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# Combined bioprocess for fermentative hydrogen production from food waste: A review

Yung-Tse Hung<sup>1,\*</sup>, Sanad AlBurgan<sup>1</sup>, Howard H Paul<sup>2</sup> and Christopher R Huhnke<sup>1</sup>

<sup>1</sup> Department of Civil and Environmental Engineering, Cleveland State University, Cleveland, Ohio, USA. <sup>2</sup> Department of Information Systems, Cleveland State University, Cleveland, Ohio, USA.

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# Abstract

Bio hydrogen is a cheaper, sustainable and safer source to produce fuel comparable to energy obtained from fossil fuels. There are many experimental methods to produce bio hydrogen using food wastes as substrates that are acted upon by specific bacterial and fungal strains. Some of the methods include batch-dark fermentation, solid-state dark fermentation, dark-anaerobic hydrogen fermentation and integrated light-dark fermentation. Different food wastes are used in these fermentation processes such as kitchen food waste, potatoes peels, sugary waste water, fish, meats, grains, cassava residues, corn pulp and starchy solution etc. These food wastes are rich source of main raw materials that are required for bio hydrogen production such as cellulose, carbohydrates, fats, proteins, lipids, starch, phosphorus, volatile solids, Published experimental and research approaches revealed that the use of mixed dark-photo fermentative bacterial consortium in flat photo bioreactors and fermenters resulted in higher yield. Combined dark-photo fermentation is an advanced and promising strategy for increasing overall yield of bio hydrogen.

Keywords: Fermentation; Hydrogen production; Combined fermentation process; Food waste; Substrates

# 1. Introduction

Usable energy is also called the golden thread. It is the dire need of this industrially revolutionized era to produce renewable and cheaper fuel. This renewable fuel should be cheaper and of higher quality than the preexisting sources. There are different fermentation types being used for biofuel production by the manipulation of the already available resources along with microbial biomass.

Two of such types are dark fermentation and photo fermentation which use acidogenic and photosynthetic bacteria to make biohydrogen, respectively. These fermentations use different substrates such as distillery wastewater, dairy wastewater, brewery industry wastewater, etc. (1). Scientific fraternity is facing the problems of energy crisis and environmental pollution for decades. They are working very hard to find out the possible solutions for tackling this issue. The development of sustainable energy production processes by applying renewable resources is under study for substituting conventionally present fossil fuels. Biogas is at the top of the list of sustainable and renewable fuels having some limitations like less heating value, low flame speed, and not being financially stable to produce. So the solution to these problems is the addition of some amount of hydrogen.

These fermentations work in a dual manner by making biohydrogen and cleaning water as well. These substrates are different from each other as they contain different nutritious compounds that are needed by the microorganisms to grow exponentially. Published experiments suggest that there is more biohydrogen production when distillery wastewater is used instead of dairy wastewater (2). Moreover, there is also the use of synthetic waster water to check different parameters as a controlled experiment. There are different drawbacks and limitations of both fermentation

<sup>\*</sup> Corresponding author: Yung-Tse Hung

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processes such as in dark, VFA are formed as an intermediate product and lowers the efficiency of acidogenic bacteria, ultimately leading to decreased production of biohydrogen. Augmentation of both fermentation processes enhances biohydrogen production by compensating for each other's limitations.

When these processes operate from a single stage, the photosynthetic bacteria metabolize volatile fatty acids produced by acidogenic bacteria and transform these fatty acids into biohydrogen as well as other products. This hybridization leads to enhanced biohydrogen production (3). Dairy and distillery industries are also using hybridized fermentation along with bio-methanation, concentration, and incineration in the process of wastewater treatment. This review article included a systematic literature review on enhanced biohydrogen production in the direction of one-stage hybridized dark-photo fermentation and its future insights.

# 2. Dark and photo fermentation phenomenon for biohydrogen production

Fermentation is the phenomenon of converting biological wastes into energy by microbes with the aid of microbial enzymes. For example, hydrogenases and nitrogenases, etc.

Nowadays, hydrogen is being produced by combustion and steaming of non-renewable fossil fuels. Around 1 billion m<sub>3</sub> of hydrogen was produced globally by the combustion of fuels like natural gas, coal, oil, and H<sub>2</sub>O splitting by electrolysis (4). This has extended the rapid lowering of fossil fuels and discharge of greenhouse gases.

The bio-based feedstock is utilized for the production of biohydrogen by a fermentation process that allows the additional growth of a bio-based economic system of a country (5). It is particularly observed that bio-based feedstock increases the production of hydrogen energy to 122kj/g, which is 2.75 times more than the hydrogen production rate in conventional methods of fermentation including the use of fossil fuels. Production of hydrogen from biological feedstock, two types of fermentation are in use such as; photo fermentation and dark fermentation.

#### 2.1. Photo fermentation

In this type of fermentation, photosynthetic bacteria are used. These bacteria are normally purple sulphur bacteria and purple non-sulphur bacteria. They are capable of doing photosynthesis as well as the metabolism of organic wastes, organic acids, biowastes and other substrates leading to the production of hydrogen and carbon dioxide. These bacteria are capable of oxidizing maleic acid, butyrate, propionate, lactic acid, acetic acid, etc. These fatty acids are formed as intermediate products during the metabolism of organic. To get a higher yield of biohydrogen two staged fermentation process is used in which dark fermentation is followed by photo fermentation (6).

# 2.2. Dark fermentation

Dark fermentative process is another method for hydrogen production by utilization of biomass. This process is carried out without light under an anaerobic environment, and it is directly related to the stage of the digestion process during acidogenesis, in anaerobic conditions. Production of fermentative biohydrogen without light is a phenomenon that happens in almost all natural anaerobic environments. That is composed in a torrent of redox reactions that should be balanced. Although the reactions are commonly thermodynamically feasible and continuous. They are additionally limited with the aid of using organic rules inside microorganisms and with the aid of interspecific interactions in different microbial populations. (7).

# 3. Bio hydrogen production from various food wastes and combination strategies to enhance biohydrogen yield

#### 3.1. Usage of kitchen waste for biohydrogen production by anaerobic bacterial pre-treatment

As people have started living in good standards of life, there is an increase in kitchen waste as well for example in China. This waste is a threatening issue for environmental pollution and also acts as a wastage of resources. There is complete research based on the composition of different kitchen wastes as they are rich in organic matter. Due to the high value of organic matter in kitchen waste, it is gathering the attention of researchers as a sustainable and utilizable resource for biohydrogen production. This biohydrogen production is done by using anaerobic fermentation and is divided into different stages such as hydrolytic acidification, methanogenesis and production of hydrogen.

After the above-mentioned steps, there is a conversion of organic matter into fatty acids that are volatile in nature by the action of acetic acid and hydrogen-producing bacterial species. Finally, the methane gas is produced by using the

fatty acids. This anaerobic fermentation can not only cause the production of methane but also cause a reduction in the overall organic matter in the kitchen waste. This methane gas can be used as a sustainable source of energy. In addition to this, there is also the production of biogas residual mass that is also utilized as a fertilizer.

There is a problem of less utilization of complete matrix system and energy recovery that can be sorted out by the combined process of fermentation. The comparison of anaerobic and aerobic fermentation using a corn stalk. It was found that there is more buffer capacity in the aerobic conditions as bacterial species can tolerate the fatty acids. This study also deals with the possible effects of the oxygen content on the improvement of gas production characteristics of kitchen waste.

In this study, we explored the effect of the micro-oxygen environment on the gas production characteristics. The kitchen waste that is used in this study included very hard-to-decompose materials such as bones and egg shells. Sludge was also added to this waste that was taken from the sewage handling plant.

The results suggested that about 20 mL of the oxygen supply promoted the decomposition and microbial utilization of the waste. The overall efficiency of the anaerobic fermentation was also found to increase.

Another research article suggested the feasible and sustainable properties of kitchen waste and its applicability as a rich substrate for biohydrogen production. Dark fermentation is applied to get maximum biohydrogen from the waste. This technique is also called as microbial technique in which organic kitchen waste rich in carbohydrates and organic nutrients is potentially used for the production of huge quantities of biohydrogen. They have also emphasized the importance of availability and its commercialization on the industrial scale. The critical role of nanomaterials is also discussed in order to enhance the overall yield and make the whole process economical (8).

#### 3.2. Usage of potato peel waste for biohydrogen production

There are high carbohydrate content and organic biodegradables present in the food waste. Food waste has more biodegradation potential in comparison to different organic debris. Therefore, the process of dark fermentation is used as a preferred method to produce biohydrogen. There are reported and published research articles describing the higher potential of carbohydrate nutrient feedstock for biohydrogen production in comparison to proteins and lipids. As carbohydrate feedstock is easier to degrade by the bacterial species and get energy out of it. There are different factors affecting the final yield of biohydrogen such as; total solids in the kitchen waste, optimum temperature and pH, moisture content, and retention time.

About 70-140,000 tons of potato peels, as waste, are generated per annum. This waste is coming straight from the food industries and kitchens. It was mainly used for feeding animals and as a fertilizer but now many efforts are made to get energy from this carbohydrate-rich substrate. Potato peels were used as a substrate in the research study designed to get energy from peel waste and used it at bench scale level. Potato peels have less lipid and protein content and higher carbohydrate content making them a potent substrate for the production of biohydrogen.

They contain a lot of structural polysaccharides due to which digestion might become difficult and complex by the microbial species. A dark fermentation process was adopted after chemically pre-treating the peels waste (9).

#### 3.3. Strategies for increased biohydrogen generation from food waste

There is a global problem of day-by-day growing food waste from domestic as well as industrial resources. There is improper management of this gradually increasing food waste. According to the Agriculture and Food Organization of the USA, there is 1.3 M tons of food wastage. These are abundantly generated from the food processing settings. While generating in such large quantities they are also rich in nutrients and organic acids. These nutrients and acids act as a feedstock for the generation of biohydrogen production by implying the fermentation process.

There are many studies revealing the bio-hydrogen production from different settings such as restaurants, kitchen waste, food-based industries and dining halls etc.

There are different parameters that should be kept optimum in order to get a maximum yield of biohydrogen. Some of the parameters are; feedstock composition, optimized pre-treatment of waste, type of microbial culture, pH, fermentation temperature, intermediate product formation and substrate.

The production of immediate products can lead to a reduction in the overall yield of biohydrogen. The usage of cyclic and short ultrasonic waves and nanoparticles in the fermentation process can enhance the method's efficiency by many

folds. The augmentation in the process that employed ultrasonic waves has proved to be beneficial due to the process of cavitation. The following are the advantages of using cavitation;

Cavitation bubbles affect the medium chemically and physically. After the transient collapse of the cavitation bubbles there is the formation of many reactive species that play a role in the biochemical and thermochemical reaction. These cavitation bubbles lead to the enhancement of the reaction rate by annihilating complex sugars present in the food waste. They help the reduction of the overall growth of the hydrogen-inhibiting microbial species present in the feedstock.

Additionally, there are also different advantages mentioned in this study after combining ultrasound and nanoparticles with the fermentative process and enhancement of bio hydrogen production (10).

#### 3.4. Combined bioprocess for biohydrogen production using solid-state and dark fermentation

Solid state and dark fermentative processes were combined to produce biohydrogen from the food waste and the combined effect was studied. A. oryzae and A. awamori were the two fungal species used for biohydrogen production in the solid-state fermentative process. These fungal species produce protease and glucoamylase enzymes that are required for the digestion of food waste. They hydrolyze the food waste and form a hydrolysate that is rich in nutrients.

These hydrolysates were used as feedstock in dark fermentative process. Heat pretreatment was also given to the sludge and 39.14 ml of biohydrogen was produced per gram of food waste as substrate. These combined bio processes lead to the establishment of several purposes like acceleration of the hydrolysis rate, improvement in raw material utilization and enhanced biohydrogen yield (11).

Techno-economic analysis of the above-mentioned combined bio processes led to the development of the protocol that runs a plant for bioenergy production using organic food wastes. There are following five phases of the bioprocesses.

Solid-state fermentative process and pretreatment - In this phase, there is grinding of the food waste and it is consumed by the above-mentioned fungal species. This fungal digestion worked in a solid-state fermentative process.

Hydrolysis of the food waste by using enzymes - Protease and glucoamylase enzymes are used for the hydrolysis process. These solid enzymes made a hydrolysate that was rich in monosaccharides like glucose.

Inoculation of the seed culture - In this phase hydrogen-producing bacterial species were used and named as bacterium R3.

Biohydrogen production by dark fermentative process - Culture was transferred in the fermenter where these inoculated bacteria changed the hydrolysate into hydrogen gas and carbon dioxide.

Purification of biogas - Finally, this biogas was purified by passing it through the purification system.

Food processing units, hotels, and households are producing a huge amount of food waste. They are present in variable amounts with a variety of compositions. Such as kitchen waste containing meat, bones, and egg shells is rich in lipids and proteins. Kitchen waste containing thrown vegetables and rice is rich in carbohydrates (12).

There is a requirement to complete the three most important steps such as hydrolysis, pretreatment, and fermentation so that the bioenergy can be obtained from different lignocellulosic compounds. There are different polymeric sugars that have lignin, cellulose, and hemicellulose and require different combination strategies in order to obtain biohydrogen (13).

Hydrolysis can be of different methods such as; acidic, thermal, basic, enzymatic, and supercritical pre-treatment hydrolysis.

After the hydrolytic cleavage, there is the production of sugars such as lactose, glucose, and sucrose. These hydrolyzed substrates always favor the usage of solubilized compounds by the microbial species. After keeping all the conditions optimum such as soluble oxygen demand, carbohydrate concentration, optimum pH, and temperature the overall hydrogen production reached to 105.38 ml / g of hydrolysate. At pH 13, after pretreatment, it was enhanced by 2.66 times than the control. Thus, pretreatment leads to enhancement in biohydrogen yield (14).

#### 4. Conclusion

In comparison to other organic wastes, enhanced biohydrogen production is achievable by using food waste in dark fermentation. Numerous research studies are conducted in order to maximize the biohydrogen yield but due to some factors, its commercialization is still questionable. There are some limitations such as less carbohydrate content in the organic waste, unwanted side reactions in fermentation, and thermodynamic limitations. There are many attempts to increase the biohydrogen yield by coupling different fermentation strategies and to analyze which combination strategy is the most feasible and sustainable for chemical bioprocessing and maximization of valuable by-products such as biohydrogen.

#### **Compliance with ethical standards**

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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