TÉCNICO LISBOA

Biohydrogen pathways – An energy comparative review of production pathways

Natalia Buczak

Thesis to obtain the Master of Science Degree in

Energy Engineering and Management

Supervisors: Dr. Ana Filipa da Silva Ferreira

Dr. João Filipe Pinto Ribau

Examination Committee

Chairperson: Prof. Edgar Caetano Fernandes

Supervisor: Dr. Ana Filipa da Silva Ferreira

Member of the committee: Prof. Eurico Vasco Ferreira Amorim

November 2019

Acknowledgements

I would like to sincerely thank my supervisors Doctor Ana Filipa da Silva Ferreira and Doctor João Filipe Pinto Ribau for tremendous support during difficult times and few obstacles along the way. I would also like to thank my family for support and faith in me. I would like to dedicate this thesis to my beloved grandmother.

ABSTRACT

The environmental consequences of using fossil fuels-based energy systems and the rapid growth of global energy demands are currently the major concerns of sustainable development in the world. Therefore, the alternative, renewable and sustainable energy sources are in the need of extensive development and commercialisation. Hydrogen has been pointed out as a potential solution and energy carrier in the future global clean energy systems. Moreover, the biological hydrogen production is a promising technology that may bypass some of the disadvantages of conventional hydrogen production, such as fossil resources dependency or costly processes (e.g. natural gas reforming or electrolysis). Although being a promising solution, as every innovative technology, biohydrogen production is facing technological and economical challenges in terms of industrial scale application. The goal of this research is to identify and evaluate bottlenecks fulfilment occurring in biohydrogen production technology in order to present clear, wide and bright perspective of biohydrogen as a green fuel of the future. Aspects such as biomass feedstock, different methods of obtaining biohydrogen as well as biohydrogen economy, energy consumption and CO_2 emission of the production processes were reviewed and assessed in a life cycle perspective.

Although biohydrogen presents great improvements comparatively to conventional hydrogen sources (and conventional fuels), the production technology still needs further research and development in terms of cost-effectiveness and technological limitations mitigation for an industrial-scale production, in a financially viable way and with profitable yields.

Overall, biohydrogen appears as the ideal fuel of the future due to the highest energy content than any other known fuel, being less energy intensive due to ambient temperatures and pressures use in biohydrogen production processes, being environmentally friendly not only due to no contribution to greenhouse gas emissions and therefore no impact on climate change but also waste treatment contribution by using waste as the substrate.

Keywords: biohydrogen; microalgae; energy; biophotolysis; fermentation; life-cycle

Resumo

As consequências ambientais do uso de sistemas de energia, baseados em combustíveis fósseis e o rápido crescimento da procura global de energia, são atualmente as principais preocupações do desenvolvimento sustentável no Mundo. Portanto, as fontes de energia alternativas, renováveis e sustentáveis, precisam de desenvolvimento e comercialização extensivos. O hidrogénio tem sido apontado como uma solução potencial como fonte de energia nos futuros sistemas globais de energia limpa. Além disso, a produção biológica de hidrogénio é uma tecnologia promissora que pode contornar algumas das desvantagens da produção convencional de hidrogénio, como a dependência de recursos fósseis ou processos dispendiosos (por exemplo, reformação de gás natural ou eletrólise). Embora seja uma solução promissora, como toda a tecnologia inovadora, a produção de biohidrogénio enfrenta desafios tecnológicos e económicos em termos de aplicação em escala industrial. O objetivo deste trabalho é identificar e avaliar os principais problemas ocorridos em toda a cadeia de produção do biohidrogénio, de modo a apresentar uma perspetiva clara e ampla do biohidrogénio como combustível 'verde' do futuro. Foi feita uma extensa revisão e avaliação sobre a produção de biohidrogénio considerando a matéria-prima (biomassa), as diferentes tecnologias de conversão e os consumos de energia e emissões de CO_2 durante toda a cadeia de produção, numa perspetiva de ciclo de vida. Embora o biohidrogénio apresente vantagem comparativamente às fontes convencionais de hidrogénio e combustíveis convencionais, a tecnologia de produção ainda necessita de mais investigação e desenvolvimento em termos de custo-eficiência e redução de limitações tecnológicas para uma produção em escala industrial, para se tornar economicamente viável e com rendimentos lucrativos. No geral, o biohidrogénio aparece como o combustível do futuro devido ao seu elevado teor de energia, consumindo menos energia devido às temperaturas e pressões ambiente utilizadas em certos processos de produção de biohidrogénio. É um combustível ecológico, não só pelo facto de não emitir gases de efeito de estufa aquando da sua utilização, mas também pelo facto de utilizar resíduos como substrato.

Palavras-chave: biohidrogénio; microalgas; energia; biofotólise; fermentação; ciclo da vida

Table of Content

List of Figures

List of Tables

List of Abbreviations

Acetyl-CoA	Acetyl coenzyme A
ACK	Acetyl coenzyme A kinase
ADH	Alcohol dehydrogenase
ADP	Adenosine diphosphate
AFBR	Anaerobic fluidised bed reactor
AnGSB	Anaerobic granular sludge bed
ATP	Adenosine triphosphate
BCaLG	Biomass-based calcium looping gasification
BCCLP	Biomass-based-co-production looping process
BCLG	Biomass chemical looping gasification
BCLP	Biomass chemical looping process
BES	Bio-electrochemical system
Btu	British thermal unit
CAGR	Compound annual growth rate
CEM	Cation exchange membrane
CLP	Chemical looping process
COD	Chemical oxygen demand
COP21	21st Conference of the Parties
COT	Carbon organic total
CSTR	Continuous stirred tank reactor
EDTA	Ethylenediaminetetraacetic acid
EGSB	Expanded granular sludge bed
F/M ration	Food-to-microorganism ratio
FAME	Fatty acid methyl ester
FBR	Three-phase fluidised bed reactor

Fd_{ox}	Oxidised ferredoxin
Fd_{red}	Reduced ferredoxin
FHL	Formate hydrogen lyase
GDP	Gross domestic product
GHG	Greenhouse gas
Gt	Giga tonnes
HRT	Hydraulic retention time
IBRCS	Integrated biohydrogen reactor clarifier system
LCA	Life cycle assessment
LCI	Life cycle inventory
LDH	Lactate dehydrogenase
MEC	Microbial electrolysis cell
MFC	Microbial fuel cell
non-OECD	Non-Organisation for Economic Co-operation and Development
NREL	National Renewable Energy Laboratory (USA)
NT	Nitrogen total
OC	Oxygen carrier
OECD	Organisation for Economic Co-operation and Development
PFL	Pyruvate Formate lyase
PFOR	Pyruvate ferredoxin oxidoreductase
Pi	Inorganic phosphate
PNS	Purple non-Sulphur
POME	Palm oil mill effluent
PSA	Pressure Swing Adsorption
R&D	Research & Development
SE	Soxhlet
SFE	Supercritical fluid extraction
SRT	Solids retention time

TSS	Total suspended solids
UASB	Upflow anaerobic sludge bed reactor
USD	United States Dollar
VFA	Volatile fatty acids
VSS	Volatile suspended solids
WGS	Water gas shift
WTT	Well-to-Tank analysis

1. Introduction

1.1. Energy context

Today we – as a global society – are facing severe energy crisis. The problem is extremely complex in its nature with many uncertainties and variables such as economic, environmental and political. In terms of environmental impacts, the modern world is facing climate change problems with global warming being the biggest concern. Unless the energy system changes in almost every aspect, the greenhouse gases emitted at the current rate will lead to average global temperature increase of around 4°C. Raising sea levels, climate zones shift as well as more and more frequently occurring extreme weather conditions and droughts would be consequences of such situation.

It is projected that global climate will be affected by current global energy situation as close as in the coming 50 to 100 years (1). In December 12, 2015 during United Nations climate change conference in Paris, 195 countries agreed and sign a legally binding agreement that aims at keeping global warming well below 2°C. The goals are ambitious and current efforts and plans agreed during COP21 are insufficient. With global CO_2 emissions of 37.1 Gt per year in 2018, the limit will be reached by 2050 (2). Figure 1.1 shows how drastically CO_2 emissions need to decrease in every sector in order to meet the effort of keeping the global temperature rise below 2 °C.

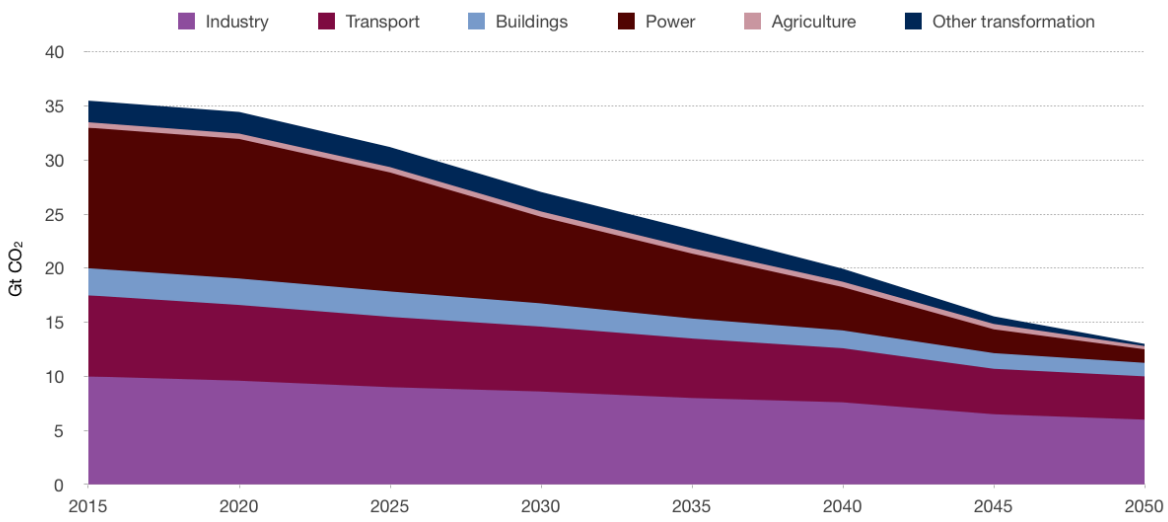


Figure 1.1 - Energy-related CO_2 emissions by sector under 2DS modelling. Adapted from (3).

Nowadays, fossil fuels account for 82% of primary energy consumption, renewable sources for 14% and nuclear resources for 4%. The population growth is forecasted to increase by 2050 from 7.3 billion to over 9 billion people, this with global economic growth will result in 50% increase in fuel demand (4). Population growth and GDP will affect the energy demand increase by 16%. Figure 1.2 shows that the worldwide energy consumption is expected to rise by 28% between 2015 and 2040 with most of the

increase by 41% occurring in non-OEDC countries due to strong economic growth, rapidly growing populations and increased access to marketed energy (5). In comparison energy consumption is expected to increase by 9% in OEDC countries. The increase of energy consumption is forecast to reach 663 quadrillion Btu by 2030 and 736 quadrillion Btu by 2040 (figure 1.2).

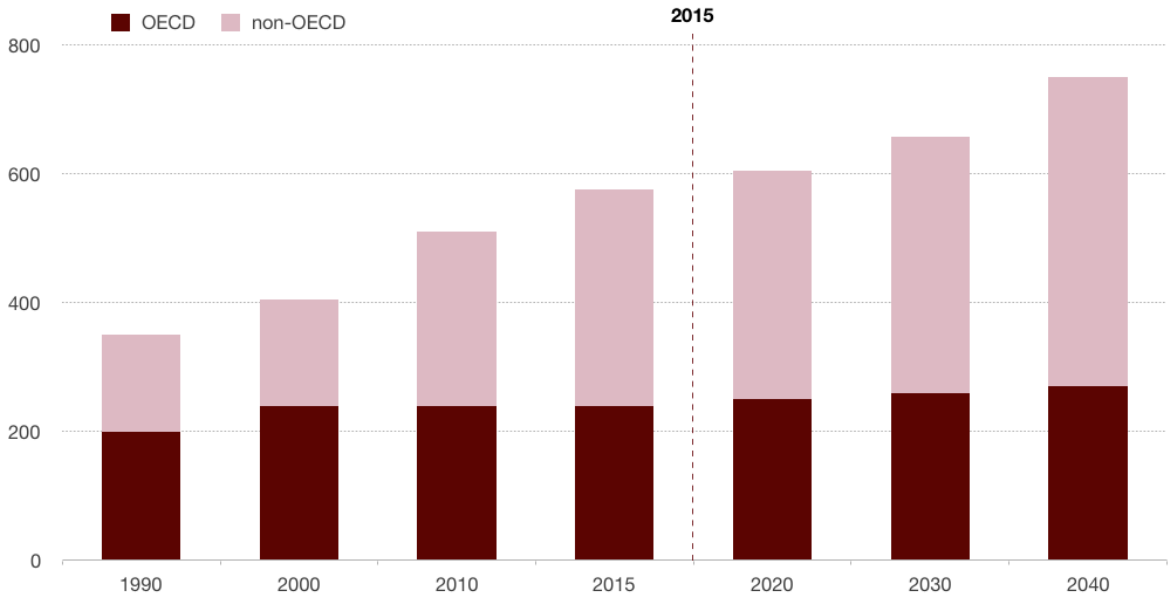


Figure 1.2 - World energy consumption. Adapted from (5).

Renewables resources are expected to increase their share in energy mix by 3 to 5 times the current amount (1). Figure 1.3 presents world energy consumption by source, as it can be seen, energy consumption is projected to increase for all fuels apart from coal. Even though renewables are the fastest growing energy source, fossil fuels are expected to continue meet most of world's energy demand (5). As we can see, petroleum and other liquid fuels will remain the largest source of energy, it is expected to decline in electric power sector but increase in transportation and industry sectors.

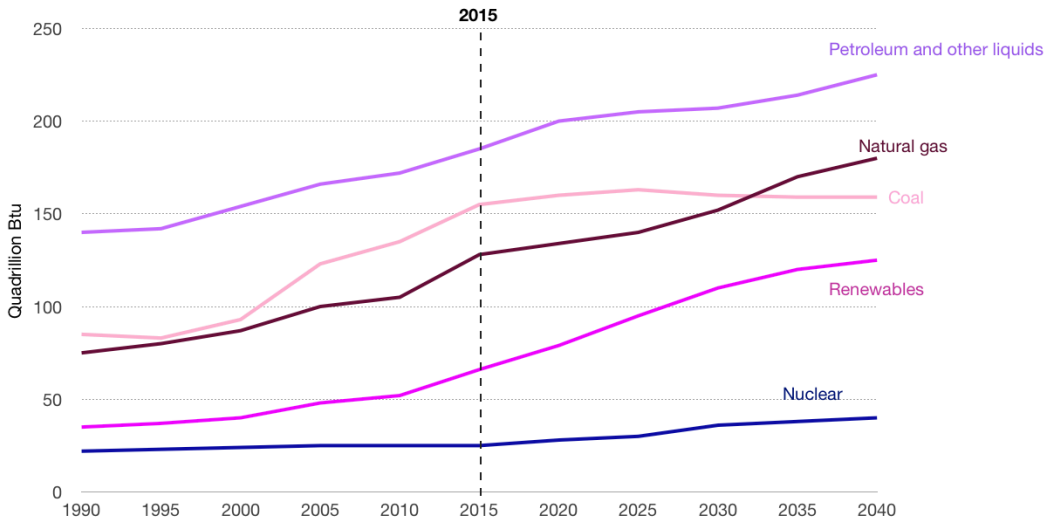


Figure 1.3 - World energy consumption by energy source. Adapted from (5).

In terms of petroleum and other liquid fuels, consumption is expected to grow by 18% between 2015 and 2040 mostly due to growth in non-OECD countries, which is presented in figure 1.4. Non-OECD Asia will contribute in more than 80% of total increase of liquid fuels consumption due to increased demand for transportation and rapid industrial growth in China and India. Liquid fuels consumption will grow slowly or decrease in OECD countries between 2015 and 2040.

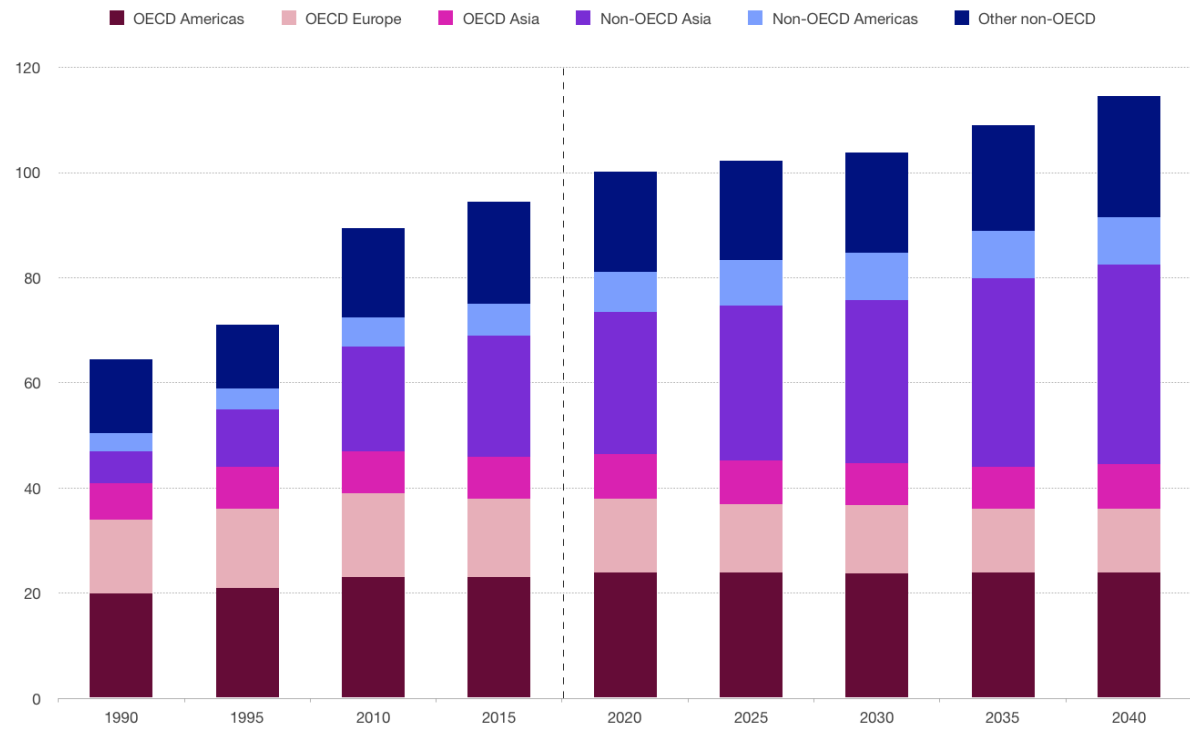


Figure 1.4 - Petroleum and other liquid fuels consumption. Adapted from (5).

Even though the general consumption is projected to increase, the share of liquid fuels holds relatively constant across sectors. Transportation sector will remain the largest consumer of petroleum and other liquid fuels which is shown in figure 1.5.

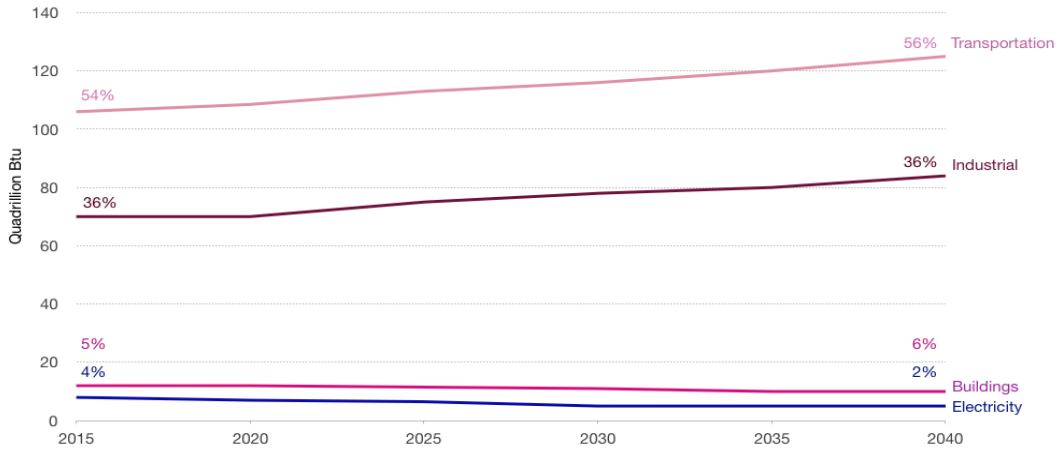


Figure 1.5 - Refined petroleum and other liquid fuels consumption by end-use sector. Adapted from (5).

On the basis of the aforementioned, major reduction in fossil fuels dependency, GHG emission mitigation and decarbonization of liquid fuels become primary issues in today's global energy system. Biofuels concept comes up as a very promising solution for this issue, especially for the decarbonization of the transportation sector. Therefore, great efforts in biofuels research and development are in demand in order to allow biofuels to play a significant role in meeting the goals of COP21 agreement. The importance of biofuels and their development will be discussed more widely in the next chapter.

1.2. Development of biofuels resources

Major drivers for biofuels development are the urgent need for decarbonization of transport sector and reduction in oil dependency. Biofuels have potential of change to low-carbon, non-petroleum fuels infrastructure without major changes in vehicle stocks and distribution. Biofuels will need to play significant role in liquid fossil fuels replacement especially for planes, marine vessels and any other heavy transport that cannot be electrified. In figure 1.6 social, economic and environmental aspects of biofuel and bioenergy production are presented.

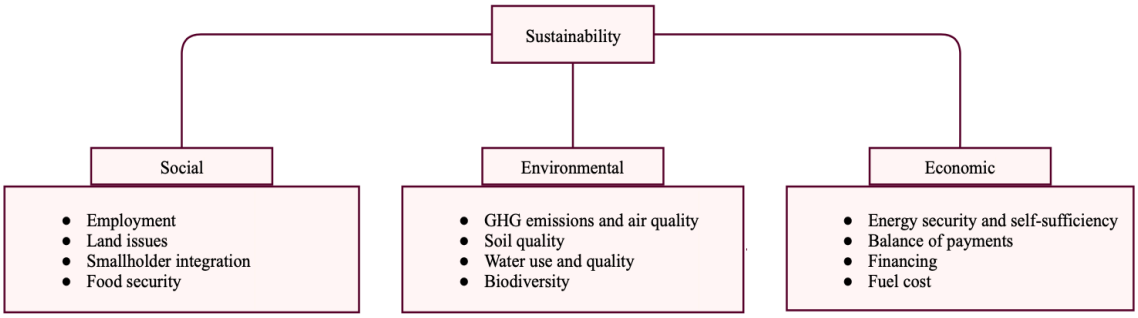


Figure 1.6 - Environmental, social and economic aspects of bioenergy production. Adapted from (6).

Biofuels are liquid or gaseous fuels derived from organic matter. Although the biofuels classification is debatable, the most common classification is biofuels division into 1st, 2nd and 3rd generation. In order to address facing energy related issues, biofuels appear as promising alternative energy carriers towards sustainable clean future of energy (6). The uniqueness of biomass among renewable energy sources is its capability to be converted directly into liquid fuels which are named as biofuels. This ability is especially crucial for transportation sector. Conventional biofuels technologies (1st generation) are well established processes that already operate on industrial scale (7). Biofuels like sugar and starch-based bioethanol, oil-crop based biodiesel and biogas for anaerobic digestion process (8). Advance biofuels technologies (3rd generation) are conversion technologies in pilot or demonstration phase that need more research and development (R&D), for example algae-gases biofuels (9). Figure 1.7 presents the current commercialisation status of main biofuel technologies.

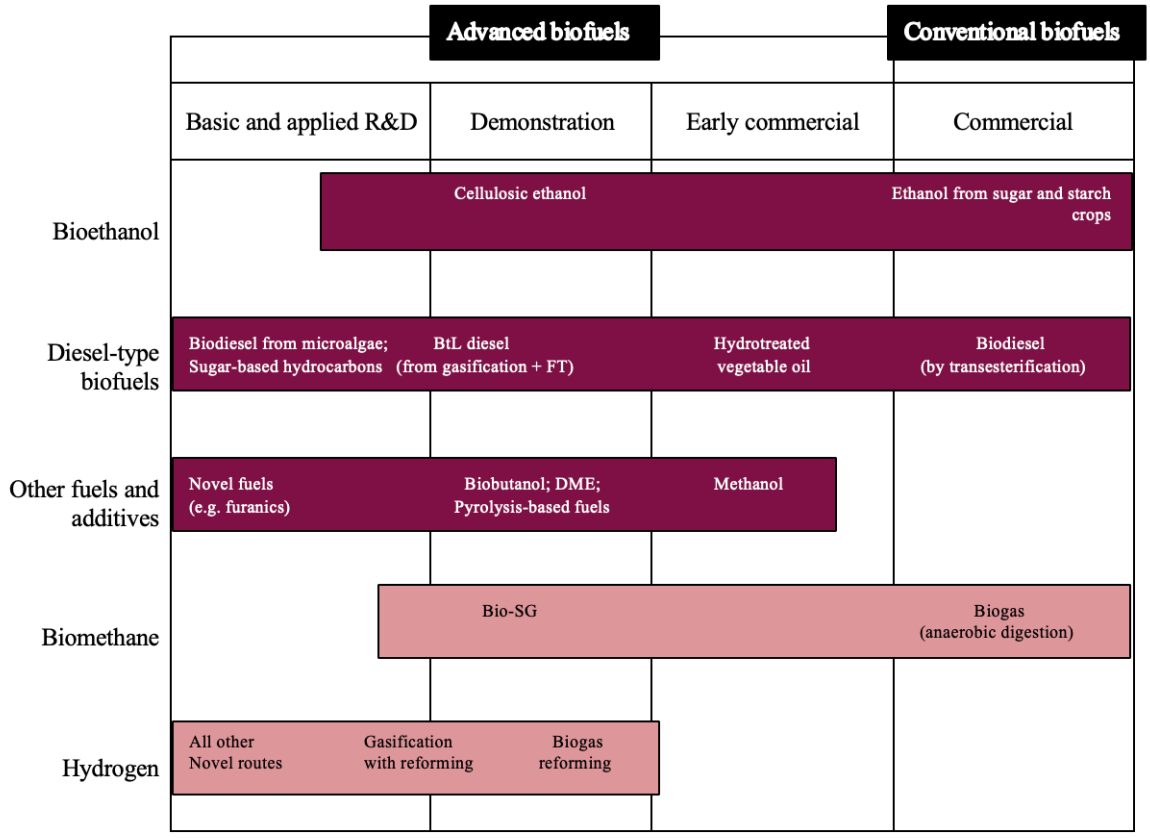


Figure 1.7 - Commercialisation status of main biofuel technologies, where BtL: Biomass-to-Liquids; FT: Fisher-Tropsch; DME: Dimethylether; Bio-SG: Bio-synthetic gas. Adapted from (6).

Conventional, as well as advanced, biofuels need development and further improvements. In both cases, conversion efficiency improvements are needed that will lead to better economics, higher land-use efficiency and better environmental performance. Advanced biofuels need to reach commercial scale rapidly in order to meet the ambitious goals of sustainable clean energy strategies. Along with conversion efficiency improvements, development of strategies for capital requirements reduction are needed. Those strategies should include integration of all process steps along whole supply chain (6). In terms of advanced biofuels specific R&D needs are crucial in order to prove industrial reliability, technical performance and operability of conversion pathways. It is very important to prevent market uncertainties by development of biofuel infrastructure like feedstock supply, conversion and end-use related infrastructure, public discussion on biofuels needs, biofuel support policies. In order for biofuels to become competitive with fossil fuels the economics of conversion processes needs improvements. This is crucial for biofuels to be widely used. Therefore, governments are obligated to create differential tax systems to reflect differing external costs of different fuels.

As it can be observed in Figure 1.7, hydrogen is least developed biofuel out of the main biofuels technologies presented as the interest in hydrogen as a potential fuel started only in the early 90's. Since then, hydrogen emerged as one of the most preferential alternative fuel for the future due to its cleanness, environmental benefits (no GHG emissions contribution, acid rain or ozone depletion and no other

environmental impacts) and high energy efficiency as hydrogen exhibits the highest energy content of 142 kJ/g or 61,000 Btu/lb (10). Due to hydrogen remarkable benefits as a fuel, it is of a great importance to put the emphasis on research and development of hydrogen or more importantly on biohydrogen technology. In the next chapters, biohydrogen technologies, resources, technological and economic limitations will be presented.

1.3. **Scope**

This work aims to provide an energy comparative insight of biohydrogen production pathways. An extensive literature review was made to this purpose. When it comes to biohydrogen production, development of the boost in discussion and clarification of its big advantages as well as awareness of its limitations and perspectives in overcoming these limitations is very much needed. Realisation of enormous potential of biological hydrogen as promising fuel and energy carrier, in order to meet global agreements regarding sustainable future of clean energy, is of much importance. Especially since in recent years we could observe minor decrease of interest in developing biohydrogen production technologies probably due to complexity of these processes as well as insufficient market and political regulations regarding biofuels implication in world's energy system structure. Limited available data of large-scale plants and therefore immense amount of research and development still needed in this area could also contribute to this slight backdown. Working with limited data and many uncertainties is always hard hence the common saying, "The hardest part is the start." The uniqueness of this work is its coverage of wide range aspects regarding biohydrogen technology. The motivation behind this thesis was identification and fulfilment of the gaps occurring in biohydrogen production field in order to present clear, wide and bright perspective of biohydrogen as a fuel of the green sustainable future. The life cycle analysis methodology was included in the review to evaluate each pathway of the hydrogen production through biological conversion process using different feedstocks.

1.4. Document structure

The present Master Thesis structure is composed by: Introduction, Biohydrogen, Resources for biohydrogen production, Biohydrogen technology, Results and comparisons of available data, Discussion, Conclusions and Future Developments. An outline of each chapter is presented below:

1st chapter: Introduction is subdivided in three sub-chapters where firstly current complexity of global energy situation is described, secondly possible solutions as an aim of this thesis are proposed, thirdly objectives and motivation for the thesis are presented and the structure ensemble of the thesis.

2nd chapter: The aim of this chapter is introduction of hydrogen as chemical compound as well as its potential as promising clean energy carrier. Social, economic and environmental benefits of hydrogen as a fuel such as: no contribution to climate change, reduction of greenhouse gas emissions, lack of adverse combustion products etc. Hydrogen technology limitations and hydrogen applications are also addressed. Current hydrogen production situation is presented. Afterwards biohydrogen is introduced as carbon-neutral fuel and more environmentally-friendly alternative to conventional hydrogen. The difference between biological hydrogen and conventional hydrogen is explained, which stands for the differences between pathways of hydrogen production and used resources for its production. Methods of biohydrogen and conventional hydrogen production are distinguished. Lastly current biohydrogen production developments are introduced in the world and in Europe.

3rd chapter: Resources for biohydrogen production: term biomass and its specification are introduced. Bioenergy in a form of biofuels can be produced from biomass and we can distinguish first-, second- and third-generation biofuels. This division is explained with introduced examples as well as short characterisation for all three types. Main criteria in raw material selection for hydrogen production are disclosed. Best theoretical raw materials for biohydrogen production are discussed. After the introduction, most promising resources of biohydrogen are discussed: microalgae, waste and wood. Their specifications, advantages and disadvantages in biohydrogen production application are presented.

4th chapter: Biohydrogen technology: there are many technologies for obtaining biohydrogen production. They can be divided into two groups thermochemical conversion of biomass-based biohydrogen and biological conversion. Thermo-chemical processes include biomass pyrolysis, biomass gasification and supercritical water gasification as the most favourable for biomass utilisation. Among biological processes we can distinguish light and dark fermentation, direct and indirect biophotolysis and biological water gas shift reaction. All mentioned processes are explained with their specifications, advantages and limitations as well as chemical pathways.

5th chapter: Results and comparison of available data: The aim of this chapter is comparison of different data available in terms of energy needed for production of biohydrogen as well as associated CO_2 emissions. Some figures and numbers are introduced in order to illustrate the actual perspectives of biohydrogen production potential.

6th chapter: Summary and Discussion regarding biological hydrogen production. The goal of this chapter is summarised on what was said before as well as discussion and an attempt to evaluate the best method at the present state.

7th chapter: Conclusions and future developments regarding comprehensive biohydrogen production potential, its obstacles are discussed.

8th chapter: Future work needed and my future contribution in the research and development in the field are discussed.

2. Biohydrogen

2.1. What is biohydrogen

Hydrogen name origins from Greek: “hydro” and “genes” which means water generation and was named by Antoine Laurent de Lavoisier who found out the gas produces water when burnt (11). Hydrogen is the most common element on Earth, it doesn’t occur in elemental form but rather in molecular forms such as water or organic compounds (12). Hydrogen is odourless, colourless and tasteless gas sparingly soluble in water. The atomic weight of hydrogen is 1.00797 and its atomic number is one which makes it first on the periodic table and it has 1s1 electronic configuration. There are three isotopes of hydrogen: 1H1 (hydrogen), 2H1 (deuterium) and 3H1 (tritium) with 1,2 and 3 atomic weight respectively. Hydrogen is the lightest element on the periodic table with density of 0.0695 with respect to air (11). Dihydrogen molecule consists of two atoms where nuclei in both atoms are spinning and depending on the spin two types can be distinguish: ortho hydrogen where nuclei spins are in the same direction and para hydrogen where nuclei spins are in the opposite direction. The equilibrium mixture of ortho and para hydrogen constitutes normal hydrogen. Physical properties for hydrogen are listed in table 2.1.

Table 2.1 - Physical properties of hydrogen (11).

	Unit	n-Hydrogen
Triple point		
Temperature	K	13.957
Pressure	KPa	7.2
Density (solid)	kg/m^3	86.71
Density (liquid)	kg/m^3	77.21
Density (vapour)	kg/m^3	0.130
Boiling point	(101.3Kpa) K	20.39
Heat of vaporization	J/mol	899.1
Liquid phase		
Density	kg/m^3	70.96
C_p	J/mol/K	19.7
C_V	J/mol/K	11.6
Enthalpy	J/mol	548.3
Entropy	J/mol/K	34.92
Viscosity	m×Pa×s	13.3×10^{-3}
Velocity of sound	m/s	1101
Thermal conductivity	W/m/K	100×10^{-3}
Compressibility factor	-	0.01698
Gaseous phase		
Density	kg/m^3	1.331
C_p	J/mol/K	24.60
C_V	J/mol/K	13.2
Enthalpy	J/mol	1447.4
Entropy	J/mol/K	78.94
Viscosity	m×Pa×s	1.11×10^{-3}
Velocity of sound	m/s	357
Thermal conductivity	W/m/K	16.5×10^{-3}
Compressibility factor	-	0.906
Critical point		
Temperature	K	33.19
Pressure	KPa	1.325
Density	kg/m^3	30.12
Properties at STP	(273.15K, 101.3 Kpa)	
Density	kg/m^3	0.0899
C_p	J/mol/K	28.59
C_V	J/mol/K	20.3
Viscosity	m×Pa×s	8.34×10^{-3}
Velocity of sound	m/s	1246
Thermal conductivity	W/m/K	173.9×10^{-3}
Compressibility factor	-	1.00042
Dielectric constant	-	1.000271
Prandtl number	-	0.680

Extensive physical characteristics of hydrogen are shown in Table 2.2.

Table 2.2 - Combustion and explosion properties of hydrogen (11).

Properties	**Units**	**Hydrogen**
Density at STP	kg/m^3	0.084
Heat of the vaporization	J/g	445.6
Lower heating value	kJ/g	119.93
High heating value	kJ/g	141.8
Thermal conductivity at std. condition	mW/cm/K	1.897
Diffusion coefficient in air at std. condition	cm^2s	0.61
Flammability limits in air	vol%	4.0-75
Detonability limits in air	vol%	18.3-59
Limiting oxygen index	vol%	5.0
Stoichiometry composition in air	vol%	29.53
Minimum energy of ignition in air	Mj	0.02
Auto ignition temperature	K	858
Flame temperature in air	K	2318
Maximum burning velocity in air at std. condition	m/s	3.46
Detonation velocity in air at std. condition	km/s	1.48-2.15
Energy of explosion mass related g TNT	g	24.0
Energy of explosion volume related g TNT	m^3 STP	2.02

Interest in hydrogen as a potential fuel started in the early 90's. Hydrogen as a fuel has many social, economic and environmental benefits. Those benefits include:

- Reduction of fossil fuels use as an energy carrier.
- Reduction of greenhouse gas emissions, if produced from renewable sources.
- No contribution to climate change, if produced from renewable sources.
- Hydrogen can serve as long-term carbon-free seasonal storage medium.
- Increase of renewable raw materials share in the overall raw material balance.
- Increase in energy security at local and country levels.
- Lack of adverse combustion products (water as a final product of combustion).
- Hydrogen can be centralized or decentralized source of primary or backup energy.
- The biofuels use in line with sustainable development policy.
- Energetic conversion of high efficiency hydrogen in fuel cells (on level of 45-60%).
- Organic waste recycles into more environmentally-friendly product.

However, transition to hydrogen energy-based economy is burden with many technical and technological challenges, for instance the fundamental challenge of obtaining hydrogen by biological routes is the urgent need to increase the efficiency of these transformations, from the cost-effective point of view this efficiency needs to be significantly increased. This is possible by processing the substrates used for biohydrogen production to make them more readily accessible microorganisms involved in the process. Among other obstacles we can name (1):

- The absence of mechanisms to mitigate as well as share the long-term risks of the initial large-scale investments.
- Lack in action coordination among stakeholders.
- Lack of fair economic treatment of a developing technology.
- Insufficient recognition of hydrogen importance for the energy transition.
- Limited standards of technology to drive economies of scale.

Acquiring hydrogen by biological pathway in comparison with other methods seems to be the most economically justified method. Therefore, most studies focus on hydrogen production using microorganisms.

Hydrogen is a key substrate in the industry with wide range of applications. Today hydrogen is produced in the industry as chemical feedstock for production of chemicals within the most important is ammonia manufacturing and methanol production. Hydrogen makes also a key feedstock in petroleum reforming processes, such as hydrocracking of heavy petroleum fractions among many, as well as in petrochemicals production. By 2023, the petroleum reforming sector is expected to dominate the hydrogen production market (13). Figure 2.1 pictures share of hydrogen in the industry.

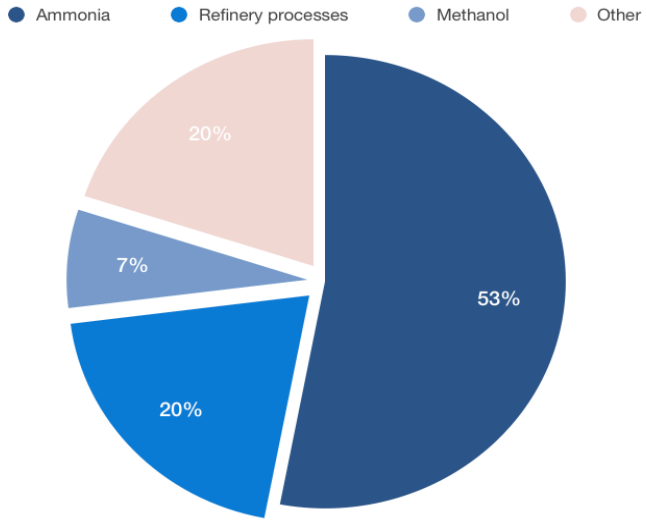


Figure 2.1 - Share of hydrogen in industry. Adapted from (14).

2.2. Hydrogen production: World vs. Europe

Hydrogen production market size was valued at 135.5 Billion USD worth in 2018 and growing at a CAGR of 8.0%, it is forecasted to be worth USD 199.1 Billion by 2023 (13). The main factors driving global hydrogen production market are mainly rising demand for hydrogen as a transportation fuel and government's regulations for desulfurization of petroleum products (15). Relatively constant growth of hydrogen generation market in recent years is associated with increasing demand of hydrogen in oil and gas refineries as well as growing demand for fuel cells in transportation and power generation sectors (13). Hydrogen annual production is estimated at 50 millions tones for the 2015 year (14). Currently global hydrogen production exceeds 1 billion m^3 per day (16). Global utilization of hydrogen is expected to increase more than 300 billion m^3 through 2019 with annual growth rate of 3.5%. The highest hydrogen consumption market is expected to continue in USA although the maximum share of growth in 2019 is most likely to occur in China. Asia Pacific is estimated to be the largest hydrogen production market from 2018 to 2023 (13). The highest number of scientific papers published about biohydrogen belongs to China followed by USA and India (figure 2.2). Moreover, from published papers we can observe certain trends in interest depending on the regions such as Asian countries seems to focus more on dark fermentation process while European countries tend to evaluate dark and photofermentation processes.

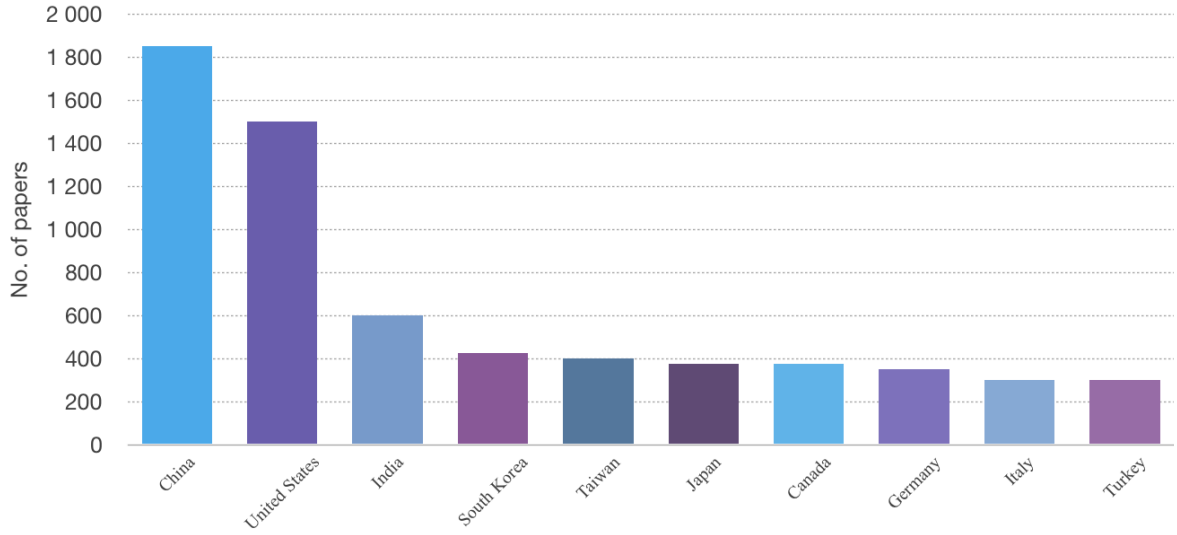


Figure 2.2 - Top ten countries publishing research on biohydrogen. Adapted from (16).

As we can see in figure 2.3 since 2003, we experience major boost in exploration and evaluation in biohydrogen production field.

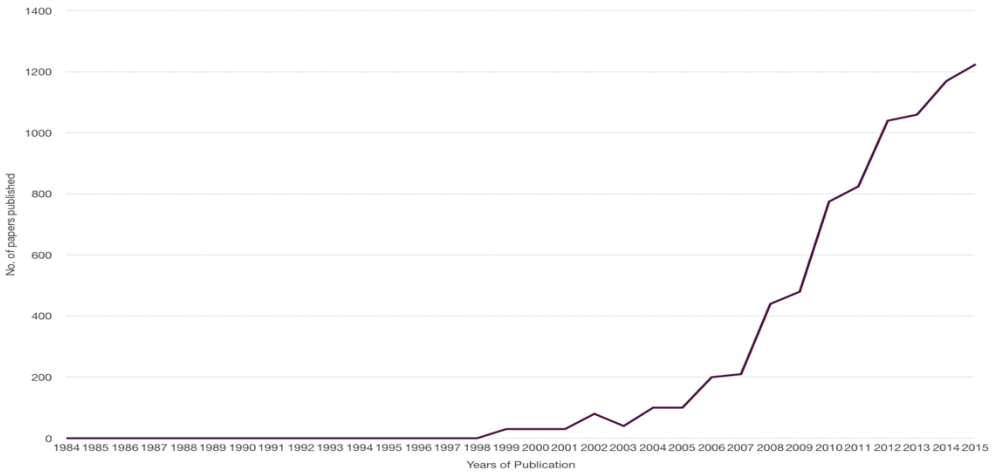


Figure 2.3 - Number of papers published on biohydrogen. Adapted from (16).

Middle East is projected to be the fastest growing market for hydrogen production due to raising demand for ultra-low-sulphur diesel fuel in Saudi Arabia, Iran and Qatar. Figure 2.4 shows the projected hydrogen production market by region in 2022.

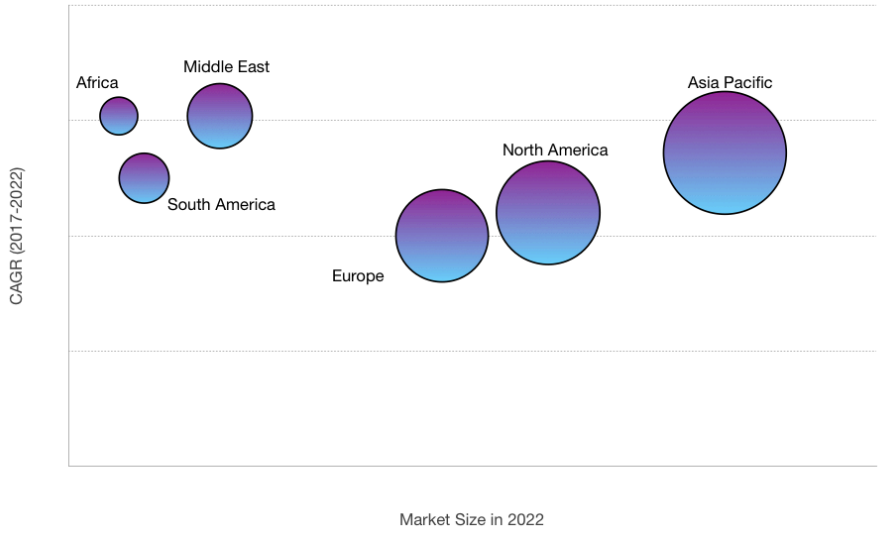


Figure 2.4 - Hydrogen generation market by region in 2022 (USD billion). Adapted from (15).

Tables 2.3, 2.4 and 2.5 show current attempts and research on biohydrogen production in European countries. As we can observe researchers used variable organic substrates and chose dark fermentation as favourable method of obtaining biohydrogen.

Table 2.3 - Current status for biohydrogen production in European countries (16)

Country	Substrate	Technology	Selected microbial utilisation	Hydrogen production
Ireland	Glucose and mannitol (volatile solid)	Co-fermentation	Microalgae (Laminaria digitata)	85.0 mL/g VS
UK	Food waste or wheat feed	Anaerobic digestion	-	84.2 L H_2 kg^{-1}
Italy	Food waste	Dark fermentation (DF), photofermentation (PF), and anaerobic digestion (AD)	Rhodobacter sphaeroides	1.75-fold
France	Buffalo slurry co-fermentation with cheese whey and crude glycerol	Mixture design	Microbial community (F210)	117 mL H_2/g VS
Sweden	$BG11_0$ medium	Photofermentation	ΔhupW strain of Nostoc PCC 7120 (filamentous heterocystous)	4.85 mL $H_2L^{-1}h^{-1}$
Turkey	Acetate VSS (volatile suspended solid)	Photofermentation processes	Rhodobacter capsulatus DSM 1710	1.04 mmol/$L_{reactor*h}$
Portugal	Sugars and small amounts of furfural and HMF (hydroxymethylfurfural)	Sequential batch fermentation	Spirogyra biomass and the subsequent fermentation by Clostridium butyricum DSM 10702	2.59 mol/mol
Turkey	Effluent of sugar beet thick juice	Batch and continuous photobioreactors	Mutant strains of rhodobacter capsulatus	34% (0.49 mmol/(Lch)
France	NH_4^+ and glucose	Electrochemical and biological processes	Anaerobic bacteria (sludge from wastewater treatment plant)	0.35 mol H_2 mol^{-1} glucose
USA	Duckweed (fermentation feedstock)	Anaerobic fermentation	-	75.3 mL H_2 per g dry duckweed in 7 days
France	35 g/L NaCl	Microbial electrolysis cell (MEC)	Proteobacteria, Bacteroidetes, Firmicutes and Spirochaetes and Actinobacteria	201.1±7.5 $L_{H2}/m^2_{Cathode*}{}^d$
Belgium	Fermentative media (LM-H, FHM, marine LB)	Hydrogen-driven remediation strategies	Pseudoalteromonas sp. BH11	2.01±0.05 mmol L^{-1} day^{-1}

Table 2.4 - Current status for biohydrogen production in European countries (16).

Country	Substrate	Technology	Selected microbial utilisation	Hydrogen production
Turkey	Modified media	Photofermentation	Rhodobacter capsulatus wild type (DSM 1710)	0.326 mol H_2/mol substrate
Belgium	Glucose	Anaerobic sequencing batch reactor	C. butyricum CWBI 1009	278 ml h^{-1}
Greece	Cottonseed cake	Dark fermentative hydrogen production	Thermophilic bacterium calidicellulosiruptor saccharolyticus	84-112%
Sweden	Wheat straw	Anaerobic digestion	Calidicellulosiruptor saccharolyticus	5.2 L H_2/L/Day
Turkey	Thick juice dark fermentor elluent	Photofermentation (solar tubular photobioreactor)	Rhodobacter capsulatus (PNS)	0.4 mol h_2/mol acetic acid
Finland	Crude glycerol	Bioconversion	Clostridium species	1.1±0.1 mol H_2/mol-$glycerol_{consumed}$
Italy	Crude glycerol	Dark fermentation	Microbial mixed culture	0.96 mol H_2/mol
Turkey	Thermophilic dark fermentor effluent of sugar beet thick juice	Photofermentation (solar fed-batch panel photobioreactor)	Rhodobacter capsulatus YO3 (hup^-)	1.12 mmol H_2/L_c./h
UK	Propane	Sorption-enhanced steam reforming (SESR)	-	9.1 mol hydrogen per mole of propane
Turkey	Molasses dark fermentation effluents	Sequential dark and photofermentation	Rhodobacter capsulatus wild type (DSM 1710) and Rhodobacter capsulatus YO3 (hup^-)	0.50 mmol H_2/L_c./h
Denmark	Wheat straw (hemicellulose rich)	Upflow anaerobic sludge bed (UASB)	-	212±24.1 mL-H_2/g-sugars

Table 2.5 - Current status of biohydrogen production in European countries (16).

Country	Substrate	Technology	Selected microbial utilisation	Hydrogen production
Turkey	Sugar beet molasses	Dark fermentation and sequential dark and photofermentation	Extreme thermophile (Calidicellulosiruptor saccharolyticus) and photodynthetic bacteria (Rhodobacter capsulatus ,Rhodobacter capsulatus hup mutant and Phodopseudomonas palustris)	4.2 mol H_2/mol sucrose and 13.7 mol H_2/mol sucrose
Sweden	Potato steam peels	Dark fermentation	-	1.37-3.48 mmol H_2/mole glucose
Turkey	Hydrolysed and untreated potato steam peels and glucose	Starch fermentation	Extreme thermophile (Calidicellulosiruptor saccharolyticus and Thermotoga neapolitana)	2.4-3.8 mol H_2/mol glucose
Netherlands	Carrot pulp and (mixture of) glucose and d-fructose	Dark fermentation	(Thermophilic bacteria) Calidicellulosiruptor saccharolyticus and Thermotoga neapolitana	2.7-2.8 mol H_2 (mol hexose)$^{-1}$
Turkey	Volatile fatty acids (VFAs) individually (malate, acetate, propionate, butyrate and lactate)	Photofermentation	Rhodobacter sphaeroides	24 $ml_{hydrogen}/l_{reactor}h$
Hungary	Wheat straw, maize leaves, sweet sorghum, sugarcane, bagasse and silphium	Dark fermentation	Calidicellulosiruptor saccharolyticus	3.8 mol H_2/mol glucose
Turkey	Olive mill wastewater (OMW)	Column photobioreactor	Rhodobacter sphaeroides OU 001	13.9 l_{H2}/l_{OMW}
Turkey	Pretreated sugar refinery wastewater (SRWW)	Column photobioreactor	Rhodobacter sphaeroides OU 001	2.67 l_{H2}

Currently, many hydrogen projects are being introduced worldwide. With ambitious projects like modernisation of entire railway network in one state by changing current one to hydrogen fuel cells-based by 2025 or hydrogen infrastructure development with 400 hydrogen refuelling stations by 2023, Germany is the leading country in hydrogen production development in Europe. Other European countries like U.K. also are introducing hydrogen energy into their energy structures with £2 billion budget „hydrogen city" project taking place in the city of Leeds. Japan is another country highly investing in hydrogen infrastructure with their plans of developing 160 hydrogen refuelling stations by 2020 and 320 stations by 2025. USA is also highly involved in hydrogen economy with $14 millions in funding for advancement

of hydrogen fuel technologies and by opening 50 hydrogen fuel stations nationwide in 2016. Hydrogen city is currently being built in China with promotion of research and development of fuel cells, building of hydrogen station and achieving mass production of fuel cell vehicles by 2020 (13). Hydrogen production development is especially hot topic in transportation sector with many big names in the automobile market like BMW, Toyota, Hyundai or Honda having fuel cell vehicle lines in production (17).

With the high demand for hydrogen as well as environmental restrictions for obtaining fuels from non-fossil resources arises the need for biological hydrogen generation pathways. One of the most crucial factors in terms of efficiency of the process as well as cost effectiveness of the process is obtaining the best substrates for biohydrogen production. In the chapter three, most promising biohydrogen resources will be discussed.

3. Resources for biohydrogen production

Biomass by the definition is organic matter derived from plants and animal waste and it's the oldest renewable source of energy known to mankind as well as the fourth largest source of energy in the world. Due to diverse nature of its composition, biomass vary significantly in physical and chemical properties. Bioenergy in a form of biofuels can be produced from biomass. Biofuels can be in a solid, gaseous or liquid form and can be divided into primary and secondary biofuels, where primary biofuels like pallets or firewood are used in unprocessed form, whereas secondary biofuels such as bioethanol and biodiesel are a result of biomass processing. Among secondary biofuels we can distinguish 1st, 2nd and 3rd generation. The 1st generation biofuels are produced from food crops like corn, wheat, sugar beet, rapeseed, soybeans etc. Even though high hydrogen yields can be obtained from 1st generation biofuels, they are associated with major limitation of utilization of arable land to produced energy crops instead of crops for food industry (16). The 2nd generation biofuels are derived from non-food crops such as wood, organic waste, therefore they are cheap, abundant and most importantly don't compete with food production as in the case of 1st generation biofuels. The major disadvantage of 2nd generation biofuels for biohydrogen production on an industrial scale is high cost of pre-treatment. The 3rd generation biofuels are based on energy crops such as algae and microalgae and currently are most promising type of biofuels as they don't have 1st and 2nd generation biofuels limitations.

Many different potential resources have been investigated under the feasibility of biohydrogen production. Criteria like cost, availability, fermentability and carbohydrate content are said to be most important in terms of raw material selection for biohydrogen production. Another important criteria are related to pre-treatment complexity which is required by the biomass content, therefore the simplest and less complex pre-treatment the more desired the biomass source (16).

The main source of hydrogen are carbohydrates, therefore the feedstock rich in sugars, like food industry waste, sewage sludge from wastewater treatment, microalgae, wood and wood waste, is applicable. The best raw material for biohydrogen are simple sugars such as glucose, lactose and sucrose due to

their easy biodegradation. Simple sugars are expensive raw materials on an industrial scale, more complex feedstock like waste is economically more viable (18). The most promising resources of biohydrogen will be discussed in this chapter.

3.1. Microalgae

Algae and microalgae are 3rd generation feedstock for biofuels. Algae is an organism with chlorophyll a and a thallus that is not differentiable into roots, leaves and stem. We can distinguish macroalgae that can grow up to tens of meters long and microalgae which size is in the range of a few micrometres to few hundreds of micrometres. Microalgae is a more interesting feedstock as energy carrier due to higher energy conversion efficiency with respect to photosynthesis in comparison with macroalgae. In terms of chemical composition of microalgae, we can recognize proteins, carbohydrates, lipids and inorganic compounds like ash. The ratio of these chemical compounds in microalgae is important in processing route selection. Proteins are major contributors to the relatively high amount of molecular nitrogen present in algae. Proteins are the most abundant element in green algae and make up to 70% of dry weight. The general formula of carbohydrates is $(CH_2O)_n$ and they are produced through photosynthesis from CO_2 and H_2O. Sugars (monosaccharides) are formed and polymerized to polysaccharides. Lipids present in microalgae are storage lipids such as acylglycerols and membrane lipids such as glycolipids or phospholipids. There are several microalgae species that are capable of biohydrogen production like *Chlorella vulgaris, Chlorella pyrenoidosa, Spirulina platensis, Platymonas subcordiformis and Chlamydomonas reinhardtii* (19). There are two types of microalgae feedstock for biofuels production, prokaryotic microalgae also known as cyanobacteria which lack of any membrane-bound organelle and eukaryotic microalgae like green algae (*Chlorophyta*), red algae (*Rhodophyta*) and diatoms (*Bacillariophyta*). Algae can be divided into autotrophic, which in order to grow require inorganic compounds like CO_2, salts and light as energy source, and heterotrophic, which require external source of organic compounds and nutrients. There also exist mixotrophic algae, which can act like autotrophic and heterotrophic algae. Potential microorganisms capable of producing hydrogen are presented in Figure 3.1.

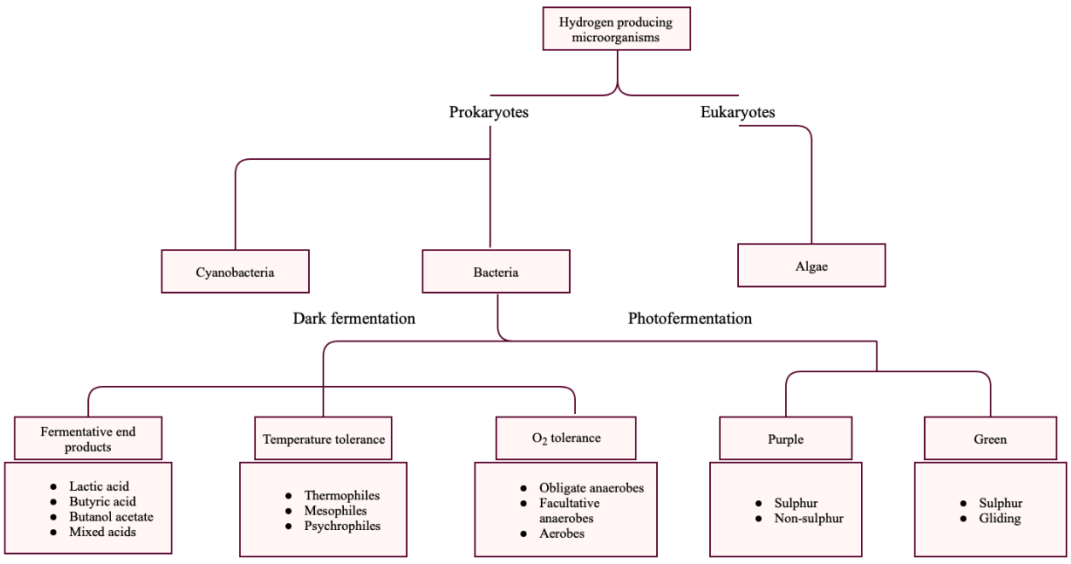


Figure 3.1 - Scheme representing potential biohydrogen microorganisms. Adapted from (20).

Microalgae when proceed can provide variety of valuable products like bioethanol, biodiesel, biomethane and biohydrogen. One of the biggest advantage of microalgae in comparison to first and second generation biofuels feedstock is their ability to grow rapidly all year round, e.g. biodiesel yield of

12,000 l ha^{-1} for microalgae in an open pond production in comparison with biodiesel 1190 l ha^{-1} from rapeseed (8). Another advantage over first- and second-generation feedstock is algae capability of growing in aqueous media, which means that no arable land is needed for microalgae cultivation, therefore microalgae don't compete with food production which is the major limitation for 1st generation biofuels. Microalgae require less water than terrestrial crops. Microalgae are capable of CO_2 bio-fixation, this ability is extremely promising way of CO_2 sequestration and could potentially be a sufficient solution for significant reduction of this greenhouse gas in the atmosphere. It is said that provision of CO_2 to microalgae cultivation process could increase microalgae growth rate as well as promote photosynthesis (21). There is a significantly rapid growth potential in microalgae and in a short period of time like 3.5 h the exponential growth rates can double their biomass. The cultivation of microalgae does not require any pesticides nor herbicides. Due to absence of lignin and hemicellulose the cost of pre-treatment, which occur in 2nd generation biofuels, is reduced. Microalgae can also be used to produce valuable co-products such as proteins and residual biomass that can be used as fertilizers as well as feed. Biohydrogen production is possible through photobiological process (9). Table 3.1 presents advantages and disadvantages of solar energy dependent biohydrogen production processes in Cyanobacteria and green microalgae.

Table 3.1 - Summary of advantages and disadvantages of light-dependent hydrogen production processes in cyanobacteria and eukaryotic biomass (16).

Microalgal groups	**Preferable light-dependent metabolic pathway for h_2 production**	**Advantages**	**Disadvantages**
Cyanobacteria	Indirect biophotolysis through nitrogenase	H_2 evolution is separated from O_2 evolution	High-energy-dependent process
		Spatial separation in heterocystous N_2-fixing cyanobacteria	Biosynthesis and maintenance of heterocysts
		Temporal separation (light/dark) in nonheterocystous cyanobacteria	Significant ATP requirement for nitrogenase. The presence of uptake hydrogenase
			Reoxidize produced molecular hydrogen
Green microalgae	Direct and indirect biophotolysis through bidirectional hydrogenase	Hydrogen-economy strategy based on a virtually limitless and renewable source	Production of O_2 and H_2 simultaneous
		Energy cycle is carbon-free	Inhibition of hydrogenase by O_2
		Theoretical energy efficiency is much higher for hydrogen production from biophotolysis (40%) compared to hydrogen production from biomass (1%)	
		One of the most promising processes due to separate production of O_2 and H_2	

As in every technology despite good potential there are limitations in industrial scale application and in viability of the process. Technological constrains are major limitations for microalgae such as (16):

- Main limitation is hydrogenase's high sensitivity to oxygen.
- Reverse nature of hydrogenase which leads to H_2 consumption under high partial pressure of H_2.
- Low level at which light saturation occurs in photosynthesis.
- Hydrogenase expression low level.
- Due to existence of competing metabolic pathways there is low reductant availability for hydrogenase activity.
- Downregulation of photosynthetic electron transport.
- Lack of data for larger scale plants.

In table 3.2 major challenges in microalgae application are presented with possible solutions.

Table 3.2 - Major limitation and remedies for biohydrogen production in cyanobacteria and green microalgae (16).

Microalgal groups	**Challenges**	**Strategies**
Cyanobacteria	Low h_2 production rate of [NiFe] hydrogenases	Requires constant sparging of inert gas
		Expressing the bidirectional, oxygen-tolerant [NiFe] hydrogenase genes, hudS and hydL in cyanobacteria
		Incorporation of [FeFe] hydrogenase into heterocysts
Green microalgae	Light conversion efficiency to H_2 (theoretically about 10%)	Truncating the chlorophyll antenna size of PSII using RNAi method
		Sulphur deprivation
	Inhibition of [FeFe] hydrogenases by oxygen production through PSII	Genetic insertion of a hydrogenase promoter proton channel into the thylakoid membranes

In terms of microalgae utility as biofuels raw material, the main challenge is a realistic projection of the potential of microalgae considering present state as well as future targets. Table 3.3 is a summary of main topics in application of microalgae as biofuels feedstock matter with present situations and future targets. In case of microalgae itself, nowadays only few strains are in focus and the interest is coming more from sectors like pharmaceutical or cosmetics industry, therefore there is a need of more interest from energy sector as well as wider research on genetically modified strains of microalgae, research on new species suitable for biofuels feedstock (22). Moreover, mixed or co-cultures of microalgae should be the main focus instead of monoculture as they appear more feasible in large-scale application. The design and use of photobioreactors is a crucial aspect of biohydrogen production commercialisation, consequently the future aim is to lower the production cost and apply technical and constructional modifications and design improvements. In terms of microalgal biofuels focus, development of biohydrogen production is directly linked with its utility in fuel cells technology and hydrogen storage, therefore the

interest in fuel cells will boost the interest in biohydrogen production. Future target for microalgae utilization needs to be biorefinery concept with its integration with other bioprocesses as well as production of variety of products for different sectors. When it comes to economy, biorefinery concept will definitely decrease the operation costs. With the depletion of petroleum resources resulting in big prices swing, the opportunity for microalgal biofuels arises in transportation sector. On the other hand, examples such as Solazyme case, which turned from promising microalgal biofuels company into food, nutrition and specialty ingredients company named TerraVia currently, show harsh economic reality and desperate need for improvement and development in microalgal biofuels applicability on an industrial scale.

Table 3.3 - The constraints, future targets and comments-opinions for microalgal biofuel realisation (19).

Focus	Today	Target	Comments/opinions
Microalgae	Few strains in focus, variable productivities, more interest from other sectors like pharmaceutical, aquaculture etc., rather than energy	Selection of new strains and routes for maximised productivities with regard to biofuel	Genetically modified strains can have a chance for a higher production but the key is to control their possible effects on the nature and the determination of their applications with legislations. New species will also be discovered any used in the researchers for progress. Also, rather than monoculture processes, mixed or co-cultures of microalgae with other microorganisms will increase the chance of application
Photobioreactors	Limited large-scale productions, high investment costs depending on the technical and constructional needs	Improved designs considering outdoor productions interactive with environment, lower production cost	Outdoor open ponds will be used in the future but with the improving materials and construction techniques, they can be transformed to closed systems with feasible modifications for increased productivity with diverse species. On the other hand, two basic designs tubular and panel will also find commercial application as a support unit that may be used for more sophisticated by-products to decrease the production cost of the biofuels produced by pond systems
Microalgal biofuels	Limited usage but increasing interest with the progress in a fuel cell technology, engines, hydrogen storage, microalgae oil/ethanol production and extraction, several production steps	Higher productivities with single step processes, leading to daily life usage in areas like transportation	Considering its potential, biodiesel will be the dominator over the other microalgal biofuels. but keeping in mind the bioethanol and biomethane production through integrated processes they can be a support for the economic feasibility of the biodiesel process by using its residues and recycle streams. Biohydrogen can be classified separately with its potential to be used in fuel cells
Biorefineries	Beginning of the spread usage with biorefinery concept	Stronger biorefinery attitude with the corporation of residential and industrial facilities supported with production of biomass and biofuels as by-products	Like petroleum refineries gaining all the possible products will be an advantage for microalgae that has diverse by-products in addition to biofuel important for different sectors. The key is the integration with other conventional bioprocesses like fermentation, digestion and waste treatment
Economy	Variable feasibility depending on the production process and technology	Feasible production considering improved downstream processes, new photobioreactor designs and carbon credits	Other than the economic profit from the biofuel, producing by-products through biorefinery concept will help to decrease the operational costs. Possible integration with other commercial processes excess emissions, energy and waste can be used in the productions serving as an economic advantage. Also transporting microalgae through conventional pipelines can be an advantage for the production in suitable climates and refinement on the raw biomass in specified facilities elsewhere will decrease the cost of downstream processes. This way the producers can send their product to centralise refinement facilities to eliminate extra infrastructure similar to the refineries in the petroleum industry. Microalgae based biofuels will also act as a backup to control the prices of especially petroleum when the reserves start to deplete resulting in big price sway with regard to elevated oil concession and drilling costs
Energy	Limited share in renewables and limited daily life usage	Higher share, widespread usage	Energy from coal, hydro and nuclear will continue to dominate the massive demand. But depending on the petroleum prices microalgal biofuels can increase their share especially in the area of transportation. Biodiesel and bio ethanol will get the main attention as blends with petroleum whereas biohydrogen with fuel celled cars

3.2. Waste

Agriculture waste has a huge potential as it is rich in carbohydrates feedstock. Agriculture-based and food industry waste such as sugarcane and potato peels are a good feedstock for biofuels due to relatively high amount of carbohydrates (23). Waste can be divided into municipal waste, industrial waste and agricultural waste. Agriculture-based waste as well as waste from food industry is rich sources of starch, protein and cellulose.

Starch is notably easy to process for biohydrogen production, firstly it can be hydrolysed to glucose and maltose either by acid or enzymatic hydrolysis, secondly carbohydrates are converted to organic acids and later to biohydrogen (18).

The biggest advantages of waste as biohydrogen production feedstock is its abundance and recycle-like approach where waste is revalued into promising resource for conversion into valuable product. Waste also doesn't compete with food industry. As it was mentioned previously the complex nature of this type of feedstock can affect negatively its biodegradability and therefore bring technological challenges into process development. Mostly due to the presence of cellulose which requires pre-treatment process in order to improve biodegradability and to use it in biohydrogen production (12,24). Lignocellulosic biomass is second most abundant component on Earth and consists of cellulose, hemicellulose and lignin (25). Agricultural wastes are ought to chemical or mechanical pre-treatment. Despite many years of research in lignocellulose pre-treatment, no significant trend has emerged. Current pre-treatment methods are usually high energy consuming, the approach to integrate different methods of pre-treatment and therefore, integration of advantages of individual methods, appears promising (26). After necessary pre-treatment cellulose, hemicellulose or lignocellulose contained in waste undergoes hydrolysis to carbohydrates and after the organic acid is processed to biohydrogen (Figure 3.2).

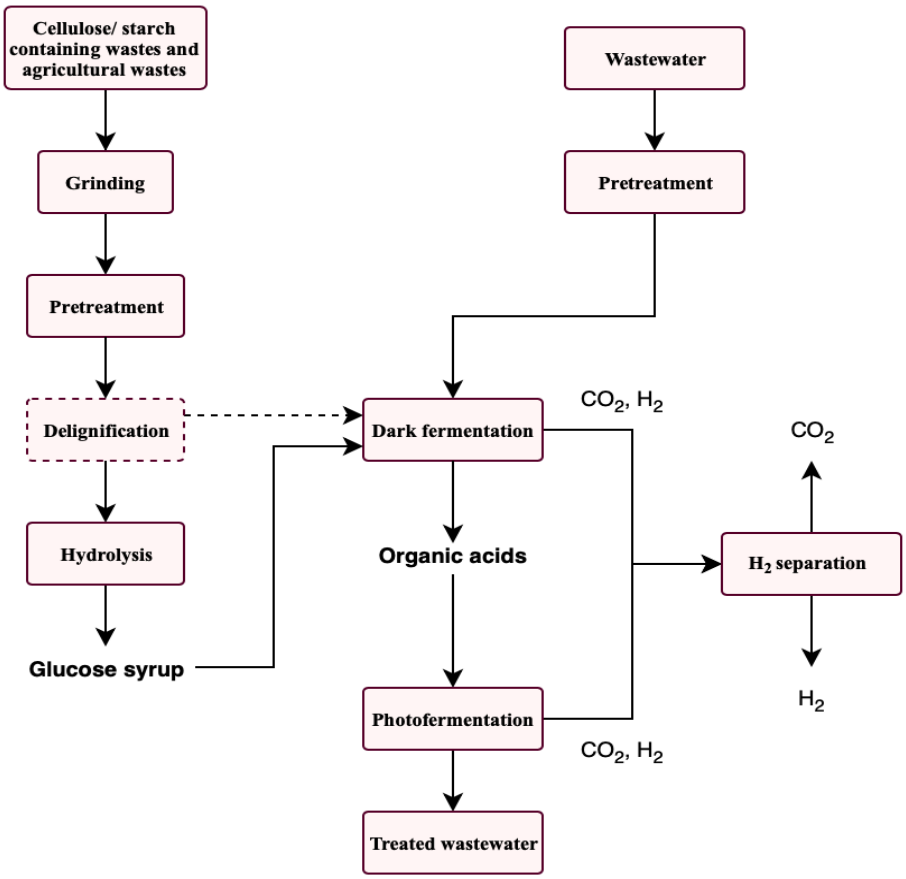


Figure 3.2 - Schematic diagram for biohydrogen production from cellulose/starch containing agriculture wastes and food industry wastewater. Adapted from (18).

The yields of biohydrogen that can be obtained from food industry waste or wastewaters are comparable to yields from pure carbohydrates and range from 0.68 mol H_2/mol hexose up to 2.70 mol H_2/mol hexose. (27). Complex solid wastes like waste from kitchen, food processing waste or municipal wastes could also be potential feedstock for biohydrogen production, but due to high fat and protein content their conversion to biohydrogen is relatively lower in comparison to rich in carbohydrates wastewaters. It was conducted that carbohydrate-based waste has 20 times higher production potential than fat or protein-based waste (27).

Although combustion of municipal solid waste contributes to greenhouse gases emissions, 70% of this waste is organic fraction that can be bioprocessed for biohydrogen production. Municipal solid waste exhibits great contaminants concentration and diversity, therefore it is one of the most technically challenging feedstock with costly pre-treatment, however with the right approach and lots of research, it can be applicable for biohydrogen production (28). Fruit and vegetable wastes are suitable for biohydrogen generation due to high carbohydrates content, but other kitchen waste as well as slaughterhouse waste including meat and fish residues, rice, noodles and variety of sauces are mostly not suitable for biohydrogen production due to high lipids and proteins content. However, this type of waste can find application in methane generation (29). Wastewater from dairy industry, olive mill, baker's yeast and breweries

can be used as raw material for biohydrogen production due to its rich carbohydrates content (18,30). In dairy industry, whey is a by-product of cheese production and it is considered a dangerous waste. Dry matter of whey consists of 70-80% lactose, 8-20% of minerals and trace components and 9% of proteins, which makes it suitable feedstock for biohydrogen. (31,32). Manure can also be used as feedstock. More than 1500 million tons of animal manure are produced every year in European Union itself. Animal manure waste include: slurry or liquid manure from live stock or poultry, solid manure or farmyard manure as well as wastewaters (16). Currently animal manure waste cannot be recognized as ideal substrate for dark fermentation rather as co-substrate or enhancing supplement with another carbohydrate rich substrate due to low hydrogen yield. Co-digestion of different complementary wastes could be a good solution to improve overall hydrogen production efficiency. Table 3.4 presents obtained biohydrogen yields with process conditions from co-digestion of manure with different substrates.

The concept of biorefinery where biohydrogen production is combined with bioethanol and biogas production comes to discussion as a solution to overcome technological and cost limitations for industrial scale development.

Table 3.4 - Comparison of optimised conditions and results studied dealing with biohydrogen production from co-digestion of manure with different substrates (16).

Substrate/(composition %)	**Type of inoculum**	**Process conditions ($g.l^{-1}$/°C/pH)**	**Type of process**	**H_2 production (ml H_2/gvs^{-1})**
Substrate pre-treatment				
Buffalo manure/LPCW*/crude glycerol/(20/70/10)	Selected from lagoon sediments	8/37/6.7	Batch	170±3
Sterilisation				
Buffalo slurry/cheese whey/(33VS/67VS)	Selected from lagoon sediments	2.06%VS/37/6.5	Batch	117±22
Sterilisation				
Liquid swine manure/beet molasses/90.75/10g/L sugar)	Liquid swine manure	12/37/5.4	Sequencing batch	1.57 mol H_2. mol^{-1} sugar
Sieved and boiled for 30min				
Liquid cow manure/cheese whey/olive mill wastewater/(5/40/55)	Anaerobic sludge from acclimatized	63.52/37/6	Batch	23.8 (0.64 mol $H_2.mol^{-1}$ glucose)
No				
Liquid cow manure/cheese whey/olive mill wastewater/(5/40/55)	Anaerobic sludge from acclimatized	84.69/37/6	Continuous	0.54 mol $H_2.mol^{-1}$ glucose
No				
Cattle manure/slaughterhouse risk material/(90(wt fry matter)/10(wt dry matter))	Cattle manure	40/55/7.1	Batch	33
Heated at 90℃ for 3h				
Cow manure/milk waste/(30/70)	Cow manure	40/55/6.5	Batch	59.5
Sieved and heat-treated/-				
Swine manure/fruit-vegetable waste/(35(w/w)/65(w/w))	Seed from 10L H_2-producing reactor	20/55/5.45	Semi-continuous	126
Sieved/shredded in a blender				

Glycerol is a big by-product in chemical industry as well as in biodiesel production. Although glycerol has wide range of applications like cosmetics industry with the development of biodiesel production and other chemical production the yields of glycerol will become bigger and it could be potentially used as biohydrogen feedstock.

Sewage sludge from wastewater treatment plants is rich in carbohydrates and proteins and therefore can be used as biohydrogen and methane production raw material. The major constrain of sewage sludge in biohydrogen application is its low carbon to nitrogen ratio, which results in lower hydrogen production yield (33).

Table 3.5 presents biohydrogen yields from the variety of agriculture-based feedstock that was pre-treated by various methods.

Table 3.5 - Potential biohydrogen yield of various agriculture-based wastes (16).

Feedstock	**H_2 yield**	**Units**
Apple processing	0.9	L H_2 L^{-1} medium (0.1 L H_2 g^{-1} COD
Maize stalk	20-176	ml H_2 g^{-1} VS
Maize stalk	3-150	ml H_2 g^{-1} TVS
Fodder maize	62	ml H_2 g^{-1} TS added
Fruit peel waste	459	ml H_2 g^{-1} VS destroyed
Olive pulp	0.19	Mmol H_2 g^{-1} TS
Olive pulp	0.321-1.6	mmol H_2 g^{-1} TS added
Pineapple waste	5.92	mmol H_2 g^{-1} COD
Poplar leaves	15-44.9	ml H_2 g^{-1} TS
Potato processing	2.1	L H_2 L^{-1} medium or 0.1 L H_2 g^{-1} COD
Potato waste	30	ml H_2 g^{-1} TS
Wheat straw	0.5-68.1	ml H_2 g^{-1} TS
Sugar wastewater	2.6	mol H_2 mol^{-1} hexose
Rice winery	2.14	mol H_2 mol^{-1} hexose
Rice slurry	346	ml H_2 g^{-1} carbohydrate
Starch wastewater	92	ml H_2 g^{-1} starch
POME (palm oil mill (effluent)	2.3-6.33	L H_2 g^{-1} raw POME
POME	0.42	L H_2 g^{-1} COD reduced

4. Biohydrogen technology

There are many technologies for obtaining biohydrogen production. In general, we can divide them into two groups thermochemical conversion of biomass-based biohydrogen and biological conversion. Thermochemical processes are series of chemical reactions generating hydrogen as a product, where we can distinguish biomass pyrolysis and its variations, biomass gasification and its variations and supercritical water gasification as the most favourable for biomass utilization. Biological processes of biohydrogen depends on hydrogenase enzyme.

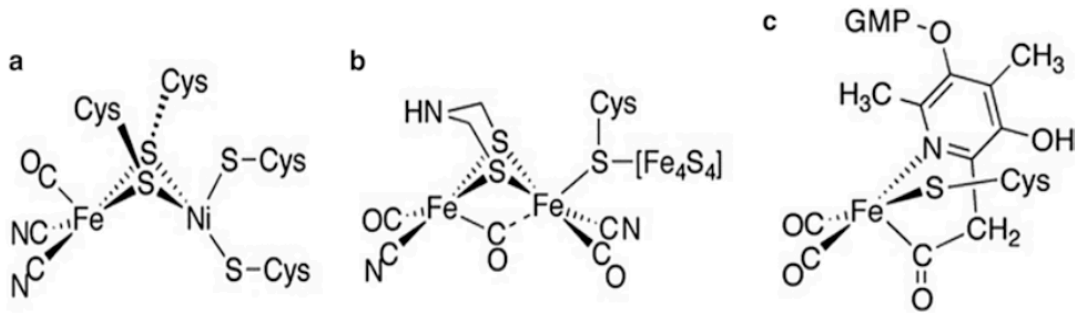


Figure 4.1 - Structure of three types of hydrogenase enzymes: (a) [NiFe] hydrogenase, (b) [FeFe] hydrogenase, (c) [Fe] hydrogenase (16).

Three enzymes are being involved in biohydrogen production: nitrogenase, Fe-hydrogenase and NiFe-hydrogenase, these enzymes work as catalysts for the biohydrogen production reactions (figure 4.1). Nitrogenase is a protein containing iron-sulphur cluster and molybdenum, less frequently vanadium. Simplified chemical reaction of biohydrogen formation is presented below [1], where ATP stands for adenosine triphosphate, ADP is adenosine diphosphate and Pi is inorganic phosphate (34):

$$4ATP + 2H^+ + 2e^- \rightarrow H_2 + 4ADP + 4P \quad [1]$$

Hydrogenase is an enzyme working as catalyst for reversible oxidation of molecular hydrogen reaction and it functions as acceptor and reversible hydrogenase in most photosynthetic microorganisms.

[FeFe]- and [NiFe]-hydrogenases are two main types of hydrogenase determined by the type of metal clusters at their catalytic sites (35). [FeFe]-hydrogenase type appears to be more active in terms of hydrogen production. In the acceptor hydrogenase the main metal clusters are [NiFe] and [NiFeS] which consume molecular hydrogen that is produced during nitrogen reduction according to the reaction (34):

$$H_2 \rightarrow 2H^+ + 2e^- \quad [2]$$

Depending on reaction conditions reversible hydrogenase has ability to consume as well as create molecular hydrogen:

$$H_2 \leftrightarrows 2H^+ + 2e^- \quad [3]$$

The properties of two main enzymes controlling all biological processes in biohydrogen production are listed in table 4.1:

Table 4.1 - The properties of nitrogenase and hydrogenase enzymes (34).

Properties	Nitrogenase	Hydrogenase
Substrates	ATP. H^+ or nitrogen electrons	H^+, hydrogen
Products	H_2, NH_4^+	ATP, H^+, hydrogen electrons
Number of proteins	2 (Mo-Fe and Fe)	1
Metal components or sulphur	Mo. Fe	Ni, Fe, S
Optimal temperature	30ºC (A. vinelandii)	55ºC (R. rubrum); 70ºC (R. capsulatus)
Optimal pH	7.1-7.3 (A. vinelandii)	6.5-7.5 (R. sulfidophilus)
Inhibitors	N_2, NH_4^+, O_2, high N/C ratio of H_2 production	CO, EDTA, O_2, some organic compounds
Stimulators.	light	Absence of organic compounds (R. rubrum, R. capsulatus)

Diagram of biohydrogen production pathways is pictured in figure 4.2. The most promising technologies of biohydrogen production pathways will be discussed in this chapter.

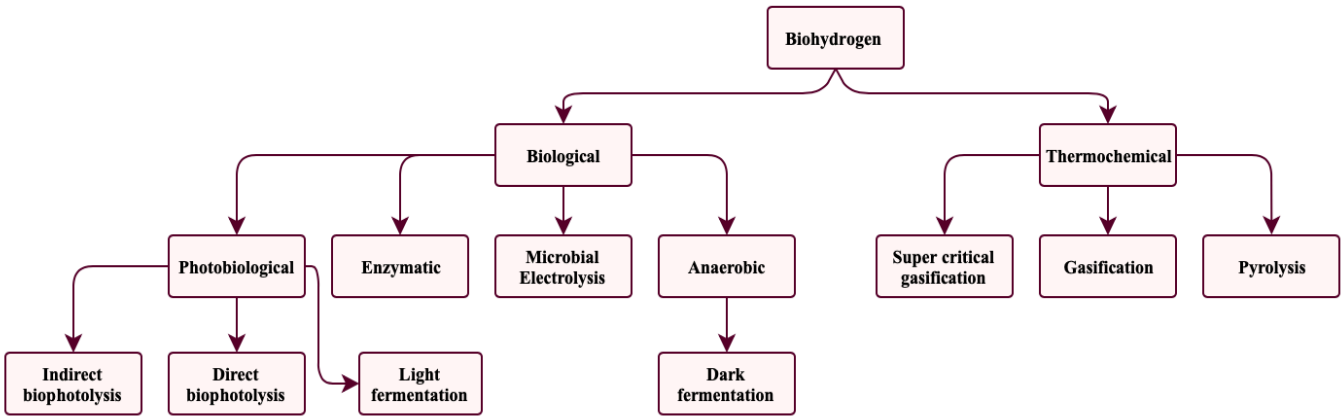


Figure 4.2 - Biohydrogen production pathways. Adapted from (12).

4.1. Fermentation

Biohydrogen can be produced by photoheterotrophic (light fermentation) and anaerobic (dark fermentation) microorganisms using biomass rich in carbohydrates as a renewable resource.

4.1.1. Dark fermentation

Dark Fermentation (light independent) is recognized as one of the most promising biological pathway for biohydrogen production (36). During dark fermentation process anaerobic bacteria grow on carbohydrates-rich biomass feedstock without access to light producing biohydrogen.

Bacteria species like Enterobacter, Bacillus and Clostridium are known to produce biohydrogen (37). Two catabolic stages take place in dark fermentation, first step is decarboxylation of pyruvate into acetyl coenzyme A (acetyl-CoA) where reduced ferredoxin (Fd_{red}) is formed which takes a role of a direct electron donor for hydrogenase and is the primary hydrogen catalysing enzyme in *Clostridium* sp.

First stage reaction in dark fermentation:

$$\text{Pyruvate} + \text{CoA} + 2\text{Fd(ox)} \rightarrow \text{Acetyl CoA} + 2\text{Fd(red)} + CO_2 \quad [4]$$

$$2\text{Fd(red)} + 2H^+ \rightarrow 2\text{Fd(ox)} + H_2 \quad [5]$$

In the second stage pyruvate and CoA is converted into formate and acetyl-CoA, after formate catalysed by formate hydrogen lyase (FHL) is decomposed, which in facultative anaerobes like *Klebsiella* sp. is H_2 production dominant mechanism.

Second stage reactions in dark fermentation:

$$\text{Pyruvate} + \text{CoA} \rightarrow \text{Formate} + \text{Acetyl CoA} \quad [6]$$

$$\text{Formate} \rightarrow CO_2 + H_2 \quad [7]$$

Later bacteria use protons as the final electron sink for electrons generated during glycolysis (38).

The general biochemical pathway for dark fermentation:

$$2H^+ + 2e^- \rightarrow H_2 \quad [8]$$

Dark fermentative metabolism overall biochemical pathway is pictured in figure 4.3.

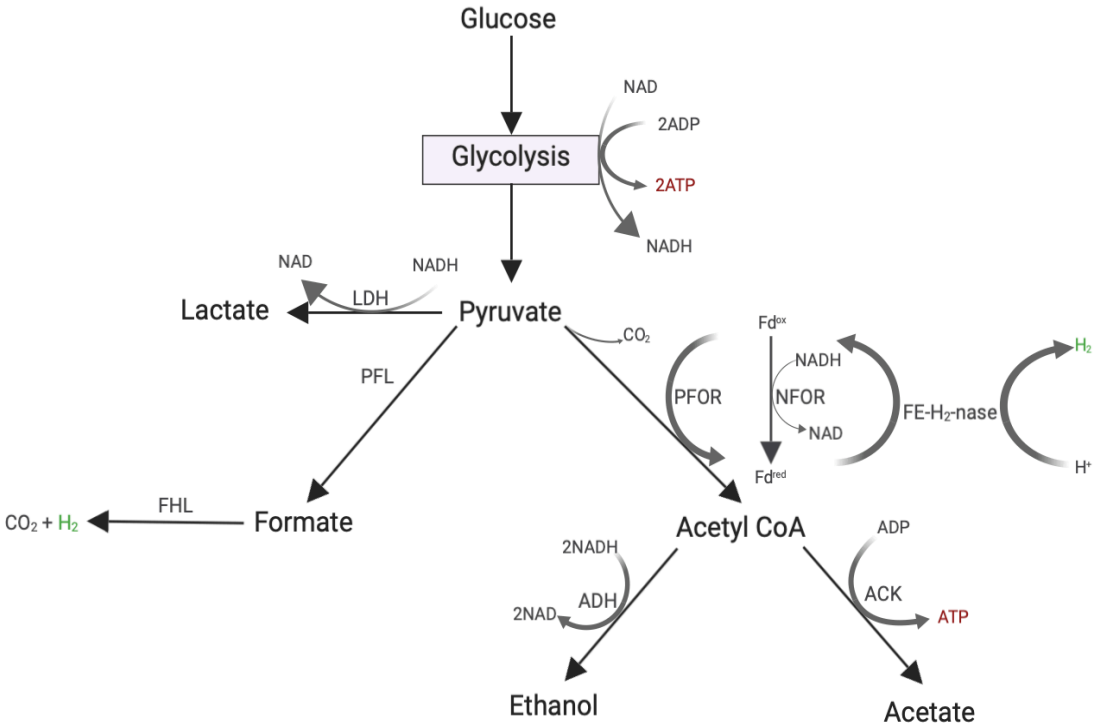


Figure 4.3 - Overall biochemical pathway for dark fermentative metabolism (LDH: lactate dehydrogenase; PFOR: pyruvate ferredoxin oxidoreductase; PFL: pyruvate formate lyase; FHL: formate hydrogen lyase; ADH: alcohol dehydrogenase; ACK: acetyl CoA kinase; NFOR-NADH: ferredoxin oxidoreductase.). Adapted from (38).

Dark fermentation reactions can be operated in various of temperature conditions: mesophilic (25-40°C), thermophilic (40-65°C), extreme thermophilic (65-80°C) and hyper thermophilic (>80°C). The end product is a biogas mixture containing primarily H_2 and CO_2 but can also contain minor amounts of H_2S, CH_4 and CO, this composition brings problematic technical challenges such as separation and purification process application. Depending on fermentation pathway and end-products, different feedstock yields are obtained with different amounts of H_2 per mole of glucose. Therefore, when the end-product is acetic acid, according to stoichiometry we can obtain maximum of 4 mole H_2 per mole of glucose by facultative anaerobic bacteria:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2 \quad [9]$$

On the other hand, when the end-product is butyrate we can obtain theoretical maximum of 2 moles H_2 by strict anaerobic bacteria:

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_2CH_2CH_2COOH + 2H_2 + 2CO_2 \quad [10]$$

In oxygen environment facultative anaerobic bacteria can switch to aerobic respiration. Facultative anaerobes are less sensitive to oxygen and are considered better option in fermentative hydrogen production processes in comparison to strict anaerobes. From stoichiometry we can conclude that the highest theoretical yield of H_2 is connected with acetate being the end-product of fermentation. In practice, however it was concluded that the highest yields of H_2 are in fact connected not only to acetate end-product but the mixture of acetate and butyrate products, the lowest yields of H_2 are said to be associated with products like lactic acid and alcohols (37). Fermentation end-products greatly depend on environmental conditions in which bacteria grow. Biohydrogen production highly depends on the key factors such as gas partial pressure, pH and hydraulic retention time (HRT) which is associated with metabolic balance. For continuous synthesis of H_2 especially crucial factor is H_2 partial pressure. With the increase of H_2 concentrations the H_2 synthesis decreases and metabolic balance aims towards unfavourable products like lactate, ethanol, butanol, acetone and alanine. However, the increase in temperature makes favourable reactions less affected with H_2 concentrations. In order to maintain continuous H_2 synthesis the following conditions should be applied: partial pressure H_2 < 50 kPa at 60℃, < 20 kPa at 70℃ and < 2 kPa at 98℃ (37). It was conducted that in general, pH for biohydrogen generation should be in the range of 5-7(26), optimal at pH 5.5 (39). Although pH value in photosynthesis and fermentation processes depend also on the type of microalgae species and therefore ideal pH needs to be examined and adjusted individually (40). Fermentation process should be directed away from alcohol products (ethanol, butanol) and reduced acids (lactate) towards volatile fatty acids (VFA) in order to maximise the H_2 yield. Dark fermentation potential mass production is promising due to relatively simple process, wide spectrum of potential feedstock, high rate of biohydrogen production and no light sources requirement. Moreover, fermentation process operation is easy as fermentation reactor technology and bioprocess control are all well known since other fermentation processes exist on the industrial scale (41). There are many inhibitors of dark fermentation that can be classified as pre-process inhibitors which are already present in microflora or substrates before the process or in-process inhibitors which appear along the dark fermentation process. One of the solution for pre-process inhibition problem is use of mixed microflora which in general appears to be more practical and economically viable on a large scale than pure cultures with the main advantage of being capable of operating in non-sterile environment, unlike pure cultures, as well as the ability of using wider spectrum of feedstock, on the other hand the downside of mixed cultures is possibility of containing variety of microorganisms that compete for substrates with hydrogen-producing bacteria or it may contain hydrogen-consuming bacteria consequently lowering biohydrogen production yield (42–44). Dark fermentation process is intensely studied in terms of integration with other technologies in order to enhance biohydrogen production rate and to overcome the process limitations (45), for example (46) propose integrating dark fermentation onto dark fermentation biorefinery with valorisation of volatile fatty acids (the by-product of dark fermentation) into high added-value omega-3 fatty acids. Table 4.2 presents H_2 yields obtained with mono-cultures and mixed cultures.

Table 4.2 - H_2 yields for Mono- and Co-culture Studies (47).

Mono/Co-culture	Substrate	H_2 yield (mol H_2/mol_{hexose})
C. butyricum	Starch	0.49
Cirobacter freundii	Starch	0.00
C. butyricum + *Cirobacter freundii*	Starch	0.73
C. butyricum	Glucose	0.97
C. pasteurianum	Glucose	0.66
C. felsineum	Glucose	0.62
C. butyricum + *C. pasteurianum*	Glucose	1.33
C. butyricum + *C. felsineum*	Glucose	1.02
C. pasteurianum + *C. felsineum*	Glucose	1.61
C. thermocellum	Cellulose	0.80
C. thermocellum + *Th. thermosaccharolyticum*	Cellulose	1.80
C. thermocellum	Cellulose	0.17
C. thermocellum +*C. thermopalmarium*	Cellulose	1.36
C. acetobutylicum	Microcrystalline cellulose	0.58
C. acetobutylicum + *Ethanoigenes harbinese*	Microcrystalline cellulose	1.40

One of the major hydrogen consuming bacteria present in mixed cultures is hydrogenotrophic methanogens. Homoacetogens are another group of bacteria that can potentially inhibit biohydrogen production by consuming H_2 for acetate synthesis (48). Other inhibiting agents in mixed microflora constitute of propionate producers, sulphate-reducing bacteria, nitrate-reducing bacteria and lactic acid bacteria. Currently the main solution strategy for inhibition is inoculum pre-treatment, principle used in mentioned pre-treatment uses the principle that many H_2 producers such as *Clostridium* sp. are able to form spores under sever temperature, pH and radiation conditions, where most H_2 consumers don't display such ability (48). Metal ions, although essential for dark fermentation process due to their role in assisting bacterial metabolism, enzyme and co-enzyme activation and cell growth, can inhibit and suppress biohydrogen production in too high concentrations (49). For example, magnesium ions present in cellular walls and membranes are cellular protein builders but in high concentrations they may accelerate glycolysis which leads to increase of glycolytic metabolites and therefore decreases availability and production of pyruvate which causes inhibition of biohydrogen production (48,49).

4.1.2. Light fermentation

Photofermentation (light fermentation) is a process of light energy to biomass conversion where photosynthetic bacteria degrade various of substrates in order to produce H_2 and CO_2. Usually the relation is

nearly stoichiometric. For this process purple non-sulphur (PNS) photosynthetic bacteria like *Rhodobacter* species are used for organic acids (acetate, lactate and butyrate) conversion in anaerobic and anoxic conditions. In this process hydrogen production is driven by nitrogenase where redox balancing of metabolism is needed, since nitrogenase is oxygen sensitive there is no problem during photofermentation due to ability of the purple non-sulphur bacteria of anoxygenic photosynthesis where no oxygen evolution is present. Therefore, no inhibition effect to nitrogenase activity is observed which is a major advantage over direct biophotolysis process discussed later on. During the process, bacteria capture solar energy, Adenosine triphosphate (ATP) is formed through mechanism of photosynthesis in bacteria, to convert organic acids into H_2 through nitrogenase in the absence of ammonium ions (20). The overall reaction is following:

$$CH_3COOH + 2H_2O + \text{solar energy} \rightarrow 4H_2 + 2CO_2 \quad [11]$$

Theoretically, process of photofermentation is capable of complete conversion of organic matter into H_2, reaching relatively high yields of H_2. Another big advantage is the capability of wide variety of feedstock use by fermentation bacteria like industrial wastewater and agricultural waste such as dairy food, olive mill waste or molasses. Even with the high conversion to biohydrogen in the process there are several limitations for practical application, including low-light conversion efficiency that leads to need for extraordinary large surface areas for any bioreactors, low volumetric rates of production (12), low photosynthetic conversion efficiency. One of the investigated enhancements for biohydrogen production via photofermentation is various chemicals addition in appropriate amounts. Iron and molybdenum addition to the medium results in biohydrogen rates and yields improvement, since those chemicals increase nitrogenase activity. Similar effect can be obtained by EDTA, vitamins, buffer solution, ethanol, nanoTiO_2 addition to the medium. This effect needs to be further studied to fully understand the chemicals addition enhancement effect, since depending on the concentration of added chemicals, biohydrogen generation can also be inhibited (50).

Ever more frequently photofermentation is discussed in terms of possibility of dark fermentation and photofermentation two-stage hybrid process, where photofermentation would be a second stage of the process to extract more biohydrogen from dark fermentation by-products (51). Maximisation of utilisation of the substrates in this process synergy, without this hybrid process, is impossible to achieve due to thermodynamics barriers. First stage of this combination process is thermophilic dark fermentation where biomass is fermented to acetate, biohydrogen and carbon dioxide. In the second stage acetate from first stage is converted into biohydrogen and carbon dioxide in a separate photobioreactor (52,53). The hybrid system is expected to reach the theoretical maximum production of 12 mol of H_2 (mol glucose)$^{-1}$ equivalent in a following reactions (54):

Stage I — dark fermentation (facultative anaerobes)

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \text{ [12]}$$

Stage II — photofermentation (photosynthetic bacteria)

$$2CH_3COOH + 4H_2O \rightarrow 8H_2 + 4CO_2 \text{ [13]}$$

Some studies on the hybrid system were performed using glucose as substrate and *Enterobacter cloacae* DM 11 strain. In the first stage hydrogen yield 3.4 mol H_2/mol of glucose was achieved, in the second stage about 1.52-1.72 mol H_2/ mol of acetic acid. The overall yield of biohydrogen achieved in the two-stage hybrid system was higher in comparison with single stage process (55). Two-stage fermentation for biohydrogen production scheme is pictured in figure 4.4.

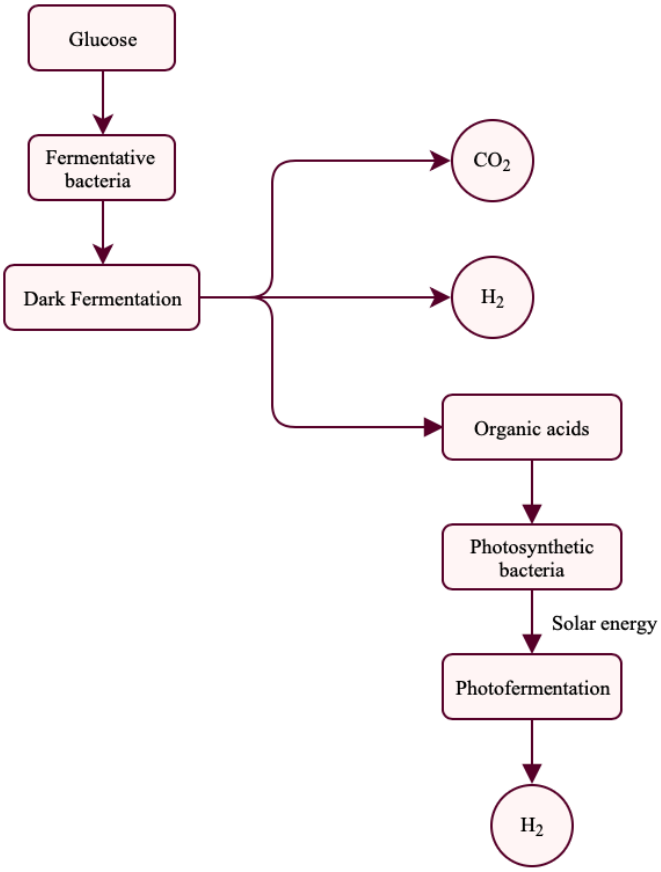


Figure 4.4 - Two-stage fermentation for biohydrogen production scheme.
Adapted from (55).

The three-stage integrated process for biohydrogen production as presented in figure 4.5 is also being discussed. The integrated process consists of photosynthesis, dark fermentation and photofermentation, where green algae absorb visible light and photosynthetic bacteria absorb infrared portion of solar radiation (55).

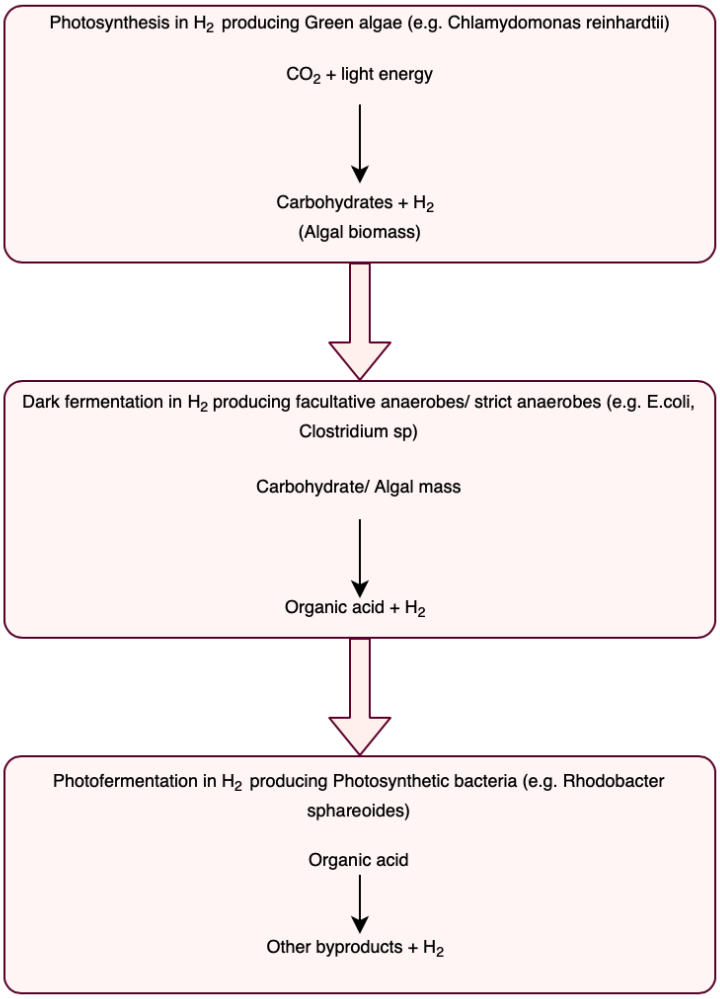


Figure 4.5 - Combination of photosynthetic, dark fermentative and photo-fermentative biohydrogen production processes can achieve higher yield of hydrogen. Adapted from (55).

Another approach is one step hybrid dark and photofermentation system, where both groups of dark- and photofermentation bacteria are co-cultured in a single bioreactor. The advantages of this approach could be simpler manipulation of the process, reduction of fermentation time and potential high H_2 yields from complex substrates (56).

4.2. Anaerobic digestion

In general, as it can be seen in figure 4.6 below, the anaerobic process is of a quite complex nature. Anaerobic digestion is a process of conversion of organic matter into biogas composed of methane and carbon dioxide, where microorganisms break down biomass in the absence of oxygen. This process consists of four stages: hydrolysis phase where polymer substrates are introduced containing carbohydrates, fat and protein, next there is acidification phase where further break down to alcohol, H_2, CO_2 and volatile fatty acids occurs, further in the process products of previous acidification phase break down to acetate in acetogenic phase. Last step in the process in methanogenic phase with methane and CO_2 production.

Hydrolysis phase is the most time limiting phase of overall process and requires long SRT/HRT. Anaerobic digestion is suitable process for highly moist (80-90% moisture) substrates and therefore is promising in terms of using wet algal biomass as feedstock. Acidogenesis phase is the biohydrogen production stage. Acidogenic fermentation can be classified according to microbial proportions into: butyric-type fermentation, where butyric acid, acetic acid, CO_2 and H_2 are produced; ethanol-type fermentation, where ethanol, acetic acid and small amount of CO_2 and H_2 are produced; propionic-type fermentation, where propionic acid, acetic acid, valeric acid with no significant amount of CO_2 and H_2 are produced (57).

Anaerobic digestion is widely used on commercial scale in biogas and therefore biomethane production. In general, biological processes of biomass are dedicated to methane production as methane is the only energy carrier that is produced in nature by conversion of organic matter (16). Hydrogen from technological point of view has higher economic value than methane due to its variable range of applications which is a motor of biohydrogen production investigation. As it can be seen in figure 4.6, during anaerobic digestion process hydrogen is a by-product of acidification phase. While in nature, hydrogen is not released due to bacteria that consume it, in controlled environment we can direct the process into biohydrogen production by for example, physical separation of hydrogen in anaerobic digestion reacting chain. Hydrogen consuming bacteria are hydrogenotrophic microorganisms that are involved in the methanogenic phase and are responsible form converting hydrogen and carbon dioxide into methane. Hydrogen consuming bacteria in hydrogenotrophic methanogenesis include sulphate reducing bacteria and nitrate reducing bacteria, which are responsible for consuming biohydrogen as electron donor (57). The process can also be manipulated into more biohydrogen generation by for example, introduction of microorganisms that leads to expansion of hydrogen production with simultaneous fade away of H_2 consuming bacteria. Anaerobic digestion is base principle for fermentative biohydrogen technologies.

Biohythane is a very promising alternative fuel that contains a mixture of biomethane and biohydrogen. This fuel is extensively researched and its production is based on two-stage process consisting of dark fermentation to generate biohydrogen and the methanogenesis of anaerobic digestion for biomethane production (58). It can be used commercially as vehicle fuel or as energy storage material (59).

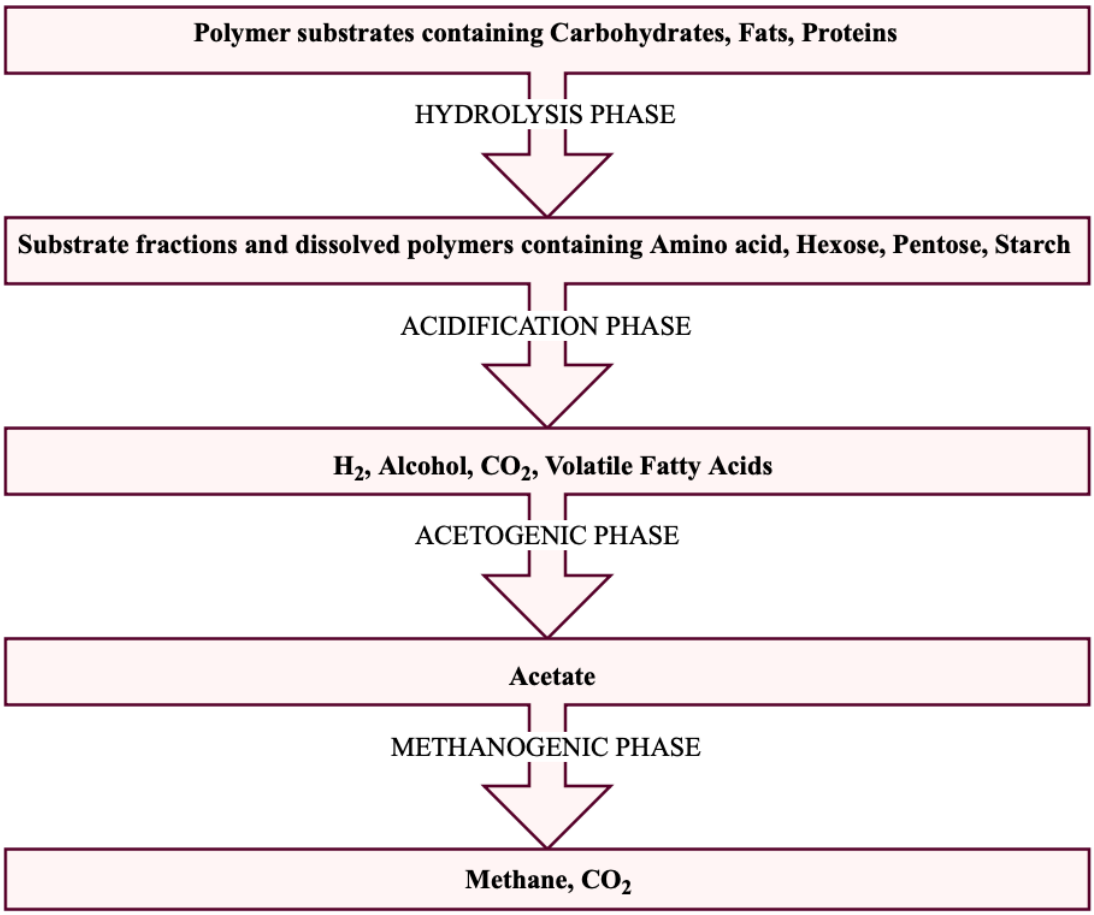


Figure 4.6 - Simplified diagram of anaerobic digestion process. Adapted from (60).

4.3. Gasification

Gasification is a very well-known thermochemical process in which carbonaceous materials are converted to syngas by means of partial oxidation with air, oxygen, carbon dioxide and steam. Gasification converts any carbon-containing material like coal, petroleum coke and biomass into synthesis gas which is composed primarily of carbon monoxide and hydrogen. The chemistry of gasification is of a very complex nature where the series of physical transformations and chemical reactions occur in the gasifier. The feedstock in gasifier undergoes the process of dehydration where any free water is evaporated, living dry material and evolving water vapour which possibly will get involved in two chemical reactions later on. Next step is pyrolysis where the breakage of weaker chemical bonds occurs along with volatile gases like methane and hydrogen release and production of high molecular weight char. Combustion is third step in the process where volatile products and some of the char react with limited oxygen access creating CO_2 and CO. Combustion is exothermic reaction, therefore during this step the necessary heat for subsequent gasification reaction is produced. Last step is gasification reaction, known as water-gas shift reaction, where remaining char reacts with CO_2 and steam producing syngas consisting of CO and H_2.

Gasification reactions:

Combustion

$$C + 1/2\ O_2 \rightarrow CO \qquad (-111\ MJ/kmol) \quad [14]$$

$$CO + 1/2\ O_2 \rightarrow CO_2 \qquad (-283\ MJ/kmol) \quad [15]$$

$$H_2 + 1/2\ O_2 \rightarrow H_2O \qquad (-242\ MJ/kmol) \quad [16]$$

Water-Gas Shift Reaction

$$C + H_2O \leftrightarrow CO + H_2 \quad (+131\ MJ/kmol) \quad [17]$$

Depending on the feedstock composition and gasification conditions like temperature and pressure, chemical reactions of gasification can progress in different extents. Due to the fact that oxidation is limited and feedstock is partially oxidised only minor amount of carbon is completely oxidised to CO_2.

Gasification process can be divided by different oxidation agents into air, steam or oxygen gasification. Air gasification is the most widely performed due to economic reasons. Biomass undergoes the process at 900-1100 ℃ with high efficiency producing low heating value gas of 4-6 MJ/Nm^3 containing up to 60% of N_2. Another type is oxygen gasification in a temperature range of 1000-1400 ℃ producing better quality gas with heating value of 10-15 MJ/Nm^3. The limitation for this technology is cost and safety issues related to oxygen supply. Steam is another oxidation agent used in steam gasification process, limitation for this process is associated with tar as a by-product which can cause problems with corrosion, catalysts poisoning and overall efficiency decreasing. Biomass can be converted into rich hydrogen fuels gas in supercritical water gasification process. The process is carried out over water super critical

point (temperature >374℃, pressure >220bar) where water is capable of dissolving any organic compounds and gases by acting like homogeneous non-polar solvent with high transport and diffusivity properties. The biggest advantage of this process is ability of direct deal with high moisture content of biomass (>50%) which eliminates problematic and costly drying pre-treatment of biomass (61). Steam gasification appears to be most suitable for biohydrogen production and it is one of the most promising thermochemical biohydrogen production pathway.

4.4. Pyrolysis

Pyrolysis of biomass is a conversion of biomass feedstock into gaseous, liquid and solid fractions in a direct heating of biomass in the oxygen absence at around 500-600°C. The conditions vary depending on biomass composition. Biomass pyrolysis is endothermic process that can obtain hydrogen-rich gas.

The overall reaction (62):

$$\text{organic matter} + \text{heat} \rightarrow H_2 + CO + CH_4 + \text{other products} \quad [18]$$

Fast pyrolysis is favoured process over slow pyrolysis in biohydrogen production (62). There are three ways for producing gas rich in biohydrogen. First method would be by steam reforming of pyrolysis liquid product. The second method contains two stages of pyrolysis and it involves the use of catalyst, usually dolomites and Ni, high temperatures, steam and oxygen as well as removal of tar from pyrolysis gaseous product. Third method incorporates two stages from previous method into one by lowering the temperature (<750℃) of pyrolysis process and directly use of catalyst during the pyrolysis of the biomass. This process is considered most economically profitable nowadays. In terms of thermochemical conversion process for biohydrogen production pyrolysis is said to be one of the most promising pathway (16). Since the process is taking place in the absence of oxygen, by-products like dioxins are eliminated, moreover the formation of carbon oxides is minor, pyrolysis is noticeably decreased in problematic emissions, simplicity of the process as well as adaptability of the fuel are advantages of this technology (62).

4.5. Biophotolysis

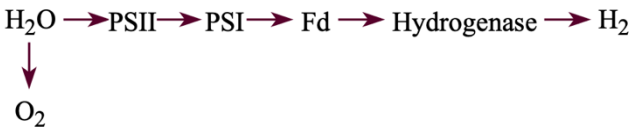
Biophotolysis is a process of oxygenic photosynthesis where solar energy is captured and water splitting coupling to proton reduction occurs. In theory this process has essentially limitless substrate supply and availability of potential energies with a total irradiance of 1.74×10^{17} W (12). Feedstock like green algae and photoautotrophic cyanobacteria break down water to biohydrogen and oxygen in the presence of light, converting therefore, solar energy to biochemical energy. Two types of biophotolysis can be recognised: direct and indirect.

4.5.1. Direct biophotolysis

Direct biophotolysis is a process of simple water splitting producing biohydrogen by either green algae or cyanobacteria. Very high energy input from solar radiation is demanded in water splitting reaction. As presented in the following reaction (63):

$$2H_2O + \text{solar energy} \rightarrow \quad 2H_2 + O_2 \qquad \Delta G^{0'} = +749 \text{ kJ} \cdot \text{mol}^{-1} \text{ [19]}$$

Direct biophotolysis is the same process that can be found in algal photosynthesis and plants but instead of adapting biomass containing carbon the process is manipulated for biohydrogen generation. Biohydrogen production in direct biophotolysis appears very attractive as it uses highly available sources, for this reason direct biophotolysis appears to allow for unlimited biohydrogen production from the most abundant resources on Earth which are sunlight and water. In this technology two photosynthetic systems: photosystem PSI (reductant production for CO_2 reduction) and photosystem PSII (splitting water and evolving oxygen) occur with complex sets of reactions. Oxygen and hydrogen ions generation of algae such as *Chlamydomonas reinhardtii* and biohydrogen production by cyanobacteria are used by the systems (34). The most important media used in the process are reverse hydrogenase, ferredoxin and reduced ferredoxin (Fd), the pathway is presented in the simplified reaction (34):



[20]

Unfortunately, major limitation for this process is simultaneous production of oxygen. In order to use green algae as a resource for biohydrogen production, crucial first step is needed for the synthesis as well as activation of enzymes mainly hydrogenase enzyme, this step is obtained by few minutes up to several hours of anaerobic incubation without access of light. As it was mentions previously reversible hydrogenase, the key enzyme that catalyses the biohydrogen production in algae is very sensitive to presence of oxygen. Therefore, oxygen is an inhibitor in biohydrogen production process, which results in 1.5% of general conversion efficiency from solar energy to biohydrogen. If oxygen is removed immediately the conversion efficiency can increase to 3-10%. This can be achieved by genetic engineering methods as well as manipulation of concentrations of certain metals ions since they can influence the enzyme activity in biohydrogen production (16). The oxygen level content needs to be kept below 0.1% in order to achieve successful biohydrogen production (34,64). Several other solutions have been introduced such as the use of oxygen tolerant hydrogenase enzymes as well as irreversible and reversible

O_2 absorbers. Most promising solution appears to be a development of microalgae with insensitive oxygen hydrogenase reaction. We can notice other challenges both biological and engineering in obtaining direct biophotolysis like gas separation, since the process requires production and immediate capture of oxygen and biohydrogen in one enclosed photobioreactor, managing H_2/O_2 mixture in large volumes and areas is necessary, which appears to be economically inviable and highly impractical (65). Another problem challenging this process is low light conversion efficiency. Light-to-biohydrogen conversion efficiencies in direct biophotolysis are below 0.1% which appear to be insufficient and impractical in terms of commercial use (65).

4.5.2. Indirect biophotolysis

Indirect biophotolysis was design to address the oxygen inhibition of biohydrogen production problem in direct biophotolysis. This process involves two stages: biohydrogen production and oxygen separation in space or time. Oxygen separation in space method consist of two phases, where in first phase the photosynthesis into carbohydrates and oxygen from atmospheric CO_2 is taking place in an open pond. In the second phase anaerobic and dark conditions are applied in closed bioreactor where carbohydrates are degraded to acetic acid and biohydrogen. The chemical pathway is presented in the following reactions:

$$6H_2O + 6CO_2 + \text{light} \rightarrow C_6H_{12}O_6 + 6O_2 \quad [21]$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2 \quad [22]$$

$$2CH_3COOH + 4H_2O + \text{light} \rightarrow 8H_2 + 4CO_2 \quad [23]$$

$$\text{Overall reaction: } 12H_2O + \text{light} \rightarrow 12H_2 + 6O_2 \quad [24]$$

Figure 4.7 describes the pathway for organic acids and glucose in purple non-sulphur bacteria for photoheterotrophic biohydrogen generation.

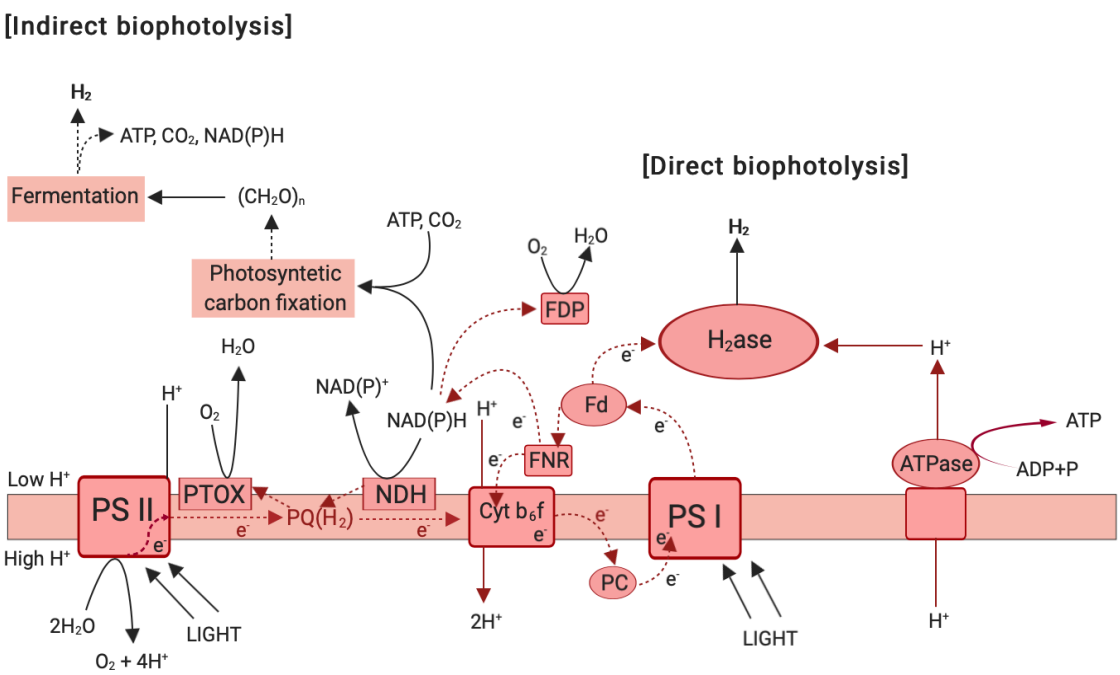


Figure 4.7 - Photoheterotrophic H_2 production metabolic pathway for organic acids and glucose in purple non-sulphur bacteria where: AC: acetate; ACCOA: acetyl-CoA; D-MAL: D-malate; Fd: ferredoxin; FHL: formate: hydrogen lyase; FOR: formate; GLC: glucose; HydC: ferredoxin-dependent hydrogenase; LAC: lactate; N2ase: nitrogenase; OR: oxidoreductase; PFL: pyruvate: formate lyase; PFOR: pyruvate: ferredoxin oxidoreductase; PRO: propionate; PS: photosystem; PYR: pyruvate; SUC: succinate; UQ: ubiquinone. Adapted from (12).

The oxygen separation method in time consist of two steps, where first step is photosynthesis with oxygen production and carbohydrates storage. The second step is either lack or deprivation of sulphur in growing media, therefore the suppression of photosynthesis and oxygen evolution following by favouring biohydrogen production through mitigation of hydrogenase activity (16). This method becomes limited with time due to the fact that after 60h of production hydrogen yield starts to level out (9). In cyanobacteria, there are cultures that are able to fix nitrogen like marine bacteria cyanobacteria *Calothrix* sp., *Oscillatoria* sp. and non-marine *Anabaena* sp. as well as non-fixing nitrogen bacteria for example Synechococcus sp., Gloebacter sp.(18). The following reactions present biohydrogen production by nitrogenase (34):

with nitrogen:

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi \quad [25]$$

without nitrogen:

$$16ATP + 8H^+ + 8e^- \rightarrow 4H_2 + 16ADP + 16Pi \quad [26]$$

The main advantage of this process is separation of O_2 in H_2/O_2 mixture, another asset is ability of by-product conversion into biohydrogen. The major limits include hydrogenase uptake consumption of H_2 as well as overall low production rate, problematic on a large-scale continuous need of light source presence (66). Indirect biophotolysis conversion efficiencies are <1%. Sulphur limitation was a huge breakthrough in biohydrogen evolution increase due to deactivation of photosystem PSII and therefore limitation of oxygen generation (67).

In table 4.3 little summary of biological pathways for obtaining biohydrogen is presented with main advantages and disadvantages for biological processes mention in this chapter.

Table 4.3 - Advantages and disadvantages of various biological processes for biohydrogen production (16).

Process	Microorganism	Advantages	Disadvantages
Direct biophotolysis	Green algae	Hydrogen is produced directly from water and sunlight	High light intensity is required
		Solar conversion energy increased by tenfold in comparison to crops and trees	Oxygen can be dangerous for the system
Indirect biophotolysis	Cyanobacteria	Produce hydrogen from water	Removal of hydrogenase enzymes to avoid degradation of hydrogen
		Ability to fix nitrogen from atmosphere	Lower photochemical efficiency
			Oxygen presence at 30%
			Inhibitory effect of oxygen on nitrogenase
Dark fermentation	Fermentative bacteria	Wide variety of feedstock	Gas mixture needs cleaning and updated from presence of CO_2
		Hydrogen production under dark	Relatively low biohydrogen yields
		Metabolites produce added value products	
Photofermentation	Photosynthetic bacteria	Use of wide-spectrum light energy	Low light conversion efficiency
		Wide variety of feedstock	Presence of oxygen inhibits hydrogenase

4.6. Electrohydrogenesis

The most recent technology introduced in biohydrogen production pathways group is biocatalysed electrolysis which is used in microbial electrolysis cells (MECs) and bio-electrochemical systems (BESs). The principle of biocatalysed electrolysis is biochemical conversion obtain with addition of low voltage from external power supply therefore it's a variation of the microbial fuel cell (MFC). MEC consist, like conventional batteries and water electrolysis cells, of two chambers separated by semi-permeable membrane in order to avoid hydrogen diffusion to the anode chamber (63). The principle of the process is what follows: organic matter (e.g. acetate Ac^-) microbes transfer electrons to the anode through oxidation either by direct contact or indirect electron shuttle, after at the anode the shuttle is reoxidised and returns to the culture. At the cathode, H^+ protons diffused through the cation exchange membrane (CEM), together with cathode electrons are formed into hydrogen. In this process electrons, protons and carbon dioxide are created (34). Figure 4.8 pictures microbial electrolysis cell principle.

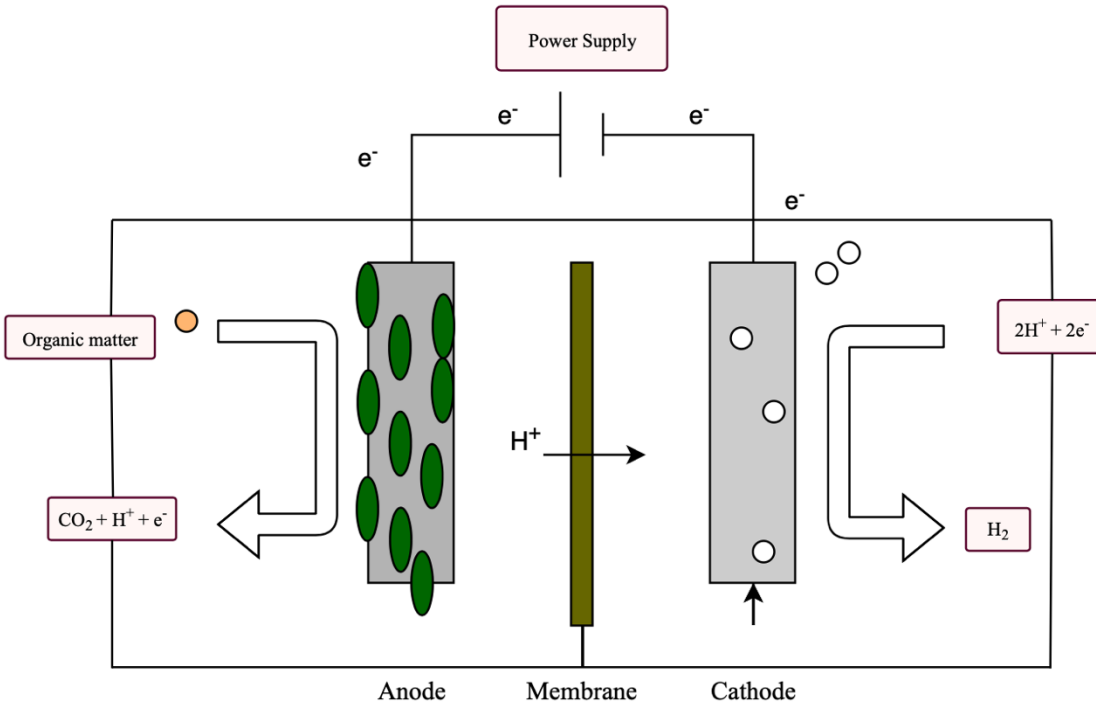


Figure 4.8 - Principle of microbial electrolysis cell. Adapted from (68).

Environment needs to be anaerobic due to potential oxygen interference with either of chambers. Low voltage supply is required to drive this reaction due to potentials difference where anode reduction potential is higher than cathode one. The minimum theoretical voltage needed is within 0.2-0.8 V range, although due to ohmic resistance, in practice higher voltage is applied. The anode and cathode reactions for hydrogen production by electrolysis of acetate are presented below (34,63):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 [27]$$

Anode:

$$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^- \quad E^{0'} = -0.28\ V\ [28]$$

Cathode:

$$8H^+ + 8e^- \rightarrow 4H_2 \qquad E^{0'} = -0.42\ V\ [29]$$

As in any technology, there are variety of factors affecting biohydrogen production in MEC, one of the major factors determining MEC performance is pH as it influences both the kinetics and thermodynamics of the reaction. According to Nernst equation the cell potential is directly related to pH by the equation (69):

$$E_{cell} = -0.059 \times pH\ [30]$$

Therefore, with the increase of pH, the anodic potential decreases by -0.059 V and E_{cell} is inversely proportional (69). At pH 7 the hydrolysis of acetate potential is approximately -0.28 V. Due to relation where pH increases with anodic potential decrease, we conclude that with the increase of anolyte pH the overall difference in potentials increases. According to studies conducted by (70) for biohydrogen production the optimal pH is around 9. The pH is also determined by the kind of bacteria used in the process, in general most bacteria operate in alkaline pH but there are exceptions such as fungi requires more acidic pH to perform. The use of catalyst in the process increase overall biohydrogen yield. The most common catalyst used for cathode reaction are noble metals mainly platinum due to its inertness, impressive catalytic properties, stability and low over potential. Unfortunately, platinum has one big disadvantage which is high cost as well as possibility of poisoning. The search for more sustainable catalyst material approaches towards biocathode idea, where cathode reactions are catalysed by microorganisms. In case of anode material, carbon-based materials are most widely used since they cover most of the requirements although their conductivity is relatively low and they are not always economically viable. Therefore, there are research conducted towards optimal anode material among the most promising we can distinguish carbon nanotubes with great stability, conductivity and large specific areas, unfortunately their potential bacterial toxicity is a major drawback. Graphene is a material highly tested for anode production (71). Stainless steel and nickel alloys appear to be good platinum replacement for anode material since they are low cost and don't exhibit performance loss (63). When it comes to microorganisms used in MEC research, most come from wastewater treatment or other sediments undefined microbial consortia. Interestingly, when pure bacteria culture (mainly *Geobacter sulfurreducens*) were tested, it resulted in similar biohydrogen production yields (63). Most conducted studies use membrane application in MEC, however it was investigated that membrane cause proton diffusion towards cathode creating more resistance as well as leakage of H_2 to the anode, moreover membrane application causes substantial potential loss due to pH gradient formation (63). Taking above into account, removal of the membrane might improve the overall process and lower the cost of biohydrogen production, although in this case additional gas upgrading step is required due to mixing of O_2 with H_2. Electrohydrogenesis exhibits many advantages such as utilisation of lignocellulosic biomass, acetate and bu-

tyrate, industrial wastewaters and pure substrates such as proteins and sugars, moreover this technology is able to carry complete substrate conversion with high rate. It is relatively new technology, therefore many challenges need to be overcome such as exoelectrogenic strains development with better electron releasing capacity, reduction of production costs, performance enhancement of MEC to overcome energy losses and methane generation prevention that hinders the MEC performance affecting efficiency, rate and yield of biohydrogen production (72). Last but not least, MEC and dark fermentation process integration emerged, since typical by-products of dark fermentation mainly acetate operates the best in MEC process. Integration of MEC with dark fermentation appears to be promising approach since both processes exhibit high biohydrogen yields and both are in similar technology development scale (73). An overall H_2 recovery of 96% was obtained by treating molasses feedstock with MEC and dark fermentation integrated system (74). It is a fresh concept and only few researches have been performed, therefore the integration concept needs more development and research.

5. Results and comparison of available data

The goal of this chapter is to present available data on biohydrogen production potential regarding different pathways, biohydrogen economy as well as energy consumption and CO_2 emissions.

5.1. Energy consumption and CO_2 emissions

There are few studies regarding energy consumption by biohydrogen production as well as CO_2 emissions. Ferreira A., Ribau J. & Silva C. (75) analysed the biohydrogen production pathways from sugarcane and potato peels, their LCA Wheel-to-Tank (WTT) analysis showed that biohydrogen from potato peels LCA is 0.49-0.61 MJ/MJ_{H2} for energy consumption and 59.78-70.39 gCO_2/MJ_{H2} for CO_2 emission. In sugarcane case the results were 0.30-0.34 MJ/MJ_{H2} for energy consumption and 23.74-31.06 gCO_2/MJ_{H2} for CO_2 emission. All obtained results are shown in figure 5.1.

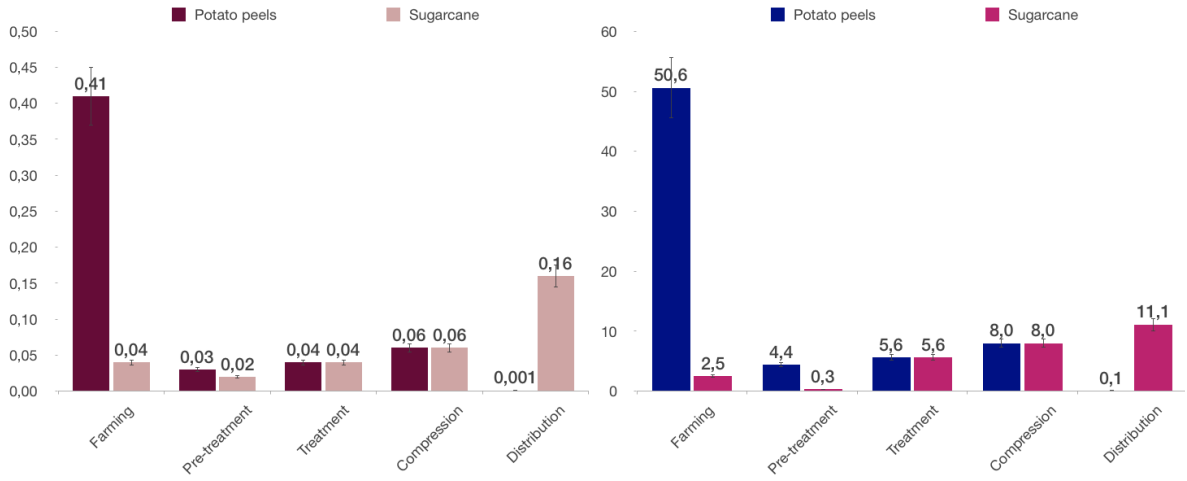


Figure 5.1 - Energy consumption and CO_2 emissions regarding biohydrogen production from potato peels and sugarcane. Results obtained by Monte Carlo simulation. Adapted from (75).

This research showed, regarding WTT, that feedstock used have a potentially lower energy consumption and CO_2 emissions than hydrogen produced from natural gas reforming and electrolysis (75). Sugarcane as feedstock exhibits lowest values for energy consumption and CO_2 emission. Farming in potato peels case stands for 75% of total energy consumption and CO_2 emissions, however if potato peel would be considered as by-product of potato production, farming should be excluded form LCA and consequently the WTT energy consumption and CO_2 emissions values would drop to 0.13-0.28 MJ/MJ_{H2} and 14-25 gCO_2/MJ_{H2} respectively (75). Energy costs and requirements of biohydrogen production were also considered by researches and they concluded a potato peels biohydrogen cost of 1.1-1.7 €/kg H_2 and a sugarcane biohydrogen cost 0.5-0.7 €/kg H_2. This research show that kind and availability of feedstock is crucial factor not only in optimisation and sustainability of biohydrogen production process but also in CO_2 emission and energy consumption of the process, which directly influence economic viability of bioH_2 technologies.

Another research regarding energy consumption, CO_2 emissions and economics analyses by Ferreira A., Ribeiro L., et al. (76). was conducted. In this research, three biorefineries and five pathways were analysed:

#1 path oil extraction by Soxhlet (oil SE);

#2 path oil and pigment extraction and fractionation by supercritical fluid extraction (oil and pigment SFE);

#3 path hydrogen production by dark fermentation from the leftover biomass after Soxhlet extraction (biological H_2 via SE);

#4 path hydrogen production by dark fermentation from the leftover biomass after supercritical fluid extraction (biological H_2 via SFE);

#5 path hydrogen production from the whole biomass by dark fermentation (biological H_2 via whole biomass)

Where #1 and #3 paths are biorefinery 1, biorefinery 2 consist of #2 and #4 paths, where else #5 path is direct biohydrogen production. The results from this research are shown in table 5.1 and table 5.2.

Table 5.1 - Energy input and CO_2 emission in different processes in the biorefinery (76).

	Oil SE (Path #1)						**Oil and pigment SFE (Path #2)**								
	BioH₂ via SE (Path #3)						**BioH₂ via SFE (Path #4)**			**BioH₂ via direct biomass (Path #5)**					
	Soxhlet	Evaporation	CO_2 pump	EtOH pump	Bath heating	Cooling	Fermentation medium	Sterilisation	Fermentation (incubation)	Fermentation medium	Sterilisation	Fermentation (incubation)	Fermentation medium	Sterilisation	Fermentation (incubation)
Energy (MJ)	0.760	0.252	0.947	0.285	0.594	0.259	0.002	0.002	0.050	0.002	0.002	0.050	0.004	0.004	0.100
Min	0.612	0.203	0.763	0.230	0.453	0.209	0.0019	0.001	0.0 40	0.0019	0.001	0.040	0.0040	0.003	0.081
Max	0.847	0.281	1.056	0.318	0.636	0.289	0.0023	0.002	0.056	0.00 23	0.002	0.056	0.0051	0.004	0.112
CO_2 (g)	49.6	16.4	61.8	18.6	38.8	16.9	0.16	0.12	3.26	0.16	0.12	3.26	0.33	0.24	6.53
Min	44.3	14.7	55.2	16.6	32.7	15.1	0.15	0.11	2.92	0.15	0.11	2.92	0.31	0.22	5.83
Max	53.1	17.6	66.1	19.9	39.9	18.1	0.18	0.13	3.49	0.18	0.13	3.49	0.35	0.26	6.99

Table 5.2 - Total energy consumption and CO_2 emissions for each pathway and biorefineries and respective uncertainty (76).

	E (MJ/MJ_{prod})	Min	Max	CO_2 (g/MJ_{prod})	Min	Max
Path #1	220	177	245	14,320	12,774	15,357
Path #2	262	210	291	17,123	15,167	18,258
Path #3	147	119	164	9665	8645	10,369
Path #4	168	136	187	11,020	9858	11,820
Path #5	9058	7285	10,123	591,112	527,022	634,402
Biorefinery 1 (Path #1 + Path #3)	214	172	239	13,982	12,471	14,994
Biorefinery 2 (Path #2 + Path #4)	258	206	286	16,800	14,881	17,913

From the results presented in the tables 5.1 and 5.2 we can see the energy requirement for biorefinery 1 was 2.67–3.70 MJ. Energy produced was 0.015 MJ of biodiesel and 0.0004 MJ of biological H_2. Energy consumption resulted in 172–239 MJ/MJ produced and in terms of CO_2 emissions 12,471–14,994 g CO_2/MJ produced. In biorefinery 2 total energy consumption was 206–286 MJ/MJ produced, and CO_2 emissions resulted in 14,881–17,913 g CO_2/MJ produced. The merge of pathways (products and co-products) results in small energy efficiency improvement of 2.7% in biorefinery 1 and 1.9% in biorefinery 2 (76). Microalgal residues used as substrate for biohydrogen production in this research exhibits higher biohydrogen yields than biohydrogen yield obtained by the whole *Nannochloropsis* sp. dried biomass. Path #5 exhibits higher energy consumption and CO_2 emissions, even with the elimination of artificial illumination and drying step, which would correspond to drops in energy input to 8195 MJ/MJH_2 produced and in CO_2 emissions to 534,503 gCO_2/MJH_2 produced, still in comparison to biorefinery 1 and 2 the values are significantly higher. In biohydrogen production from the whole biomass, the most energy consuming and consequently most CO_2 emitting stage was the microalga culture due to high requirement for water and nutrients following by the harvest and fermentation medium stages. In case of biohydrogen production from biomass residues, the fermentation stage exhibits highest values for energy consumption and emissions (76). Path #5 among all studied dark fermentation pathways (Path #3, #4 and #5) is the most expensive one. Path #4 proved to be the most economically viable (76). This research shows that biorefinery concept significantly lowers energy consumption and CO_2 emissions for biohydrogen production.

Different research performed by Ferreira A., Ortigueira J. at al. (77) regarding energy consumption and CO_2 emissions, during fermentation of microalgae in biohydrogen production process, showed energy requirement of 71-100 MJ/MJ H_2 and CO_2 emission of 5-6 kg CO_2/MJ H_2 (77). The results are presented in table 5.3. The biohydrogen production yield obtained in this research was 7.3 g/kg of S. obliquus dried biomass. Taking into consideration energy consumption and emissions with biohydrogen yield obtained, the results are considerably high and unsustainable to meet pilot/industrial scale requirements (77).

Table 5.3 - Energy and CO_2 emission of whole processes of biohydrogen production (77).

	Energy (MJ)	Min	Max	CO_2 (g)	Min	Max
Microalga culture						
Nutrients	0.002	0.001	0.002	0.12	0.10	0.15
Water	0.001	0.000	0.002	0.05	0.02	0.10
Paddle wheels	0.005	0.004	0.005	0.31	0.28	0.33
Centrifugation	0.040	0.032	0.045	2.63	2.35	2.82
Drying	0.001	0.001	0.001	0.05	0.04	0.05
Pre-inoculum preparation						
Degasification	0.006	0.005	0.006	0.37	0.33	0.40
Sterilisation	0.004	0.003	0.004	0.26	0.23	0.28
Inoculum preparation	0.001	0.001	0.002	0.10	0.09	0.10
Basal medium (BM1)						
Nutrients	0.0002	0.0001	0.0002	0.01	0.01	0.01
Phosphate buffer	0.0021	0.0017	0.0024	0.14	0.12	0.15
N_2 degasification	0.006	0.005	0.006	0.37	0.33	0.40
Sterilisation	0.004	0.003	0.004	0.25	0.23	0.27
Stock solution preparation						
Yeast Nitrogen Base (YNB)	0.004	0.003	0.005	0.27	0.24	0.29
Incubator						
Incubation	0.005	0.004	0.005	0.32	0.29	0.34

The highest energy consumption and emissions values were obtained in microalga culture stage, where centrifugation unit is most contributing to those high values. In biohydrogen production, electricity is the biggest (95%) contributor in energy consumption, therefore the high values of energy consumption and CO_2 emissions can be decreased by using renewable sources of electric energy (77). Similar to the results obtained in previously mentioned research by (76) biomass as a by-product results in around 38% decrease in energy consumption and emissions due to exclusion of microalgal biomass production from LCI (77). Researchers also examined the potential scale-up scenario that resulted in energy consumption around 6-8 MJ/MJ H_2 and with very promising negative values of (-716) to (-613) g/MJ H_2 for emissions (77).

There are other studies using different biomass feedstock, as mentioned before.

San- Martín M.I., Alonso R. M., Pelaz G., Escapa A. (78) conducted a study of semi-pilot microbial electrolysis cell (MEC) for biohydrogen production using pig slurry as a feedstock. The aim of the study was to examine biohydrogen production yield, organic matter degradation rates and energy consumption. For the experiment, two-chamber MEC with membrane has been used with anodes made from 5 mm-thick graphite felt and cathodes made of a stainless-steel mesh. Projected surface area of both electrodes was 2226 cm^2, the electrodes were wired to power source with applied voltage of 1V between

cathode and anode. The catholyte consisted of a phosphate buffer (PBS 0.1M) and anolyte was consisting of the treated pig slurry and they were separated by cationic exchange membrane (CEM) for NH_4^+ cations to flow from the anolyte to the catholyte. In table 5.4 pig slurry characterisation used as anolyte in this study is given.

Table 5.4 -- Pig slurry characterisation (78).

Parameter	Units	Value
COT	g/L	2,81
Acetate	mg/L	173
NT	g/L	2,03
Phosphate	mg/L	32
Sulphate	mg/L	34
Chloride	g/L	2,06
TSS	g/kg	17.3
VSS	g/kg	7.6
%VSS	%	44
pH	-	8
Conductivity	mS/cm	20.4

After inoculation of the anodes the electrical current was gradually increasing until stabilisation occur after 16 days at current density of 3.5 A per m^2 of anode. After stabilisation reactors started to operate at batch mode. The anode was fed with 20% of diluted pig slurry at initial time and after the dilution rate was gradually increased. With the increase of pig slurry in the feed, hydrogen production rate was increasing until it reached maximum of 0.16 L of hydrogen per litre of reactor and per day (0.16 $L_{H2}(L_R\,d)$) at dilution rate of 70%. Energy consumption was improving with dilution rate until reaching a minimum of ~3 kWh per m^3 of hydrogen, which fits the range of energy consumption by conventional hydrogen technologies (78). After dilution rate reached 80%, hydrogen production rate started to decrease and energy consumption increased, most probably due to organic matter overload or ammonia inhibition. Since pig slurry is characterised by relatively high ammonia content, NH_4^+ cations were recovered with the 57% efficiency. Due to low biodegradability of pig slurry, efficiency of organic matter removal was quite low (below 20%) which negatively influenced the overall hydrogen production rate as well as ammonia recovery rate. This effect could be potentially limited by adding more biodegradable carbon-rich feedstock for example cheese whey which leads to the possibilities of combining different wastes and that would be beneficial approach for industrial scale in the future (78). Energy consumption and hydrogen production rate as a function of the amount of slurry in the feed in this research is given in the figure 5.2.

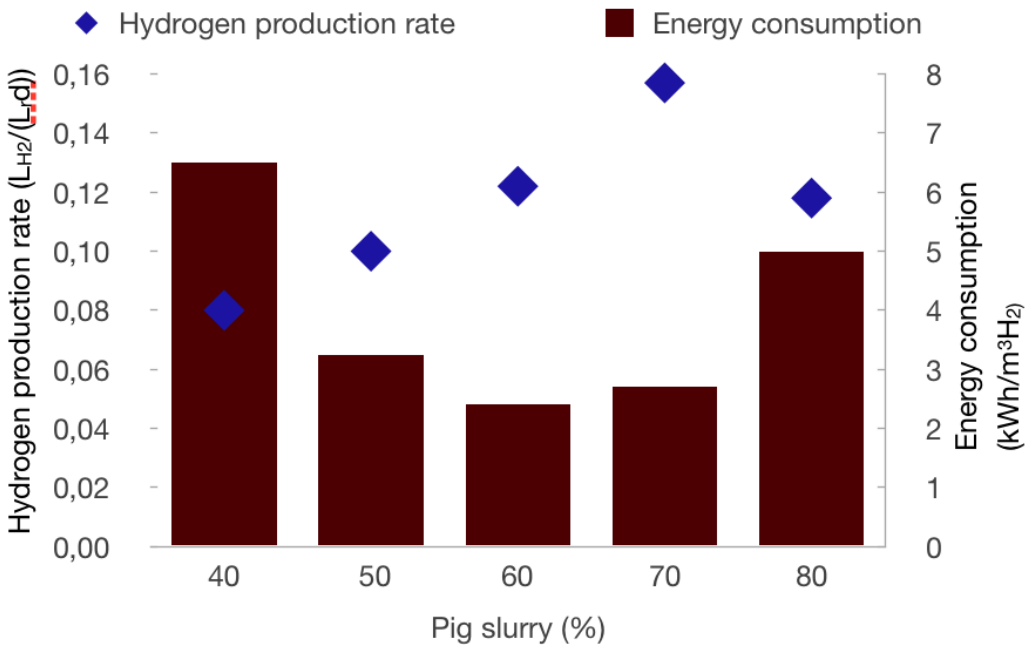


Figure 5.2 - Energy consumption and hydrogen rate as a function of the amount of slurry in the feed. Adapted from (78)

Aall Ø. (79) performed a scenario-based life cycle assessment of environmental impacts of large-scale adoptions of hydrogen as energy carrier. In this study, dark fermentation and photofermentation were examined with biomass feedstock. In terms of the greenhouse gases emission, the highest share of emissions was contributed by fermentation (45%), pre-treatment was responsible for 29% of emissions share and biomass feedstock contributed to 26% of emissions. The study results in the figure 5.3, show that the main GHG emissions contributor is electricity-use in the hydrolysis process, enzyme treatment and fermentation of biomass process, where 93% comes from fermentation process and only 7% from pre-treatment. The electrical-use was for running machines and pumps during fermentation process. The second highest contribution in emissions is steam from pre-treatment which is used to heat the biomass for enzyme treatment as the energy used for heat is fossil gas.

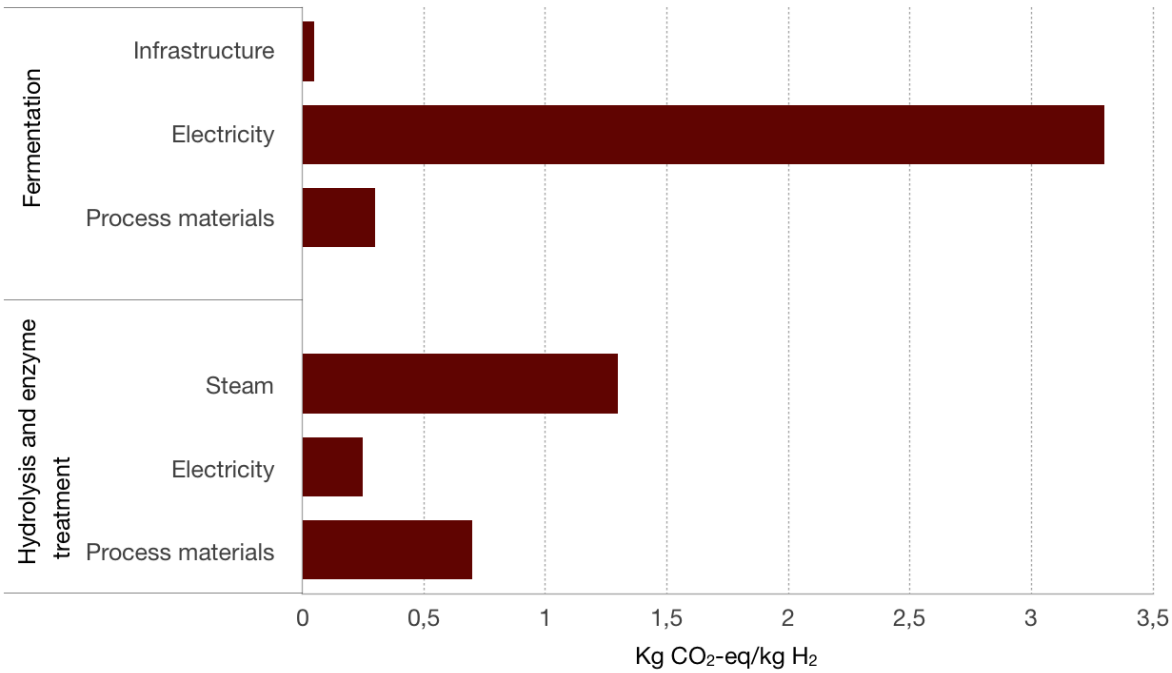


Figure 5.3 - GHG emissions from pre-treatment and fermentation of biomass. Adapted from (79).

In case of dark fermentation and microbial electrolysis combination, the distribution of GHG emissions presents what follows: the highest share of emissions is from biohydrogen production with 69%, the pre-treatment accounts for 16% and biomass feedstock for 15%. In Figure 5.4 we can observe that, as in previous case, the highest contributor to GHG emissions is electricity-use for MEC with 7.4 kg CO_2-eq per kg H_2 which accounts for 56% of total emissions. This is the result of high consumption of electricity (15 kWh/kg H_2) and the emissions from electricity production in Europe (0.492 kg CO_2eq/kWh) (79). The second highest emissions are due to use of steam.

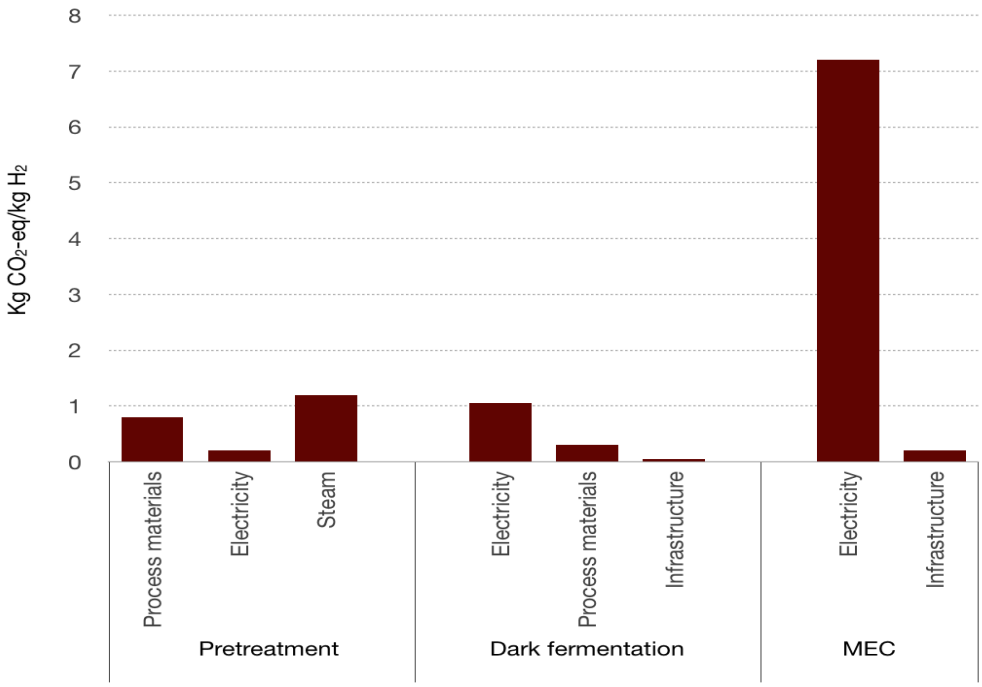


Figure 5.4 - Process emissions from dark fermentation and MEC of biomass. Adapted from (79).

This study also conducted a Scenario for 2050 case for electricity mix and heat, where it was assumed that all the processes, which include use of steam and heat using fossil fuels as a source, use biomass instead. The assumption was a reduction of GHG emissions by 37% per kWh in European electricity mix in comparison to present emissions level. Similar conclusions were achieved in research conducted by Ferreira A., Ortigueira J., et al. (77).The results of using biomass as energy carrier for steam and heat are presented below in figure 5.5.

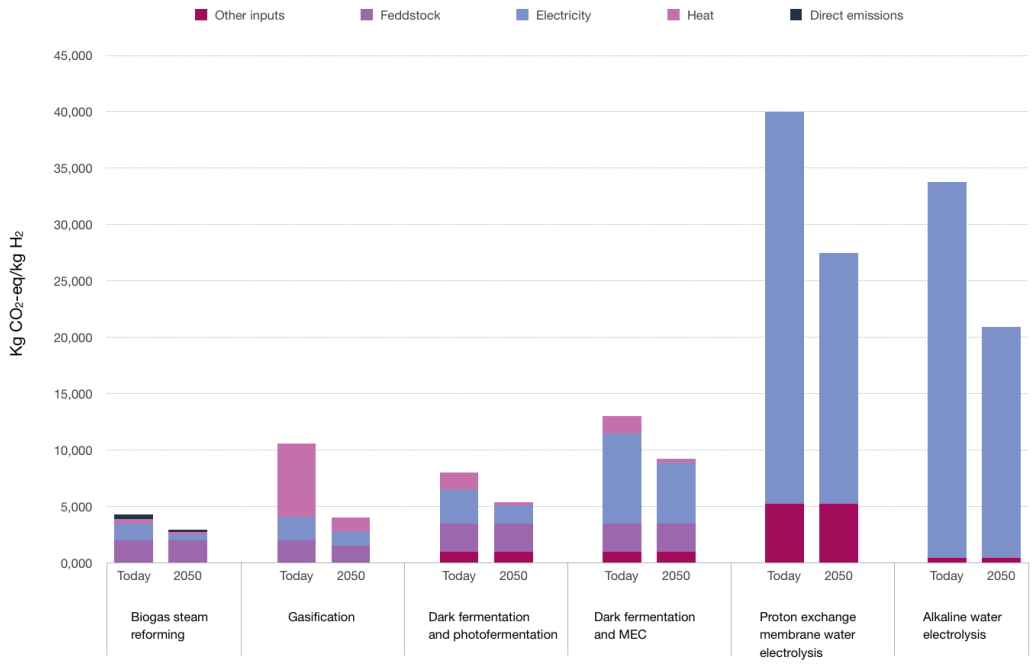


Figure 5.5 - Scenario2050 for GHG emissions per hydrogen unit for various methods. Adapted from (79).

Figure 5.5 shows that most promising results in Scenario 2050 are obtain by biogas steam reforming with 2.6 kg CO_2-eq/kg H_2 emissions followed by gasification with 4.9 kg CO_2-eq/kg H_2 emissions and dark fermentation integrated with photofermentation with 5.5 kg CO_2-eq/kg H_2.

5.2. Biohydrogen economy

In every technology development, economic viability of the process is crucial aspect in terms of industrial scale application.

Amos W. (80) conducted an estimation regarding cost of algal biohydrogen production for 300kg^{-1} stand-alone system with pond area of 110,000 m^2. Table 5.5 presents the estimations.

Table 5.5 - Biohydrogen production from algae capital costs (80).

Units	Cost [$]
Algae Ponds	1,100,000
PSA Compressor	359,000
PSA Unit	121,000
Storage Compressor	578,000
High-Pressure Storage	913,000
Total Equipment Cost	**3,071,000**
Engineering and Construction	1,423,000
Contractor Fees and Contingency	674,000
Total Capital Investment	**5,168,000**

Cost of biohydrogen production in comparison to conventional methods was analysed by (81), the analysis showed that hydrogen production cost, except co-pyrolysis of coal with biomass, is higher in comparison to conventional methods of fuel production. Therefore, more research and development are needed to make biohydrogen production more cost-effective. The cost comparison is presented in table 5.6.

Table 5.6 - Unit cost comparison of hydrogen production processes with conventional processes (81).

Name of the process	Raw materials	Energy content of the fuel (MJ kg^{-1})	Unit cost of energy content of the fuel (US \$/ $MBTU^{-1}$)
Photobiological H_2 hydrogen	H_2O, organic acids	142	10
Fermentative hydrogen	Molasses	-	10
Pyrolysis for hydrogen production	Coal, biomass	-	4
Hydrogen from water electrolysis	H_2O	-	11
Hydrogen from nuclear energy	Electrolysis and water splitting	-	12-19
H_2 by biomass gasification	Biomass	-	44-82
H_2 from wind energy	Wind mill	-	34
H_2 from photovoltaic power station	Solar energy	-	42
H_2 from thermal decomposition of steam	H_2O	-	13
H_2 from photochemical	Organic acids	-	21
Gasoline	Crude petroleum	43.1	6
Fermentative ethanol	Molasses	26.9	31.5
Biodiesel	Jatropha seed	37.0	0.4
Natural gas	Raw natural gas	33-50	10

In table 5.7 conversion efficiency, production costs and CO_2 emissions for different biohydrogen production pathways are presented. As it can be seen biological pathways are higher in cost production than non-biological routes. Natural gas reforming is the most economically viable hydrogen production process nowadays but it's not free from carbon footprint. Data in the table show clearly the two major challenges facing biological pathways for hydrogen production, which are efficiency increase and cost decrease in order to become competitive on the energy market. However, what is not considered is short and long-term environmental consequences and their cost as well as direct and indirect health costs. Manipulation in biomass feed like using waste biomass seems to be solution for economical constrains.

Table 5.7 - Comparison of biohydrogen production costs, conversion efficiency and net CO_2 emissions, where NA- Not available (82).

Production processes	Conversion efficiency (per cent)	Production costs (US$/Nm^3 H_2)	CO_2 emissions (kg/Nm^3 H_2)
Natural gas reforming	75	0.45	0.8
Electrolysis of water with conventional electricity	NA	0.32	1.8
Electrolysis with electricity from wind turbines	~75	0.35	0
Steam-reforming of bio-methane	~20	0.45	0
Photobiological hydrogen	~10	~10 US$/MBTU	0
Electrolysis with electricity from photo-voltaic cells	NA	4.13	0
2-stage bioprocess for hydrogen from bi-omass	15	0.35	0
Indirect microalgal biophotolysis	~7	10 US$/GJ	NA
Cyanobacterial biophotolysis	NA	15 US$/GJ	NA
Fermentative hydrogen	~22	~40 US$/MBTU	NA
Hydrogen from coal/biomass	NA	4 US$/MBTU	NA

Highly interesting techno-economic analysis were conducted by Randolph K. And Studer S. from U.S. Department of Energy (83). The aim of analysis was projection of cost of biohydrogen production via dark fermentation of biomass (corn stover). Two projected cases were considered, projected Current Case based on 2015 technology and projected Future Case based on projected technological advance-ments by 2025 (83). The cost analysis was performed using the Hydrogen Analysis version 3.101 model, the production capacity was assumed at 50.000 kg H_2/day. The capital cost was taken from a 2013 NREL report on hydrocarbons production from lignocellulosic compounds. Since there is no commercial biohydrogen production via dark fermentation plant which could be used as base system for the analysis, the techno-economic inputs were taken from hypothetical system. In table 5.8 cost projections are given in both case studies.

Table 5.8 - High volume cost projections for biohydrogen production (83).

Case Study	Optimistic Value [2007$/kg H_2]]	Baseline [2007$/kg H_2]	Conservative Value [2007$/kg H_2]
Current Case (2015)	59.76$	**67.71$**	75.67$
Current Case (2015) with by-product credit	40.88$	**51.02$**	61.16$
Future Case (2025)	7.68$	**8.56$**	9.43$
Future Case (2025) with by-product credit	3.40$	**5.65$**	7.91$

One of the technological improvements in Future case study introduced was microbial consortium, which shows ability to enhance biohydrogen yields. The fermentation reactor was projected at 55ºC for given batch time. The maximum fermentation period was limited to 74 hours as maximum conversion is achieved at that time according to NREL data. This fermentation time is also said to be corresponding to the minimum cost for both Current and Future case study. In Figure 5.6 and 5.7 optimisation curves for both cases are given.

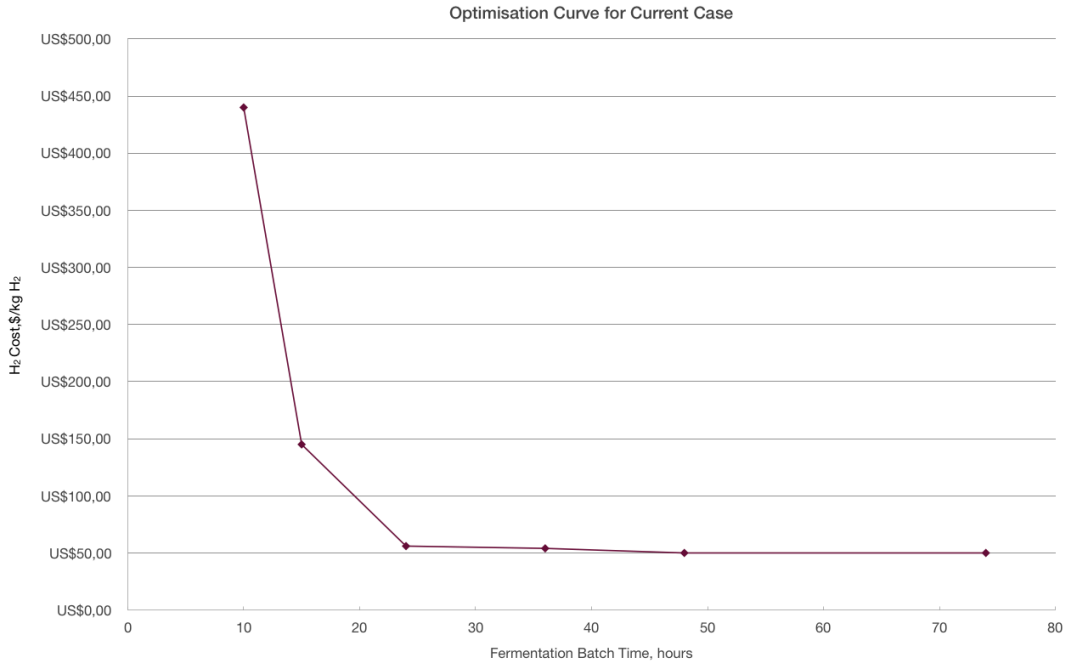


Figure 5.6 - Optimisation curve for Current case study based on the modelled corn stove loadings and variables. Adapted from (83).

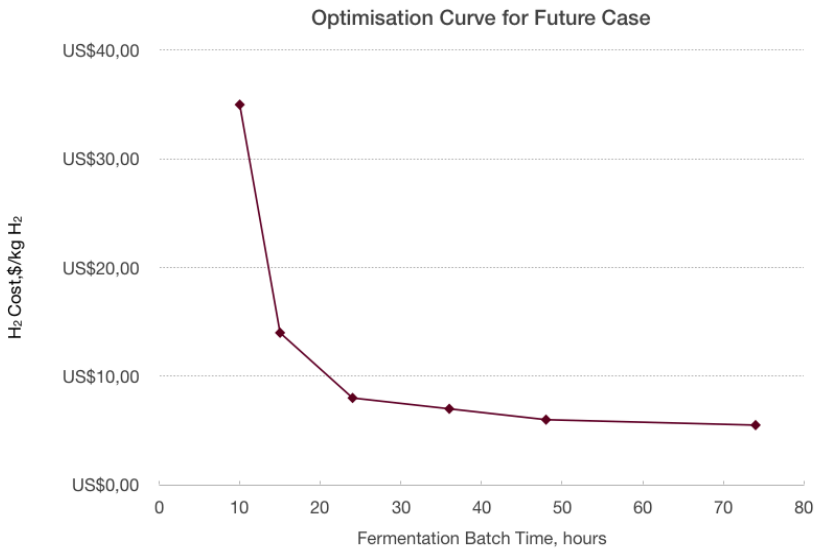


Figure 5.7 - Optimisation curve for Future case study based on the modelled corn stover loadings and variables. Adapted from (83).

After fermentation, Liquid waste are redirected to wastewater treatment, which consist of anaerobic digestion process, where methane is produced as a by-product. Methane is later combusted along with lignin and, as a thermal energy, it is used to heat the system. In case of projected Current, the thermal energy excess is converted into electricity and is sold to the grid for by-product credit equivalent to $11.93 per kg of hydrogen produced, whereas for projected Future it is $8.19 per kg of hydrogen produced (83). In case of no by-product scenario, hydrogen price is higher due to lack of revenue from electricity sales. However, in that case the capital cost of the system is decreased as gas turbine for electricity production is no longer needed (83). During the analysis, three major differences were found between Current and Future case study:

- The change of the capability of only hexose sugars conversion from *Clostridium thermocellum* in Current case to the capability of hexose and pentose sugars conversion from microbial consortium in case of Future projection.
- Concentration of broth fermentation increase from 12.8 g/L in Current case to 175 g/L in Future case.
- Molar peak conversion of sugars to biohydrogen increase from 1.16 mol H_2/mol sugar in Current case to 3.2 mol H_2/mol sugar in Future case.

During the experiment, sensitivity analysis was performed for projected Future case. Table 5.9 shows the range of parameter values used within the H2A v3.1 sensitivity analysis where all parameters were fixed at their baseline case values. Tornado chart pictured in figure 5.8 is graphical projection of results shown in table 5.9.

Table 5.9 - Sensitivity analysis results from projected Future case with by product credit (83).

Projected Future Central	Units	Parameter Values for Lower Bound Cost	Baseline Parameter Value [5.65$/kg]	Parameter Values for Upper Bound Cost
Installed Capital Cost	millions $	470 [3.39$/kg H_2]	627	784 [7.90$/kg H_2]
Feedstock Cost	$/dry metric ton	56.53 [4.67$/kg $H_{2]}$	75.37	94.21 [6.63$/kg H_2]
Broth Concentration	g/L	300 [5.42$/kg H_2]	175	100 [6.14$/kg H_2]
Electrical Generator Efficiency	%	55 [4.85$/kg H_2]	50	45 [6.52$/kg H_2]
PSA Recovery	%	96 [5.34$/kg H_2]	88	80 [6.06$/kg H_2]
Fermentation Time	hours	24 [5.34$/kg H_2]	74	74 [5.65$/kg H_2]

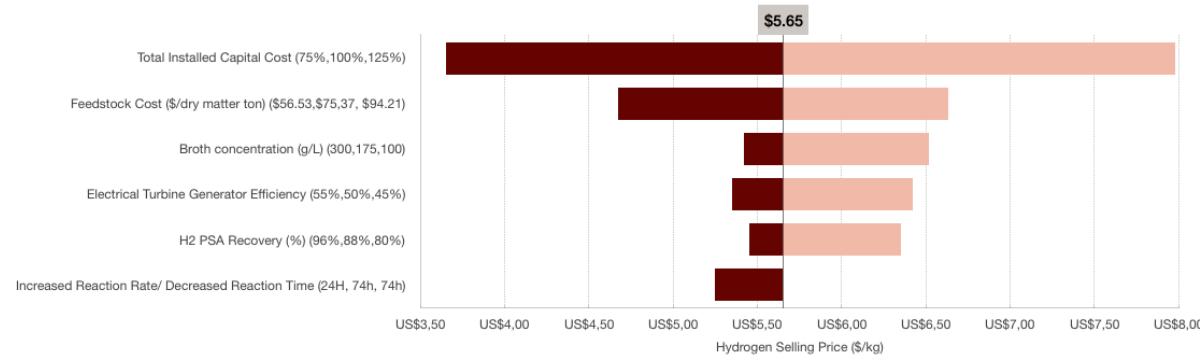


Figure 5.8 - Tornado chart showing parameter sensitivities for projected Future central fermentation case with by-product credit. Adapted from (83).

The tornado chart presents the H_2 production cost sensitivity to variations such as total installed cost, feedstock cost, broth concentration, electrical turbine generator efficiency and reduced fermentation time due to an increased reactor rate (83). The tornado chart is organised from the most to the least sensitive analysed parameter. Burgundy red colour indicates an increase and beige pink colour indicates a decrease from the baseline hydrogen cost (5.65$/kg) as a result of change in input parameter value (83). The tornado chart shows significant dependence between fermentation process and the capital cost of the system which varied +/-25% based on capital cost estimation. The analysis clearly shows that in order to become feasible production pathway, biohydrogen production via dark fermentation from biomass needs technological developments, mainly molar yield of the biomass to biohydrogen conversion needs to increase.

Study conducted by Sathyaprakasan P. & Kannan G. (84) shows comparison of costs between dark fermentation, photofermentation, indirect biophotolysis as well as direct biophotolysis. The highest cost estimated in direct biophotolysis process is the cost of the labour, which is directly associate with the

large land requirement. The estimations were made for bioreactors occupying 1,924,000 ha. Large capital and operating costs are drawbacks in direct biophotolysis process due to significant land requirements. Therefore, direct biophotolysis is not favourable choice for biohydrogen production method, coming out as costly and inefficient for industrial scale. Indirect biophotolysis appears to be more economically viable solution due to higher biohydrogen production rate and less area of land requirement, although it is burden with many technological limitations. The main drawbacks for photofermentation process are scaling up difficulties as well as significantly high gas separation cost. Among these four methods, dark fermentation appears to be most efficient with high biohydrogen production rate and small footprint. The highest cost-driving parameter in dark fermentation is requirement of glucose as a substrate for the process. Therefore, in order to significantly lower the total cost of this technology, it is crucial to identify cheap sources of biomass. Consequently, if the cost of substrate is successfully decreased, the cost of dark fermentation process becomes similar to photofermentation process.

In figure 5.9, water, power and gas separation cost comparison between indirect biophotolysis, photofermentation and dark fermentation is presented.

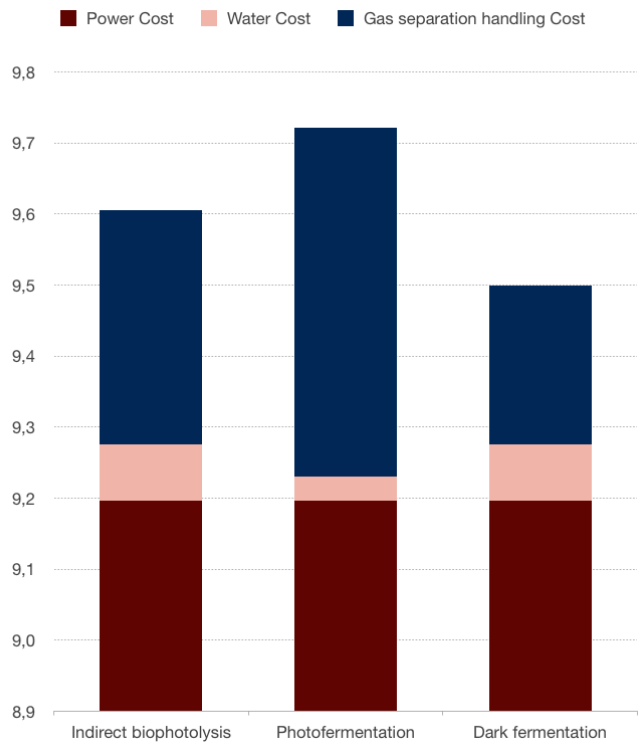


Figure 5.9 - Water, power and gas separation cost comparison in indirect biophotolysis, photofermentation and dark fermentation. Adapted from (84).

In figure 5.10 labour, general supplies and culture production costs are being compared. The cost is significantly higher in indirect biophotolysis than in dark fermentation or photofermentation due to characteristic of the process, which requires more supplies as well as large land area which leads to high labour cost and therefore directly influences the capital cost of the process.

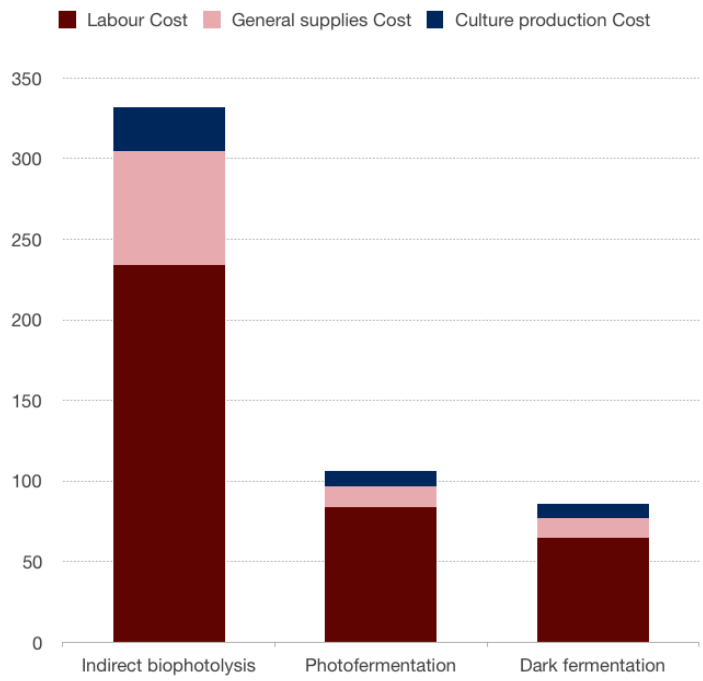


Figure 5.10 - Labour, general supplies and culture production cost comparison. Adapted from (84).

Table 5.10 - Biohydrogen cost comparison (84).

Process	Direct biophotolysis	Indriect biophotolysis	Photofermentation	Dark fermentation
Cost in million USD				
Power cost	2.40	2.50	2.50	2.50
Water cost	0.03	0.03	0.0	0.03
Labour cost	50,469.30	65.10	23.03	17.83
General supply cost	7760.66	17.32	3.51	2.70
Culture production cost	5807.97	8.22	2.70	2.01
Glucose substrate	0.0	0.0	144.19	867.18
Gas separation and handling cost	23.03	0.08	0.14	0.05
Subtotal cost	64,063.38	93.25	176.10	892.31
Contingency 10%	6406.35	9.31	17.62	89.22
Total operating cost	70,469.73	102.56	193.69	981.53
Cost per gigajoule of hydrogen	11,185.67	16.28	30.74	155.79
Cost per kilogram of hydrogen	1342.27	1.96	3.70	18.70

Table 5.10 presents costs comparison between direct and indirect biophotolysis as well as dark fermentation and photofermentation. The results clearly show that the biggest cost is represented by direct biophotolysis, whereas indirect biophotolysis shows the lowest cost per kilogram of biohydrogen.

5.3. Biohydrogen production potential

Many studies have been conducted to investigate biohydrogen production potential. Biohydrogen production potential from various agricultural waste is presented in table 5.11.

Table 5.11 - Biohydrogen production potential from various agriculture waste (85).

Substrate	Type of inoculum/pre-treatment	Process conditions ($g.1^{-1}$/°C/pH)	Type of process	H_2 production (ml $H_2.g^{-1}$)
Beet pulp	Seed sludge	20/35/6.0	Batch	90.1 ml $H_2.g_{COD}^{-1}$
Leaf shape of mixed vegetable and potatoes	Indigenous microflora	-/37/6.7	Batch	19 ml $H_2.g_{TS}^{-1}$
Lettuce	Heat-treated anaerobic sludge	20/37/5.5	Batch	48 ml $H_2.g_{TS}^{-1}$
Potatoes	Heat-treated anaerobic sludge	20/37/5.5	Batch	102 ml $H_2.g_{TS}^{-1}$
Rice straw	Anaerobic sludge	90/55/6.5	Batch	24.8 ml $H_2.g_{TS}^{-1}$
Soybean straw	Cracked cereal acclimated in continuous stirred tank reactor (CSTR)	-/37/7.0	Batch	5.46 ml $H_2.g_{VS}^{-1}$
Sunflower stalks	Anaerobic digested sludge	5/35/5.5	Batch	2.3 ml $H_2.g_{VS}^{-1}$
Wheat bran	Activated sludge paper mill	100/36/7.0	Batch	50.6 ml $H_2.g_{TVS}^{-1}$
Wheat bran	Digested sludge paper	100/36/7.0	Batch	28.6 ml $H_2.g_{TVS}^{-1}$
Wheat bran	Mill cornstalk compost	100/36/7.0	Batch	22.6 ml $H_2.g_{TVS}^{-1}$
Wheat bran	Wheat straw compost	100/36/7.0	Batch	17.7 ml $H_2.g_{TVS}^{-1}$
Wheat stalks	Anaerobic digested activated sludge	60/35/6.5	Batch	23 ml $H_2.g_{VS}^{-1}$
Wheat straw	Seed sludge from H_2 producing CSTR	6/35/-	Batch	5.69 ml $H_2.g_{VS}^{-1}$
Wheat straw	Mesophilic anaerobically digested sludge	4/37/5.5	Batch	10.52 ml $H_2.g_{VS}^{-1}$
Wheat straw	Clostridium butyricum	40/35/7.2	Batch	9 ml $H_2.g^{-1}_{substrate}$
Wheat stalks	Anaerobic digested dairy manure	60/35/6.5	Batch	37 ml $H_2.g_{VS}^{-1}$
Wheat straw	Cow dung compost	25/36/7.0	Batch	0.5 ml $H_2.g_{TVS}^{-1}$

This results show that lignocellulose waste exhibits low yields, which is directly associated with low accessibility of carbohydrates as well as its resistance to biodegradation (16). Hemicellulose and lignin protect the easily fermentable glucose that is ingrained into cellulose micro fibrils (86). Therefore, pretreatment of lignin solubilisation and removal leading to sugar rich feedstock is needed (24,87).

Table 5.12 presents biohydrogen yields and the effect of F/M ratio on biohydrogen production from food waste in dark fermentation process. The F/M ratio is the food-to-microorganism ratio that determines the number of microorganisms needed for proper decomposition of the organic matter. It is one of the most significant design and operation parameter in active sludge systems. (88) performed an experiment in batch reactors where each reactor consisted of seed sludge concentration of 2.5 g VS/L, food

waste concentration of 25 g COD/L and 10 mL of nutrient solution, reactors were later filled with distilled water to the volume mark. Experiment consisted of three steps where firstly the initial pH influence was investigated at 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0. The initial F/M ratio was fixed at 10.0 and mesophilic (35±1ºC) and thermophilic (55±1ºC) condition were examined. Secondly, the F/M ratio was investigated at 4.0, 6.0, 8.0, 10.0 and 12.0 under pH at 8.0 in thermophilic conditions. Lastly iron concentration influence was investigated. The composition of waste used is given in table 5.13.

Table 5.12 - Effect of F/M ratio on biohydrogen production from food waste (88).

F/M ratio	H_2 yield [mL H_2/g COD]	Acetate (A) [mg/L]	Butyrate (B) [mg/L]	Propionate (P) [mg/L]	B/A ratio	Final pH
4	42.51	402.97	850.60	8.31	2.11	4.5
6	34.96	408.89]	801.89	7.23	1.96	4.5
8	31.33	406.16	791.54	12.17	1.95	4.5
10	29.99	430.40	788.01	5.85	1.83	4.5
12	27.95	403.31	629.64	5.30	1.56	4.5

Table 5.13 - Characteristic of food waste and seed sludge (88).

Parameter [unit]	Value	
	Food waste	Seed sludge
Total solid [g/L]	334.55	89.29
Volatile solid [g/L]	318.81	59.64
Chemical oxygen demand [g/L]	186.67	31.60
Total Kjeldal nitrogen [g/L]	3.49	1.54
Protein [g/L]	62.50	-
Carbohydrate [g/L]	142.68	-
Total lipid [g/L]	26.57	-
pH	4.11	7.47

In terms of pH influence, biohydrogen was not produced at initial pH values of 4.0-5.0 under both conditions. The bacteria involved were unable to sustain metabolic activity at pH value lower than 5.0 and complete inhibition of the process was observed at pH value range of 4.0-5.0. At the mesophilic condition the highest biohydrogen yield of 29.32 mL H_2/g COD_{add} was achieved. The highest biohydrogen yield under thermophilic conditions of 30.69 mL H_2/g COD_{add} was achieved with initial pH value of 8.0. The maximum of biohydrogen yield of 42.51 mL H_2/g COD_{add} was achieved at initial F/M ratio of 4.0 with COD removal efficiency at 65.83%. From the results presented in table 5.13, it can be observed that hydrogen production decreases with the F/M ratio increase. This can be due to VFA distribution interference with hydrogen-producing bacteria during fermentation (88). Researchers reported that different results were obtained in another study, where highest biohydrogen yield was achieved at F/M ratio of 6.0 and 1.5. Those differences occurred due to difference of substrate and mixed microflora used in research. Nevertheless, F/M ratio is an important parameter in enhancing fermentative biohydrogen production.

In tables 5.14-5.17 raw materials and methods of hydrogen production are given. The substrates for hydrogen production can be classified as direct and indirect sources (direct marked as D, indirect as ID), where direct stand for one-stage process and indirect consist of two or more stages in a process. In the last stage of indirect process there is a mixture of simple compounds that can be used in direct processes.

Table 5.14 - Substrates for various hydrogen production routes (89).

Material	Chem.Form.	Method	Type	Remark
Methanol	CH_4O	Plasmolysis	D	High conversion 87.1% for 5% solution of CH_4O in argon
Hydrogen Sulfide	H_2S	Thermal Dissociation	D	Method designed for Sulphur production
		Plasmolysis	D	Conversion 87.1% for 5% H_2S in nitrogen
Hydrogen Chloride	HCl	Copper-Chloride cycle	D	High costs of substrate
Hydrogen Iodine	HI	Sulphur-Iodine Hydrogen production cycle (Sulphur iodine Thermochem. water splitting)	D	It uses sulphur dioxide as substrate, can be used to prevent its emission
Acetic Acid	$C_2H_4O_2$	Photofermentation	D	Hydrogen yield 69 ml of H_2 per g of $C_2H_4O_2$ (R. spareoides RV)
Ethanol	C_2H_6O	Plasmolysis	D	Hydrogen yield 17.2% for 0.08% C_2H_6O in CO_2
Propionic Acid	$C_3H_6O_2$	Photofermentation	D	Hydrogen yield 60 ml of H_2 per g of propionic acid (R. palustris A7)
Lactic Acid	$C_3H_6O_3$	Photofermentation	D	Hydrogen yield 138 ml of H_2 per g of butyric acid (R. monas)
Isopropanol	C_3H_8O	Plasmolysis	D	Hydrogen yield 23.5% for 0.11% C_3H_8O in CO_2

Table 5.15 - Substrates for various hydrogen production routes from glycerol and low organic acids (89).

Material	Chem.Form.	Method	Type	Remark
Glycerol	$C_3H_6O_3$	Dark fermentation	D	Hydrogen yield 172.87 ml of H_2 per g of glycerol (E. aerogenes)
		Photolysis	D	Hydrogen yield 0.086 ml of H_2 per g of glycerol
Butyric Acid	$C_4H_8O_2$	Photofermentation	D	Hydrogen yield 209 ml of H_2 per g of butyric acid (R. spareoides RV)
Malefic Acid	$C_4H_8O_5$	Photofermentation	D	Hydrogen yield 100 ml of H_2 per g of maleic acid (R. spareoides RV)
Succinic Acid	$C_4H_6O_4$	Photofermentation	D	Additive fermentation to low organic acids and sugars

Table 5.16 - Substrates for various hydrogen production routes from 5 and 6-membered carbohydrates (89).

Material	Chem.Form.	Method	Type	Remark
Xylose	$C_5H_{10}O_5$	Dark fermentation	ID	Hydrogen yield 117.97 ml of H_2 per g of glucose (E. aerogenes strain HO-39)
Arabinose	$C_5H_{10}O_5$	Dark fermentation	ID	Hydrogen yield 120.96 ml of H_2 per g of arabinose (E. aerogenes strain HO-39)
Hexane	C_6H_{14}	Plasmolysis	D	Conversion 95% in 5% water solution
Rhamnose	$C_6H_{12}O_5$	Dark fermentation	ID	Hydrogen yield 69.69 ml of H_2 per g of rhamnose E. aerogenes strain HO-39)
Galactose	$C_6H_{12}O_6$	Dark fermentation	ID	Hydrogen yield 118.218 ml of H_2 per g of galactose E. aerogenes strain HO-39)
Glucose	$C_6H_{12}O_6$	Dark fermentation	D	Hydrogen yield 124.45 ml of H_2 per g of glucose E. aerogenes strain HO-39)
		Photofermentation	D	Hydrogen yield 33 ml of H_2 per g of glucose (R. palustris ATCC RV)
		Plasmolysis	D	Conversion 95% in 5% water solution
		Photolysis	D	Hydrogen yield was 7% with lantan perovskite catalyst and 1.84% with home-made titanium oxide
Fructose	$C_6H_{12}O_6$	Dark fermentation	D	Hydrogen yield 121.952 ml of H_2 per g of fructose (E. aerogenes strain HO-39)
Mannose	$C_6H_{12}O_6$	Dark fermentation	D	Hydrogen yield 121.96 ml of H_2 per g of mannose (E. aerogenes strain HO-39)
Mannitol	$C_4H_6O_4$	Dark fermentation	D	Hydrogen yield 206.76 ml of H_2 per g of mannitol (E. aerogenes strain HO-39)

Table 5.17 - Substrates for various hydrogen production routes from carbohydrates, isooctane and citric acid (89).

Material	Chem.Form.	Method	Type	Remark
Citric Acid	$C_6H_8O_7$	Photofermentation	D	Hydrogen yield 36 ml of H_2 per g of citric acid (R. plaustris AT7))
Isooctane	C_8H_{18}	Plasmolysis	D	Process occurs in 5-15% solution in water
Sucrose	$C_{12}H_{24}O_{11}$	Dark fermentation	D	Hydrogen yield 109.40 ml of H_2 per g of sucrose (E. aerogenes strain HO-39)
Sucrose	$C_{12}H_{24}O_{11}$	Photofermentation	D	Hydrogen yield 33 of H_2 per g of sucrose (R. plaustris AT7))
Maltose	$C_{12}H_{22}O_{11}$	Dark fermentation	D	Hydrogen yield 140.65 ml of H_2 per g of maltose E. aerogenes strain HO-39)
Lactose	$C_{12}H_{22}O_1$	Dark fermentation	D	Hydrogen yield 37.767 ml of H_2 per g of lactose E. aerogenes strain HO-39)
Starch	$(C_6H_{12}O_5)_n$	Dark fermentation	ID	Common food waste component
Cellulose	$(C_6H_{12}O_5)_n$	Dark fermentation	ID	Promising material due to its abundance
		Pyrolysis	ID	Dehydration is quite expensive

It can be concluded, while analysing data from tables 5.14-5.17, that most of biological methods for hydrogen production use substrates in water solution. More interestingly, more substrates have even number of hydrogen atoms than odd numbers. In short-chained organic compound (hydrogen sources especially for dark fermentation) the ratio of oxygen to carbon O/C is 1. The O/C ratio for starch and cellulose in molecule is 0.83 and for sucrose, maltose and lactose is 0.92 (89).

In table 5.18, biohydrogen production rate is presented regarding different reactors configurations in dark fermentation process (where CSTR: continuous stirred tank reactor; UASB: up flow anaerobic sludge bed reactor; EGSB: expanded granular sludge bed; AFBR: anaerobic fluidised bed reactor; Three-phase FBR: three-phase fluidised bed reactor; IBRCSs: integrated biohydrogen reactor clarifier systems; AnGSB: anaerobic granular sludge bed).

Table 5.18 - Hydrogen production under different operation strategies using reactors of different configurations (90).

Reactor type	Substrate	Optimal index	Bio-growth mode	Inoculum
CSTR	Molasses	Max H_2 production rate 390 mmol/L/d	Suspension	Municipal sewage sludge
CSTR	Glucose-peptone	Max H_2 production rate 1247 mmol/L/d	Suspension	Anaerobic sludge
CSTR	Starch-peptone	Max H_2 production rate 412 mmol/L/d	Suspension	Anaerobic sludge
CSTR	Tofu-waste and anaerobic digester sludge	Max H_2 yield 2.3 mol/mol glucose	Suspension	Sludge from hydrogen producing CSTR
CSTR	Sucrose	Max H_2 yield 3.74 mol/mol sucrose	Suspension	Mixed cultures with mainly *clostridium pasteurianum* bacteria
CSTR	Lactose	Max H_2 yield 2.8 mol/mol lactose	Suspension	Anaerobic granular sludge from a full-scale UASB
CSTR	Molasses	Max H_2 production rate 6.65 L/d/L	Suspension	Municipal sewage sludge
CSTR	Molasses	Max H_2 production rate 9.72 L/d/L	Attachment	Municipal sewage sludge
CSTR	Starch	Max H_2 yield 2.8 mol/mol glucose	Suspension	Anaerobically digested sludge
CSTR	Molasses	Max H_2 production rate 249 mmol/L/d	Suspension	Municipal sewage sludge
UASB	Starch	Max H_2 yield 1.7 mol/mol glucose	Granular	Sludge from hydrogen producing CSTR and granular from a full-scale UASB
UASB	Coffee drink wastewater	Max H_2 yield 1.29 mol/mol hexose	Granular	Anaerobic digester sludge
EGSB	Glucose	Max H_2 production rate 1.36 L/gVSS/d	Immobilized and granular	Municipal sewage sludge and strain B49
EGSB	Glucose	Max H_2 production rate 1.10 L/gVSS/d	Granular	Municipal sewage sludge
EGSB	Glucose and L-arabinose	Max H_2 production rate 2.7 L/L/d	Granular	Sludge from H_2 producing CSTR and granular from full-scale UASB
AFBR	Glucose	Max H_2 yield 2.49 mol/mol glucose	Attachment	Sludge from UASB
AFBR	Glucose	Max H_2 yield 2.29 mol/mol glucose	Attachment	Sludge from UASB
Three-phase FBR	Sucrose	Max H_2 yield 4.26 mol/mol sucrose	Attachment	Municipal sewage sludge
IBRCSs	Glucose	Max H_2 yield 2.8 mol/mol glucose	Suspension	Municipal sewage sludge

Many developments of bioreactors have been made in order to optimise their performance. CSTR reactor is most commonly used reactor in continuous fermentative hydrogen from variety of substrates (90). Different configurations of bioreactors can have a significant impact on fermentative processes especially through biomass hydraulic retention time, which is related to the amount of feedstock that can be handled per unit time and thus, HRT influences economic feasibility of the process. Attached-sludge CSTR can maintain higher concentration of biomass and therefore is more stable than suspended-sludge CSTR. AnGSB reactor can obtain higher biohydrogen yields than traditional CSTR reactor (90).

In terms of biohydrogen production from microalgae, the pre-treatment as well as type of pre-treatment of the microalgae plays a significant role in obtained biohydrogen yields. Table 5.19 shows biohydrogen yields obtained without microalgae pre-treatment.

Table 5.19 - Hydrogen production from microalgae without pre-treatment (91).

Substrate	Substrate concentration (g/L TS)	Inoculum	Operational conditions (pH/°C/type of process)	Hydrogen yield (mL H_2/g VS)	Comments
Chlorella vulgaris	5	Anaerobic sludge	7.0/37/batch	10.8	Due to the activities of satellite bacteria associated with algal cultures, hydrogen can be produced with and without inocula. Addition of BESA inhibited both hydrogen production and methane production
Chlorella vulgaris	5-30	Anaerobic sludge	7.5/60/batch	1.75-19	Combination of hydrogen production from microalgae and methane production from hydrogen fermentation residues was investigated. Effects of different enzymatic pre-treatment on hydrogen and methane yield were examined
Chlorella vulgaris	3-117	Anaerobic sludge	4.2-9.8/35/batch	14.6-31.2 mL H_2/g TS	Hydrogen production from microalgae biomass via dark fermentation was optimized by response surface methodology (CCD). The optimal condition was found at 76 g TS/L and initial pH of 7.4
Chlorella sp.	4-40	Anaerobic sludge	6.5/35/batch	0.37-7.13	Influences of inoculum-substrate ratio, VFAs and NADH on anaerobic hydrogen production from chlorella sp. Were examined. Results showed that inoculum-substrate ratio and NADH had a negative correlation with hydrogen production and increase of VFA formation was accompanied with increased hydrogen production. 3D EEM fluorescence spectrometry was used to determine NADH
Nannochloropsis sp. NANNO-2	25-10	Enterobacter aerogenes ATCC 13048	-/30/batch	26.4-60.6 mL H_2/g TS	Hydrogen was produced from *Nannochloropsis sp.* biomass before or after lipid extraction. Higher hydrogen yield was obtained from lipid extracted microalgae biomass
Nannochloropsis oceanica	50	Anaerobic sludge	6.0/35/batch	2	The flue gas-cultivated microalgae biomass *(N. oceanica)* is efficiently used as feedstock to cogenerate hydrogen and methane through a novel three-stage method comprising dark fermentation, photofermentation and methanogenesis
Scenedesmus sp. (lipid extracted)	18g/LVS	Anaerobic sludge	6.3/37/batch	16.99	Different treatment methods on hydrogen production from microalgae biomass were examined. Including base, heat and combination of base heat treatment. Treatment methods except base treatment all led to a significant increase in hydrogen production from microalgae biomass
Scenedesmus sp. (lipid extracted)	45-45g/LVS	Anaerobic sludge	5.0-7.0/37/batch	0.42-40.27	Effects of inoculum treatment, inoculum concentration, initial pH and concentration on hydrogen production were investigated. Optimum condition was determined to be initial pH 6.0-6.5, heat treated inoculum concentration of 2.35 g VSS/L and the microalgae biomass concentration of 36 g VS/L
Chamydomonas reinhardtii	50	Clostridium butyricum NCBI 9576	6.0/37/batch	16.6 mL H_2/g TS	Anaerobic hydrogen production from *Chlamydomonas reinhardtii* biomass was followed by photofermentation, increase hydrogen yield from 2.58 mol H_2/mol starch-glucose to 8.30 mol H_2/mol starch-glucose equivalent algal biomass
Dunaliella tertiolecta	5	Anaerobic sludge	7.0/37/batch	12.6	The high salinity of the *D. tertiolecta* slurry was prohibitive to methanogens, resulting in low methane production and high hydrogen yield

For biohydrogen production without pre-treatment, the most common microalgae used is *Chlorella vulgaris* from which the highest biohydrogen yield is achieved at 14.6-31.2 mL H_2/g VS. Biohydrogen yields without pre-treatment ranges from 0.37 to 19 mL H_2/g VS. The highest biohydrogen yield was obtained from *C. vulgaris*, following by lipid extracted *Scenedesmus* sp. (91). Table 5.20 presents biohydrogen yields obtained with microalgae chemical and physical pre-treatment.

Table 5.20 - Hydrogen production from microalgae with physical and chemical pre-treatment methods (91).

Treatment methods	Substrate	Substrate concentration (g/L TS)	Inoculum	Operational conditions (pH/°C/type of process)	Hydrogen yield (mL H_2/g VS)	Comments
Milling	*Scenedesmus obliquus*	10-50	Clostridium butyricum DSM 10702	7.0/37/batch	28.1-35.0	Pure culture showed better hydrogen production than mixed culture
Milling	*Scenedesmus obliquus*	10-50	Anaerobic sludge	7.0/37/batch	5.4-34.8	Hydrogen production by mixed culture showed lower H_2/CO_2 ratio than pure culture
Milling	*Scenedesmus obliquus*	10-50	Anaerobic sludge	7.9/58/batch	0.7-15.3	Higher hydrogen production was achieved at higher temperature
Milling	*Scenedesmus obliquus*	10-50	Anaerobic sludge + Clostridium butyricum DSM 10702	7.9/58/batch	32.7-48.9	Co-culture of microorganisms achieved the highest hydrogen yield
Heat: 100°C, 8h	*Scenedesmus* sp. (lipid extracted)	18 g/L VS	Anaerobic sludge	6.3/37/batch	35.38	Hydrogen production from microalgae biomass was increased by over 2 times after heat treatment at 100°C for 8h
Heat: 121°C, 15min	*Scenedesmus obliquus*	2.5-50	Enterobacter aerogenes ATCC 13048	6.8/30/batch	10.8-56.5	With the increase of substrate concentration, hydrogen yield decreased while cumulative hydrogen production and hydrogen production rate increased. Better hydrogen production was obtained from wet biomass than dried microalgae
Heat: 121°C, 15min	*Scenedesmus obliquus*	2.5-50	Clostridium butyricum DSM 10702	6.8/37/batch	94.3-113.1	Hydrogen yield, cumulative hydrogen production and hydrogen production rate increased with the increase of substrate concentration. Better hydrogen production was obtained from wet biomass than dried microalgae
Heat: 121°C, 20min	*Chlorella sorokiniana*	14	Anaerobic sludge	6.5/60/batch	338	Different treatment methods on hydrogen production from microalgae biomass were examined. XRD and SEM were used to examine the rupture effect on cells by different treatment methods
Heat: 121°C, 4h	*Scenedesmus* sp. (lipid extracted)	18 g/L VS	Anaerobic sludge	6.3/37/batch	35.58	Increasing treating temperature from 100°C to 121°C can achieve similar hydrogen production but shorter treating time was needed
Base: NaOH 8 g/L, 24h	*Scenedesmus* sp. (lipid extracted)	18 g/L VS	Anaerobic sludge	6.3/37/batch	16.89	Based treatment alone showed little effect on hydrogen production from microalgae biomass
Chemical: H_2O_2 2%, 12h	*Chlorella sorokiniana*	14	Anaerobic sludge	6.5/60/batch	63	H_2O_2 showed better effect in treating microalgae biomass than sonication, but not as effective as other methods like heat and heat acid treatment
Sonication: 130W, 10min	*Chlorella sorokiniana*	14	Anaerobic sludge	6.5/60/batch	52	Sonication showed little effect on cell disruption, and hydrogen production from sonication treated microalgae was not obviously increased

Hydrogen producing bacteria usually have low hydrolytic enzymatic activity. To enhance the biohydrogen generation efficiency, the pre-treatment is usually required for the hydrolysis of algal biomass to release the organic substances from the algal cells (91). Physical and chemical pre-treatment methods such as heat, ultrasonic, mechanical, base, acid and ozonation are used to break microalgal cell wall in order to enhance carbohydrates hydrolysis and release the organic substances from the cells, making it biodegradable and therefore increasing biological conversion process (91). Consequently, higher biohydrogen yields are obtained in physical and chemical pre-treated microalgae than in non-treated microalgae, as it can be observed in table 5.20. Relatively high yields were obtained by heat treatment of microalgae (94.3-338 mL H_2/g VS), where the highest yield was obtained by heat treatment of *Chlorella sorkainiana* (91).

Another method of pre-treatment is biological pre-treatment where microbes and enzymes are used to break cell walls. Enzyme selection is determined by the composition of cell wall. Microalgae cell walls consist of cellulose, mucopolysaccharide and peptidoglycan so biological pre-treatment methods focus on using macerozyme. For cyanobacteria cell wall, lysozyme is found to be effective in pre-treatment. Cellulases are found to be suitable for breakage of *Chlorella sorokiniana* cell wall (91). As it can be observed in table 5.21, biohydrogen yields varies in the range of 11-135 mL H_2/g VS. Enzyme treated microalgae exhibit higher biohydrogen yields in comparison with microbial consortium treated microalgae. Mix of enzymes used in pre-treatment can greatly increase biohydrogen yield (91). Summary of biological pre-treatment of microalgae is given in table 5.21.

Table 5.21 - Hydrogen production from microalgae with biological pre-treatment methods (91).

Treatment methods	Substrate	Substrate concentration (g/L TS)	Inoculum	Operational conditions (pH/°C/type of process)	Hydrogen yield (mL H_2/g VS)	Comments
Biological: Onozuka R-10 enzyme	*Chlorella vulgaris*	10	Anaerobic sludge	7.5/60/batch	39	Onozuka R-10 enzyme treatment increased hydrogen production from Chlorella vulgaris biomass from 19 to 39 mL/g VS
Biological: macerozyme R-10 enzyme	*Chlorella vulgaris*	10	Anaerobic sludge	7.5/60/batch	62	Macerozyme R-10 enzyme showed better effect on hydrogen production from Chlorella vulgaris biomass than Onozuka R-10 enzyme
Biological: Onozuka R-10 enzyme + macerozyme R-10 enzyme	*Chlorella vulgaris*	10	Anaerobic sludge	7.5/60/batch	135	Combination of Onozuka R-10 enzyme and macerozyme R-10 enzyme treatment resulted in significant increase in hydrogen yield from Chlorella vulgaris biomass than single enzyme treatment
Biological: microbial consortium TC60, 60°C, 10days	*Chlorella vulgaris*	0.14 g/L VS	TC60 from compost	7.0/60/batch	11	Chlorella biomass showed recalcitrance to anaerobic digestion by tc60, and hydrogen was produced by satellite heterotrophs from C. vulgaris
Microbial consortium TC60, 60°C, 10days	*Dunaliella tertiolecta*	0.094 g/L VS	TC60 from compost	7.0/60/batch	13	Hydrogen yields increased at least 10% after biological treatment process. Digestion of Dunaliella tertiolecta provided additional nutrients for cellulolytic activity

In order to optimise the pre-treatment process, combined pre-treatment methods are used. The most widely used combined methods are physical and chemical ones, within them heating and acid treatment combination is mostly used. Heat pre-treatment can also be combined with variety of other methods, for example base treatment or enzymatic treatment. All of the combined pre-treatment methods increase

biohydrogen production rate (91). Biohydrogen yield varies significantly between 33.56 and 958 mL H_2/g VS. Among many combinations of pre-treatment, the most promising combined method in increasing biohydrogen production from microalgae appears to be the combination of acid and heat (91). Tables 5.22-5.23 present biohydrogen production using combined pre-treatment.

Table 5.22 - Hydrogen production from microalgae with combined pre-treatment methods (91).

Treatment methods	Substrate	Substrate concentration (g/L TS)	Inoculum	Operational conditions (pH/°C/type of process)	Hydrogen yield (mL H_2/g VS)	Comments
Acid: HCl 2.0%, 12h; Heat 121°C, 20min	*Chlorella sorokiniana*	10	*Enterobacter cloacae* IIT-BT 08	7.0/37/batch	201.6 mL H_2/g COD	Algal biomass of *C.sorokiniana* was produced by CO_2 sequestration in continuous mode and then used as substrate for anaerobic hydrogen production. Substrate concentration was optimized to enhance the hydrogen yield from *C. sorokiniana*
Acid-heat: HCl 5%, 121°C, 20min	*Chlorella sorokiniana*	14	Anaerobic sludge	6.5/60/batch	760	Better hydrogen production was achieved from microalgae biomass treated by combined treatment than single treatment method including autoclave, sonication and H_2O_2 treatment
Acid-heat: HCl 20%, 121°C, 20min	*Chlorella sorokiniana*	14	Anaerobic sludge	6.5/60/batch	958	Hydrogen yield was increased from 760 to 958 mL/g VS when HCl concentration was increased from 5 to 20%
Acid-heat: H_2SO_4 0.1 mM, 108°C, 30min	*Chlorella vulgaris*	20	*Clostridium acetobutylicum* B-1787	6.8/37/batch	2.24 mL H_2/g TS	Immobilized *Clostridium acetobutylicum* cells were used from hydrogen production from various microalgae species
Acid-heat: H_2SO_4 0.1 mM, 108°C, 30min	*Nannochloropsis sp. rsemsu-N-1*	20	*Clostridium acetobutylicum* B-1787	6.8/37/batch	0.90-9.52 mL H_2/g TS	Different microalgae species were used as substrate and highest hydrogen yield was obtained from wet *Nannochloropsis sp.* biomass
Acid-heat: H_2SO_4 0.1 mM, 108°C, 30min	*Arthrospira platensis*	20	*Clostridium acetobutylicum* B-1787	6.8/37/batch	2.24-8.06 mL H_2/g TS	Heating temperature range of 100-121°C, with and without acid addition were applied in treating microalgae biomass, most efficient treatment condition was determined to be 108°C, 30 min with 0.1 mmol/L H_2SO_4
Acid-heat: H_2SO_4 0.1 mM, 108°C, 30min	*Dunaliella tertiolecta*	20	*Clostridium acetobutylicum* B-1787	6.8/37/batch	0.22-1.46 mL H_2/g TS	Immobilized *Clostridium acetobutylicum* cells were used for hydrogen production from various microalgae species
Acid-heat: H_2SO_4 0.5 mol/L, 100°C, 30min	*Scenedesmus obliquus*	-	*Clostridium butyricum*	7.0/37/batch	2.9 mol/H_2/mol sugar	Potential of H_2 production from microalgae biomass and the respective energy consumption and CO_2 emissions on the bioconversion process were evaluated. Energy consumption of 7270 MJ/MJH_2 and 670 CO_2/MJH_2 were achieved, 98% of which owed to microalgae culture process due to the use of artificial lighting
Acid-heat: H_2SO_4 0.5%, 121°C, 60min	*Spirulina platensis*	10	*Bacillus firmus* NMBL-03	6.5/38/batch	0.38 mol/H_2/mol sugar	A wide variety of substrates (glucose, xylose, arabinose, lactose, sucrose and starch and carbohydrate rich waste products (bagasse hydrolysate, molasses, potato peel and cyanobacterial mass) were used for dark fermentative hydrogen production. Abundant VFA were present in spent medium of hydrogen production from cyanobacterial mass, which can be further used as substrate for photo fermentative hydrogen production

Table 5.23 - Hydrogen production from microalgae with combined pre-treatment methods (91).

Treatment methods	Substrate	Substrate concentration (g/L TS)	Inoculum	Operational conditions (pH/°C/type of process)	Hydrogen yield (mL H_2/g VS)	Comments
Acid-heat: H_2SO_4 1 %, 135°C, 15min	Chlorella pyrenoidosa	20	Clostridium butyricum	6.0/35/batch	81.2	Heat acid treated Chlorella pyrenoidosa biomass was used as substrate for hydrogen production. Energy was further removed through following photo hydrogen production and methane fermentation
Acid-heat: H_2SO_4 1 %, 135°C, 15min	Chlorella pyrenoidosa	10 (additional cassava starch 10g/L)	Clostridium butyricum	6.0/35/batch	276.2	Hydrogen production from microalgae biomass was significantly increased from 81.2 to 276.2 mL/g VS by the addition of cassava starch to get an optimum C/N ratio
Acid-heat: H_2SO_4 3 %, 121°C, 60min	Lipid extracted algae cake (collected from a lake)	5 g/L COD	Anaerobic sludge	6.0/29/batch	122 mL H_2/g TS	Comparison o hydrogen production from algae untreated, liquid fraction of treated algae, solid fraction of treated algae and treated algae mixture was examined. Best hydrogen and VFA generation were achieved from liquid fraction of treated algae
Acid-microwave: H_2SO_4 0-2.0 %, 80-180°C, 5-25min	Nannochloropsis oceanica	50	Anaerobic sludge	6.0/35/batch	39	Hydrogen production from microalgae biomass was significantly increased by combined acid and microwave treatment
Base-heat: NaOH, 8 g/L, 100°C, 8h	Scenedesmus (lipid extracted)	18 g/L VS	Anaerobic sludge	6.3/37/batch	45.54	For the combined treatment lower temperature and longer treating time was preferred than higher temperature and shorter time
Base-heat: NaOH, 8 g/L, 121°C, 4h	Scenedesmus (lipid extracted)	18 g/L VS	Anaerobic sludge	6.3/37/batch	37.42	Better hydrogen production was achieved from microalgae biomass treated by combined treatment than single treatment method
Acid-heat: pH 1.4,140°C, 15min; biological cellulase 0.05 g/g TVS, 48h; glucoamylase 0.05 g/g VS, 24h	Mixed algae (collected from algae bloom in Taihu Lake)	25	Anaerobic sludge	6.0/35/batch	33.56-43.84	Steam with acid treatment showed better reducing sugar release than steam with alkaline treatment. The energy conversion efficiency was significantly increased through 3-stage process: dark fermentation, photofermentation and methanogenesis
Acid-microwave: pH 1.4,140°C, 15min; biological cellulase 0.05 g/g TVS, 48h; glucoamylase 0.05 g/g TVS, 24h	Mixed algae (collected from algae bloom in Taihu Lake)	25	Anaerobic sludge	6.0/35/batch	42.4-47.07	Microwave with diluted acid treatment degraded algal cells into smaller fragment (<5mm) and resulted in higher saccharification efficiency of microalgae
Acid-microwave: H_2SO_4 0.2 mL, 140°C, 15min, biological: glucoamylase 0.2%	Arthrospira platensis	10-40	Anaerobic sludge	6.5/35/batch	86.5-96.6 mL H_2/g TS	Hydrogen yield was significantly enhanced from 96.6 to 337.0 mL H_2/g DW using a combination of dark fermentation and photofermentation. Removal of harmful byproducts from hydrolysis pretreatment and dark fermentation can further enhance the overall hydrogen yield

6. Discussion

The aim of this chapter is a critical summary of the biohydrogen pathways as well as an attempt to distinguish the best possible pathway at present by comparing biohydrogen production methods. Criteria such as environmental impact, yields of biohydrogen, cost of production, maturity and feedstock will be considered in order to identify most promising technology. Table 6.1 presents the essence of previously discussed biohydrogen production methods.

Table 6.1 - Summary of biohydrogen pathways.

Technology	Reactions	Organisms	Advantages	Disadvantages	References
Dark Fermentation	$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2$	Anaerobic bacteria mesophile or thermophile	Light independent process therefore continuous production process No 0_2 inhibition problem due to anaerobic nature of the process Wide range of carbon sources feedstock Variety of bacteria applicable Fermentation is a well-known process By products constitute of valuable metabolites such as lactic, acetic and butyric acids Simplicity of the process	Process becomes thermodynamically unfavourable with the increase of H_2 yield Gas separation needed Relatively low rates of H_2	(82,92)
Photofermentation	$CH_3COOH + 2H_2O$ + solar energy→ $4H_2 + 2CO_2$	Purple non-sulphur bacteria	Variety of substrates applicable High conversion rates of organic acids	Very low light conversion efficiency Hydrogenase enzyme inhibition by O_2 Light dependent process Large surface area requirement High energy demand from nitrogenase enzyme	(82,92,93)
Direct biophotolysis	$2H_2O$ + solar energy → $2H_2 + O_2$	Green microalgae	Produce H_2 directly from water and sunlight No requirement of any organic carbon substrates No greenhouse gases emission	Low light conversion efficiency Hydrogenase enzyme inhibition by O_2, therefore requires separation of H_2 and O_2 Light dependent process Large photobioreactors requirement Necessity to keep O_2 content at level below 0.1% Design improvements for efficient photobioreactors	(65,82,92,94)
Indirect biophotolysis	$12H_2O$+light→$12H_2$ +$6O_2$	Cyanobacteria, Green microalgae	Produce H_2 from water N_2 fixation ability	Hydrogenase enzyme inhibition by O_2 Hydrogenase uptake consumption of H_2 Low production rate Light dependent process Design improvements for efficient photobioreactors	(82,92,94)
Gasification	$C + 1/2\ O_2 \rightarrow CO$ $CO + 1/2\ O_2 \rightarrow CO_2$ $H2 + 1/2\ O_2 \rightarrow H_2O$ $C + H_2O \leftrightarrow CO + H_2$		Mature technology Fast process Variety of feedstock Utilization of entire biomass Economically viable Cost-effective	Separation and purification of the gas needed Biomass moist content restriction (<35%) Tar formation Impurities and seasonal availability of feedstock results in H_2 content variation Char formation logistic	(95,96)
Pyrolysis	organic matter + heat →H_2 + $CO + CH_4$ + other products		Variety of feedstock Economically profitable Fast process Utilization of entire biomass Mature technology	Separation and purification of the gas needed Tar formation Impurities and seasonal availability of feedstock results in H_2 content variation Char formation logistic	(95,96)

In table 6.2 cardinal scientific and technical limitations for biohydrogen production are presented., where low yields of hydrogen and high cost of production are two main limitations in biohydrogen production.

Table 6.2 - Scientific and technical barriers for biohydrogen production (54).

Type of barrier	Barrier	Putative solution
Basic science		
Organism	Bacteria do not produce more than 4 mol H_2mol glucose naturally	Isolate more novel microbes and combinational screen for H_2 production rates yields durability Genetic manipulation of established bacteria
Enzyme (hydrogenase)	Hydrogenase overexpression not stable O_2 sensitivity H_2 feedback inhibition	Greater understanding of the enzyme regulation and expression Mutagenic studies Low H_2 partial pressure fermentation
Fermentative		
Feedstock	High cost of suitable feedstock (glucose) Low yield using renewable biomass	Renewable biomass as feedstock Co-digestion/use of microbial consortia which can increase the yield
Strain	Lack of industrial-suitable strain	Development of industrially viable strain(s)/consortia
Process	Commercially feasible product yield Incomplete substrate utilisation Sustainable process Sterilisation	Hybrid system (photo + dark fermentation) Link fermentation to a second process that makes both economically possible Application and utilisation of fermentation tools such as continuous culture Development of low-cost stream sterilisation technology/process that can bypass sterilisation
Engineering		
Reactor	Lack of kinetics/appropriate reactor design for H_2 production Light intensity in case of photo-bioreactor	Incorporation of process engineering concept to develop a suitable reactor for the defined strain/process Flat panel or hollow tube reactor can be employed
Thermodynamic	Thermodynamic barrier NAD(P)H→H_2 (+4.62 kJ/mol)	Reverse electron transport to drive H_2 production past barrier
Hydrogen	H_2 purification/separation Storage	Selection absorption of CO_2/H_2S Basic studies on H_2 storage

As it was previously stated, there are two possible pathways to obtain biohydrogen. One of the pathways would be biomass-based thermochemical routes and the second one would be biological pathways. The definition of biohydrogen in literature is incoherent, some sources provide the definition where biohydrogen is hydrogen obtained via biological and photo-biological way, where other sources broaden the definition by including biomass-based thermochemical hydrogen production processes.

Nevertheless, this paper takes into the account thermochemical processes due to their smaller environmental impact than conventional technologies obtaining hydrogen on industrial scale nowadays. One of the major advantages of thermochemical processes is their scalability to industrial scale due to their similarity to well-known processes already implemented in oil refineries. Thermochemical processes exhibit high potential to be economically viable and competitive on a large scale in near future. Moreover, thermochemical routes, in comparison with biological pathways, exhibit lower costs as well as higher overall efficiencies (thermal to hydrogen) (96,97). Hydrogen production from biomass via thermochemical pathways is by far more developed and approachable in the near perspective than biological ways. Biomass as a feedstock is challenging to work with due to impurities as well as its variable nature mainly variable moisture and chemical content. Therefore, hydrogen yields obtained are rather low since the hydrogen content in biomass is quite low to begin with (96). High oxygen content in biomass directly influences hydrogen yields obtained from the processes and low energy content since almost half of the hydrogen from the processes comes from water splitting in the steam reforming reaction. Biomass transportation in a form of raw material or formed gaseous products is another issue in the overall cost of the thermochemical processes, therefore in order to lower the cost of transportation, the gasification plant requires to be implemented in areas with biomass wide availability (96). Nevertheless, thermochemical processes will play a major role in global energy system in the future of power and heat generation as well as fuels and chemicals production. Gasification and pyrolysis are considered the most promising among thermochemical processes for commercialization of biohydrogen production from biomass. The

hydrogen costs are estimated to be in the range of 1.25-2.20$/kg for both pyrolysis and gasification, in comparison hydrogen from steam methane reforming costs are 2.08$/kg and 2.27$/kg without CO_2 sequestration cost and with CO_2 sequestration, respectively (95). Biomass can be transferred either to bio-oil or synthetic gas via pyrolysis and gasification, these products due to their complex composition and low hydrogen concentration are usually used as low-quality fuels. Therefore, in order to increase hydrogen yields, reforming technologies over catalysts are required such as steam reforming, auto-thermal reforming, partial oxidation, dry reforming and chemical looping reforming. The most developed industrial process that has the lowest operating cost and highest hydrogen yield is steam reforming (97).

Among many variations of gasification process, steam as gasifying agent exhibits enhancement in hydrogen formation and its purity. Steam gasification highly endothermic nature increases the energy costs compared to air gasification as well as produces maximum tar yield, in comparison with other gasification variations, but eliminates costs of oxygen separation process (98). Air gasification is not a favourable process since it produces syngas with low hydrogen yield and low heating. Moreover, steam gasification exhibits higher hydrogen content than pyrolysis and air gasification. In steam gasification process, primary tar is formed in pyrolysis step which is unstable mix of ketones, furans, alcohols, phenols, acids and saccharides that in further gasification conditions cracks and is reformed into secondary and tertiary tars consisting of more stable aromatic structures (99). Tar formation is a serious limitation for the gasification process due to fouling, corrosion, blockage of pipes, particle filters and heat exchangers, consequently one of the most challenging constrains in gasifier design is the increase of tar cracking performance (most stable tertiary tars elimination requires residence time above 0.5s and temperature above 1250°C) (99). The gasification of char formed in the pyrolysis phase is the limitation step in biomass gasification, even though char is gasified by steam gasification and Boudouard reactions, the rates of these reactions are low (99). With the temperature increase the yield of hydrogen also increases and the tar content decreases, nevertheless the economics will be a limiting agent in selection of the temperature of steam gasification process. The products obtained from biomass steam gasification is a mixture of H_2, CO, CO_2, CH_4 and tar (100). The yields as well as qualities of the obtained gases varies and depends on many factors such as operating conditions of the process, reactor configurations, catalyst used and initial composition of biomass. The most problematic characteristics of biomass, in terms of gasification process optimization, are biomass moisture content, particle size and biomass type especially ash content. In general, high moisture content results in the loss of gasification efficiency, although gasifiers are designed to handle biomass moisture content around 10-15wt%, most gasifiers can handle feed with moisture content below 35%. High ash content directly results in high char yields as well as is causing the need for downstream removal of the matter by means of gas cleaning process, the smaller the particle size of biomass the better heat and mass transfer occurs in a process, however reduction of biomass particle size results in exponential increase in energy consumption of the process (99,101). The answer to high moisture content in biomass is supercritical water gasification that can deal with high moisture content in biomass but high energy costs and biomass feeding are severe limitations for this process and therefore not feasible on a large scale (99). The yield of hydrogen increases with the temperature increase, therefore yields obtained by steam gasification are in general higher than those obtained by pyrolysis due to higher temperature of the gasification process (101). Lower yields of

CO, CH_4, tar and char content are obtained with temperature increase (99). One of the gasification disadvantages over biological pathways is the fact that the main focus of gasification process is not biohydrogen production but syngas production where one of the obtained gases is H_2, therefore the need for multiple, cost intensive purification catalytic conversion steps, arise to enhance the biohydrogen production. The use of catalyst can be classified into primary processes, where catalyst suppresses tar formation directly in the gasifier such as dolomite or olivine, and secondary processes, where catalyst takes part in syngas cleaning process such as Ni based catalyst. The use of secondary catalyst appears to be a better solution since use of primary catalyst complicates the gasification process, increase the energy costs and doesn't sufficiently increase biohydrogen production (99). Although secondary catalyst exhibits benefit in comparison to primary catalyst, still even with use of secondary catalyst the problem with tar formation and further use of syngas in synthesis remains (102). In order to reduce tar content in biomass, chemical looping with CO_2 capture has been proposed as a novel technology in biomass treatment. Chemical looping appears to be a promising solution for tar minimalization due to process operation temperatures above the condensation point of tar as well as the oxygen carrier ability to decompose tar content into small organic matter (103). The principle of biomass chemical looping gasification (BCLG) system is integration of biomass gasification and water gas shift reaction in two solid loops. In the first loop, biomass is gasified and syngas is produced. The second loop is in water shift gas reactor where steam reacts with CO and converts it to H_2 and CO_2. The WGS reaction is shifted in favourable way due to CO_2 absorption by circulating solid. The oxygen carrier (OC) usually has chemical composition of M_xO_{y-1} and is solid, metal-based compound in the form of single metal oxide such as copper, nickel or iron oxide or a metal oxide supported on a high surface area substrate like alumina or silica (104). Nonetheless, metal oxides just like catalyst in catalytic reforming are prone to deactivation as a consequence of coke deposition, sintering as well as some contamination by sulphur, alkali metals and chlorine. The other way chemical looping can be used in order to enhance H_2 production is as a part of conventional gasification system, where synthetic gas is produced in a gasifier and after gasification process, syngas is fed to a chemical looping process to convert CO into CO_2 for additional H_2 production and removal. Typically, in this system there is a fuel reactor for biomass gasification process and air reactor for residual char combustion and oxygen carrier regeneration (104). One of the advantages of BCLG comparing to conventional gasification is better syngas quality since oxygen carrier provides the oxygen for gasification, therefore there is no direct contact between fuel and air. Moreover, the OC is not only an oxygen carrier but also thermal carrier, therefore provides good heat balance between reactors. The oxygen carrier also provides catalytic effect for biomass tar cracking since it is a metal oxide. The use of oxygen carrier brings many improvements; consequently, the key factor in successful chemical looping technology is the right selection of oxygen carrier (97). Iron based OC comparing to other metal oxides appears to be a good option with its high melting temperatures, good physical strength and less environmental concerns (105). Apart from BCLG there are many other chemical looping technology approaches being investigated. One of them is biomass-based co-production looping process (BCCLP), where hydrogen is produced in gasifier, added between air reactor and fuel reactor, rather than in fuel reactor (106). This process is focused on H_2 production rather than syngas. Second ap-

proach is biomass-based calcium looping gasification (BCaLG). The focus of this process is also hydrogen production as CO concentration in the produced gas is low. The CaO addition main role is CO_2, HCl and H_2S in situ capture inside the gasifier (106). This process exhibits many potential advantages due to CaO additional ability to act as catalyst for gasification process and tar reforming, improving rate of the reaction and decreasing tar content as well as CO_2 in situ capture promotes reactions that enhance hydrogen production (107). Another looping technology is biomass-based chemical looping pyrolysis-gasification process, where the main chemical reactions and therefore fuel reactor are divided into biomass pyrolysis zone and biomass gasification zone. Biomass is introduced downstream in pyrolysis zone of fuel reactor and once the solid fuel releases volatile matter fully, these volatiles go up to gasification zone, where large hydrocarbons are decomposed into smaller molecules with stream being introduced in that zone. Syngas is the product of this process (108). All biomass chemical looping processes are novelty, promising approaches far from commercialisation in comparison with conventional thermochemical processes and fossil-fuelled CLPs, therefore there are many challenges for the technology scale up. Many technical issues need to be addressed, widely researched and optimised, for example deactivation of oxygen carriers during biomass conversion due to attrition in fluidised bed, agglomeration, carbon deposition, sulphur present in the fuels, ash deposition or in case of BCaLG calcination (106). Second major issue is fouling associated to complex biomass chemistry due to tar formation, biomass ash melting, fouling and corrosion. Other issues are associated with system complexity and scale such as biomass pre-treatment, biomass ash and oxygen carrier separation, high solid recirculation rate (biomass ash is cycled together with oxygen carrier and therefore the recirculation rate is expected to be high which increases the energy costs).

Another noteworthy thermochemical pathway for biohydrogen production is biomass pyrolysis. The gaseous products of pyrolysis standalone process exhibit too low hydrogen concentration for industrial scale. Therefore, the need for methods maximizing hydrogen yields arises. Increase in H_2 concentration can be achieved by applying catalyst, high temperature and long residence time in processes such as bio-oil reforming obtained via fast pyrolysis and pyrolysis and in-line catalytic reforming process. Under fast pyrolysis conditions (short residence time and high heating rates) high yields of bio-oil are obtained (around 60-75wt%) (99), that bio-oil is further manipulated by catalytic reformation steps to produce biohydrogen as the main product. The main advantage of bio-oil over biomass itself is up to ten times higher energy density, which naturally decreases the costs of transportation. Bio-oil properties such as low heating value, high corrosiveness, high viscosity, high oxygen and water content, bio-oil aging, due to thermal and chemical instability, therefore causing problematic storage, are main drawbacks and challenges for this process development (101). Due to limitations mentioned, mainly in order to minimize the carbon deposition, there is a need for an upgrade. The main focus of upgrading the process is oxygen content decrease by deoxygenation, hydrodeoxygenation or decarboxylation (109). This can be done by changes in parameters of the process like temperature, steam to carbon ratio, O_2 or H_2 addition to feed or use of catalyst, which is most efficient and researched solution. Ni-based catalyst are most used in catalytic steam reforming of bio-oil. Many catalyst support materials such as $MgAl_2O_2$, CeO_2, $CeZrO_2$, Al_2O_3 were investigated. Catalyst promoters mostly Ce, La, Mg and Ca were studied in order to increase stability and activity of catalyst in bio-oil aqueous fraction and raw bio-oil reforming (99,110).

Nevertheless, the coke deposition is inevitable. Some catalysts exhibit high H_2 yields but also high coke deposition, some exhibit satisfactory coke deposition minimalization but hydrogen yields are not sufficient enough. Therefore, at present there is no stable and efficient enough catalyst available. At the present state of knowledge, the fundamental understanding, the kinetics and mechanism of bio-oil steam reforming complex reactions is very limited. Most research are based on single compound models, therefore more work on real bio-oil steam reforming is needed. Carbon deposition leading to catalyst deactivation is major burden in bio-oil steam reforming development on a large scale, the other major issue on an industrial scale application might be bio-oil polymerization forming gum and carbon deposit causing clogging of pipes, due to bio-oil thermal and chemical instability, in traditional heat exchanger feed heating system. The solution for this limitation would be spraying cold bio-oil into the reactor, but it requires high energy input. The energy demand could be lower by O_2 addition, however providing oxygen on an industrial scale is very expensive as well as oxygen addition lowers H_2 yield. Sometimes the bio-oil steam reformation is referred as bio-oil steam gasification as those processes are not well distinguished from one another in a literature.

Over last couple of years, pyrolysis and in-line catalytic reforming of volatiles gained interest as an alternative for H_2 production in thermochemical biomass conversion processes. This is very interesting approach for H_2 production via biomass that eliminates some of the drawbacks of biomass steam gasification and fast pyrolysis and bio-oil reforming. This hydrogen production pathway is two-step continuous process, where the first step is biomass pyrolysis and the second one is catalytic steam reforming of the volatiles obtained from the pyrolysis step. Pyrolysis reactor (conical spouted bed reactor being the favourable one) and catalytic reforming reactor (fluidized bed reactor being the favourable one) are connected in series and operating in continuous regime with the separate control (111). This separation of pyrolysis and catalytic reforming steps brings few practical advantages, both from an operational point of view as well as for the performance of the catalytic reforming. The main advantage of pyrolysis and in-line catalytic reforming process over gasification is rich H_2 gas production free of tars, which is considered main burden of gasification process (100). In order to assure gas free of tars the pyrolysis-reforming process needs to be conducted with relatively high space time. In terms of hydrogen focus production, the pyrolysis and in-line reforming process appears to be more favourable comparing to gasification since hydrogen is the main product of pyrolysis-reforming process, whereas syngas (CO and H_2 mixture) is the main product of gasification, therefore as it was mentioned previously gasification is not a process focused mainly on hydrogen production itself (100). The catalytic steam reforming step is crucial in this process as it improves the gas composition in comparison to gasification by increasing hydrogen and CO_2 concentrations and simultaneously decreases CH_4 and CO concentrations. Pyrolysis and in-line catalytic steam reforming requires lower temperatures than gasification, therefore there is increase in energy efficiency, higher tar removal and hydrogen production. Pyrolysis and in-line catalytic steam reforming process exhibits significant advantage over bio-oil reforming because entire volatile fraction (the whole bio-oil and gases) is reformed, only char fraction is not treated, whereas bio-oil reforming process is barely applied to the whole bio-oil as most research focus on bio-oil aqueous fraction as a feed. The loss of potential feed in bio-oil reforming also occurs due to incomplete vaporization of

bio-oil as well as due to raw bio-oil problematic phase separation and phenolic compounds repolymerization, which exhibit great H_2 production potential (100,111).

Thermochemical processes are very promising pathways for hydrogen production from biomass. As in every technology there are challenges and possible solutions that need to be extensively researched and further developed. One of the main issues regarding all thermochemical processes involve biomass complex and variable nature that leads to inconsistency in biomass conversion, product behaviour and selectivity. This inconsistency makes it very difficult to scale-up from the laboratory stage to industrial pilot plants. Extensive research on kinds of biomass and multi-scale and multistage methods by integration of the technologies need to be introduce to improve the performance with high selectivity of hydrogen production. Hydrogen production and biomass conversion are limited in conventional thermochemical processes due to thermodynamics of the reversible water gas shift reaction. Thermochemical processes for hydrogen production are highly energy intensive due to endothermic chemical reactions present in those processes. Biomass chemical looping technologies are proven to provide the energy input for hydrogen production system, there is no CO_2 emissions from external combustion, less carbon formation occurs, less catalyst is required, less steam to syngas production, exhibit high reaction rate and can produce pure hydrogen or syngas. Therefore, BCLP appears to be promising solution for burdens associated with conventional thermochemical technologies, but also exhibits issues such as poisoning stability of catalysts and sorbents and attrition performance. In summary, chemical looping technologies as well as pyrolysis and in-line catalytic reforming appear to be most promising in sustainable development of hydrogen production via thermochemical pathways.

Biological hydrogen production as a natural by-product of microbial metabolism is alternative and very exciting pathway that potentially can become sustainable technology from renewable resources. Biological processes exhibit few major advantages over thermochemical pathways, they are more environmentally friendly, they contribute to waste recycling as they utilize renewable energy resources and less energy input is required as most of those processes operate at ambient temperature and pressure (112–114). Biological hydrogen production plantations are capable of complete by-product CO_2 absorption through photosynthesis (26). Biological pathways also fit perfectly into the global green ideology and environmentalism movement, that raised exponentially in recent years in all areas of modern society lifestyle on a global scale. Biohydrogen can be produce by three types of microorganisms: fermentative bacteria, anaerobic bacteria and cyanobacteria. Dark fermentation, light fermentation, direct and indirect biophotolysis are processes that most researches are devoted to. Fermentative processes like dark fermentation and photofermentation appear to be most promising biological methods for biohydrogen production. Dark fermentation is a process that operates under anaerobic conditions, therefore the biohydrogen production can run continuously and independently from light without extensive land requirement as well as there is no O_2 limitation that other biological processes struggle with. Fermentation processes are more stable and efficient comparing to biophotolysis processes. They use simple control system therefore operational costs are minimised on an industrial scale. Dark fermentation and photofermentation can use wide variety of organic wastes from various origins (manure, glycerol after FAME production, cheese whey, olive mill wastewater, sewage sludge, distillery wastewater, molasses, whey

etc.) as substrate providing not only energy generation but also simultaneously waste treatment (115). However, in terms of industrial waste as feedstock for photofermentation, the colour of wastewaters can be a significant problem as it can hinder and severely limit light penetrations, potential content of heavy metals in waste can also be problematic and toxic, therefore pre-treatment is most likely required. Dark fermentation process produces butyric and acetic acids as by-products which are valuable metabolites, it's also best understood process out of all biological hydrogen generation processes. Dark fermentation has a potential to be combined into wastewater treatment facilities to generate H_2 and can also be combined with biogas (methane) production in such facilities. The main constrain of dark fermentation technology is relatively low H_2 yields for industrial application due to the principle that with the H_2 yield increase hydrogen fermentation becomes unfavourable thermodynamically. The need of fermentation products removal is also a burden in keeping constant production level on a large scale (116). In order to develop dark fermentation into competitive technology on an industrial scale, comprehensive research is needed, especially finding new strains of bacteria, improving and isolating already existing ones that are able to convert lignocellulosic materials into biohydrogen (26). Promising approach in biohydrogen yields increase and process cost reduction are genetic engineering tools used for microorganism's metabolism manipulation (10). Use of mixed cultures has a potential to simplify otherwise imperative special treatment as well as lower the cost of overall process. Photofermentation process obtains similar yields of biohydrogen as biophotolysis processes which are very low. Therefore, studies are focused on photofermentation process in an integrated two-steps system with dark fermentation rather than standalone technology. In an integrated system, first stage is dark fermentation process and the second stage uses acetate from the first step to produce more biohydrogen and therefore increases the biohydrogen yield (26). This technology is fast and simple and can use variety of organic wastes as feedstock. It combines advantages of both photofermentation and dark fermentation therefore, it is currently the most promising biological pathway. According to studies, estimated cost of biohydrogen is 2.57$/kg for dark fermentation and 2.83$/kg for photofermentation, for integrated system the cost is not available but it is assumed to be lower than for individual processes (95). Fermentative biohydrogen production and integrated processes fit well into biorefinery concept. Biorefinery uses biotechnological processing of biomass for wide spectrum of valuable products such as energy, chemicals, feed, materials and food (117). Sustainability and cascading principles are two fundaments for biorefinery concept. Sustainability and environmental friendliness principle is about meeting the needs of present requirements without compromising the future generation needs, as it was defined by the Brundtland Report (117). Cascading principle is about sequencing processes for maximisation of product yields and profits (117). There are many possible configurations in biorefinery approaches. In terms of biohydrogen, especially dark fermentation integrated with either bioelectrogenesis, photofermentation, biomethane or combination of all three is considered for biorefinery system (118). In biorefinery concept not only biotechnologies need to complement each other, by for example using dark fermentation by-products as a feed for photofermentation or biomethane generation, but also utility of feedstock needs to be maximised, for example by using microalgae as feedstock, firstly lipid content can be utilised for biodiesel and after the residual biomass can be used for biohydrogen generation (119). This approach was conducted

by (76) as previously discussed, where lipids-to-pigment-to-biohydrogen biorefinery approach was studied and concluded that biorefinery concept was more economically viable than single process.

Both direct and indirect biophotolysis are able to produce biohydrogen directly from water and sunlight. These technologies utilise water as renewable source and consume CO_2, a known air pollutant. The biggest drawback for photobiological processes is that the evolution of H_2 generation is inhibited by O_2. Moreover, in order to collect sufficient amount of light in algal H_2 production, significant surface area is required (120,121). Indirect biophotolysis was design to overcome this limitation through separation (into two stages) of H_2 and O_2 generation reactions. This system integrates biophotolysis with dark fermentation through CO_2 fixation. Major burden for this solution is the continuity of this process since it was observed that after 100h of the process the algae need to rejuvenated (122) also the cost of photobioreactors is quite high and the overall rate of the process is low. In both direct and indirect biophotolysis the biohydrogen yields do not exceed 10% (26).

Thermochemical biomass conversion is the best approach for biohydrogen production on an industrial scale in the near future (2020-2030), where biological processes are pathways for long-term biohydrogen production strategy for rather small-scales, more locally oriented production systems. Distinguishing whether biological or thermochemical pathway is optimal solution for biohydrogen generation is not the best approach, due to complexity of the matter, but rather establishing best possible option considering multiple variables for specific area to meet environmental and hydrogen production needs. Given this, biological processes especially dark fermentation in an integrated system can ideally exhibit the best compromise between production efficiency, energy, emissions, policy and cost at present state of knowledge.

7. Conclusions

- We are facing today a rapid acceleration of global population growth and economic growth which directly increases global energy demand. Fossil fuels are limited energy resources, their use is not sustainable and burden with severe environmental consequences on a global scale. Therefore, the need for alternative, environmentally friendly and sustainable energy sources is clear.

- Biohydrogen with its high energy content and no contribution to climate change has huge potential to become one of the future clean energy system main energy carrier.

- In order to commercialise biohydrogen production technical and economic issues needs to be addressed and overcome. Therefore, next step for biohydrogen production processes is scale up and optimisation of reactor configurations. In order to optimised and provide direct feedback for finding sustainable solutions, simultaneous extensive techno-economic studies and LCA must be performed. Thus, engaging in those challenges requires work by multidisciplinary teams which itself can be a challenging factor.

- Two main limitations for biohydrogen production are relatively low yield of hydrogen and the cost of the production.

- The principal in all biological hydrogen production methods is extraction of hydrogen from biomass by using microalgae or bacteria in sunlight, external heat and electricity conditions. Fermentative processes use fermentative microorganisms for biohydrogen production like dark fermentation which operates in thermophilic or mesophilic conditions using external heat or photofermentation with use of sunlight energy.

- In terms of economic viability and cost-effectiveness of biohydrogen process there are three key factors to overcome for industrial scale production: decrease of photobioreactor cost by using less expensive materials, appropriate storage system and increasing electron transfer rate in bacteria and other microorganisms in order to increase biohydrogen production rate, therefore more research and development is needed in genetic engineering area.

- The reason why no single one biological hydrogen production process moved beyond pilot-scale are microbial, technical and physical challenges that need to be faced with minimal impact on environment.

- Due to low light conversion efficiencies in biophotolysis, bioreactors used in the process require grand surface areas which is associated with high investment (material and land area) and operation costs.

- In the matter of biophotolysis processes indirect biophotolysis appear to be better pathway for biohydrogen production with sulphur deprivation despite requirement for more complex process operation as well as reactor configuration in comparison to direct biophotolysis.

- Due to hydrogenase inhibition caused by produced oxygen as well as high capital and operating costs, direct biophotolysis is not economically viable for commercial use for the time being. However, use of microorganisms that are capable of oxygen removal through respiration in specially designed co-cultures with oxygenic photosynthetic microorganisms can increase the efficiencies in biohydrogen production through direct biophotolysis. For example, (124) conducted a successful co-cultivation of *Chlamydomonas reinhardtii* with *Bradyrhizobium* japonicum which resulted in an increase of biohydrogen production yield by 14 times and 26% higher growth in co-cultivation than in cultivation of the algal strain alone.

- In terms of products obtained in the biological hydrogen production pathways, in biophotolysis processes biohydrogen is one and only end product, while in dark fermentation process apart from biohydrogen, carbon dioxide is also produced as well as volatile fatty acids.

- Biohydrogen production via dark fermentation or photofermentation exhibit relatively high speeds when comparing to biophotolysis.

- Dark fermentation is currently one of the most promising pathways for biohydrogen productions mainly due to ability to use variety of feedstocks from food industry waste, agriculture waste, crops and wood feedstock as lignocellulosic biomass as well as municipal waste, moreover it's relatively low in energy intensity. Additionally, the effluent from dark fermentation process can be used as substrate for photofermentation process or microbial electrolysis cell mainly due to high volatile fatty acids content as byproducts of DF process. Still main limitations of the process

such as relatively low biohydrogen yield, gas purification and separation challenges, instability need to be addressed for future improvements.

- Integrated biological methods appear to be promising approach like dark-fermentation combined with photofermentation can, not only resolve difficulties with feedback inhibition of substrate during dark fermentation but can also lead to maximum overall hydrogen yield.

- Although MEC technology is in a very early stage of development being discovered only in 2005, the current state of knowledge of the microbiology and reactor configurations is already highly promising in future real applications. In order to develop and scale up the MEC technology two major challenges are needed to be addressed in near future i.e. low-cost replacement of cathode material for too expensive platinum and more knowledge of methods to increase the hydrogen productivity (Q_{H2}) which is proportional to the volumetric current density and the cathodic hydrogen recovery. The search for low-cost material replacement of cathode is ongoing and studies suggest that a good replacement can be low-cost stainless steel and nickel alloys with no performance loss.

- Biogas steam reforming, steam gasification with chemical looping technologies, dark fermentation and photofermentation combination at present are the most promising biohydrogen production pathways with average of 39% GHG emission reduction potential facing 2050 due to fossil fuels replacement to biomass as energy source of pre-treatment needs, electricity-use, heat and steam production in biohydrogen production process as well as projected situation of future lower emissions in European electricity mix.

- In extensive studies, where the combination of assumptions of using most promising biohydrogen production methods overcoming the limitations of today and unlimited maximum availability of low-cost biomass feedstock for biohydrogen production were made, biohydrogen production of 93 200 000 tons H_2 per year with an estimated average emission of 4kg CO_2-eq per kg of H_2 was calculated.

- Biological pathways especially dark fermentation preferably combined with photofermentation as well as indirect biophotolysis are less pollutant than thermochemical pathways. With the use of renewable sources of energy needed for microalgae farming, biohydrogen produced from microalgae as feedstock has an advantage due to CO_2 absorption properties and can potentially

absorb more CO_2 than the process emits. Although it is still long way to go for microalgae to be competitive feedstock with waste in terms of CO_2 emissions and energy consumption.

- Thermochemical processes like steam gasification and pyrolysis are at present more efficient than biological pathways, although biological pathways have a potential to become efficient, sustainable, profitable and the best biohydrogen production option.

- All biological hydrogen production pathways are big field of research at the time being where advantages and disadvantages of each single pathway create the complexity of this subject. Furthermore, one of the main limitations of biohydrogen production processes is the inverse relation between biohydrogen production yield (Y_{H2}) and biohydrogen productivity (Q_{H2}), therefore improvement of one influence directly the other. In practical approach another question emerges, whether the goal of biohydrogen production is fast or efficient production. Fast production can be obtained by simpler setup where efficient requires more efficient control. Furthermore, appropriate process selection will depend on feedstock cost and if the applicable process would become part of a biorefinery process or not. Taking this into the account as well as the complex characteristics of biological treatment of biomass the most fundamental conclusion emerge where for every single one specific waste every biohydrogen production process needs to be tailor-made.

8. Future work

Biohydrogen production pathways, as every innovative and young technology, are in need of extensive research and development in order to reach its full potential of becoming one of the solutions to all major energy policy objectives. The magnitude of the possible solutions to biohydrogen generation challenges is overwhelming, therefore it is of a great importance to attempt setting directions for future work in the field. Direction focused on multidisciplinary research on biohydrogen generation integrated with waste management and CO_2 bio-fixation. In terms of thermochemical pathways, development of inexpensive catalyst with high conversion efficiencies, inexpensive biomass pre-treatment as well as biomass kind and availability research in order to enhance biomass overall performance are needed in future work. Furthermore, in the matter of biological pathways, future efforts in metabolic engineering, culture selection and its enrichment, immobilisation of microbial culture, process conditions optimisation, hydrogen energy system development and adaptation and fuel economy are just some of the most important issues to overcome.

Another primary issue in biohydrogen production that needs to be addressed is the need of separation and purification of hydrogen after being obtained from any pathway discussed above. The need for inexpensive, efficient and low in energy consumption, separation and purification technology is crucial aspect in overall viability of any pathways. Technologies like PSA, chemical absorption and organic or inorganic membranes are currently being researched (64). PSA being the most applied method in biohydrogen purification due to its advanced level of development and high efficiency. Membrane technology is extensively studied as a very promising purification method as it is easy to scale-up and exhibits low energy consumption (64). Another idea for possible purification of biohydrogen might be using algal biomass production, this solution is currently studied for obtaining high purity biomethane (123).

Regarding my personal future contribution in the field, I would like to put the efforts in developing fermentative biohydrogen in biorefinery concept from microalgal or waste biomass, focusing on pre-treatment research and process conditions optimisation.

9. References

(1) Hydrogen Council, *How hydrogen empowers the energy transition 2017*, 2017.

(2) R. B. Jackson, C. Le Quéré, R. M. Andrew, J. G. Canadell, J. I. Korsbakken, Z. Liu, G. P. Peters and B. Zheng, *Environmental Research Letters*, 2018, **13**, 120401.

(3) IEA, *Energy, Climate Change and Environment*, OECD, Paris, 2016.

(4) M. Oey, A. L. Sawyer, I. L. Ross and B. Hankamer, *Plant Biotechnology Journal*, 2016, **14**, 1487–1499.

(5) EIA, *U.S. Energy Information Administration*, 2017, **IEO2017**, 143.

(6) IEA, *Technology Roadmap: Biofuels for Transport*, OECD Publishing, Paris, 2011.

(7) T. Suganya, M. Varman, H. H. Masjuki and S. Renganathan, *Renewable and Sustainable Energy Reviews*, 2016, **55**, 909–941.

(8) P. M. Schenk, S. R. Thomas-Hall, E. Stephens, U. C. Marx, J. H. Mussgnug, C. Posten, O. Kruse and B. Hankamer, *BioEnergy Research*, 2008, **1**, 20–43.

(9) L. Brennan and P. Owende, *Renewable and Sustainable Energy Reviews*, 2010, **14**, 557–577.

(10) B. Bharathiraja, T. Sudharsanaa, A. Bharghavi, J. Jayamuthunagai and R. Praveenkumar, *Fuel*, 2016, **185**, 810–828.

(11) R. C. Saxena, D. Seal, S. Kumar and H. B. Goyal, *Renewable and Sustainable Energy Reviews*, 2008, **12**, 1909–1927.

(12) A. Pandey, J. S. Chang, P. C. Hallenbeck and C. Larroche, *Biohydrogen*, Elsevier B.V., 1st edn., 2013.

(13) MarketsandMarkets, Hydrogen Generation Market by Generation, Application, Technology, Storage, and Region - Global Forecast to 2023, https://www.marketsandmarkets.com/Market-Reports/hydrogen-generation-market-494.html, (accessed 11 May 2019).

(14) Centre for Industry Education Collaboration, EssentialChemicalIndustry - Hydrogen, http://www.essentialchemicalindustry.org/chemicals/hydrogen.html, (accessed 14 May 2019).

(15) MarketsandMarkets, Hydrogen Generation Market, https://www.marketsandmarkets.com/Market-Reports/hydrogen-generation-market-494.html, (accessed 19 September 2017).

(16) A. Singh and D. Rathore, *Biohydrogen Production: Sustainability of Current Technology and Future Perspective*, 2017.

(17) D. Nagarajan, D.-J. Lee, A. Kondo and J.-S. Chang, *Bioresource Technology*, 2017, **227**, 373–387.

(18) I. K. Kapdan and F. Kargi, *Enzyme and Microbial Technology*, 2006, **38**, 569–582.

(19) S. S. Oncel, *Renewable and Sustainable Energy Reviews*, 2013, **26**, 241–264.

(20) K. Chandrasekhar, Y.-J. Lee and D.-W. Lee, *International Journal of Molecular Sciences*, 2015, **16**, 8266–8293.

(21) J. R. Benavente-Valdés, C. Aguilar, J. C. Contreras-Esquivel, A. Méndez-Zavala and J. Montañez, *Biotechnology Reports*, 2016, **10**, 117–125.

(22) S. Rittmann and C. Herwig, *Microbial Cell Factories*, 2012, **11**, 115.

(23) N. H. M. Yasin, T. Mumtaz, M. A. Hassan and N. Abd Rahman, *Journal of Environmental Management*, 2013, **130**, 375–385.

(24) C. L. Cheng, Y. C. Lo, K. S. Lee, D. J. Lee, C. Y. Lin and J. S. Chang, *Bioresource Technology*, 2011, **102**, 8514–8523.

(25) P. Sivagurunathan, G. Kumar, A. Mudhoo, E. R. Rene, G. D. Saratale, T. Kobayashi, K. Xu, S. H. Kim and D. H. Kim, *Renewable and Sustainable Energy Reviews*, 2017, **77**, 28–42.

(26) R. Łukajtis, I. Hołowacz, K. Kucharska, M. Glinka, P. Rybarczyk, A. Przyjazny and M. Kamiński, *Renewable and Sustainable Energy Reviews*, 2018, 91, 665–694.

(27) K. Y. Show, D. J. Lee, J. H. Tay, C. Y. Lin and J. S. Chang, *International Journal of Hydrogen Energy*, 2012, **37**, 15616–15631.

(28) M. Materazzi, R. Taylor and M. Cairns-terry, *Waste Management*, 2019, **94**, 95–106.

(29) W. Cieciura-Włoch and S. Borowski, *Journal of Environmental Engineering and Landscape Management*, 2019, **27**, 101–113.

(30) A. Ghimire, L. Frunzo, F. Pirozzi, E. Trably, R. Escudie, P. N. L. Lens and G. Esposito, *Applied Energy*, 2015, 144, 73–95.

(31) K. Kozłowski, A. Lewicki, K. Malińska and Q. Wei, *Journal of Ecological Engineering*, 2019, **20**, 146–160.

(32) A. K. Patel, N. Vaisnav, A. Mathur, R. Gupta and D. K. Tuli, *Renewable Energy*, 2016, **98**, 221–225.

(33) D. Prabakar, V. T. Manimudi, S. Suvetha K, S. Sampath, D. M. Mahapatra, K. Rajendran and A. Pugazhendhi, *Renewable and Sustainable Energy Reviews*, 2018, **96**, 306–324.

(34) O. Bičáková and P. Straka, *International Journal of Hydrogen Energy*, 2012, **37**, 11563–11578.

(35) J. W. Peters, G. J. Schut, E. S. Boyd, D. W. Mulder, E. M. Shepard, J. B. Broderick, P. W. King and M. W. W. Adams, *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 2015, **1853**, 1350–1369.

(36) K. Y. Show, Y. G. Yan and D. Lee, in *Biohydrogen*, Elsevier, 2019, pp. 391–411.

(37) D. B. Levin, L. Pitt and M. Love, *International Journal of Hydrogen Energy*, 2004, **29**, 173–185.

(38) N. Khanna and D. Das, *Wiley Interdisciplinary Reviews: Energy and Environment*, 2013, **2**, 401–421.

(39) M. L. Chong, V. Sabaratnam, Y. Shirai and M. A. Hassan, *International Journal of Hydrogen Energy*, 2009, **34**, 3277–3287.

(40) N. Rashid, M. S. U. Rehman, S. Memon, Z. Ur Rahman, K. Lee and J.-I. Han, *Renewable and Sustainable Energy Reviews*, 2013, **22**, 571–579.

(41) S. N. A. Rahman, M. S. Masdar, M. I. Rosli, E. H. Majlan and T. Husaini, *American Journal of Chemistry*, 2015, **5**, 13–23.

(42) J. Wang and Y. Yin, *Critical Reviews in Environmental Science and Technology*, 2019, **49**, 825–865.

(43) P. Sivagurunathan and C.-Y. Lin, *Waste and Biomass Valorization*, , DOI:10.1007/s12649-019-00606-z.

(44) J. B. Veeramalini, I. A. E. Selvakumari, S. Park, J. Jayamuthunagai and B. Bharathiraja, *Bioresource Technology*, 2019, **286**, 121402.

(45) A. J. Guwy, R. M. Dinsdale, J. R. Kim, J. Massanet-Nicolau and G. Premier, *Bioresource Technology*, 2011, **102**, 8534–8542.

(46) A. Chalima, A. Hatzidaki, A. Karnaouri and E. Topakas, *Applied Energy*, 2019, **241**, 130–138.

(47) N. El-Sayed Nasr, University of Western Ontario, 2017.

(48) M. A. Z. Bundhoo and R. Mohee, *International Journal of Hydrogen Energy*, 2016, **41**, 6713–6733.

(49) E. Elbeshbishy, B. R. Dhar, G. Nakhla and H.-S. Lee, *Renewable and Sustainable Energy Reviews*, 2017, **79**, 656–668.

(50) P. M. Budiman and T. Y. Wu, *Energy Conversion and Management*, 2018, **165**, 509–527.

(51) C. Sambusiti, M. Bellucci, A. Zabaniotou, L. Beneduce and F. Monlau, *Renewable and Sustainable Energy Reviews*, 2015, **44**, 20–36.

(52) P. Mishra, S. Krishnan, S. Rana, L. Singh, M. Sakinah and Z. Ab Wahid, *Energy Strategy Reviews*, 2019, **24**, 27–37.

(53) S. Ghosh, R. Chowdhury and P. Bhattacharya, *International Journal of Hydrogen Energy*, 2018, **43**, 2091–2107.

(54) S. Meher Kotay and D. Das, *International Journal of Hydrogen Energy*, 2008, **33**, 258–263.

(55) P. Sinha and A. Pandey, *International Journal of Hydrogen Energy*, 2011, **36**, 7460–7478.

(56) R. Zagrodnik and M. Łaniecki, *Bioresource Technology*, 2017, **224**, 298–306.

(57) M. A. Khan, H. H. Ngo, W. Guo, Y. Liu, X. Zhang, J. Guo, S. W. Chang, D. D. Nguyen and J. Wang, *Renewable Energy*, 2018, **129**, 754–768.

(58) F. M. S. Silva, C. F. Mahler, L. B. Oliveira and J. P. Bassin, *Waste Management*, 2018, **76**, 339–349.

(59) G. Kumar, S. Shobana, D. Nagarajan, D.-J. Lee, K.-S. Lee, C.-Y. Lin, C.-Y. Chen and J.-S. Chang, *Current Opinion in Biotechnology*, 2018, **50**, 136–145.

(60) M. C. Gould, in *Bioenergy*, Elsevier, 2015, pp. 297–317.

(61) Y. Kalinci, A. Hepbasli and I. Dincer, *International Journal of Hydrogen Energy*, 2009, **34**, 8799–8817.

(62) O. Bičáková and P. Straka, *Acta Geodynamica et Geomaterialia*.

(63) E. W. J. van Niel, in *Springer International Publishing Switzerland 2016*, 2016.

(64) S. N. A. Rahman, M. S. Masdar, M. I. Rosli, E. H. Majlan, T. Husaini, S. K. Kamarudin and W. R. W. Daud, *Renewable and Sustainable Energy Reviews*, 2016, 66, 137–162.

(65) P. HALLENBECK, *International Journal of Hydrogen Energy*, 2002, **27**, 1185–1193.

(66) A. Sharma and S. K. Arya, *Biotechnology Reports*, 2017, **15**, 63–69.

(67) A. Melis, L. Zhang, M. Forestier, M. L. Ghirardi and M. Seibert, *Plant Physiology*, 2000, **122**, 127–136.

(68) A. Kadier, Y. Simayi, P. Abdeshahian, N. F. Azman, K. Chandrasekhar and M. S. Kalil, *Alexandria Engineering Journal*, 2016, **55**, 427–443.

(69) M. Z. Khan, A. S. Nizami, M. Rehan, O. K. M. Ouda, S. Sultana, I. M. Ismail and K. Shahzad, *Applied Energy*, 2017, **185**, 410–420.

(70) Y. P. Liu, Y. H. Wang, B. S. Wang and Q. Y. Chen, *International Journal of Hydrogen Energy*, 2014, **39**, 14191–14195.

(71) A. Escapa, R. Mateos, E. J. Martínez and J. Blanes, *Renewable and Sustainable Energy Reviews*, 2016, **55**, 942–956.

(72) P. Parkhey and P. Gupta, *Renewable and Sustainable Energy Reviews*, 2017, **69**, 1085–1099.

(73) A. Marone, O. R. Ayala-Campos, E. Trably, A. A. Carmona-Martínez, R. Moscoviz, E. Latrille, J.-P. Steyer, V. Alcaraz-Gonzalez and N. Bernet, *International Journal of Hydrogen Energy*, 2017, **42**, 1609–1621.

(74) Y.-M. Yun, M.-K. Lee, S.-W. Im, A. Marone, E. Trably, S.-R. Shin, M.-G. Kim, S.-K. Cho and D.-H. Kim, *Bioresource Technology*, 2018, **248**, 79–87.

(75) A. F. Ferreira, J. P. Ribau and C. M. Silva, *International Journal of Hydrogen Energy*, 2011, **36**, 13547–13558.

(76) A. F. Ferreira, L. A. Ribeiro, A. P. Batista, P. A. S. S. Marques, B. P. Nobre, A. M. F. Palavra, P. P. da Silva, L. Gouveia and C. Silva, *Bioresource Technology*, 2013, **138**, 235–244.

(77) A. F. Ferreira, J. Ortigueira, L. Alves, L. Gouveia, P. Moura and C. Silva, *Bioresource Technology*, 2013, **144**, 156–164.

(78) M. A. San-Martín M.I., Alonso R. M., Pelaz G., Escapa A., in *European Hydrogen Energy Conference 2018*, Costs del Sol, 2018, p. 2.

(79) Ø. K. Aall, Norwegian University of Science and Technology, 2017.

(80) W. a Amos, *Updated Cost Analysis of Photobiological Hydrogen Production from Chlamydomonas reinhardtii Green Algae Milestone Completion Report*, 2004.

(81) K. Pandu and S. Joseph, *International Journal of Advances in Engineering & Technology*, 2012, **2**, 2231–1963.

(82) K. Nath and D. Das, *Journal of Scientific & Industrial Research*, 2004, **63**, 729–738.

(83) K. Randolph, S. Studer, H. Liu, A. Beliaev and J. Holladay, *DOE Hydrogen and Fuel Cells Program Record Title: Hydrogen Production Cost from Fermentation*, 2017.

(84) P. Sathyaprakasan and G. Kannan, *International Journal of Environmental Science and Development*, 2015, **6**, 352–356.

(85) A. Singh and D. Rathore, *Biohydrogen production: Sustainability of current technology and future perspective*, Springer India, New Delhi, 2017.

(86) N.-Q. Ren, L. Zhao, C. Chen, W.-Q. Guo and G.-L. Cao, *Bioresource Technology*, 2016, **215**, 92–99.

(87) D. Nagarajan, D. Lee and J. Chang, *International Journal of Hydrogen Energy*, 2019, **44**, 14362–14379.

(88) J. Wongthanate and K. Chinnacotpong, *Environ. Eng. Res*, 2015, **20**, 121–125.

(89) G. Sołowski, *International Journal of Bioprocessing and Biotechniques*, 2018, **2018**, 22.

(90) N. Ren, W. Guo, B. Liu, G. Cao and J. Ding, *Current Opinion in Biotechnology*, 2011, **22**, 365–370.

(91) J. Wang and Y. Yin, *Microbial Cell Factories*, 2018, **17**, 22.

(92) P. T. Sekoai and M. O. Daramola, *International Journal of Renewable Energy Research*, 2017,

7, 259–378.

(93) M. M. Arimi, J. Knodel, A. Kiprop, S. S. Namango, Y. Zhang and S.-U. Geißen, *Biomass and Bioenergy*, 2015, **75**, 101–118.

(94) S. Shah, Aalto University, 2014.

(95) P. Nikolaidis and A. Poullikkas, *Renewable and Sustainable Energy Reviews*, 2017, **67**, 597–611.

(96) H. Balat and E. Kırtay, *International Journal of Hydrogen Energy*, 2010, **35**, 7416–7426.

(97) B. Dou, H. Zhang, Y. Song, L. Zhao, B. Jiang, M. He, C. Ruan, H. Chen and Y. Xu, *Sustainable Energy and Fuels*, 2019, **3**, 314–342.

(98) E. Shayan, V. Zare and I. Mirzaee, *Energy Conversion and Management*, 2018, **159**, 30–41.

(99) A. Arregi, M. Amutio, G. Lopez, J. Bilbao and M. Olazar, *Energy Conversion and Management*, 2018, **165**, 696–719.

(100) A. A. Joaristi, Universidad del Pais Vasco, 2017.

(101) P. Parthasarathy and K. S. Narayanan, *Renewable Energy*, 2014, **66**, 570–579.

(102) J. Watson, Y. Zhang, B. Si, W. T. Chen and R. de Souza, *Renewable and Sustainable Energy Reviews*, 2018, **83**, 1–17.

(103) X. Tian, P. Niu, Y. Ma and H. Zhao, *Biomass and Bioenergy*, 2018, **108**, 178–189.

(104) J. D. Figueroa, T. Fout, S. Plasynski, H. McIlvried, R. D. Srivastava, I. Y. Mohammed, M. Samah, A. Mohamed, G. Sabina, L. S. Fan, D. O. E. N. A. De-fe and A. Levasseur, *International Journal of Greenhouse Gas Control*, 2013, **2**, 9–20.

(105) M. Luo, Y. Yi, S. Wang, Z. Wang, M. Du, J. Pan and Q. Wang, *Renewable and Sustainable Energy Reviews*, 2018, **81**, 3186–3214.

(106) X. Zhao, H. Zhou, V. S. Sikarwar, M. Zhao, A. H. A. Park, P. S. Fennell, L. Shen and L. S. Fan, *Energy and Environmental Science*, 2017, **10**, 1885–1910.

(107) J. Udomsirichakorn, P. Basu, P. A. Salam and B. Acharya, *International Journal of Hydrogen Energy*, 2013, **38**, 14495–14504.

(108) J. Zeng, R. Xiao, H. Zhang, Y. Wang, D. Zeng and Z. Ma, *Fuel Processing Technology*, 2017, **168**, 116–122.

(109) R. Trane-Restrup, Technical University of Denmark, 2013.

(110) J. Chen, J. Sun and Y. Wang, *Industrial and Engineering Chemistry Research*, 2017, **56**, 4627–4637.

(111) A. Arregi, G. Lopez, M. Amutio, I. Barbarias, J. Bilbao and M. Olazar, *RSC Advances*, 2016, **6**, 25975–25985.

(112) K. Y. Show, Y. Yan, M. Ling, G. Ye, T. Li and D. J. Lee, *Bioresource Technology*, 2018, 257, 290–300.

(113) C. Y. Lin, T. M. L. Nguyen, C. Y. Chu, H. J. Leu and C. H. Lay, *Renewable and Sustainable Energy Reviews*, 2018, 82, 4215–4220.

(114) B. Bin Hu, M. Y. Li, Y. T. Wang and M. J. Zhu, *Chemical Engineering Journal*, 2018, **335**, 979–987.

(115) M. Y. Azwar, M. A. Hussain and A. K. Abdul-Wahab, *Renewable and Sustainable Energy Reviews*, 2014, **31**, 158–173.

(116) B. S. F. Boodhun, A. Mudhoo, G. Kumar, S. H. Kim and C. Y. Lin, *International Journal of Hydrogen Energy*, 2017, **42**, 27471–27481.

(117) H. M. Poggi-Varaldo, K. M. Munoz-Paez, C. Escamilla-Alvarado, P. N. Robledo-Narváez, M. T. Ponce-Noyola, G. Calva-Calva, E. Ríos-Leal, J. Galíndez-Mayer, C. Estrada-Vázquez, A. Ortega-Clemente and N. F. Rinderknecht-Seijas, *Waste Management & Research*, 2014, **32**, 353–365.

(118) G. C. Premier, J. R. Kim, J. Massanet-Nicolau, G. Kyazze, S. R. R. Esteves, B. K. V. Penumathsa, J. Rodríguez, J. Maddy, R. M. Dinsdale and A. J. Guwy, *Renewable Energy*, 2013, **49**, 188–192.

(119) M. Mitra, C. Salt, S. Mishra and C. Salt, in *Application of Microalgae in Wastewater Treatment*, eds. S. K. Gupta and F. Bux, Springer International Publishing, Cham, 2019, pp. 123–145.

(120) C. Acar and I. Dincer, in *Comprehensive Energy Systems*, Elsevier, 2018, vol. 3–5, pp. 1–40.

(121) M. S. Kim, D. H. Kim and J. K. Lee, *Comprehensive Biotechnology, Second Edition*, 2011, **3**, 115–125.

(122) Z. Xu, in *Bioprocessing for Value-Added Products from Renewable Resources*, Elsevier, 2007, pp. 527–557.

(123) A. F. Ferreira, A. Toledo-Cervantes, I. de Godos, L. Gouveia and R. Munõz, *Algal Research*, 2019, **44**, 101668.

(124) S. Wu, X. Li, J. Yu and Q. Wang, *Bioresource Technology*, 2012, **123**, 184–188.