Pretreatment Studies of Biohydrogen Production from Agro Industrial Waste

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Abstract:

This study evaluates the production of biohydrogen from agro industrial waste. The worldwide energy demand is increasing exponentially and the reserves of fossil fuels are depleting, the combustion of fossil fuels has the effect on environment because of CO_2 emission. Hydrogen generation market size is forecast to cross 180 billion by 2024, according to a new research report by global market. For the production of biohydrogen. we had chosen groundnut shell as our source, using Tween80 as a surfactant we had undergone pre-treatment studies for (10min,20min,30min,40min,50min) we had estimated the content of cellulose, protein, carbohydrates at (1%,2%,3%,4%,5%) and obtained the optimum value in the form of graph. The production of hydrogen is done by using the rumen fluid of the cow and the quantity of the hydrogen produced by this process is identified by using the analytical instrument Gas Chromatography.

KEY WORDS: *Bio hydrogen, photo-fermentation, agro-waste*

Introduction:

Biohydrogen is a type of biofuel, the biofuels are referred to those kinds of fuels, which are incorporated biomass derived from plant-based products being processed to form a sustainable energy source (Pandey 2008). Different kinds of biofuels generate a wide variety of fuel types (in forms of liquid, solid and gaseous) for producing heat, electricity, chemicals and other materials (Jaccard 2006). Biofuels seems as a promising new source of energy with some similar properties to petroleum fuels of which the transportability is the most important. This property would make biofuels superiority in comparison to other renewable energy sources, which is the potential to incorporate biofuels as driving force for the transportation. Regarding the environmental concerns and greenhouse effect, due to plant-based origin of biofuels, burning this kind of fuels would not lead to increase in the net amount of carbon dioxide in the atmosphere because the stored carbon materials inside biofuels had been added through photosynthesis from adsorption of carbon dioxide from the atmosphere (Stevens and Verhé 2004). In general, and due to origin of biomass which has been used for production of biofuels, the different kinds of biofuels are being classified in three generation. In following sections, the differences of each generation of biofuels along with the origin and advantages and disadvantages would be discussed. these biofuels are derived from organic biomass natural or synthesized by 2050 the world would need energy equivalent to 50 cubic miles of oil annually (currently 3.1 CMOs). At low rates of energy consumption, our lives do not continue to improve significantly as we consume more energy beyond approximately 4 kW/person. These are sobering numbers. By approximately 2025, global population will be about 8 billion. If everyone were to consume primary energy at a rate of 4 kW/person (e.g., the energy consumption rate of Portugal), the global energy consumption rate would be 32 terawatts (TW). Currently, we consume about 16 TW, so if we were to raise the average global living standard to the equivalent of Portugal, we would need to double primary energy production by 2025.The hydrogen is used for transport fuel can be produced from fossilfuels as well as renewable sources Biological hydrogen production methods can be classifying as below, Direct bio photolysis , Indirect bio photolysis , Photo fermentation ,Dark fermentation ,Two stage process integration of dark and photo fermentation, Bio catalyzed electrolysis, Steam Reforming, Microbial fuel cells .The goal of the pretreatment process is to remove lignin and hemicellulose, reduce the crystallinity of cellulose, and increase the porosity of the lingo cellulosic materials. Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by hydrolysis, (2) avoid the degradation or loss of carbohydrate, (3) avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes, and (4) be cost-effective. Pretreatment methods can be roughly divided into different categories: physical (milling and grinding), physicochemical (steam pretreatment/auto hydrolysis, hydro thermolysis, and wet oxidation), chemical (alkali, dilute acid, oxidizing agents, and organic solvents), biological, electrical, or a combination of these. The following pretreatment technologies have promise for cost-effective pretreatment of lingo cellulosic biomass for biological conversion to fuels and chemicals. Hydrogen is used in many chemical industries to

produce ammonia (27%) for agricultural fertilizers, methanol (11%) and cyclohexane. During oil refining process (33%) the sulfur is removed from fuels by using hydrogen. In many industries hydrogen can be used as liquid & gas especially petroleum industry. It's used for industrial application such as food processing, refining, as rocket fuel etc. The main application of hydrogen includes as a reactant in hydrogenation processes, as an O2 scavenger, as a fuel rocket engine and as a coolant in the electrical generation to take advantage of its physical properties

Materials and Methods:

The objective of the paper is to review the bio hydrogen production from lingo cellulosic biomass(rice, corn and wheat residues) and the pre- treatment involve the concentrated or dilute acids include hydrochloric acid (HCL) and sulphuric acid (H2SO4).The biohydrogen production ranged from 12 to 7019 mL H2/L(Juliana Ferreira Soares etal., 2020).In this study,the date seed waste were valorised for this production and the effect of surfactants is triton X-100, surfactin and PEG 1000. The maximum yield of biohydrogen is 103.97 mmol/L(Rambabu etal.,2019). In DF the hydrogen production is enhanced by anaerobic process. Hydrogen-producing bacteria in sugarcane vinasse (Lucas Tadeu Fuess.et al.,2017), garden waste(Abreu AAa.et al,.2019), mushroom farm waste hydro lysate (Li 54 et al., 2011) in DF has got highest hydrogen yield. A mechanism behind galactose-based fermentative hydrogen production from macro-algal biomass studies reveals that pre-treatment enhances the bio hydrogen production (Sang-Hyoun Kim.et al.,2019). The pre-treatment methods include physical, chemical and biological processes (Preethi etal 2019). Here are the some of the literature review for Production of bio hydrogen in recent years.

Therefore, various pre-treatment methods can be used, mainly mechanical treatment like ultra-sonication and chemical treatment like acetic acid, hydrochloric acid and sulfuric acid. It can be used to increase the yield of biohydrogen production(Rajesh banu etal., 2019). Paper waste is one of the municipal and industrial solid waste. It was obtained by using dilute acid method in steam sterilizer for 121°C in 1hr and the yield of production is 1.45mmol H2 L^{-1}(Anna poladyan etal., 2019). In the organic fraction of municipal solid waste is investigated under thermophilic conditions and the addition of tween 80 and polyethylene glycol is to improve the production of biohydrogen and the yield is 109.99±7.1 and 113.8±7.7ml H2/g (Elsamadony etal., 2015). Hydrogen-producing bacteria in sugarcane vinasse (Lucas Tadeu Fuess.et al.,2017), garden waste(Abreu AAa.et al,.2019), mushroom farm waste hydro lysate (Li 54 et al., 2011) in DF has got highest hydrogen yield.

Selection of substrate for bio hydrogen production

The substrate selection of biohydrogen production is important because they contain high amount of protein, cellulose and carbohydrates. In many review paper the substrate can be used such as lingocellulosic sources include agroindustrial and agriculture residues like rice straw, wheat residues, sewage sludge, cassava pulp, food waste, beef manure, crude glycerol, household

solid waste, oil mill effluent, pulp and paper waste,sugarcane bagasse, sugarcane molasses , sugarcane vinasse, cornstalk, corn Stover, corn cobs, corn bran, wheat straw, sorghum rusk, sorghum leaves, sorghum Stover, sugar beet juice,mushroom farm waste, cashew apple bagasse, rice bran, rice husk, oat straw, date seed, forestry waste, wood, grass, marine macro algae, water hyacinth, livestock manure waste, eucalyptus.

In this project we had used Peanut shell as agro waste materials. Peanut shells are the leftover product obtained after the removal of peanut seed from its pod. This is the abundant agro-industrial waste product which has a very slow degradation rate under natural conditions (Zheng et al., 2013). However, peanut shells contain various bioactive and functional components such as cellulose, hemicellulose and lignin which are beneficial for mankind. It can be convert into various bio products such as biohydrogen, bio diesel, bioethanol.

Preparation of inoculum for bio hydrogen production

Rumen fluid is used as inoculum source. The cattle rumen fluid is collected from the cow meat shop, The potential of application of rumen microorganisms as inocula has been explored so far, mainly for the conversion of groundnut shell (Hu and Yu, 2005;Sutherland and Varela, 2014; Sawatdeenarunat et al., 2015; Li et al., 2017). Rumen is a well-adapted microbial community, on which ruminants rely on to convert feed into energy-yielding products, such as VFAs, used by the host as an energy source. Rumen contains two groups of prokaryotes (bacteria, dominated by the phyla Firmicutes and Bacteroidetes, and Archaea) and two groups of eukaryotes (protists and fungi) and their strong metabolic interactions characterize its environment. It is stored in refrigerator and maintained a temperature at -20^0C to -80^0C.

Pretreatment of agrowaste for biohydrogen production:

Effect of physical methods for agro/ligno-cellulosic waste

In this method, milling and grinding are mostly used for the physical pre-treatment process. It reduces the size of ligno-cellulosic bio hydrogen and increase the surface area to the reaction. This method can be applied to corn Stover, wheat straw, oat straw, rice straw, paper waste, sorghum leaves, sugarcane bagasse. The effect of rice husk particle size such as< 2000 µm, <300µm, 74µmon bio hydrogen by solid state fermentation has studied by velioglu and his co-workers. A production yield of 3.99 mLH2/g substrate was obtained with particle size of 6 <300µm. This study also reveals that the combinative inoculum usage has achieved better digestibility when compared to the individual consortia (Velioglu Tosuner etal.,2018). Food waste can be pre-treated as different types of radiation such as gamma rays. Electron beam, ultrasound and microwaves (rajeshbanu et al., 2019).

Effect of chemical methods for agro/ligno-cellulosic waste

The chemical pre-treatment involves alkaline, acid, ozonation, organic solvents, ionic liquids, metal chlorides and plasma etc. Acid pre-treatment is one of the most effective pre-treatment used for ligno-cellulosic biomass. It involves the use of concentrated or dilute acids mainly sulphuric acid (H2SO4) and hydrochloric acid (HCl) to break down the structure of ligno-cellulosic materialprecipitating lignin, releasing sugars and inhibitory compounds.(Moodley e Kana) and (Rorke and Kana) compared as the three acid pre-treatments like (HCl, H2SO4, and HNO3) for the recovery of glucose and xylose from sorghum residue and sugarcane leaf waste.(Vavouraki et al) are also found the concentration of sugars increased to 120% by using 1.12% HCL for 94 min as compared to control. This process can be produced phenolic compounds and carboxylic acid that can restrict the production of biogas. Alkaline pre-treatment is found to be efficient than acid treatment (pinanong Tanikul et al.,2018) because the side product formed have less effect on pH of the digestion process and for this process neutralization is not required. In a study of ozonation aided mesophilic bio hydrogen production from palm oil effluent, the maximum Hydrogen production was found to be 43.1 mL/h. This pre-treatment method significantly enhanced the biodegradability of the substrate and improved the production of acetic and butyric acid. A study was performed by incorporating ferrous ion in the bio hydrogen production of macro algae (Laminaria japonica). This reveals that Ferrous ions improved the enzymatic process during bio hydrogen production (Yanan Yina et al ,2019).

Effect of biological methods for agro/ligno cellulosic waste

In this pre-treatment is also based on delignification of lignocellulose from microorganisms or enzymes. The most effective micro-organisms for delignification are fungi. They can be produce enzyme to degrade the hemi-cellulose, lignin and polyphenols are also present in the biomass. (Shanmugam et al) has evaluate laccase from *Trichoderma asperellum* on sweet sorghum Stover biomass for production of bio hydrogen. An optimal removal of lignin is76.93% leading to biohydrogen production is (402.01 mL) 3.26 times higher than control without enzymatic pre-treatment.(Saratale et al) has been isolated from actinomycetes (Streptomyces sp. and Nocardiopsis sp.) and fungi (Phanerochaete chrysosporium) grown in agriculture wastes like sorghum husk, rice husk, Vigna mungo harvesting waste, soybean straw, and waste tea powder forthe cellulase and xylanase production under solid state fermentation.The biological pre-treatment is more advantages than others, because of less toxic product formation. On the other hand, the disadvantages include high production cost and slow process time. However, the incorporation of combinative treatments can be more effective and economical (Mohal et.al) investigated the combinative treatment process using heat shock and chemical. An approach of incorporation of Nano-surfactant pre-treatment methods will improve the digestibility of the substrate. The application and the effects of Nano-material for cellulosic fermentative hydrogen

was reviewed by (neha srivastava et al,.2018). This has given a new insight of incorporation of sono-coupled Nano-surfactant for the production of bio hydrogen.

Preparation of substrate for bio hydrogen production

Size Reduction:

Ball mills are one of the most commonly used milling equipment approximately 20% of the energy generated by the mill is utilized for actual grinding of the material. The crushing efficiency is due to various The groundnut shell ash was then placed in the high energy mill A Retsch Planetary Ball Mill PM 400. The objective of this milling is to reduce the groundnut shell ash particle size and blending of particles in new phases (size Reduction of Groundnut Shell by Ball Mill and Estimation of Breakage Parameters Using Population Balance Model S.M. Mubashera**,**et.al 2016).

The grinding jars of the Retsch Planetary Ball Mill are arranged eccentrically on the sun wheel of the planetary ball millfactors such mill load, rotation speed, type of milling as well as the size of the balls. The grinding balls in the grinding jars are subjected to superimposed rotational movements, the so-called Coriolis forces. The difference in speeds between the balls and grinding jars produces an interaction between frictional and impact forces, which releases high dynamic energies. The interplay between these forces produces the high and very effective degree of size reduction of the planetary ball mill. The balls fall freely and impact the groundnut shell ash and balls beneath them. The kinetics of the process depends on the energy transferred to the powder from the balls during milling. In order to obtained the optimal milling time to produced the optimal particles size the mechanical milling was done at time of 2, 4, 6, 8, 10, and 12 h (Synthesis of groundnut shell nanoparticles: characterization and particle size determination S. A. Yaro1 et.al.,2016). Many researchers investigated various materials size reduction using different types of mechanical crushers. Sakthivel and Pitchumani4 investigated effect of operating parameters such as ball loading , solid mass fraction , pH of the suspension and grinding time on particle size (silica) distribution for the production of nano mineral particles in a stirred ball mill using response surface modeling. The particle size was decreased with the increase in ball loading, pH and grinding time, but not solid mass fraction.

Sieving:

The peanut shell is washed and dried for shadow drying, after crushing and then powdered the shell. By using the sieving machine at the range of 14 to 15 the sample is sieved finely and the size of sieved groundnut shell is 0.5mm.

Effect of surfactant on pre-treatment for agro/ligno-cellulosic waste

Tween 80 is used as a surfactant for this pre-treatment studies, it is also known as polysorbate80. It is a non-ionic surfactant and the emulsifier is also derived from oleic acid and poly ethoxylated

sorbate. It is water soluble yellow liquid form. It has a several chemical properties and useful various biological activities. it is viscous, non- toxic, hydrophilic, non- immunogenic and highly flexible.

The molecular formula of tween 80 is C64H124O26. During the bio hydrogen production of organic fraction of municipal solid waste(OFMSW), the effect of tween 80 on the H2 production, the dosage of Tween 80 from 0.0% to 2.8% increased and volumetric hydrogen production from 455.5±37.5 to 719.5±51.2 ml, and from 2.5±0.2 to 4.0±0.3L H2/ L Substrate. the maximum production of hydrogen is 92- 93%(Elsamadony et al, 2015).

During the bio hydrogen production of palm oil mill effluent(POME),2ml of tween 80 is dissolved in distilled water filled up 100ml of volumetric flask and the stock solution is arrived at 2%. stock solution was prepared of 4 concentrations is 0.05%, 0.1%, 0.2%, 0.5%, it was also based on (feng et al 2006) Tween 80 concentration from 0.1% to 0.7%. In 220ml of palm oil mill effluent treated with 22ml of tween 80 with different concentration and it is incubated at 37°C at 45rpm for 6hrs in rotary shaker. the pH is maintained at 6 and 7. The hydrogen production for palm oil mill effluent is recorded at the pH 7 for 0.20% tween 80 at 2.76 mol g^{-1}, and the COD is 5.83% more than hydrogen production obtained at same concentration at pH 6(Emmuanuel pacheco et al 2012).

During the production of bio hydrogen in ligno-cellulosic biomass (sugarcane bagasse, pine tree wood pellet, waste wheat powder, waste paper, palm kernel cake, rice bran, sorghum), if it is treated with sulphuric acid and hydrochloric acid. in this study HCL provides highest recovery of sugars, 52.02 g/L of xylose and 18.42 g/L of glucose from sorghum residue and 78 g/L of xylose and 11.48 g/L of glucose from sugarcane leaf waste (soares et al, 2020). Bio hydrogen production of food waste, if it is treated with sulphuric acid, hydrochloric acid, hydrogen peroxide, acetic acid is mainly used. The sugar is increased 120% using 1.12% HCL for 94 min compared to control.In this process produced carboxylic acid and phenolic compound that restrict the biogas production. If the enzymatic pre-treatment improves the volatile fatty acids production. The hydrolysis of food waste with acid pre-treatment PH2 increasing the H2 production as much as 3 times versus normal process.

Pre-treatment of peanut by shaker:

Shaker is used to mix, blend and to agitate the substrate in the conical flask by shaking them. It contains oscillating board is also used to place the flask. Pre-treatment of peanut shell plays a vital rolein biohydrogen,it is used to break the lignin in the cell wall by using some chemicals, Nano surfactant, mechanical methods and optimized physio chemical methods.A non-ionic surfactant Tween80 is selected for the pretreatment of peanut shell.

Table.1

Source	Water	Tween 80	Time duration
1g	100ml	1% - 1ml	10 min
1g	100ml	2% - 2ml	20min
1g	100ml	3% - 3ml	30min
1g	100ml	4% - 4ml	40min
1g	100ml	5% - 5ml	50min

Estimation of protein:

To estimate the amount protein present in the groundnut shell by using lowry's method. It is determining the protein concentration lies in the reactivity of the peptide nitrogens with the copper ions under alkaline conditions and the subsequent reduction of the folin ciocatteau phosphomolybdic phosphotungstic acid to hetro polymolybdenum blue by the copper catalyzed oxidation of aromatic acids. It is very sensitive to PH of assay solution should be matained at 10-10.5. The reagents are used in protein estimation is Na_2Co_3, N_aOH, NaK tartarate, $CUSO_4$, $5H_2O$.Measure the absorbance at 660nm and calculate the amount of protein in the groundnut shell using standard graph.

Pretreatment Studies of peanut shell using Tween 80 (OD at 625nm)

Time of Exposure	OD at 625nm					Time of Exposure	Concentration of Protein(mg/ml)				
	1	2	3	4	5		1	2	3	4	5
1	0.48	1.17	1.16	2.10	2.20	1	0.2	0.5	0.4	0.8	0.9
2	1.41	2.18	2.10	2.19	3	2	0.6	0.9	0.8	0.9	1.2
3	1.31	2	2.32	2.00	1.66	3	0.5	0.8	0.9	0.8	0.7
4	1.20	1.71	3	1.7	1.10	4	0.5	0.7	1.2	0.7	0.4
5	0.22	1.57	1.9	0.56	0.79	5	0.1	0.6	0.8	0.2	0.3

Estimation of cellulose:

To estimate the amount of cellulose content present in the peanut shell by using anthrone method. Cellulose is a major structural of polysaccharides in plants. It is also composed of linear chain of glucose molecules it is linked by beta glycosidic bond. If the nitric/acetic reagents forming the acetylated cellodextrins which hydrolyzed and dissolved from glucose molecules with 67% H_2so_4.The dehydrated of glucose molecules to form the hydroxymethyl furfural and formed green color. Measure the absorbance at 630nm and calculate the amount of cellulose present in the groundnut shell using standard graph.

Pretreatment Studies of peanut shell using Tween 80 (OD at 625nm)

Time of Exposure	OD at 625nm					Time of Exposure	Concentration of cellulose(mg/ml)				
	1%	2%	3%	4%	5%		1%	2%	3%	4%	5%
10	0.488	1.174	1.162	2.104	2.203	10	0.21	0.50	0.49	0.89	0.94
20	1.416	2.189	2.102	2.194	3	20	0.60	0.93	0.89	0.93	1.27
30	1.312	2	2.325	2.002	1.666	30	0.56	0.85	0.99	0.85	0.71
40	1.201	1.718	3	1.7	1.106	40	0.51	0.73	1.27	0.72	0.47
50	0.224	1.572	1.94	0.569	0.797	50	0.10	0.67	0.82	0.24	0.34

Estimation of carbohydrates:

To estimate the amount of carbohydrates, present in the peanut shell by using Anthrone method.In plants, the carbohydrates are very important component of the storage and structural materials and it is also stored as a sugars and polysaccharides. Monosaccharide is the basic unit of carbohydrates. The reagents are used in the carbohydrates estimation is anthrone, glucose

reagent. Measure the absorbance at 620nm and calculate the amount of protein present in the peanut shell using standard graph.

Pretreatment Studies of peanut shell using Tween 80 (OD at 625nm)

Timeof Exposure	OD at 625nm					Timeof Exposure	Concentration of carbohydrates(mg/ml)				
	1%	2%	3%	4%	5%		1%	2%	3%	4%	5%
10	0.724	1.345	0.826	0.725	0.805	10	0.52	0.97	0.60	0.52	0.58
20	0.819	2.079	0.952	0.862	0.912	20	0.59	1.50	0.69	0.62	0.66
30	0.974	3	1.293	0.972	1.481	30	0.70	2.16	0.93	0.70	1.07
40	0.909	0.819	1.054	1.132	1.345	40	0.65	0.59	0.76	0.82	0.97
50	0.521	0.572	0.992	0.785	0.797	50	0.38	0.41	0.71	0.57	0.57

Biohydrogen Production:

Biohydrogen production is done by the process of dark fermentation, the production process is done by using the production bottle, sieved peanut shell, 60ml disposable syringe and the cattle rumen fluid. Before going to production set up the sieved peanut shell are undergone in a pre-treatment studies using Tween 80 by the ultra-sonication and by optimized orbital shaker. During these pre-treatment studies the functional compound lignin which is present in peanut shell will be eliminated by the surfactant Tween 80 and also the estimation of cellulose, carbohydrates and protein is done. Then the production is done by using the glassbottle with a steel tube get attached at the top of the bottle cap. Then the syringe is get attached to the bottle cap, the production set up is consist of two glass bottle, one is kept for control other is kept for sample. The control bottle consist of substrate+ water(100ml)+ rumen fluid(300ml) and sample bottle consist of substrate+ water(83ml)+ rumen fluid(300ml)+ Tween 80(17ml) and the two bottles are kept in a tray of water to balance the pressure during the production of biohydrogen, and stored in a room temperature.

Control

Test

Result and Discussion:

In agro waste products, different sugars and volatile fatty acids are commonly found that were also chosen as substrate for the biohydrogen production. The worldwide needed on hydrogen is increasing with a growth rate of nearly 10% per year and the contribution of hydrogen to the total energy market will be 8-10% by 2025.Hydrogen presents an interesting energy carrier for the future due to its high power content (143 MJ/kg) compared to other combustibles (methane, ethanol, and gasoline) (Rao et al. 2018)**.**This hydrogen can be produced by some of the following methods like Steam Reforming, Fermentation and Microbial fuel cells. The peanut shell is washed and dried for shadow drying, after crushing and then powdered the shell. The characterization of peanut shell indicates the presence of biopolymer like protein(0.0806mg/ml), carbohydrates(0.0730mg/ml) and cellulose (1.8117mg/ml) present in the peanut shell by using followingmethods.To compare the estimations, the cellulose content is higher than protein and carbohydrates. The pre-treatment of peanut shell plays a vital role in bio hydrogen. It is used to break the lignin from the cell wall using some chemicals. The dosage of tween 80 is 1%-5% using a shaking incubator. During this pre-treatment studies using surfactant, the concentration of protein as found to be 3% and the time of exposure is 40mins and also 5% (20mins). The concentration of carbohydrates was found to be 2.16mg/L at 3% of tween 80. The concentration of 3% and 5%, time of exposure of 20mins has high cellulose level. The pre-treatment process parameter such as exposure time and concentration of tween 80 as found to be in 3% within 30mins.

Conclusion

In agro waste, different sugars and volatile fatty acids are commonly found that were also chosen as substrate for the biohydrogen production. For the process we had chosen as a source material is peanut shell. The pre-treatment is done to break the center part of lignin in the cell wall using some chemicals(tween 80) and we had estimated the content of protein, carbohydrates and cellulose at (1%, 2%, 3%, 4%, 5%) for (10-50mins) obtained value in the form of graph. Pre-treatment is also used to increasing the yield of biohydrogen production. Rumen fluid is used as an inoculum for the hydrogen production and this process is identified using the analytical instrument Gas chromatography.

References

o   Kim, S.H., Mudhoo, A., Pugazhendhi, A.,Saratale, R.G., Surroop, D., Jeetah, P., Park, J.H., Saratale, G.D., Kumar, G., 2019. A perspective on galactose-based fermentative hydrogen production from macroalgal biomass: Trends and opportunities. Bioresour .Technol. 280, 447–458

o   Hankamer, B., Lehr, F., Rupprecht, J., Mussgnug, J., Posten, C., Kruse, O., 2007. Photosynthetic biomass and H2 production by green algae: from bioengineering to bioreactor scaleup, PhysiologiaPlantarum 131, 10–21

o   Gopalakrishnan Kumar, DinhDuc Nguyen, PeriyasamySivagurunathan, Takuro Kobayashi, Kaiqin Xu, Soon Woong, Chang Cultivation of microalgal biomass using swine manure for biohydrogenproduction(2018)

o   Lucas TadeuFuess, AntônioDjalmaNunesFerrazJúnior, Carla Botelho Machado, Marcelo Zaiat, hydrogen-producing bacteria in sugarcane vinasse dark fermentation(2017)

o   Anburajan, P., Pugazhendhi, A., Park, J.H., Sivagurunathan, P., Kumar, G., Kim, S.H., 2018. Effect of 5-hydroxymethylfurfural (5-HMF) on high-rate continuous biohydrogen production from galactose. Bioresour. Technol. 247, 1197–1200

o   Dong, L.L., Cao, G.L., Zhao, L., Liu, B.F., Ren, N.Q., 2018. Alkali/urea pretreatment of rice straw at low temperature for enhanced biological hydrogen production. Bioresour. Technol. 267, 71–76

o   Ganesh dattatraysaratale, rijutaganeshsaratale, sang hyoumkim, gopalakrishnankumar, screening and optimization of pre-treatment in the preparation of sugarcane bagasse feedstock for biohydrogen production and process optimization(2018)

o   NooshinAsadi, Hamid Zilouei Optimization of organosolvpretreatment of rice straw for enhanced biohydrogen production using Enterobacteraerogenes (2016)

o   Lili Dong, Guangli Cao, Jiwen Wu, Bingfeng Liu, Defeng Xing, Lei Zhao, Chunshuang Zhou, Liping Feng, Nanqi Ren High-solid pretreatment of rice straw at cold temperature using NaOH/Urea for enhanced enzymatic conversion and hydrogen production (2019)

o   MaktumMuharja, FitriJunianti, Dian Ranggina, TantularNurtono, AriefWidjaja∗An integrated green process: Subcritical water, enzymatic hydrolysis, and fermentation, for biohydrogen production from coconut husk 2018

o   Sang-Hyoun Kim, AckmezMudhoo, ArivalaganPugazhendhi, Rijuta Ganesh Saratale, Dinesh Surroop, PratimaJeetah, Jeong-Hoon Park, Ganesh DattatrayaSaratale, Gopalakrishnan Kumar, hydrogen production from macroalgalbiomass(2019)

o   Abreu AAa, Tavares Fa, Alves MMa, CavaleiroAJa, Pereira MAa* Garden and food waste co-fermentation for biohydrogen production(2019)

o   Octavio García-Depraect, Idania Valdez-Vázquez, Eldon R. Rene, Jacob GómezRomero, Alberto López-López, Elizabeth León-Becerril Enhanced biohydrogen production from

the dark co-fermentation of tequila vinasse and nixtamalization wastewater: Novel insights into ecological regulation by pH(2019)

o SupratimGhosha, BrajagopalDuttab, AbhikBanerjeeb, ShantonuRoyb,∗, Bio-H2 production using de-oiled cake (2018)

o Preethi, T.M. Mohamed Usman, Rajesh Banu, M. Gunasekaran, Gopalakrishnan Kumar KumarPII:S2, biohydrogen production from industrial wastewater: Advancement towards commercialisation(2019).

o Pinanong tanikkul, siriornbooyawanich, niponpisutpaisal, ozonation aided mesophilic biohydrogen production from palm oil mill effluent(2018)

o VelioglutosunerZ.,, G.G. taylan, S.Ozmiha, effect of rice husk particle size on biohydrogen production under solid state fermentation(2018)

o Tugui Yuan, SongweiBian, Jae Huanan Wu, Qiyong Xu enhancement of hydrogen production using untreated inoculum in two- stage food waste digestion(2019)

o Kuan-Yeow Show, Yuegen Yan, Ming Ling, Guoxiang Ye, Ting Li, Duu- Jong Lee hydrogen production from algal biomass(2017)

o Gopalakrishnankumar, ackmezmudhoo, periyasamysivagurunathan, dilliraninagarajan. Anish ghimre, chyi-how lay, chi-yuelin, duu-jong lee.jo-shuchang dark fermentation of biohydrogen production(2016)