercially generated [16]. Direct or indirect dependency on non-renewable energy sources, high carbon footprint and high consumption of energy are to be taken notice of as well.

As clean solutions for renewable energy, technology is under investigation. Bio-oil production by biomass gasification, pyrolysis of petroleum and hydrothermal liquefaction are some of the techniques. Hydrogen Components, Inc. first developed hythane, which gained attention only recently. The biological generation for clean gaseous energy revolves around production of biohydrogen and biomethane.

The carbon footprint of chemical processes is higher compared to the process of biohydrogen and biomethane production [17]. Compared to other biological processes, dark fermentative hydrogen production by dark fermentation is declared for its high yield and rate[18]. Biohydrogen production out of organic waste can be achieved at a suitable atmospheric pressure and temperature [19] there forwarding a sustainable waste stabilization process.

The key techniques for the production of biohydrogen are oxidation of organic acids and photolysis of water by photo-dark fermentation. Biophotolysis of water along with photo fermentation yield hydrogen production at a low rate and additional energy input is required for internal lightening. It is hard scaling up these processes as well. Meanwhile, dark fermentation needs modest process conditions, it is independent of light and consumes less energy [20]. Proving the facts, dark fermentative biohydrogen production is recognised to be the most prominent method. The output media has an enormous quantity of short-chain fatty acids, specifically, propionate, butyrate,acetate and more after dark fermentative H2 production. These volatile short chain fatty acids can be considered as substrates for methanogens. Thereupon, the biohydrogen-biomethane integration procedure under the tag of biohythane contributes in the development of renewable energy. It is crucial to adjust the pH range to 7 to 7.8 before the subjection of output media for biomethane production. Furthermore, the development of hydrogenotrophic methanogens is influenced by dissolved H2. Accordingly, the production of biohythane encourages the generation of clean energy from organic substrates rich in proteins, carbohydrates and fats.

2. APPLICATIONS

Since 1980, Hythane has been highly referred to as a vehicle fuel. Methane, though marked as a clean vehicle fuel when compared to diesel or gasoline, is restricted by factors including high ignition temperature, narrow flammability range and slow-burning speed. Poor combustion efficiency and a high requirement of energy for ignition of CNG vehicles is the output. Hydrogen plays the role to be the perfect completion for failing points of methane. Increasing the hydrogen to carbon ratio reduces GHG emissions with improved efficiency of fuel; boosting heat efficiency, enhancing flame speed and decreasing combustion

duration; bringing down quenching distance to ignite the engine with lesser input energy are the negotiations.
Observations on H2 and CH4 mixture have been made since the 1980s [10] though hythane with the advantages of both was marked later [11]. Combustion properties, ignition performance and GHG emissions highlighting optimized fuel efficiency as vehicle fuel [12] are the research keys. The importance of making H2 and CH4 into hythane contributes to hythane fueling station as the central hand in its production and utilization industry [13], although independent production of both from fossil-based substances is energy-intensive and unsustainable.

The underlined advantages of H2 and CH4 as the combination for vehicle fuel are lower flammability of methane limiting fuel efficiency and hydrogen's ability to improve lean flammability range, low flame speed of methane in lean air/fuel mixtures where hydrogen has octuple flame speed, hydrogen's ability to stimulate and accelerate methane combustion. In addition, its power as a reducing agent at lower temperatures is used for efficient catalysis.

Hydrogen combines the properties to enhance the performance of internal engines, often fed by methane from fossil-based sources, to reduce the percentage of methane in bio hythane and is linked to knock resistance. Compared to methane, hydrogen`s ignition energy is lower, hence helps to burn better. Alongside, it exposes the mixture to preignition in contact with residual gases or hot spots. Biohythane attracted the automobile sector, particularly with the highlights on these features. Toyota is one of the many car manufacturers who developed hythane vehicles pointing up the energy consumption aspect. All things noted, it can be a compelling intermediate for value-added products and liquid fuels[14].

3. BIOCHEMISTRY BEHIND BIOHYTHANE PRODUCTION

3.1 Hydrogen production using dark fermentation

Glucose is a common substrate used for the dark fermentation process as it is more preferred by many microbes. The complex substrates are hydrolyzed to convert them into simple sugars and then metabolized through a glycolytic pathway to produce pyruvate with the help of dark fermentative bacteria. Microbes obtain energy in the form of ATP in this process and also pyruvate will convert to form acetate and butyrate by leaving H2 as a byproduct.
H2 production is different in case of facultative and obligate anaerobes. The process of metabolism of obligate anaerobes include the oxidation of pyruvate by pyruvate-ferredoxin oxidoreductase enzyme to produce acetyl CoA with the help of ferredoxin (fd) reduction (equation 1). This is then oxidized to produce H2 with the help of FeFe hydrogenase(equation 2).

$$Pyruvate + CoA + 2\,Fd(ox) \longrightarrow Acetyl\ CoA + 2fd(red) + CO_2 \quad \cdots (1)$$

$$2H^+ + Fd\,(red) \longrightarrow H_2 + Fd(ox) \quad (2)$$

In the process of metabolism of facultative anaerobes like *E.coli or Enterobacter* sp. and pyruvate oxidation, they produce formate and acetyl CoA which is catalyzed by pyruvate formate lyase (equation 3) [23]. The formate produced is then split into hydrogen and carbon dioxide in the presence of formate hydrogen lyase (equation 4) [24].
So, the hydrogen production using dark fermentation has thermodynamic restriction and the product depends on oxidation of pyruvate .

$$Pyruvate + CoA \longrightarrow Acetyl\ CoA + formate \quad \cdots (3)$$

$$HCOOH \longrightarrow COL_2 + H_2 \quad \cdots (4)$$

3.2 Biomethane production (Methanogenesis)

Methyl-coenzyme M-reductases (MCMR) is an important enzyme in the biomethane production process.This process includes the reduction of activated C1 intermediates and coenzyme bound. The main substance MCM (CH3–SCoM) reacts with HS–CoB(electron donor coenzyme B) to produce methane and heterodisulfide (CoM–S–S–CoB). MCMR by methanogenic organisms catalyzes the whole reaction under optimum anaerobic conditions.[25] .
The H2 and CO2 produced by dark fermentation can be used as energy sources in methanogens metabolic pathway and these are the only energy sources which are depended by methanogenic bacterias[26,27]. This process is known as Hydrogenotrophic methanogenesis.

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ ….. (5)

The conversion of H2, CO2 and methanofuran into N-formylmethanofuran is mainly controlled by two enzymes. This includes F420-non reducing NiFe hydrogenase that catalyze the reduction of an electron acceptor and a N-formylmethanofuran dehydrogenase which is responsible for catalyzing the reduction of carbon dioxide and MFR to formyl MFR with the help of an electron donor.
In a methanogenic environment, the hydrogen partial pressure is generally 1 - 10 Pa and corresponding change in free energy of carbon dioxide and hydrogen is −20 to −40 kJ/mol. Synthesis of ATP requires 50 kJ/mol of Gibbs free energy where ATP is synthesized by ADP and other Pi [28].

4. Microbial insights on biohydrogen and biomethane production

4.1 Biohydrogen producing microorganisms

4.1.1 Mesophilic dark fermentation

In general, H2 is produced by a diverse group of microorganisms in anaerobic conditions; However, they vary in the hydrogen producing capacity. Worldwide, many species that produce hydrogen were discovered, they belonged to various domains of microbes for example, photosynthetic bacteria, methylotrophs and facultative/obligate anaerobes [29]. From the domains mentioned above, anaerobic chemoheterotrophs related to Enterobacteriaceae and Clostridia sp. have demonstrated to be the most prominent H2-producing microorganisms.

Table 1. Characteristics of microbes producing mesophilic dark fermentative hydrogen

Microbes	**Physical Characteristics**	**Remarks**
Clostridium	Gram +ve , rod-shaped with G+C content	▪ More important as they have low doubling time and can resist adverse conditions (high temperature, stress, etc.) ▪ Highest H2 yielding bacteria. ▪ Some new *Clostridium* sp. reported includes *C. welchii, C. beijerinckii, C. pasteurianum and C. butyricum*.
Enterobacter sp.	Gram -ve, rod-shaped, non-motile or motile (peritrichously flagellated)	▪ Higher growth rate in contrast to other mandatory anaerobes. ▪ Resistant to very low amounts of dissolved oxygen and use various carbon sources. ▪ Lower H2 yield compared to *Clostridium* sp.
Escherichia coli.	Gram -ve, rod-shaped, low G+C content, motile	▪ H2 produced from formate using enzyme complex formate lyase (FHL). ▪ Used as an important microbes for genetic modification for enhanced H2 production. ▪ Use glycerol as substrate.
Citrobacter sp.	Gram -ve, low G+C content.	▪ These facultative anaerobes come under the Enterobacteriaceae family. ▪ Produce H2 organotrophically and chemolihotrophically. ▪ Use glucose as a substrate.
Bacillus sp.	Gram-positive, facultative mesophilic bacterium.	▪ They survive by producing spores in higher temperature and unfavourable conditions. ▪ Many H2 producing potent microbes have been identified in *Bacillus* sp and these include *Bacillus licheniformis, Bacillus coagulans.*

Table 2. Characteristics of microbes producing thermophilic dark fermentative hydrogen

Microbes	**Physical characteristics**	**Remarks**
Thermoanaerobacterium sp.	Gram-negative straight rods, motile peritrichous flagella, low G+C content.	▪ Generate H2 by degrading xylan and they produce spores under nutritionally deprived conditions. ▪ Also produce diverse metabolic products like lactate, acetate, ethanol and carbon dioxide. ▪ Associated to *Clostridium* species
Thermoanaerobacter sp.	Irregular Gram-positive rods and obligate anaerobes.	▪ Earliest thermophilic anaerobic microbe to produce H2, carbon dioxide, ethanol, acetate and lactate along with *Thermoanaerobium.* ▪ These organisms can use different types of sugars but cannot degrade cellulose.
Thermophilic *Clostridium* sp.	Gram +ve, rod shaped, motile obligate anaerobic organism	▪ Have the ability to break down cellulose utilizing cellulase enzyme and ferment lignocellulosic biomass to generate H2. ▪ These belong to *phylum Firmicutes.*
Caldicellulosiruptor sp.	Anaerobic Gram-positive from natural habitats like lake sediments and hot springs.	▪ Use different cpmpounds like xylan, xylose ,cellulose and cellobiose utilizing hydrolytic enzymes. Therefore can produce hydrogen using lignocellulosic wastes ▪ Grows at 70 °C and forms lactate and acetate as predominant metabolite.
Thermotoga sp.	Gram ve, rod shaped, obligate anaerobes.	▪ Present in high sulfur-containing temperature or pressure conditions and can utilize thiosulfate or elemental sulfur or both as electron donor. ▪ Hydrogen,acetate and carbon dioxide are their metabolic end products (with trace amount of ethanol). ▪ Examples include *Thermotoga maritima* and *Thermotoga neoplanita.*

4.1.2 Thermophilic dark fermentation

Several industries emit higher temperature effluents that are full of organic matter for instance distillery industrial effluents, wastewater from sugar based industries and food processing industries. These are incapable of direct dischargement into water bodies to avoid environmental pollution. Also, the process of cooling is uneconomical, and loss of biological activity may occur [30]. Therefore, these higher temperature sewage is used by thermophilic bacteria to produce H2. The thermophilic bacteria can be differentiated based on their optimum growth temperature as Extremophiles (above 75 °C); True thermophiles (55 -75 °C); Moderate thermophiles (45 - 55 °C) [31].

4.2 Biomethane producing microorganisms

Compared to other bacterias, methanogens possess a characteristic capability to form methane and other hydrocarbons, these bacterias belong to the group *Archaeobacteria*. They have characteristic attributes like discrete ribosomal RNA sequences, membrane lipids containing isoprenoids ether linked to glycerol and lack of peptidoglycan possessing muramic acid [26, 32]. Methanogens can be differentiated into three categories depending upon their route of metabolism for methane production. This includes reducing aceticlastics and methylotrophs [33]. Most methanogens utilize hydrogen as the sole source of electrons.

Table 3. Characteristics of methanogens used in biohythane production

Methanogens	Physical characteristics	Remarks
Methanobacteriales	Rod-shaped,Gram-positive, methanogens.	▪ These utilize carbon dioxide as a source of energy for the reduction of methanol into methane. ▪ The diverse Methanobacteriaceae family includes various genera like *Methanobacterium, Methanobrevibacter, Methanosphaera.* ▪ They are reliable on CO2 and H2 as a source of energy and cannot use formate.
Methanococcales	Coccoid-shaped, halophilic, chemolithotrophic, marine methanogens.	▪ They use H2 and reduce CO2 or utilize formate as an energy source to produce methane. ▪ This methanogen has 3 thermophilic sp. Which are *Methanocaldococcus*,*Methanothermococcus*, and *Methanogenesis* , along with mesophilic sp. (*Methanococcus*). ▪ *Methanothermococcus thermolithotrophicus* is a kind of thermophilic species which is mainly found in hydrothermal vents at 65°C.
Methanomicrobiales	Pleomorphic, and appear as plate, rod-shaped cells.with non-uniform coccoid	▪ Acetic acid is utilized as a carbon source. ▪ Cell walls of these organisms have a protein layer which helps in osmotic sensitivity of these organisms and are susceptible to dilute detergents. ▪ Consists of 3 families, which are *Methanosarcinaceae,Methanocorpusculaceae and Methanomicrobiaceae.* ▪ The *Methanocorpusculaceae* oxidize formate, hydrogen and reduce CO2 to produce methane.
Methanosarcinales	Gram-negative, nonmotile rods, long-string-like structures.	▪ These depend upon methyl groups containing components for nutrition and therefore known as methylotrophic bacterias. Examples;methyl sulfides, methanol, methylamines. ▪ Possess the capability to dismutate trimethylamine into methane ,ammonia and CO2. ▪ Also digest methanol to produce CH4 and CO2.
Methanopyrales ord. nov	Gram-positive microbes, generally rod-shaped	▪ Contains a single species known as *M. kandleri.* ▪ These hydrogenotrophic microbes can reduce CO2 to CH4 , and need to be pretreated. ▪ Pre-treatment techniques include load shock treatment , alkaline pre-treatment, chemical treatment etc.

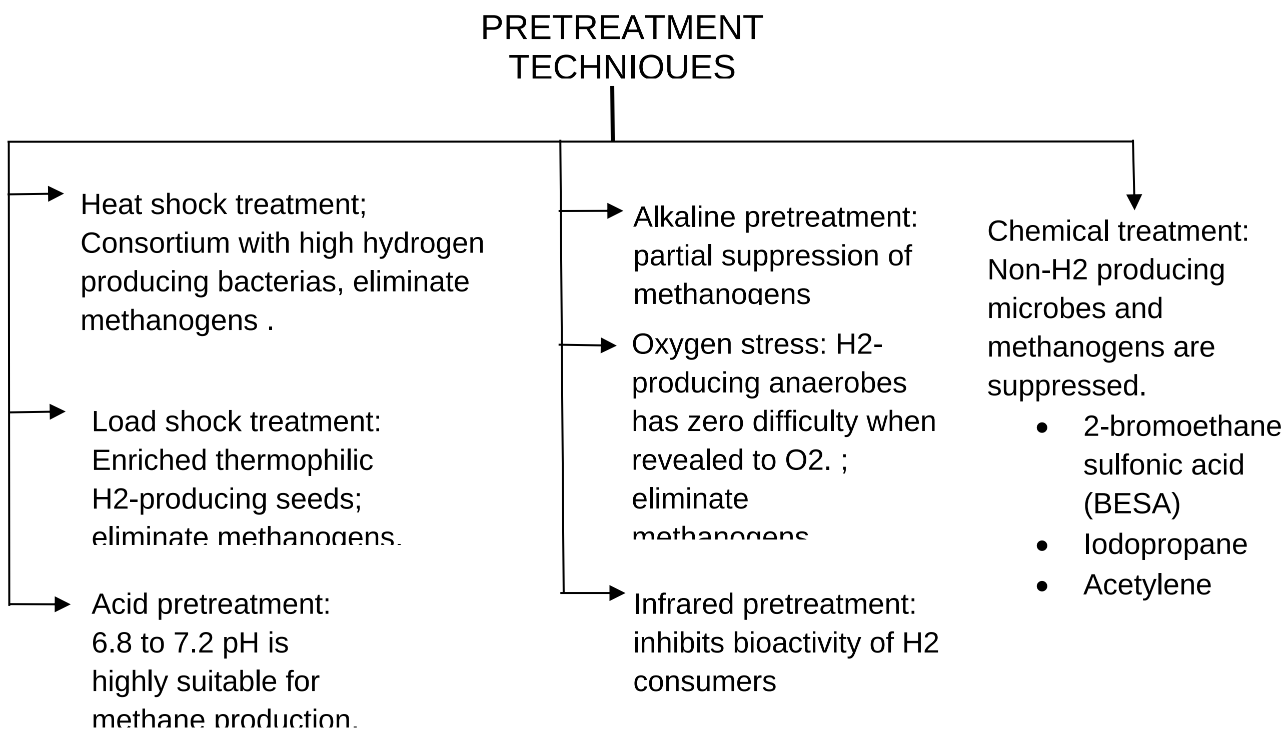

Fig 1. Pretreatment techniques for enhancement of mixed culture

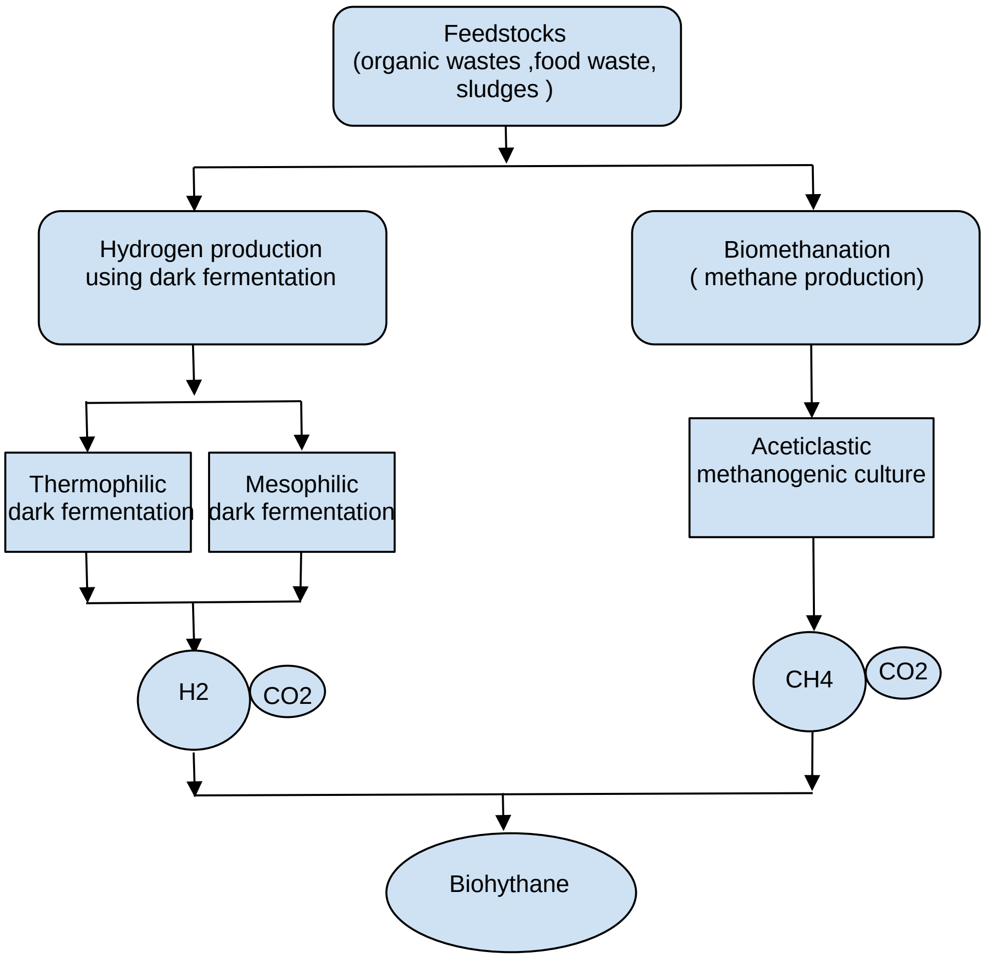

Fig 2. Outline of biohythane production procedure

5. KEY ASPECTS OF BIOHYTHANE PRODUCTION

Production of biohythane can be implemented through a two-stage AD process. Through a stage AD (one reactor) or two-stage AD (two reactors), making of biogas by Anaerobic Digestion can be carried out. Through the separation of hydrolysis and acidogenic, two-stage AD occurs, also noted as dark fermentation [1].Biogas enriched with H2 and CH4 is obtained after the acetogenic phase and methanogenic phase subsequently.

With a favourable pH and temperature, anaerobes like Thermoanaerobacterium sp., Clostridium sp., Bacillus And Enterobacter, through the chain of microbial procedures produce biohydrogen via dark fermentation [34]. Biomethanation is furnished by microbes namely Methanococcus and Methanosarcina barkeri, the more sensitive ones. Less thick cellular membrane, consequent steady temperature request, pH parameters and a delicate agitation contributes to the specified noted characteristics [35].

The complementary activity between the microorganisms points out that the methanogens utilize H2 generated from the acetogenic stage. At appropriate anaerobic parameters, the generation of CO2 and CH4 occurs. Aforementioned, the production of biohythane includes dark fermentative and acetogenic stages; each regulated by specific microbes with varying requirements of optimal condition. The balanced temperature,pH,OLR, partial pressure,nutrients and HRT are considered as well. Micronutrients at a low concentration is required for metabolism, which further is important for the accession of the two AD stages.

The end of the process proceeds with the need for cleaning of hydrogen and methane and upgrading phases to remove ammonia, CO2, siloxanes, acid compounds and water from biogas. Chemical scrubbing (22%), Water scrubbing (41%), or pressure swing absorption (PSA) (21%) are the most preferred techniques. Referring to the recent study of preference, 1/10th of the upgrading units employ membrane separation technique [36].

6. FEEDSTOCKS UTILIZED FOR PRODUCTION

6.1 Household food waste

The wide-ranging availability leads the scientific community to choose food wastes and sewage wastes for experimentation. The estimation shows that in EU countries 72% of feedstocks received is food wastes, whereas the rest 28% is sewage sludge [37]. Hemicellulose and Cellulose are uncooperative without an advance pre-treatment stage; however, proteins and carbohydrates are ultimate substrates for CH4 and CO2 production.

The pH control is another most important factor especially in the treatment of heterogeneous feedstocks such as household food wastes (HFWs). The optimum pH is 5.5 which is considered as the best for hydrogenase enzyme activity [39]. HFWs containing high protein or supplementing alkaline substances are some of the strategies to control the pH. Two other most considerable factors, Temperature and the reactor configurations need different parameters for methanogenic phase and dark fermentation.

An investigation happened concerning the production of biohythane from HFWs using different reactors, adopting CSTR for dark fermentation and AFBR for methanogenic phase. CSTR is mostly preferred because of its simplicity in design and removes a lot of AD bacteria, for example methanogens are removed by high OLR and low HRT [38]. Contradicting the fact, the 2nd stage AD is distinguished by slow and vulnerable methanogens [35].

6.2 Codigestion of HFWs and sewage sludge

Wastewater produced by a community is referred to as ‘sewage’ and sewage mainly originates from 3 sources. Household wastewater accumulated from bathrooms, toilets and other activities, Industrial wastewater with treated or non- treated effluents and lastly rainwater completes the 3 sources [40]. In the practice of optimizing the two stages of AD, the Codigestion of feedstocks having distinct composition and origin is customary. Its function helps in metabolism of bacteria by making up for lack of micro and macronutrients in substrates. Co-digestion provides continual feed even during seasonal substrates. To improve biohythane production, HFWs are co digested alongside sewage sludge.

Goberna et al a different classification of household sludge noting down variable concentrations, chemical elements and properties; mentioning pH (6.4 to 7.9), phosphorus (6 to 20 g/kg) along with water contents (70% to 87%) and nitrogen (2.4 to 8.1%), electrical conductivity (0.7 to 4 dS/m), total organic carbon (21.5% to 49.5%) [41]. It also led to the finding that concentration of heavy metals responsible for inhibition of AD are lower in municipal sewage sludge compared to industrial. The composition of microbes are not influenced by the origin of sludge. Rather, it depends on the duration of HRT and the concentration of ammonia.

Huge availability, stability of the source, high organic content (dry matter > 60%) and low cost of sewage sludge captivated the interest in fermentation of biohythane[42]. In co-fermentation for H2 and CH4 production, better substrate conditions, inhibitors dilution and balanced nutrient conditions are relevant advantages. As the performance is low regarding the production of biohythane utilizing sewage sludge, it attributes to low ratio of carbon-nitrogen with subsequent formation of ammonia [42, 43]. Even though ammonia plays the role of a crucial nutrient for microbial growth it can hinder AD, mainly in its second stage. Co-digestion with HFWs is one among the finest techniques to enhance the yield due to high carbohydrates concentration.

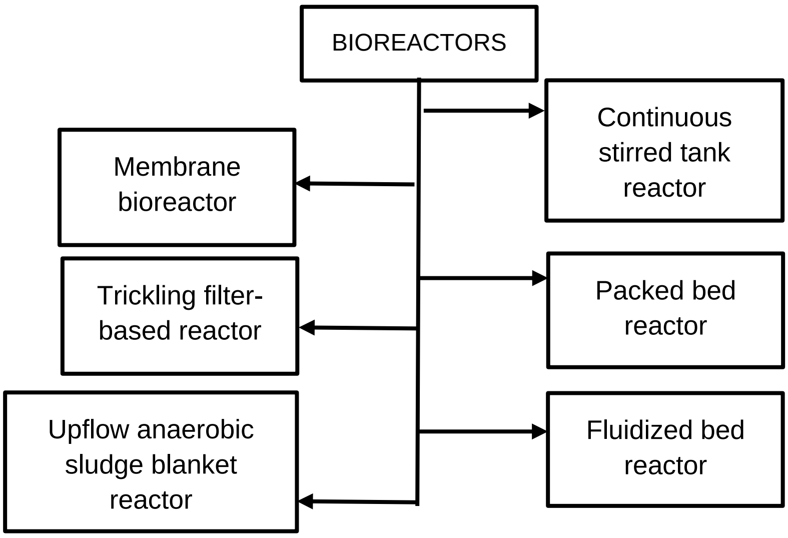

Fig 3. *Various bioreactors used for biohythane production*

7. FACTORS INFLUENCING PRODUCTION

7.1 pH

pH is one among the crucial chemical factors governing the efficiency of enzymatic machinery of the microorganisms and maintaining the oxidoreduction ability of the cells as well. Glycolytic or supporting enzymes such as Fe-Fe H2ase and formate lyase, plays a major role as enzymes involved in H2 producing metabolic pathways. As enzymes have an ideal pH for maximum activity, therefore understanding the role of pH in H2 generation is vitally important. On account of the accumulation of metabolites such as volatile fatty acids changes throughout dark fermentation. The process led to effects on the pH (3.8 to 4.2) and working of enzymes at low H2 production. The growth of microorganisms is determined by the disruption of membrane integrity.

7.2 Temperature

At an ideal range of temperature, enzyme activity is high. When the variance in temperature from optimum ranges occurs, it can lead to denaturation or inhibition of life-supporting enzymes or metabolic enzymes. Generally, enzymatic activity increases by two times when there is an increase in temperature by 10°C. This is studied till it reaches an ideal temperature and enzymatic activities decrease beyond the ideal temperature. In different temperature ranges, H2 and CH4 producing microbes are found. Therefore, metabolite formation, nutritional requirement and microbial characteristics are affected by temperature. Temperature drop can drastically affect anaerobic microorganisms' activities and cease the production of methane. Nevertheless, these microorganisms can recover with appropriate temperature stabilization.

7.3 Partial pressure

During the production activity, pathways susceptible to partial pressure of H2, gaseous products hydrogen and methane get dissolved in the fermentation broth. As accumulated in the headspace, there is an increase in H2 partial pressure in the reactor, contributing to the metabolic remodeling in the course of fermentation and further leads to form reduced products like butanol, lactate, acetone, propionate and ethanol.

7.4 Hydraulic retention time

It represents the time sequence retained by soluble nutrients and cells in the reactor. Production of H2 takes place at lower HRT level. The maximum production of H2 and conversion of substrate was noted at suitable HRT range. Considerably low HRT might increase the washout of the active cells from the reactors. Concluding that the microbial growth rate is closely linked to HRT optimization.

HRT's property to form metabolites associates with variation in bacterial profile as a result in change of HRT. Observations provide in scheming the reactor and experiment and reactor for industrial wastewater treatment, implementing low level of HRT to improve hydrogen production. This helps in removal of COD, OLR and loading rate is found to be one of the functions of HRT. Also mentioning dynamics of microbes in the bioreactors can be easily influenced by varying HRT range.

7.5 Inoculum

Certain bacteria with enriched mixed consortium can form hydrolases. Thus, hydrolases assist in degradation of large carbohydrates existing in HFWs. The generated soluble sugar is fermented for H2 production. The concept of 'biological waste to energy' is extended to develop a mixed microbial culture harbouring a set of symbiotically associated bacteria. To achieve a perfectly desired product, it is important to prepare a suitably enriched inoculum.

8. RECENT ADVANCEMENTS

The expanding attention to biohythane led to investigations regarding innovations to enhance the yield from two-stage AD. Aforementioned, molar yield of hydrogen is lower than the conceptual one. A blend of photo fermentation along with dark fermentation contributed as an alternative. Non oxygenic bacteria utilize biomass and sunlight to give rise to hydrogen in photo-fermentation. Then the dark fermentative products undergo photo-fermentation to produce hydrogen as stated by the given reaction [44]:

$CH_3COOH + 2\ H_2O \rightarrow 2\ CO_2 + 4\ H_2$ … (6)

Photo-fermentation's main disadvantages; high amount of nutrients needed by anaerobes, expensive and less efficient photochemical reactors [45].

As an alternative, the production of biohythane could be enhanced by reinforcing the pre-treatment phase. Borg et al., detailed the interest in thermal pre-treatments consisting of 17 compounds at very high temperature (more than 120°C) differing by the nature of biological compounds for half an hour [46].

Adding to the points, pre-aeration contributes to the breakdown of complex HFWs to form simple molecules. This task resulted in an enhancement of H2 conversion rate by 97% and production of VFAs by 10%. The main problem of this strategy could be the oxygen infiltration in AD methanogenic step, carried out by oxygen sensitive microbes.

To improve biohythane process from HFWs, operating in a thermophilic environment increases kinetics of microbes, thus reducing the HRT value, reactor volume, installation and management costs are also considered. Low HRTs affect substrate degradation, digestate quality and methane production even though it significantly washout methanogenic biomass.

Another well-thought strategy for the improvement of production is by supplementing Mg, Fe, Cu, Zn and P salts, their ions enhance bacterial metabolism, mainly during the digestion of a specific substrate rather than a mixture of substrates [47]. Strong influences of certain metals on biohythane production from household wastes are suggested through some recent studies.

9. Future Outcomes

Keeping in mind the importance of energy security and pollution, biohythane production is inevitable. As the world's energy requirements heightened every second, it is estimated that by 2030, it will increase by half with more than 2/3rd this increase observed from developing countries according to a survey by the International Energy Agency. An alternative for the energy for socioeconomic development, biohythane considerably forms a future energy sector.

Using food wastes and sewage sludge as substrates as research material in laboratories and pilot scales, biohythane production grasped the scientific community's attention. Worldwide availability with an estimation of 72% of the anaerobic digestion plants treating agricultural and food wastes of 80 lakh tons only in the EU is an evident reason. Zooming to the account of food waste, 38% obtained out of food processing, 42% out of households and 20% from other supply chains. Sewage sludge is adopted as a substrate for AD by the remaining 28%. As discussed, anaerobic digestion considers the reduction of disposal of food waste in landfills, an intolerable activity, still greatly accepted worldwide resulting in emission of GHG and avoidance of recovery of resources.

Hydrogen is a carbon-neutral fuel, has the potential to be a prominent replacement to fossil fuels in future taking into account the ability in reduction of CO2 and emission of NOx. As per the whole understanding, hydrogen has got the feature to improve the engine performances which are generally provided by CH4 from fossils, further reducing knock resistance and methane number. Additionally, biohythane puts forward the ability to increase the limit of lean burn due to a highly steady combustion.

10. Conclusion

Contemporarily, biohythane replaced methane fuel in the automobile industry due to the presence of hydrogen resulting in improving combustion efficiency, reduction of carbon dioxide and emission of NOx. The requirement of larger industries and investments for the expansion of biohythane fuel distribution to be utilized for vehicles is to be looked into.

The gaseous blend biohythane has hydrogen and methane v/v composition of 10-30 % and 70-90% respectively. A wide variety of microbes are associated with dark fermentative hydrogen production. Thermophilic obligate anaerobes proved a higher yield of H2 production. Numerous methanogenic microorganisms play an essential role during the second stage of biomethanation. Interaction of methanogens with acetogens is important in the reactor's performance. Exploration of various ways to enrich microorganisms generating H2 in seed culture through physical and chemical pre-treatments, for instance load shock, heat shock, alkali, acid, acetylene and iodopropane. Appreciable results were obtained for Co digestion of HFWs with sewage sludge.

Being environmental, the production of biohydrogen outway its chemical counterparts. Integrating dark fermentative hydrogen production with biomethanation can recruit the maximum recovery of gaseous energy. Concerted studies have already been attended on advancing production of biohydrogen, for example development of genetically remodeled microbes, consortium of microorganisms, designs of reactor, operating conditions of two-stage AD and various solid matrices.

There are many challenges to be directed regarding the application of the reactor configuration, pretreatment techniques and managing appropriate physicochemical factors. Waste disposal management and socio-economic concerns. A few of them were biological wastes like starchy water, distillery outflow and food industrial waste for fermentative H2 production. Scrutinizing the near future, hydrogen and methane could be promisingly transformed into electricity by the advent of fuel-cells.

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