

PAPER • OPEN ACCESS

Production of Bio-Hydrogen from Agricultural residues : A Contemporary Review

To cite this article: C Prem Balaji *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **573** 012007

View the [article online](#) for updates and enhancements.



The banner features a colorful striped border at the top. On the left, the ECS logo is displayed in a green circle. To its right, the text reads: **240th ECS Meeting**, Oct 10-14, 2021, Orlando, Florida. Below this, it says: **Register early and save up to 20% on registration costs**, Early registration deadline Sep 13, and **REGISTER NOW** in orange. On the right side of the banner is a photograph of a diverse group of people in a professional setting, with a man in a white shirt and tie clapping and smiling.

Production of Bio-Hydrogen from Agricultural residues : A Contemporary Review

Prem Balaji C¹, Mukundhan C¹, M.B. Shyam Kumar¹, R. Harish¹

¹ School of Mechanical Engineering, Vellore Institute of Technology, Chennai

Email: mukundhanvit@gmail.com

Abstract: With the increase in usage of bio-products of all sorts in the modern world, the waste obtained during the production phases has been a major concern for over a period of time. The accumulation of these wastage not only enhances various kinds of pollution, but also increases the carbon footprint. Considering these negative impacts brought about by poor maintenance of these wastages and advancements in the field of utilization of sustainable and renewable energy, it is very important to produce bio-hydrogen due to the limitations of using industry-produced hydrogen. Hydrogen, though having many advantages, like being environmentally friendly and highly efficient when used as a fuel, has been neutralized by certain drawbacks such as production of it in industrial set up is found to be expensive and the storage process is tedious because of its flammable nature. Therefore, production of bio-hydrogen is found to be vital. In our paper, we have touched upon various methods of preparation of bio-hydrogen from the by-products of the farms such as corn stover, corn straw and rotten apple. Production of bio-hydrogen from micro algae has also been stressed upon as it has been found be one of the most efficient method. But, most of the analysis has been done on corn stover based on ambient atmospheric conditions, factors that affect the process and comparison between photo fermentation, dark photo fermentation and dark fermentation has also been judged as an important criterion for enzyme saccharification. Another important method of production of production of hydrogen using tequila vinasses in presence of charcoal by dark fermentation process has also been reviewed in this paper

1. Introduction

In recent years, production of hydrogen from renewable resources has improved interest among different types of hydrogen production [1]. China being one of the largest agricultural land has highest amount of crop straw available all over the world. Cornstalk is very abundantly available biomasses on earth. It is the main kind of crop straw, which is a part of lignocellulosic biomass [2]. Dark fermentation process is focused on bio-hydrogen production from cornstalk, considering the research on photo-fermentation process are scarce and less reports study about the two-step fermentation. Cornstalk contains compounds such as hemicellulose, cellulose and lignin, are ones with high crystallization property, obstructing microbial degradation and additional other fermentative hydrogen production, while manufacturing process of the fuel from other sources depends on surrounding conditions [3].



2. Methodology and Results of Production of Bio-Hydrogen from different sources

2.1. Synthesis from corn stover and corn straw

Improvement of hydrogen production from cornstalk is done by the pre-treatment conditions of Calcium Hydroxide enzymolysis [4]. Orthogonal design method is used to optimization. With methods of dark, photo, and two-stage fermentation, hydrogen is produced by using the cornstalk hydrolysate as a substrate, and cow dung and mutant of *Rhodobacter capsulatus* used as inocula, respectively [5]. Energy Sustainability Index (ESI) is used to examine the 3 different fermentation systems in order to choose a production method which is efficient from the perspective of energy sustainability [6]. Ca (OH)₂ concentration, temperature and alkaline hydrolysis time required for sugar yield reduction were examined based on the analysis. The results showed that the optimum formulation is A₃B₅C₃, videlicet, concentration of Ca (OH)₂- 0.5%, hydrolysis time- 1.5 h, and hydrolysis temperature of 390 K [7]. The three factors which affected the production are ordered: Calcium Hydroxide concentration > hydrolysis time [8]. This implies that time required for hydrolysis reflects the similar effect as temperature required for hydrolysis. Surge in Calcium Hydroxide concentration above 0.5%, there is no increase in the amount of sugar release [9].

The objective now is to investigate the enzymatic saccharification, structural characteristics and bio-hydrogen production efficiency of straw biomass due to various pre-treatment methods. Substrate and inoculum used in this method are Corn straw and Photosynthetic bacteria HAU-M1 respectively [10]. Acid-heat and alkali-heat, hydrothermal, alkali and acid treatment are the different pre-treatment methods used in the process. Pre-treated corn straw was used to conduct the photo fermentation method in order to achieve the bio-hydrogen production [11].

2.1.1. Pre-treatment effects on the Structural Characteristics of corn straw:

Cellulose of corn straw consists of amorphous and crystalline structures, which affects corn straw' enzymatic hydrolysis. XRD analysis was used to observe the crystallinity of various pre-treated specimens given. After observing all the samples given, around $2\theta=18^\circ$ we observe the trough, and peaks of diffraction near $2\theta=22^\circ$ and 35° [12]. The positions of different peaks of pre-treated samples did not have obvious change, whereas the intensities of these varied greatly, when compared with the control [13]. These observations showed that the cellulose in crystalline form was not affected by pre-treatment processes, whereas the cellulose structure changed in different degrees after various pre-treatment processes. Particularly, the main peak's intensity rose by 33.2% in comparison to the control [14]. This implies that 2% concentration heat treatment with NaOH, broke the amorphous cellulose chain of molecules effectively, making the order degree and the freedom of cellulose of amorphous structure improved [15].

2.1.2. Effect of pre-treatment on the Enzymatic Saccharification potential of corn straw:

Glucose and Xylose are the main components in the enzymatic hydrolysate of corn straw, which are respectively due to the dissolution of hemicellulose and cellulose [16]. The glucose concentration in hydrolysate is higher than that of xylose in the pre-treated samples, because of the higher cellulose content in corn straw than of hemicellulose [17]. From the observation, the reducing sugars concentration in hydrolysate varied greatly according to the pre-treatment done. The concentration of total reducing sugars from different pre-treated samples increased from 3.94g/L to 23.07 g/L, and the efficiency of sugar conversion varied from 7.9 to 46.1 percent [18].

2.1.3. Effect of pre-treatment on the Hydrogen Production Performance of corn straw:

To evaluate the corn straw hydrogen production performance, overall hydrogen yield is a main index used for it. The cumulative hydrogen yield from different samples varied showed significant change as seen from observation. 137.76 mL/g TS is the maximum hydrogen production achieved when corn straw was pre-treated with 2% NaOH, which was 31.4% more the control (104.94 mL/g TS) [19].

It is quite important to have a comparison among the three types of fermentation, namely dark photo, photo and dark fermentation, implemented for the production of bio-gas [20]. The corn stover is initially pulverized and sieved through 60 mesh sieves. The cellulose, hemicellulose and lignin content in corn

stover was found to be 38.53%, 22.74% and 10.73% [21]. The inoculants used were found to be HAU-M1 and *Enterobacter aerogenes*. For the extraction of HAU-M1, bacterium such as *Rhodospirillum rubrum*, *Rhodopseudomonas capsulate* are cultivated at a surrounding temperature of 30°C. The light intensity was maintained at 3500lx [22]. Certain salts were also added along with yeast to favour fermentation. The *Enterobacter aerogenes* were found to be insensitive to light and supported anaerobic fermentation [23].

Process Involved: In a batch of experiments, three reactors of glass of 200 mL volume were filled with 5 g of corn stover, 40 FPU/g of cellulose and 100 mL of citrus buffer. The container was then filled with 30% inoculum. HAU-M1 embedded with sodium alginate solution and *Enterobacter aerogenes* were filled at a ratio of 3:1 [24]. Ambient conditions of pH, temperature and light intensity were moderated at 6.5, 35°C and 3500 lx respectively. The three reactors were then initiated with the different fermentation processes [25]. A micro-computer was used to determine the chemical composition and flow rate of argon at 45 mL/min was used as the carrier gas. A formula, $Y=C*V*100/m$, where Y is reduced sugar yield, C- concentration of the substrate, V- volume of the substrate and m- initial dry matter weight, was deduced, using which the results were inferred [26]. Photo fermentation process recorded as the highest producer of bio-hydrogen, with almost 59% of the fuel produced during it, followed by dark photo fermentation [27]. Dark fermentation produced the least amount of bio-hydrogen. Large amount of substrate was consumed by dark photo fermentation process, while photo fermentation consumed the lowest amount of substrate during the procedure [28].

2.2. *Synthesis from Micro-algae*

There is a large amount of CO₂ emitted in atmosphere due to the drastic augmentation in the energy demand curve, caused to enormous fossil fuel utilization, due to which green energy, in particular, hydrogen fuel is needed for the future [29]. Production of hydrogen through phototrophic microorganisms (micro-algae) is implemented due to economic feasibility.

Photosynthetic synthesis of environmental output using industrial applications operates through Micro-algae. The potential of micro-algae in the coming years is deemed to be higher, making it a higher-ranked model for research as well as industrial purposes[30]. Similar to cyanobacteria, micro-algae as well as dark fermented microorganisms could be adopted to manufacture bio-hydrogen [31].

Micro-algae are single or multi-cellular organisms, that can thrive in variegated biosphere and can be cultivated in fresh, marine and wastewater. They consist about 50% of carbon by dry mass, which is generally in the form of CO₂. Synthesis of 100 gram of biomass of microbes can replace nearly 183 g of CO₂. They consist of 5 to 60 percentage of carbohydrates, 40 to 60 percentage of proteins, 5 to 10 percentage of nucleic acids and 8 to 30 percentage of lipids [32].

Algae has recently been considered to be the third-generation inexhaustible feedstock for a continual bio-hydrogen formation. Microalgae have a greater probability of photosynthetic effectiveness and fertility with less land area required for the survival. They have more probability to transform atmospheric CO₂ into different varieties of organic amalgam [33]. They give out germane sources as unprocessed material for the synthesis of bio-hydrogen. Several microalgae like *Chlorella*, *Scenedesmus*, *Anabaena*, *Synechocystis*, *Tetraspora* harbour and *Nostoc* hydrogenase for the generation of the fuel [34]. The procedures involved in production of bio-hydrogen from microalgae are Direct and Indirect Bio-Photolysis. Hydrogenases and Nitrogenases are the couple of enzymes, that are important in cellular machinery, which is accountable for production of bio-hydrogen in photosynthetic micro-algae [35]. Cyanobacteria and micro-algae undergo formation of Hydrogenase [36]. The structural variation, activity and enzyme maturation of these hydrogenases formed can differ according to the species involved [37]. The commercialized production of hydrogen is pliable only if high bio-hydrogen production effectiveness could be achieved at a low cost compared to the existing methods [38]. Photo-bioreactors are required for production of bio-hydrogen by industrial methods using micro-algae, that are specific for their cultivation [39]. The fundamental factors that influence the design of PBR are agitation, depth of tank, and right supply of light energy. Generally, the algae growth in commercialized scale is proceeded in open ponds [40].

2.3. Synthesis from rotten apple

Another alternative method for synthesis of bio-hydrogen has been discovered from rotten apple. It has been reported that around 10% to 15% of apples get rotten owing to growing, picking, transporting and storage [41]. Direct disposal has been found to create a lot of environmental pollution, hence most of them have been used in raising livestock and are also used in production of human food, which poses greater threat to life of living beings. It is because of these reasons, synthesis of renewable fuel such as bio-hydrogen has become very evident in yesteryear [42]. Catalyst used was found to be photo synthetic bacteria (PSB) HAU-MA1 for photo-fermentation process. The PSB is a mixture of three different bacterium, *Rhodospirillum rubrum*, *Rhodobacter capsulatus* and *Rhodopseudomonas palustris* cultured at a colorless bottle, which was inoculated at 20% [43].

Process Involved: The rotten apples were crushed and the juice was extracted. They were then sieved in order to maintain same size of particles. The setup consists of incubator where, the PSB is regulated and the hydrogen production reactor was placed inside it. An incandescent lamp is also placed for facilitating photo-fermentation. Gas purifier, gas collector and gas chromatograph are used for analysis of the gases emitted during the procedure [44].

Various parameters such as temperature, material to liquid ratio, light intensity and pH are varied in order to find out the maximum efficiency. It was reported that 110.5 mL/g of bio-hydrogen was produced when the light intensity was 3029 lx. The pH of the rotten apple was found to 7.14 while the material to liquid ratio was 0.21. The setup was maintained at 30.46°C [45]. This was the maximum amount of fuel synthesized from the devised setup. Fig. 1 depicts the process through which the fuel is extracted from rotten apple

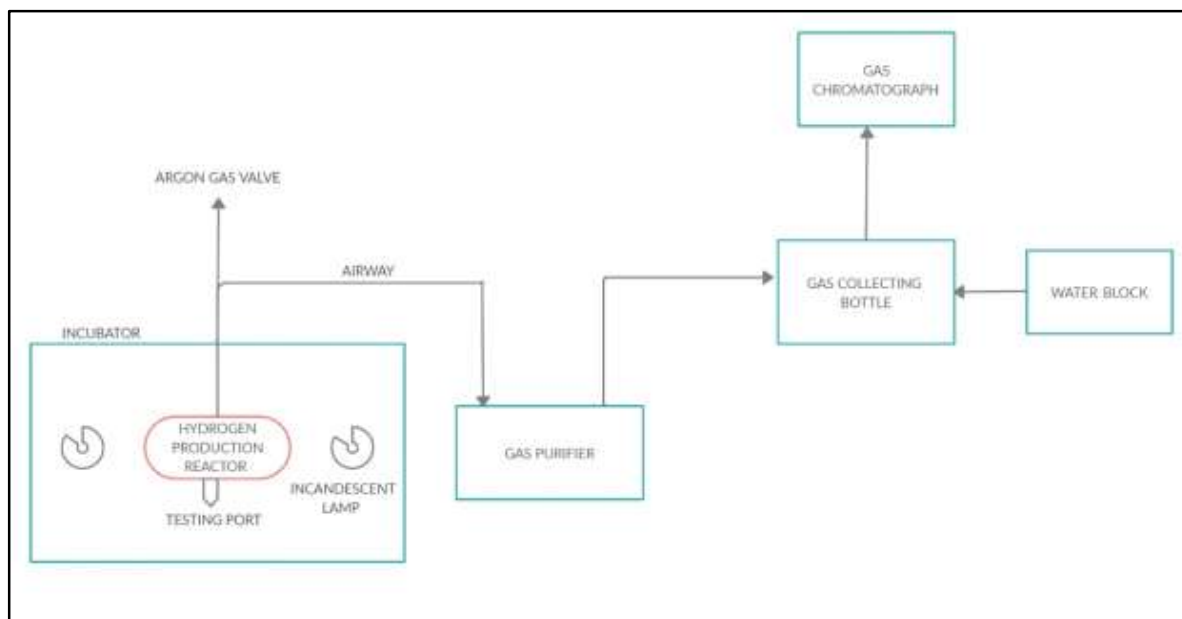


Fig. 1: Systematic representation of the process involved in extraction of bio-hydrogen from rotten apples

2.4. Synthesis from tequila vinasses

Similarly, Bio-hydrogen can also be produced tequila vinasses. Vinasses are the waste water remains after the first distillation during the production of tequila [46]. There are around 10-12 liters of vinasses produced per liter of tequila generated. This method was found be already in existence, but the output of bio-hydrogen produced was found to be less, due to which there are certain amount of nutrients expounded to the vinasses to increase the production of the fuel. Another important factor to be considered is that this method of production is not economically viable when it will be tried to fit in as an industrial setup [47].

Methodology: Two samples of vinasses were extracted. One was preserved at 4°C when centrifuged within 10 days of collection [48]. Another sample was filtered using 0.45 micrometer pore and was preserved at -15°C. The vinasses were then detoxified using activated charcoal, following which it is

stored in a freezer until at use. The nutrient mixture consists of ammonium chloride, Morpholineethanesulfonic acid, Magnesium Chloride, Iron Sulphate, Cobalt Chloride and Potassium Iodide. These salts are maintained at hydrated condition. The inoculum for dark fermentation process is powdered anaerobic green sludge [49]. All the components are mixed at a favorable ratio and are fermented [50]. The bio-hydrogen fuel is then filtered from this substrate, while the nutrients are re-used [51].

3. Conclusions

The reported pre-treatments efficiently break down corn straw composition and ameliorate the saccharification of enzymes' potential. The alkali-heat and pre-treatment depicted remarkable ascendancy in the reducing sugars release, and the largest concentration of sugar which can be reduced was found to be 23.07 gram per liter, which was validated under 2% of NaOH-Heat condition. The highest cumulative hydrogen production of 137.76 mL per gram TS was achieved from 2% of NaOH pre-treated corn straw, and 4% of NaOH-heat pre-treated corn straw had the lowest cumulative hydrogen production of 44.20 mL per gram TS.

It was also noted that photo fermentation process recorded the maximum production of bio-hydrogen among the existing process, while micro-algae serve as a dependable source for constant synthesis [5-9]. Rotten apples and tequila vinasses are alternative methods, which are not completely implemented but can be a great source for the future

References

- [1] IK Kapdan, F Kargi, "Bio-hydrogen production from waste materials"- Enzyme and microbial technology, 2006 – Elsevier
- [2] Yeqing Li, Fang Yan, Tao Li, Ying Zhou, Quan Xu, "High-solid anaerobic digestion of corn straw for methane production and pre-treatment of bio- briquette" Bioresource Technology Volume **250** February 2018, Pages 741-749
- [3] X Xu, MJ Antal Jr, "Gasification of sewage sludge and other biomass for hydrogen production in supercritical water" - Environmental progress, 1998
- [4] MF Arooj, SK Han, SH Kim, DH Kim, HS Shin, ."Continuous biohydrogen production in a CSTR using starch as a substrate"- journal of hydrogen energy, 2008 – Elsevier
- [5] S Wang, T Zhang, H Su , "Enhanced hydrogen production from corn starch wastewater as nitrogen source by mixed cultures" Renewable Energy, 2016 – Elsevier
- [6] P Khongkliang, P Kongjan, O Sompong, "Hydrogen and methane production from starch processing wastewater by thermophilic two-stage anaerobic digestion"- energy Procedia, 2015 – Elsevier
- [7] I Hussy, FR Hawkes, R Dinsdal , "Continuous fermentative hydrogen production from a wheat starch product by mixed microflora" - Biotechnology an, 2003 - Wiley Online Library
- [8] SD Chen, KS Lee, YC Lo, WM Chen, JF Wu, "Batch and continuous biohydrogen production from starch hydrolysate by Clostridium species"- Journal of Hydrogen, 2008 – Elsevier
- [9] SK Han, HS Shin, " Biohydrogen production by anaerobic fermentation of food waste" - International journal of hydrogen energy, 2004 - Elsevier
- [10] GA Weaver, JA Krause, "Cornstarch fermentation by the colonic microbial community yields more butyrate than does cabbage fiber fermentation; cornstarch fermentation rates correlate"-TL Miller,

1992 – academic.oup.com

- [11] F Vendruscolo, “Starch: a potential substrate for biohydrogen production” - International Journal of Energy Research, 2015
- [12] Tian Zhang, Danping Jiang, Huan Zhang, Doo-Jong Lee, Chenxi Xiad, “Effects of different pretreatment methods on the structural characteristics, enzymatic saccharification and photo-fermentative bio-hydrogen production performance of corn straw” Bioresource Technology Volume **30**, 4 May 2020 Article 122999
- [13] Danping Jiang, Xumeng Ge, Tian Zhang, Huiliang Liu, Quanguo Zhang, “Photo-fermentative hydrogen production from enzymatic hydrolysate of corn stalk pith with a photosynthetic consortium”, International Journal of Hydrogen Energy Volume **41**, Issue **3815** October 2016 Pages 16778- 16779
- [14] Yan-Chun Guo, Yang Dai, Yan-Xia Bai, Yan-Hong Li, Hong-Wei Hou, “Co-producing hydrogen and methane from higher-concentration of corn stalk by combining hydrogen fermentation and anaerobic digestion” International Journal of Hydrogen Energy Volume **39**, Issue **263** September 2014 Pages 14204-14211
- [15] Serena Croce, Qiao Wei, Giuliana D'Imporzano, Renjie Dong, Fabrizio Adani, “Anaerobic digestion of straw and corn stover: The effect of biological process optimization and pre-treatment on total bio-methane yield and energy performance” Biotechnology Advances Volume **34**, Issue **8** December 2016 Pages 1289-1304
- [16] Dong Li, Chenxi Jiao, Wei He, Zhiying Yan, Xiaofeng Liu, “Comparison of micro-aerobic and anaerobic fermentative hydrogen production from corn straw” International Journal of Hydrogen Energy Volume **41**, Issue **126** April 2016 Pages 5456-5464
- [17] Richen Lin, Chen Deng, Jun Cheng, Jerry D. Murphy, “Low concentrations of furfural facilitate biohydrogen production in dark fermentation using *Enterobacter aerogenes*” Renewable Energy Volume **150** May 2020 Pages 23-30
- [18] M Bao, H Su, T Tan,” Biohydrogen Production by Dark Fermentation of Starch Using Mixed Bacterial Cultures of *Bacillus* sp and *Brevumdimonas* sp.”- Energy & fuels, 2012 - ACS Publications
- [19] PR de la Piscina, N Homs,” Use of biofuels to produce hydrogen (reformation processes)” - Chemical Society Reviews, 2008 - pubs.rsc.org
- [20] Tian Zhang, Danping Jiang, Huan Zhang, Yanyan Jing, Quanguo Zhang, “Comparative study on bio-hydrogen production from corn stover: Photo-fermentation, dark-fermentation and dark-photo co-fermentation” International Journal of Hydrogen Energy Volume **45**, Issue **64** February 2020 Pages 3807-3814
- [21] H Hafez, G Nakhla, H El Nagggar, ” Biological hydrogen production from corn-syrup waste using a novel system”- Energies, 2009 - mdpi.com
- [22] ZMA Bundhoo,” Potential of bio-hydrogen production from dark fermentation of crop residues: a review”- International Journal of Hydrogen Energy, 2019 – Elsevier
- [23] JXW Hay, TY Wu, JC Jua,” Biohydrogen production through photo fermentation or dark fermentation using waste as a substrate: overview, economics, and future prospects of hydrogen usage” – Biofuels and Bioproducts, 2013 - Wiley Online Library.

- [24] B Fabiano, P Perego, “Thermodynamic study and optimization of hydrogen production by *Enterobacter aerogenes*” - International Journal of Hydrogen Energy, 2002 – Elsevier
- [25] Xin Mei Guo Eric Trably Eric Latrille H el ene Carr ere Jean-Philippe Steyer, “Hydrogen production from agricultural waste by dark fermentation: A review”
- [26] Rohit Datar Jie Huang, Pin-Ching, Maness Ali, Mohagheghi, Stefan Czernik, Esteban Chornet, “Hydrogen production from the fermentation of corn stover biomass pretreated with a steam-explosion process”- International Journal of Hydrogen Energy, 2007- Elsevier
- [27] Q Zhang, Z Zhang, Y Wang, DJ Lee, G Li, X Zhou, “Sequential dark and photo fermentation hydrogen production from hydrolyzed corn stover: A pilot test using 11 m³ reactor”- Bioresource, 2018 – Elsevier
- [28] G Davila-Vazquez, S Arriaga,” Fermentative biohydrogen production: trends and perspectives” - Science and Bio- Hydrogen Energy, 2008 – Springer
- [29] J. G. G. Jonker, A. P. C. Faaij, “Techno-economic assessment of micro-algae as feedstock for renewable bio-energy production”, Applied Energy Volume **102** February 2013 Pages 461-475
- [30] Amita Jacob, Ao Xia, Jerry D. Murphy, “A perspective on gaseous biofuel production from micro-algae generated from CO² from a coal-fired power plant”, Applied Energy Volume **148** 15 June 2015 Pages 396-402
- [31] MF Demirbas, “Biofuels from algae for sustainable development” - Applied energy, 2011 – Elsevier
- [32] Sara Pourkarimi, Ahmad Hallajisani, Asghar Alizadehdakhel, Amideddin Nouralishahi, “Biofuel production through micro- and macroalgae pyrolysis – A review of pyrolysis methods and process parameters”, Journal of Analytical and Applied Pyrolysis Volume **14** 2 September 2019, Article 104599
- [33] L. -F. Wu, M. A. Mandrand, “Microbial hydrogenases: Primary structure, classification, signatures and phylogeny FEMS Microbiology”, Letters Volume **104**, Issues **3** – 4 April 1993 Pages 243-269
- [34] Jude A. Onwudili, Amanda R. Lea-Langton, Andrew B. Ross, Paul T. Williams, “Catalytic hydrothermal gasification of algae for hydrogen production: Composition of reaction products and potential for nutrient recycling”, Bioresource Technology Volume **127** January 2013 Pages 72-80
- [35] A Sharma, SK Arya, “Hydrogen from algal biomass: a review of production process” - Biotechnology reports, 2017 - Elsevier
- [36] Y Li, M Horsman, N Wu, CQ Lan, “Biofuels from microalgae” - Biotechnology, 2008 - Wiley Online Library
- [37] JR Benemann,” Hydrogen production by microalgae”- Journal of applied phycology, 2000 – Springer
- [38] M Koller, A Muhr, G Braunegg,” Microalgae as versatile cellular factories for valued products” - Algal research, 2014 – Elsevier
- [39] Y Asada, J Miyake, “Photobiological hydrogen production” - Journal of Bioscience and Bioengineering, 1999 – Elsevier
- [40] PC Hallenbeck, JR Benemann, “Biological hydrogen production; fundamentals and limiting processes” International journal of hydrogen energy, 2002 – Elsevier

- [41] Chaoyang Lu, Zhiping Zhang, Xumeng Ge, Yi Wang, Quanguo Zhang, “Bio-hydrogen production from apple waste by photosynthetic bacteria HAU-M1”, *International Journal of Hydrogen Energy* Volume **41**, Issue **3117** August 2016 Pages 13399-13407
- [42] Dillirani Nagarajan, Duu-Jong Lee, Jo-Shu Chang, “Recent insights into consolidated bioprocessing for lignocellulosic biohydrogen production”, *International Journal of Hydrogen Energy* Volume **44**, Issue **2831** May 2019 Pages 14362-14379
- [43] V. Godvin Sharmila, S. Kavitha, Parthiba Karthikeyan Obulisamy, J. Rajesh Banu, “Chapter 8: Production of fine chemicals from food wastes”, *Food Waste to Valuable Resources 2020* Pages 163-188
- [44] Nazlina Haiza Mohd Yasin, Tabassum Mumtaz, Mohd Ali Hassan, Nor'Aini Abd Rahman, “Food waste and food processing waste for biohydrogen production: A review”, *Journal of Environmental Management* Volume **130** 30 November 2013 Pages 375-385
- [45] A Tenca, F Perazzolo, E Naldi, G Provolò,” Design and start-up of a two-stage farm-scale pilot plant for biohydrogen and biomethane production”-Energy, and biomass, 2012 - academia.edu
- [46] Manuel García-Becerra, Marcos Macías-Muro, Luis Arellano-García, Oscar Aguilar-Juárez, “Bio-hydrogen production from tequila vinasses: Effect of detoxification with activated charcoal on dark fermentation performance”, *International Journal of Hydrogen Energy* Volume **44**, Issue **606** December 2019 Pages 31860-31872
- [47] Alma Toledo-Cervantes, Francisco Villafán-Carranza, Jorge Arreola-Vargas, Elías Razo-Flores, Hugo Oscar Méndez-Acosta, “Comparative evaluation of the mesophilic and thermophilic biohydrogen production at optimized conditions using tequila vinasses as substrate”, *International Journal of Hydrogen Energy* Volume **45**, Issue **193** April 2020 Pages 11000-11010
- [48] B Budiyo, S Sumardiono,” Effect of total solid content to biogas production rate from vinasse” - *International Journal of Engineering*, 2014 - sid.ir
- [49] O García-Depraect, E León-Becerril,” Fermentative biohydrogen production from tequila vinasse via the lactate-acetate pathway: Operational performance, kinetic analysis and microbial ecology”- *Fuel*, 2018 - Elsevier
- [50] G Buitrón, D Prato-García, A Zhang,” Biohydrogen production from tequila vinasses using a fixed bed reactor”- *Water science and technology*, 2014 - iwaponline.com
- [51] “Upgrading of anaerobic digestion of tequila vinasse by using an innovative two-stage system with dominant lactate-type fermentation in acidogenesis”.