Kingdom of Saudi Arabia
Imam Mohammad Ibn Saud Islamic University
Collage of science
Department of Biology

المملكة العربية السعودية
جامعه الامام محمد بن سعود الإسلامية
كلية العلوم
قسم الأحياء

Microbial Production of Hydrogen as a Future Fuel

A Graduation Research Project Submitted to the Department Biology in Partial Fulfillment of the Requirements for the Completion of the Degree of Bachelor of Science in Biology

By:

Name	ID Number
Norah Muthwid wassam Alqahtani	442020950
Badriah Farhan Noor Almotairi	443018944
Samaher Mohammed Ghanem Algatani	440025805
Fatimah Mohammed Ali Al Rebh	443019157

Under Supervision

of

Dr. Seham Moussa

Associate Professor of Microbiology

First semester, October 2024

Microbial Production of Hydrogen as a Future Fuel

A Graduation Research Project Submitted to the Department Biology in Partial Fulfillment of the Requirements for the Completion of the Degree of Bachelor of Science in Biology

By:

Norah Alqahtani	ID:442020950
Badriah Almotairi	ID:443018944
Samaher Alqahtani	ID:440025805
Fatimah Al Rebh	ID:443019157

Examination committee

	Name	Rank	Signature
First Examiner	**Dr .Marwa Yousry**	Assistant Professor	
Second Examiner	**Dr. Badriah Al amari**	Assistant Professor	
Advisor	**Dr. Seham Moussa**	Associate Professor	

Imam Mohammad Ibn Saud Islamic University

23/10/2024, 20/4/1446

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

Table of contents

List of Tables

List of Figures

Acknowledgement

First and foremost, we thank God for giving us the knowledge and the ability to complete our study for bachelor's degree and that what will make us reach our goals towered the future by achieve more and hit more goals.

We would like to thank and express our deep appreciation to our supervisor *Dr. Seham Moussa Mohamed Hamed*, Associate Professor of Microbiology for her academic advising and her support that give us the opportunity and encourage us to complete this research and also for providing us precious guidance throughout this research, and we extending all the thanks to the committee members in which their advises are important for us, and the gratitude to all of the Department of Biology, Faculty members, and colleagues for their help and support.

Finaly, thanks are also extended to our families and friends who support us and encourage us to continue.

Abstract

The rising demand for clean and sustainable energy sources has intensified research into alternative fuels. Among myriads of biofuels, hydrogen gas is considered as an emerging and a promising candidate due to its high energy content and zero-emission potential. Microbial production of hydrogen (biohydrogen) offers a sustainable and eco-friendly pathway, utilizing various microorganisms such as anaerobes, facultative anaerobes, aerobes, methylotrophs, photosynthetic bacteria and algae.

This approach leverages renewable resources due to its simple requirements for organic waste and sunlight, making it attractive for future energy systems. To turn organic resources into hydrogen, this process depends on microorganisms like anaerobic or photosynthetic bacteria. Wastewater and agricultural waste are examples of renewable resources that can be utilized as production feedstock.

Key microbial processes include dark fermentation, photofermentation, and microbial electrolysis cells, are explored for their hydrogen production capabilities. Despite significant progress, challenges remain, including low production yields, process optimization, and scaling up for industrial applications. Advances in genetic engineering, bioprocess design, and system integration are essential to overcoming these hurdles. Also, the development of mixed culture technologies and the utilization of by-products, research is concentrating on increasing the efficiency of microbial hydrogen production, which could eventually replace conventional fuels.

This review discusses the potential of microbial hydrogen production as a future fuel source, emphasizing its environmental benefits, technological challenges, and the prospects for commercialization in a decarbonized energy landscape.

الخلاصة

لقد أدى الطلب المتزايد على مصادر الطاقة النظيفة والمستدامة إلى تكثيف البحث في مجال الوقود البديل. ومن بين عدد لا يحصى من أنواع الوقود الحيوي، يعتبر غاز الهيدروجين مرشحًا ناشئًا وواعدًا نظرًا لمحتواه العالي من الطاقة و انبعاثاته الصفرية. يوفر الإنتاج الميكروبي للهيدروجين (الهيدروجين الحيوي) مسارًا مستدامًا وصديقًا للبيئة، باستخدام العديد من الكائنات الحية الدقيقة مثل اللاهوائيات الاجبارية، واللاهوائيات الاختيارية، والهوائيات، والميثيلوتروف، والبكتيريا الضوئية والطحالب.

يستفيد هذا النهج من بعض الموارد البسيطة المتجددة فى البيئة مثل النفايات العضوية وأشعة الشمس، مما يجعله جذابًا لأنظمة الطاقة المستقبلية. لتحويل الموارد العضوية إلى هيدروجين، تعتمد هذه العملية على الكائنات الحية الدقيقة مثل البكتيريا اللاهوائية أو الضوئية. تعد مياه الصرف الصحي والنفايات الزراعية أمثلة على الموارد المتجددة التي يمكن استخدامها كمواد خام للإنتاج.

تشمل العمليات الميكروبية الرئيسية لإنتاج الهيدروجين: التخمير اللاهوائى، والتخمير الضوئي، وخلايا التحليل الكهربائي الميكروبية. وعلى الرغم من التقدم الكبير، لا تزال هناك تحديات، بما في ذلك انخفاض الإنتاجية من غاز الهيدروجين، وتحسين العمليات، وتوسيع نطاق التطبيقات الصناعية. والتقدم في الهندسة الوراثية، وتصميم العمليات الحيوية، لذا فان تكامل الأنظمة ضروري للتغلب على هذه العقبات. يركز هذا البحث على استعراض لبعض تقنيات استخدام البيئات المختلطة والاستفادة من المنتجات الثانوية لزيادة كفاءة إنتاج الهيدروجين الميكروبي، والذي قد يحل في نهاية المطاف محل الوقود التقليدي. كما يناقش هذ البحث إمكانات وطرق إنتاج الهيدروجين الميكروبي و انواع الميكروبات المنتجة له كمصدر وقود مستقبلي، مع التركيز على فوائده البيئية والتحديات التكنولوجية كمصدر للطاقة الخالي من الكربون.

List of Abbreviations

Nicotinamide adenine dinucleotide (NADH)

acetylcoenzyme A (acetyl-CoA),

Hydrogen (H_2)

Dehydrogenase (DH)

Iron (Fe^{2+})

Lead (Pb)

Nickel (Ni)

Zinc (Zn)

Chrome (Cr)

Cadmium (Cd)

Copper (Cu)

Sodium (Na^{2+})

Magnesium (Mg^{2+})

Biologival oxygen demand (BOD)

Chemical Oxygen Demand (COD)

Hydrogen Producing Bacteria (HPB)

Escherichia coli (*E.coli*)

Total Primary Energy Supply (TPES)

Carbon dioxide (CO_2)

Representative Concentration Pathway (RCP)

Low Heating Value (LHV)

High Heating Value (HHV)

Oxygen (O_2)

Dark fermentation (DF)

Microbial Electrolysis cell (MEC)

photosystem I (PSI)

photosystem II (PSII)

Photo Fermentation (PF)

Purple Non-Sulfur (PNS)

Citric Acid Cycle (TCA)

Atmosphere (atm)

Hyrdrogenase (HD)

Polyhydroxybutyrate (PHB)

Soluble Microbial Products (SMPs)

Formate Dehydrogenase (FDH)
reductase and hydrogenase enzymes (HD)
formate hydrogenlyase (FHL)
Methylene blue (MB)
Ultraviolet (UV)

List of symbols

Milliliter per gram (ml/g)

Milliliter per liter (ml/l)

Millimoles (mmol)

Millimoles per gram (mmol/g)

Mega Joules kilogram (MJ kg^{-1})

Mega Joules liter (MJ L^{-1})

kilo-joules per gram (kJ/g)

Micromole (µmole)

Aim of Work

The purpose of this review study is to outline microbial hydrogen production as a future fuel, to develop sustainable and environmentally friendly methods for generating energy from natural and renewable sources. Traditional hydrogen production methods typically rely on fossil fuels, which result in significant greenhouse gas emissions. In contrast, microbial hydrogen production relies on microorganisms such as bacteria and algae, which can produce hydrogen through biological processes like fermentation or photolysis. Overall, microbial hydrogen production may provide the following objectives

- Achieving clean and sustainable energy
- Reducing carbon emissions
- Improving production efficiency
- Future applications

The goal is to move towards a sustainable hydrogen economy that relies on clean and efficient production technologies

Chapter(I)

Introduction

1. Introduction

One of the main concerns of the twenty-first century is the requirement for fossil fuel which is keeping up with the rise in global energy demand needed for improving living standards and population expansion (Dince and Acar., 2015). For instance, in 2011 around seven billion people worldwide utilized 15 Terawatt (TW)-energy (Dince and Acar., 2015). It is anticipated that these figures would rise to 30 TW and nine billion people, respectively, by 2050 (Benterbusch and Husar., 2013; Vision., 2023).

As shown in Fig. 1, the global fuel shares for power generation, total primary energy supply (TPES), and the corresponding carbon dioxide emissions (CO_2). 85% of the world's energy supply in 2011 came from fossil fuels (Dince and Acar., 2015). Nevertheless, it is not anticipated that fossil fuels would be able to meet the rising demand for energy due to their finite supply and nonhomogeneous distribution (Dince and Acar., 2015). Furthermore, when the readily available fossil fuel reserves are depleted, the remaining reserves become harder to extract, and the political unpredictability of the nations that control the world's fossil fuel supplies contributes to the ongoing rise in fossil fuel prices (Dince and Acar., 2015).

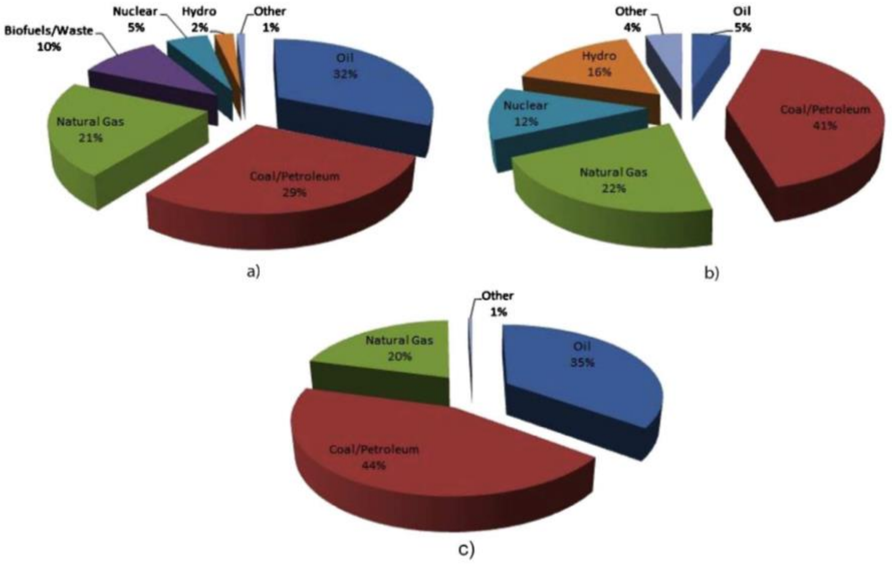

Fig. 1 shows (a) total primary energy supply (TPES), (b) electricity generation, and (c) CO_2 emissions (others include waste, geothermal, sun, wind, and heat) (Benterbusch and Husar., 2013).

1.1. Impact of CO_2 elevation on climate

Since the year 2000, there has been a noticeable increase in the rate of global climate change (Lee et al., 2020). During the industrial revolution, for instance, the atmospheric CO_2 content rose steadily to at least 400 μmol mol^{-1} from 280 μmol mol^{-1} in 1880 (Lee et al., 2020). The annual average temperature has increased by 0.8 since 1880 (NASA, 2019). According to the Intergovernmental Panel on Climate Change's Representative Concentration Pathway (RCP), the atmospheric temperature could rise by as much as 4.8 °C and the CO_2concentration might reach 940 μmol $mol^{-1}$by around 2100 (Intergovernmental Panel on Climate Change., 2014). These predicted rises in temperature and atmospheric CO_2 concentration is found to influence crop yield and quality (Yubi et al., 2019; Liu et al., 2017). Increasing CO_2 concentration may contribute to global warming and climate changes, leading to significant and unavoidable consequences such as increasing temperatures, shifting precipitation patterns, ocean acidification, rising sea levels as well as an increase in the frequency and severity of extreme weather events. Therefore, finding renewable and clean energy sources to meet the energy demand has become paramount issue.

Renewable sources for clean energy production are considered as promising feedstocks, among which, hydrogen may be created through environmentally friendly, clean techniques. Furthermore, because of its abundance, purity and accessibility, hydrogen may be the first alternative to fossil fuels as a clean energy source in the future (Aydin et al., 2021). Hydrogen can be produced using a variety of clean, continuous energy sources, including biomass, solar, hydro, wind, and ocean energy (Aydin et al., 2021). To improve productivity and cut costs, several issues with each hydrogen generation technique must be considered (Aydin et al., 2021).

Scholars and researchers have worked closely to address the shortcomings of each hydrogen generating system (Aydin et al., 2021). The potential for a clean, sustainable energy source that can be created from local energy resources wherever in the world is presented by hydrogen generated from renewable energy sources (Aydin et al., 2021). In recent years, the use of hydrogen energy has grown in popularity (Tarhan et al., 2021). Fossil fuels can be replaced with this sustainable energy source (Singh et al., 2020). Hydrogen possesses a higher energy content and a lower environmental impact than fossil fuels, making it a viable alternative (Tarhan et al., 2021). Hydrogen is a colorless and odorless atom that is typically made up of an electron and a proton. Air has a greater density than hydrogen does (Tarhan et al., 2021).

Using hydrogen as fuel is one of these more environmentally friendly approaches that shows the most promise. When utilized as a fuel, hydrogen has a gravimetric energy density of 2.5^{-3} times greater than that of the fossil fuels that are now most widely employed (see Table 1) (Tashie et al., 2021). Though there are still certain things that may be done better, hydrogen energy is clearly going to be a major energy source in the future (Tarhan et al., 2021). Hydrogen has several benefits, including: (i) high energy conversion efficiencies; (ii) emission-free production from water; (iii) abundance; (iv) various forms of storage (such as gaseous, liquid, or combined with metal hydrides); (v) long-distance transportation; (vi) ease of conversion to other forms of energy; and (vii) higher HHV and LHV (Low Heating , Value (LHV) , High Heating Value (HHV) than most conventional fossil fuels(see Table 2) (Dince and Acar., 2015).

Table 1: Common fuels' volumetric and gravimetric energy densities (Tashie et al., 2021).

Fuel	**Gravimetric Energy Density (MJ kg^{-1})**	**Volumetric Energy Density (MJ L^{-1})**
Hydrogen (liquid)	143	10.1
Hydrogen (compressed, 700 bar)	143	5.6
Hydrogen (ambient pressure)	143	0.0107
Methane (ambient pressure)	55.6	0.0378
Natural Gas (Liquid)	53.6	22.2
Natural Gas (Compressed, 250 bar)	53.6	9
Natural gas	53.6	0.0364
LPG propane	49.6	25.3
LPG butane	49.1	27.7
Gasoline (petrol)	46.4	34.2
Biodiesel oil	42.2	33
Diesel	45.4	34.6
Kerosene	46.4	36.7

Table 2: Elevated and lowered heating values of hydrogen and typical fossil fuels at 25 °C and 1 atm **(Dince and Acar., 2015)**.

Fuel	**HHV (kJ/g)**	**LHV (kJ/g)**
Hydrogen	141.9	119.9
Methane	55.5	50.0
Gasoline	47.5	44.5
Diesel	44.8	42.5
Methanol	20.0	18.1

As a result, hydrogen gas is regarded as the most promising and clean energy source of the twenty-first century (Zhang et al., 2021). Hydrogen is also a crucial chemical raw element that is utilized extensively in major industrial processes like the water-gas shift reaction, petroleum refining, and ammonia synthesis (Zhang et al., 2021). As a result, the creation of hydrogen has drawn significant attention worldwide (Zhang et al., 2021).

Water and carbohydrates are examples of source materials that contain the hydrogen element and can be used to make hydrogen, as hydrogen does not exist as a molecule in nature (Zhang et al., 2021). Currently, conventional fossil fuels provide more than 96% of the hydrogen produced worldwide (Da Silva veras at el., 2017). Regrettably, most conventional methods for producing hydrogen from fossil fuels are linked to significant energy consumption and environmental contamination (Zhang et al., 2021). As a result, with increasingly stringent and pertinent environmental protection legislation in place worldwide, more focus is being placed on the deployment of innovative technologies for hydrogen production from nuclear and renewable sources (Suman, 2018). Nevertheless, using new or conventional technologies to produce hydrogen requires a certain amount of energy and resources, which will unavoidably influence the environment (Parkinson et al., 2019).

Fig 2. represents the biological components of living objects, such as plants, which are referred to as "biomass." It is a commonly utilized energy source in both developed and developing countries (Worku et al., 2024). About 3% of the primary energy needs of developed countries and 35% of those of developing countries are met by biomass, the most sophisticated renewable energy source (Mancusi et al., 2021). Biomass can be burned directly to provide heat or indirectly by converting it into several kinds of biofuel (Worku et al., 2024). For example, deer, twigs, and other remaining biomass from forests can be converted into biodiesel and ethanol, or alternatively, methane gas, which is a valuable energy source (Worku et al., 2024).

The growing percentage of waste materials and the consequent need to decrease them has led to an explosion in popularity for the biological approach of creating hydrogen during the past 20 years (Worku et al., 2024). Biological processes are catalyzed by microorganisms that live in an aqueous medium at ambient temperature, atmospheric pressure, and both (De sales et al., 2017). These methods can be applied in places where biowaste and biomass resources are easily accessible (Worku et al., 2024). These precursors' natural availability reduces the energy and transportation costs associated with the original raw material (Worku et al., 2024). The two primary biological mechanisms used to generate hydrogen are photofermentative processes and dark fermentative hydrogen synthesis (Worku et al., 2024). Dark fermentation is one of the most well-known processes for creating bio-hydrogen (Worku et al., 2024).

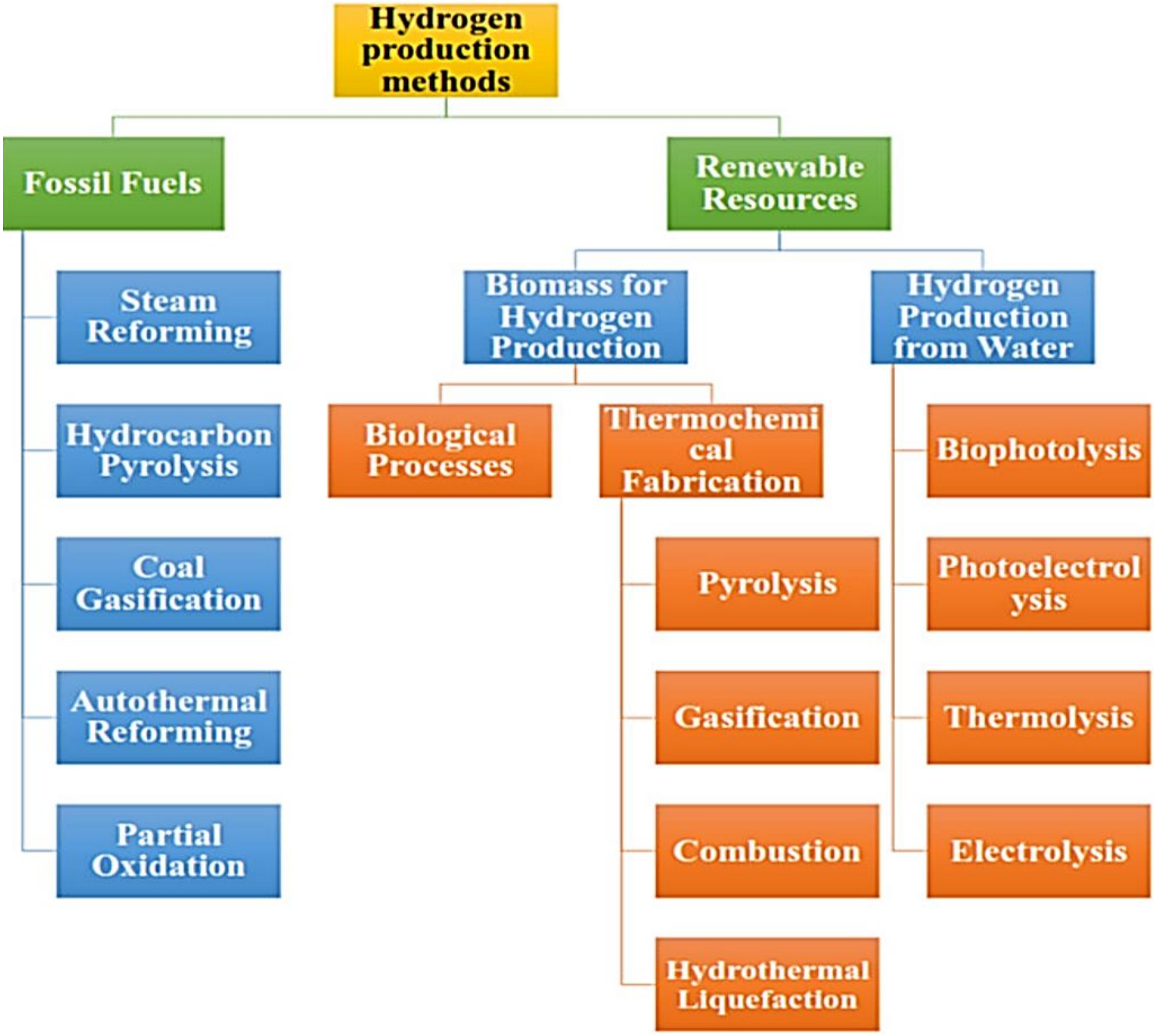

Fig. 2: Schematic diagram showing variable methods for hydrogen production (Worku et al., 2024).

Chapter (II)

Literature Review

2. Methods of Microbial Production of H_2 gas

H_2 is produced by a wide range of natural species, such as the archaea, facultative aerobic, anaerobic bacteria, cyanobacteria, and lower eukaryotes (protists and green algae) (Hallenbeck, 2012; Biochenko et al., 2004; Saifuddin and priatharsini., 2016). These organisms can work alone, in groups of related types, or in mixed cultures (Fig. 3) (Chandrasekhar et al., 2015). Typical heterotrophs in fermentation are the main biocatalysts that produce H_2 (Chandrasekhar et al., 2015). Certain dark fermentative bacteria are obligate anaerobes, which are further categorized according to their sensitivity to O_2 and their growth temperature (Fig. 3) (Chandrasekhar et al., 2015). These microorganisms can survive in environments lacking in oxygen and do not require solar energy as a source of energy (Chandrasekhar et al., 2015).

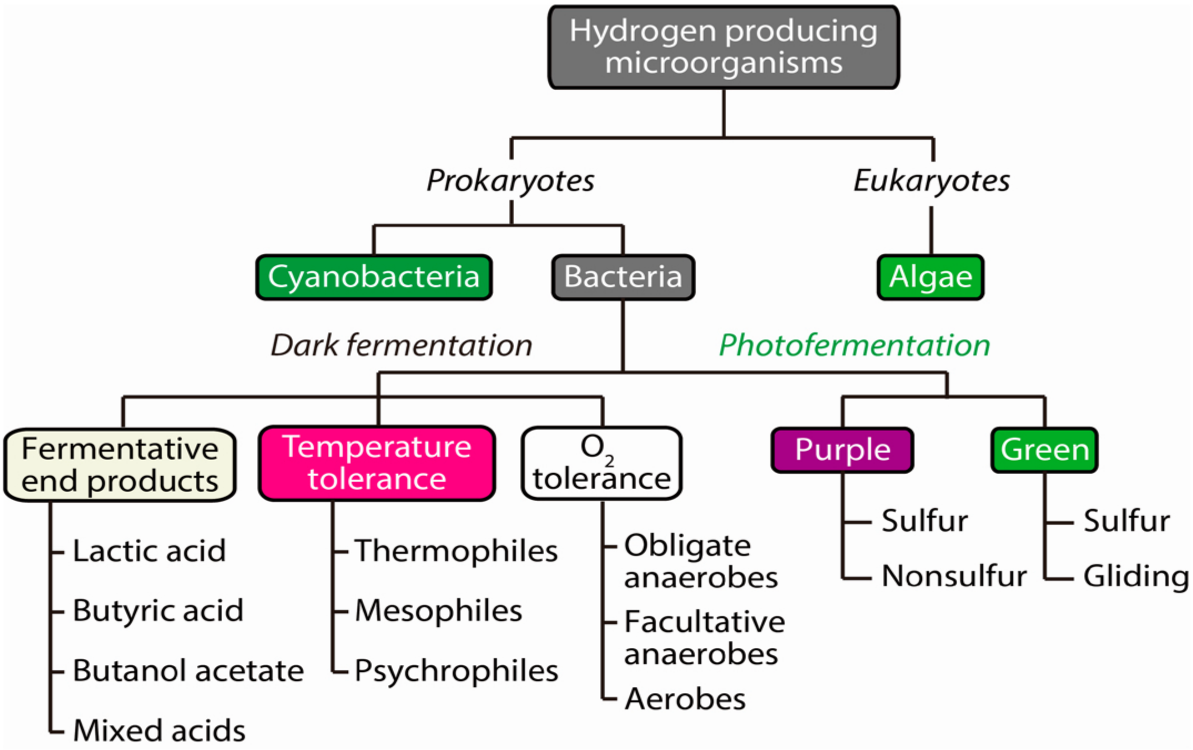

Fig. 3: Diagram illustrating the variety of H_2 generating via H_2-producing microorganisms (Chandrasekhar et al., 2015).

2.1. H_2 Production by Dark Fermentation

The most promising method for converting biomass into biohydrogen is dark fermentation (DF), which has a net energy ratio of 1.9 compared to steam methane reforming's 0.64 (Manish and Banerjee., 2008; Łukajtis et al., 2018).

Anaerobic bacteria can produce hydrogen when they thrive in a dark or carbohydrate-rich substrate (Agyekum et al., 2022). Most microbial hydrogen production is powered by the anaerobic metabolism of pyruvate, which is created through the breakdown of several substrates (Agyekum et al., 2022). Pyruvate degradation is catalyzed by one of two enzyme systems; formate lyase or Ferredoxin Oxygen Reductase (Manish and Banerjee., 2008; Łukajtis et al., 2018) as described in the following equations.

1. Pyruvate: formate lyase*:*

Pyruvate+CoA→acetyl−CoA+formate
(Abe et al., 2019)

2. Pyruvate: Ferredoxin Oxygen Reductase

$\text{Pyruvate+CoA+2Fd(ox)}\rightarrow\text{acetyl−CoA+}CO_2\text{+2Fd(red)}$
(Liu et al., 2020)

Anaerobic bacteria that are cultivated in the dark transform substrates in DF (Fig. 4) (Agyekum et al., 2022). Hydrogen is a crucial metabolic substrate for a number of anaerobic microbes (Agyekum et al., 2022). When present, these microbes can use hydrogen molecules high in gydroenergy to generate energy by exploiting the electrons released during hydrogen oxidation (Agyekum et al., 2022). In the absence of external electron acceptors, organisms produce excess electrons during metabolism as a result of protons being reduced to form hydrogen molecules (Agyekum et al., 2022). The main enzymes that control the metabolism of hydrogen are called hydrogenases (Łukajtis et al., 2018).

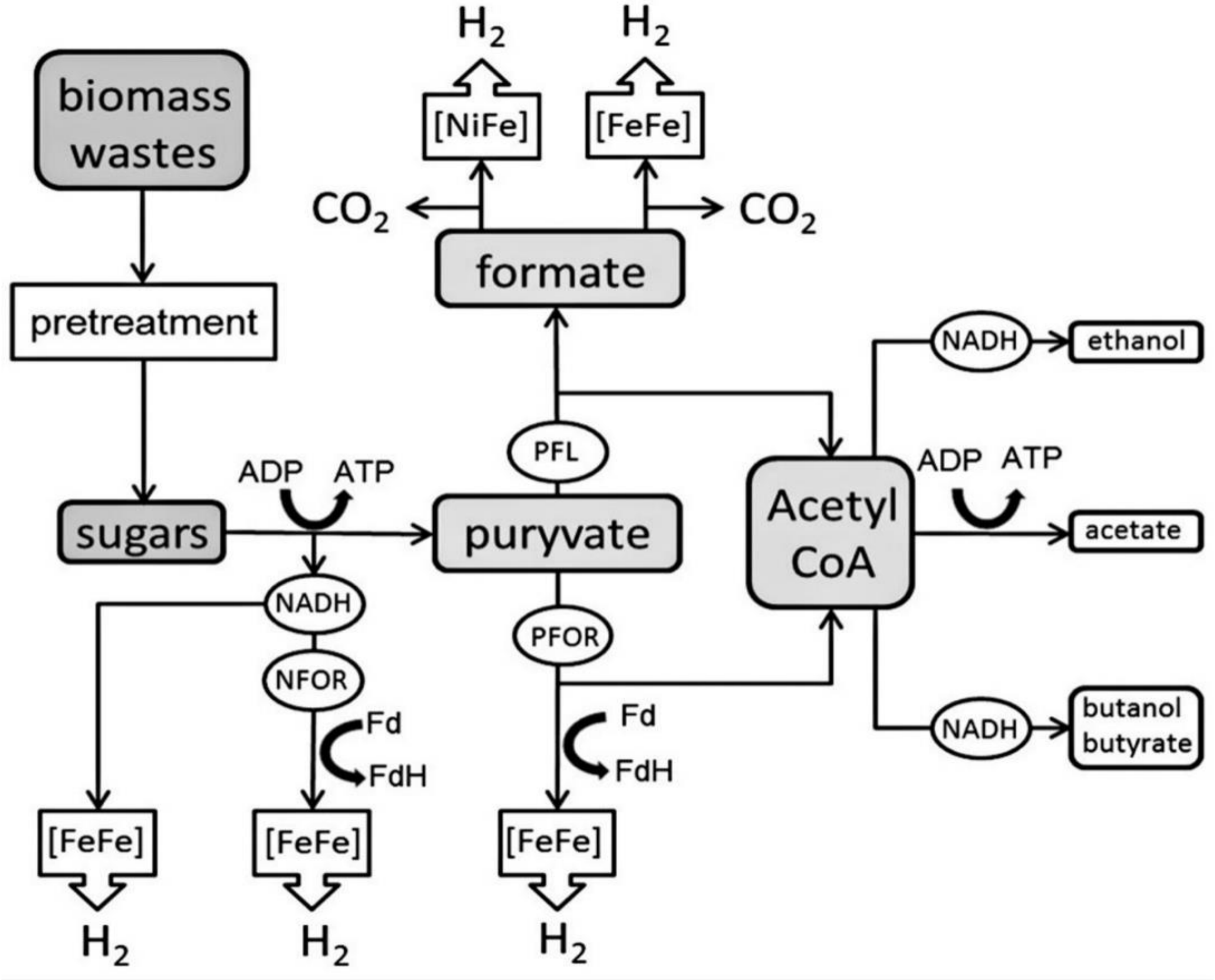

Fig. 4: Typical metabolic pathways used in dark fermentation to convert substrate to hydrogen (Łukajtis et al., 2018).

2.2. H_2 Production by Microbial Electrolysis Cell

One of the most promising methods for producing hydrogen is the microbial electrolysis cell (MEC) technology, which can also be used to manufacture hydrogen or other chemicals with added value from wastewater (Liu et al., 2005; Lu et al., 2016). When compared to alternative biologically based processes for producing hydrogen and water electrolysis, MECs offer a number of well-known advantages (Zhao et al., 2020). One the one hand, exoelectrogenic bacteria in MECs catalyze the organic compounds on the anode while requiring a far lower energy input than electrolysis, which oxidizes the stable water molecule (Azwar et al., 2014;

Chandrasekhar et al., 2015). Furthermore, MECs enhance the production of hydrogen by encouraging hydrogen capture (Zhao et al., 2020). Additionally, MECs enhance the production of hydrogen by encouraging hydrogen capture. When compared to the fermentation process (Chandrasekhar et al., 2020; Dawood et al., 2020), MEC produces a higher hydrogen yield because its performance is not light-dependent, unlike photo-fermentation, which is primarily governed by the physiology of microorganisms and physical-chemical transport processes (Hasnaoui et al., 2020; Hu et al., 2020).

2.3. H_2 Production by Biophotolysis

Algal and plant cells engage in direct biophotolysis, a process akin to photosynthesis (Da silva veras et al., 2017). In this case, the microbial photosynthesis mechanism uses a water molecule to convert solar energy into molecular hydrogen (as a chemical energy form) through a combination of biological and chemical processes (Fig. 5). As effective hydrogen-producing algae strains that use this method of hydrogen synthesis are *Chlorella* spp., *Chlorococcum* spp., and *Scenedesmus* spp. (Das et al., 2008; Paramesh et al., 2018).

$2H_2O + \text{solar energy} \longrightarrow 2H_2 + O_2$
(Chih et al., 2020)

Green algae are thought to be capable of producing biohydrogen in anaerobic environments (Sivaramakrishnan et al., 2021). Among these significant microalgal species is *Chlamydomonas* sp. (Sivaramakrishnan et al., 2021), which can both create hydrogen and use it as an electron donor (Das et al., 2008; Jiménez Llanos et al., 2020). Hydrogenase is an enzyme that is used in the biophotolysis of water to produce hydrogen (Sivaramakrishnan et al., 2021). In the process of biophotolysis, hydrogenase obtains electrons from ferredoxin, which can serve as an electron donor to help turn water into hydrogen gas (Nargarajan et al., 2017). The photosynthesis process involves two parts: photosystem I (PSI) and photosystem II (PSII) (Sivaramakrishnan et al., 2021). While PSII splits water molecules into hydrogen and oxygen, PSI is charged with reducing CO_2 (Srirangan et al., 2011; Barber., 2016). The water molecule releases two protons at the result of biophotolysis. Hydrogen can be produced when hydrogenase is present or when PSI reduces CO_2(Kim et al., 2011; Ghiasian., 2019). Because hydrogenase is absent in all green plants, PSI is involved in CO_2 reduction. In the process of harnessing solar energy, PSII produced oxygen and electrons, which the PSI ferrodoxin component then used to create hydrogen (Ni et al., 2006; Sekar et al., 2015).

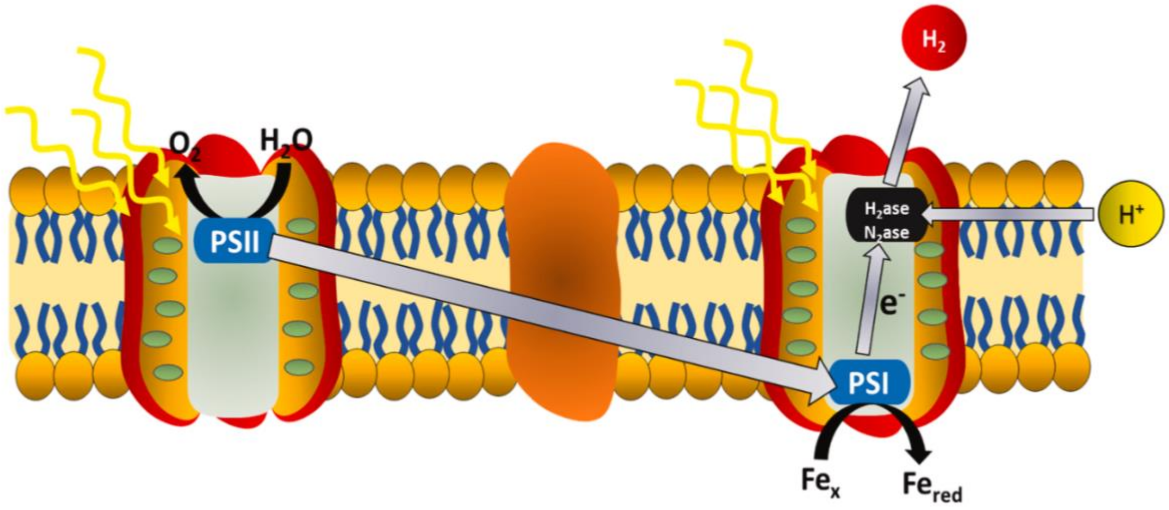

Fig. 5: Utilizing direct biophotolysis to produce biohydrogen (Sivaramakrishnan et al., 2021).

2.4. H_2 Production by Photo Fermentation (PF)

PF is the process by which photosynthetic bacteria convert organic substrates into hydrogen (Wang et al., 2009; Hallenbeck et al., 2016). PF is thought to be an effective and secure method of producing hydrogen (Redwood et al., 2009; Kumar et al., 2017). The mechanism of photochemical oxidation (PF) involves the use of electrons from water molecules by PSII (Sivaramakrishnan et al., 2021). These electrons are then used by [Fe]-hydrogenase in the direct biophotolysis process, which results in the creation of photosynthetic hydrogen. In order to produce hydrogen by photofermentation, photosynthetic purple non-sulfur (PNS) bacteria use organic acids like butryrate, acetate, succinate, and malate as well as carbon dioxide from the atmosphere as a carbon source (Li et al., 2009; Doğan and M., 2016). Therefore, PF can produce hydrogen using a variety of substrates, such as organic acids, organic acid-rich wastewater, or organic acid-rich biomass (Basak et al., 2007; Mohan et al., 2019). Gram-negative PNS bacteria are commonly thought to be responsible for PF, including *Rhodobacter sulfidophilus, R. sphaeroides, R. capsulatus, Rhodospirillum rubrum,* and *Rhodopseudomonas palustris*.

Other environmental elements that affect the photosynthesis process include light intensity, carbon sources, and the level of anaerobiosis (Park et al., 2018). Nitrogenase and hydrogenase enzymes carry out PF in PNS bacteria via the TCA cycle (citric acid cycle) (Sivaramakrishnan et al., 2021). The nitrogenase enzyme is necessary for the TCA cycle-mediated photofermentative hydrogen generation process (Sivaramakrishnan et al., 2021). The process of oxidizing carbon substrate to CO_2 and H^+, which are then transported via the TCA cycle, is the hydrogenase enzyme-linked photofermentative hydrogen generation process (Akhlaghi et al., 2020). As a result, nitrogenase and N_2molecules are essential to the photofermentative hydrogen generation process, which is predominantly carried out by PNS bacteria (Sivaramakrishnan et al., 2021).

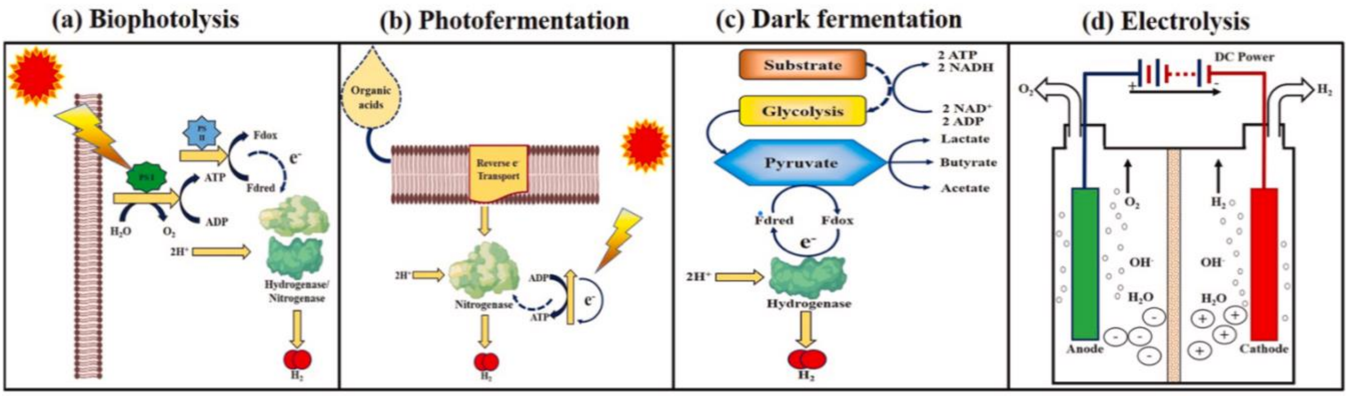

Fig 6. Schematic diagram illustrates variable methods of biological generation of hydrogen. a) biophotolysis, b) photofermentation, c) dark fermentation, and d) electrolysis (Sivaramakrishnan et al., 2021).

3. Hydrogen Production by Anaerobes

3.1 *Clostridia*

Clostridium strains are rod-shaped, gram-positive hydrogen-producing bacteria, commonly found in soil, water, and animal intestines. The majority of *Clostridium* strains are strictly anaerobic, the genus *Clostridium* is frequently employed in mixed-culture and pure-culture systems for dark-fermentative hydrogen production because of its great efficiency in hydrogen synthesis. Pretreatment is typically employed to inhibit the bacteria that consume hydrogen when mixed cultures are utilized as inoculum (Wang, and Yin, 2017).

Previous studies indicated that various pretreatment techniques such as base, aeration, ionizing radiation, acid, and heat shock influence *Clostridium* growth. Where microbial structure was found to be enriched under all pretreated groups. Furthermore, ionizing radiation and acid pretreatment were found to be more effective in enriching the genus *Clostridium*, with the highest occupation of 50-90% (Yin and Wang, 2016; Yang et al., 2019).

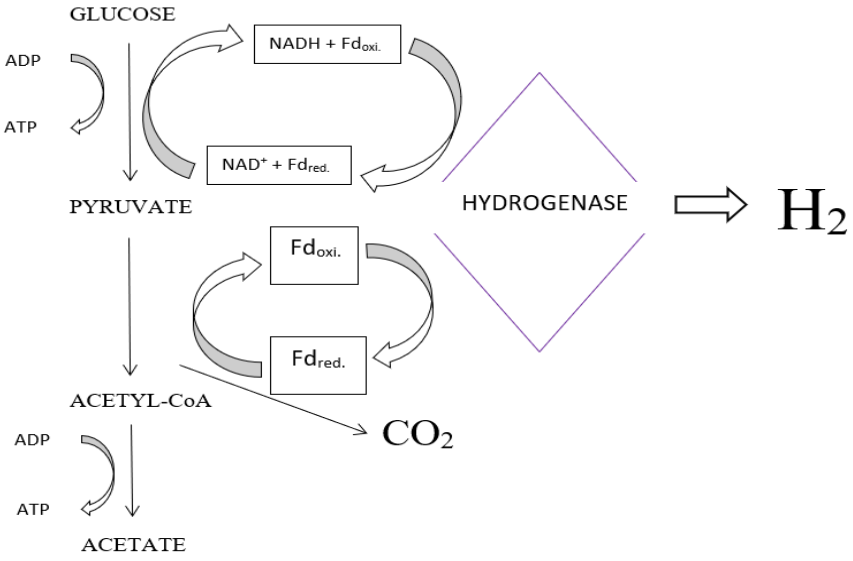

Fig 7. Diagram of hydrogen production by *Clostridium* sp. (Wang and Yin, 2021).

3.2 *Methylotrophs*

When NAD dependent Formate Dehydrogenase (FDH) was initially discovered from a methylotrophic bacterium in 1979 (Egorov et al., 1979; Tishkov, et al., 2023). This finding suggested a mechanism for NADH regeneration or H_2 production using organic fuel. A further study by Kawamura et al., (1983) and AlSayed et al., (2018) used the anaerobic bacteria *Methylomonas albus* BG8 and *Methylosinus trichosporium* OB3b to investigate the generation of H_2 by CH4. They looked at how H_2 is produced from a variety of organic substrates, including pyruvate, formate, formaldehyde, methanol, and methane.

Formate proved to be the most appropriate of these substrates for the anaerobic generation of H_2. Following a 5-hour incubation period, *M. albus* and *M. trichosporium* generated 2.45 and 0.61 μmol H2/μmol of formate, respectively. It was proposed that NAD-dependent FDH and HD, which are constitutive and soluble enzymes for the strain, are part of the H_2 generating system. *Pseudomonas* AMI, another methanol-using bacterium, was also examined for its ability to produce H_2, however no H_2 evolved that was comparable to another strain, *Pseudomonas methylica*, that had previously been described (Gogotov et al., 1975; Sapountzaki et al., 2023).

Table 3. Growth parameters of different methylotrophic bacteria (Mosin, 2013).

Bacterial strains	The molar yield of dry biomass, g/mol of methanol	The specific growth rate, h^{-1}	The level of carbon conversion of methanol, %	The quantity of consumed nitrogen, %
Ribulose-5-monophosphate pathway of carbon assimilation				
Pseudomonas C.	17.3	0.49	67.5	13.2
Pseudomonas methanolica	17.0	0.63	66.5	11.0
Methylomonas methanolica	15.7	0.52	62.0	11.7
Serine pathway of carbon assimilation				
Pseudomonas 1	12.1	0.17	47.5	11.37
Pseudomonas 135	12.1	0.14	47.5	11.48
Pseudomonas AM1	9.8	0.09	37.6	11.20
Pseudomonas M-27	13.1	0.11	51.5	9.40
Pseudomonas roseus	13.1	0.15	51.0	10.60

Fig. 8. Electron micrograph of rod-shaped obligate methylotrophic bacterium *Methylomonas methanicm* (Whittenbury et al 1970)

3.3 *Methanogenic* Bacteria

Since fossil oil is set to run out in the next decades, the flammable gas methane is thought to be a good substitute in the future (Ren et al., 2008; Qi et al., 2022). Methanogens are biocatalysts that may help to find solutions to future energy-related issues, by generating methane as a storable energy carrier. Methane generation is a characteristic shared by a very diverse set of *Archaeal methanogens* (Balch et al., 1979).

Methane has multiple applications such as energy storage, automobile fuel, electricity generation, and synthesis base chemical. Several nations have established natural gas networks (Ren et al. 2008; Shah, 2017). Methanotrophic bacteria can use methane as a carbon and energy source to create biomass, enzymes, polyhydroxybutyric acid (PHB), or methanol, depending on the required shift from chemical to biological processes (Strong et al., 2015). Biological methanation is already widely utilized in sewage water plants and biogas plants. It naturally occurs in wetlands, animal digestive tracts, oil fields, and other habitats (Garcia et al. 2000; Holmes and Smith 2016).

Even while methanogens are finding new uses, such electromethanogensis, there is still a lot of fundamental research being done on this extremely diverse and special group of methanogens, including strain characterisation and the creation of basic genetic tools (Blasco-Gomez et al. 2017). The group known as methanogenic archaea is incredibly diverse, and certain strains of the bacteria may thrive in harsh environments such as extremely high or low temperatures, high osmolarities, or pH levels. Thus, it is desirable to design and optimize methanogen-using procedures that are suitable to industry. This is true in many ways than only methane generation as a fuel with technical significance (Kondusamy and Kalamdhad, 2014).

3.4 Rumen Bacteria

The anaerobic fermenter known as rumen is home to a wide variety of bacteria, protozoa, fungus, and archaea. According to estimates made by Kim et al., (2011) and Owens and Basalan, (2016) only a tiny portion of rumen bacteria have been grown and studied. The advancement of molecular biology techniques, like sequencing small subunit rRNA genes (Edwards et al., 2004; Hoggard et al., 2018) and more sophisticated metagenomic analysis (McCann et al., 2014), is rapidly expanding our understanding of this ecosystem. Many researchers have employed these modern technologies to explore the ruminal microbial community at various taxonomic levels throughout the years (McCann et al., 2014).

These technologies can offer a deeper description of the numerous microbial species in the rumen. The ruminal microbiota and its collective genomes have over 100 times more genes than the genomes of the host animal, providing them with genetic and metabolic capabilities, such as the ability to digest substances that are inaccessible to the host, that the host has not evolved on its own (McSweeney and Mackie, 2012). The food, breed, season, location, feeding techniques, feed additives, intake level, growth stages, and physiological conditions of the host animals, among other things, can all have an impact on the diversity, density, and functions of the ruminal microbiota (Zanon et al., 2022).

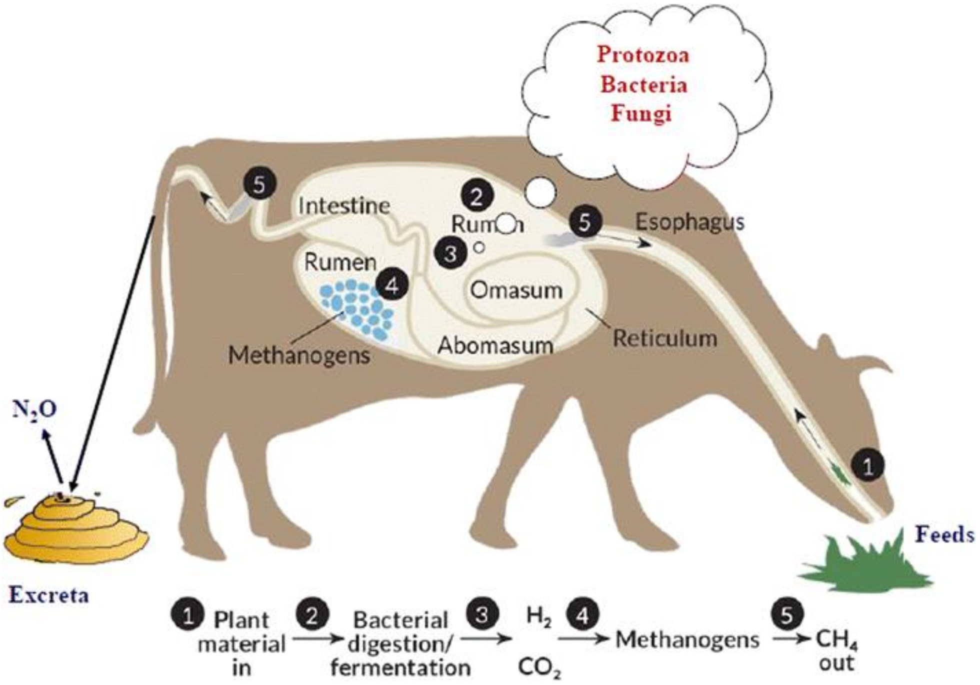

Fig 9. Hydrogen (H_2) and methane (CH_4) production by natural rumen bacteria

The ecological redundancy and resilience of the microbial community to withstand and bounce back from external or internal shocks, however, contribute to its general stability. While this consistency is important for animal nutrition and metabolism, it also presents significant obstacles to rumen function engineering through external intervention (Weimer, 2015). The rumen's microbial community ferments feed, which produces methane as a byproduct of biochemistry. The microbial community of ruminants with varying feed efficiency has been the subject of increasing investigation due to its strong correlation with reduced feed efficiency. According to studies by Myer et al., (2015), distinct microbial communities have been discovered in the animals that vary in methane output or feed efficiency. Gaining a more comprehensive understanding of the microecosystem that is related to methane generation in the rumen would provide targeted regulation of methane output. The environment and animal production are impacted by the products that are produced during rumen fermentation.

The release of CH_4 to the atmosphere, which is the primary [H] sink in rumen fermentation, represents an energy loss of 2–12% of ingested gross energy (Johnson and Johnson, 1995; Ungerfeld 2020). This was recognized early in ruminant nutrition research as an energy inefficiency in rumen fermentation and a chance to increase animal productivity (Cammack et al., 2018). Recently, there has been a greater focus on reduction of ruminant CH_4 emissions due to growing worries about climate change.

Enteric CH_4 emissions are thought to make up roughly 6% of all anthropogenic greenhouse gas emissions, or CO_2-eq, which is the amount of all greenhouse gas emissions divided by the potential for global warming (Gerber et al., 2013). Additionally, the host animal's post-absorptive metabolism is impacted by the volatile fatty acid (VFA) profile that is absorbed from the rumen (Bedford et al., 2020). The primary glucose precursor in ruminants, propionate, can be produced through fermentation by inhibiting methanogenesis (Buccioni et al., 2015).

For animals with high glucose requirements, like high-producing dairy cows during the early stages of lactation, increased propionate production may be beneficial. Conversely, propionate, a satiety signal in ruminants, has been shown to reduce milk fat content (Maxin et al., 2011; Allen, 2014). Accordingly, the percentage of milk fat rises when there is a greater supply of acetate (Maxin et al., 2011).

3.4 *Archaea*

Iron-sulfur proteins are the only type of microbial hydrogenase. These enzymes include reductase and hydrogenase enzymes (HD) connected to the oxidation or evolution of molecular H_2, while nickel-containing hydrogenase connected to membrane-bound electron transport systems typically oxidize hydrogen (Rousset, 2014; Rosenbaum and Müller, 2021). HD is a nickel lacking soluble enzyme that has been connected to ferredoxin or low potential cytochromes.

On the other hand, the hyperthermophilic archeon *Pyrococcus furosus* generates H_2 from peptide and carbohydrates and has soluble nickel that contains hydrogenase (Adams, 1990 ; McTernan, 2015; Fiala and Stetter,1986 ; Basak, 2020. Krishnan et al., (2023) investigated the isolated *P. furiosus* enzymes' ability to produce H_2 from pyruvate. They demonstrated that pyruvate-ferredoxin oxido-reductase participated in the generation of H_2 from pyruvate, and that electrons were then transferred from reduced ferredoxin to NADP using NADPH as an electron donor, the ferredoxin enzyme, also known as NADPoxido-reductase (sulfide dehydrogenase), also decreased elemental sulfur.

Dhydrogenase is a sulfur reductase or sulfhydrogenase, facilitated the conversion of NADPH into H_2. Comparing P. *furiosus's* H_2 evolving system to those found in other bacterial systems, it seemed peculiar. This microorganism was said to grow best at 100 °C and make organic acid, CO_2, and H_2 from peptides or carbohydrates.

4. Hydrogen Production by Facultative Anaerobes

4.1 *Escherichia coli*

In-depth research on *E. coli's* anaerobic breakdown of formate into H_2 and CO_2 was done between 1929 and 1933 by Stickland (1929; Dey et al., 2013). It was discovered that *E. Col*. is capable of inducing the enzymatic activity known as "formate hydrogenlyase" (FHL), which breaks down formate anaerobically into equimolcular quantities of H_2 and CO_2 in the cell suspension (Stickland,1929; Metcalfe, 2022). On the other hand, formate broke down in the presence of O_2 or methylene blue without releasing H_2.

Aeration inhibited induction but had no influence on the FHL system's catalytic activity (Stephenson and Stickland,1932; Xu et al., 2018). Subsequent research revealed that the FHL system is a membrane-bound multienzyme system made up of an HD and an FDH connected by unknown electron carriers (Al Lawati, 2018; O'Hara et al.,1967). Unlike other FDH that can reduce methylene blue (MB), the FDH associated with H_2 generation was active on the one-electron dye benzyl viologen (BV).

The non-energy producing process was catalyzed by Cole and Wimpenny, (1966). Without producing H_2, formate could be oxidized by FDH (MB) and connected to various anaerobic reductase systems (NO_3 ---> NO_2^- and fumarate → succinate) with the creation of ATP as energy (Pinske et al., 2015; Ruiz-Herrera and Alvarez,1972; Haddock and Jones,1977).

Promoting the use of the formate hydrogenlyase (FHL) system for the synthesis of H_2 from formate and the transportation of H_2 as formate in the reversible reaction catalyzed by the bacterial FHL system HCO. It has been found that the immobilization of the *E. Coli* FHL system induces the continuous stoichiometric conversion of formate into H_2 and CO_2 (Nandi et al., 1992; Simon et al., 2015). They demonstrated the 96-hour synthesis of H_2 from 1.15 M formate, with a 25% efficiency loss per cycle.

A tiny amount of glucose, which was transformed into succinct acid, was necessary for the system to work. Additionally, formate (224 mg/g wet cell) was produced by the immobilized cell from a combination of H_2 and CO_2 Peck and Gest (Peck and Gest, 1957; Peiro, 2021). It has been previously reported that the addition of carbohydrates or C_2 chemicals related to carbohydrate metabolism was necessary to activate the FHL system for the activity of FHL to be present in the cell-free lysate of *E. Coli*. This study indicated that inhibiting other anaerobic reductases found in *E. coli* was necessary for the continuous lysis of formate (Nandi and Sengupta, 1996; Roger et al., 2021).

They postulated that there may be an electron leakage from the FHL system to the reductase due to an overlap between the redox potentials of the electron transport carrier as present in FDH to HD and those present in the anaerobic reductase systems (Fumarate → Succinate, Tetrathionate → Thiosulfate). In addition to preventing leakage, the presence of succinate or thiosulfate as the terminal reduction product promoted stoichiometric or prolonged formate lysis.

Stickland observed that washed *E. Coli* cells produced hydrogen gas (H_2) from carbohydrates (Mai and Adams, 1994; Hafez et al., 2014). It can also catalyze lactose, galactose, arabinose, glycerol, and mannitol to produce H_2 at a lower yield. Compared to the anaerobic breakdown of glucose, fructose, and mannose, which was comparable to that of formate. However, they suggested that formate had not been the intermediary in the H_2 generation from glucose (Mai and Adams, 1994; Hafez et al., 2014).

Nevertheless, Ordal and Halvorson, (1939) and Wang et al., (2017) examined the creation of H_2 from sugars and formic acid by regular and mutant strains of *E. coli* and demonstrated that the source of H_2 from glucose was unquestionably formic acid, which is an intermediary in the bacterial production of H_2. Since formate is not the only byproduct of glucose, the anaerobic generation of H_2 from carbohydrates by *E. coli* cells had low conversion rates. Growing *E. coli* produced 90 mmol of ethanol and acetate, 90 mmol of H_2 and formate, and 15 mmol of CO_2 and succinate from 100 mmol of glucose, according to observations on carbon balance (Blackwood et al., 1956; Bohnenkamp et al., 2021)

In the absence of any electron acceptor, such as nitrate or fumarate, it was discovered that pyruvate produced from glucose metabolized in two phases (Ruiz-Herrera and Alvarez, 1972; Schubert and Unden, 2023; Schuber and Unden, 2023)

4.2 *Enterobacter*

Enterobacter aerogenes has been identified to generated maximum production of 0.20 to 0.21 H_2/h/1 in a medium comprising of salts, glucose, and peptone at 38 to 40 °C (Pachapur et al., 2017; Tanisho et al., 1983). A later identification of the strain as *E. aerogenes* led to the optimization of H_2 production to 0.52 l H_2/h/l of medium over a 23-hour period. The generation of H_2 followed a mol/mol glucose stoichiometry.

78% of studies were also conducted on the effects of pH and biomass yield on the strain's ability to produce H_2. The maximum H_2 evolution rate at 38 °C was 13 mmol H_2/g dry weight cell/h when glucose was the carbon source (Tanisho et al., 1989; Łukajtis et al., 2018).

At a mol/mol conversion rate, the strain produced H_2 using glucose, galactose, fructose, and mannose. For the same conversion rate, the strain also produced H_2 using dextrin. Moreover, production was seen in continuous culture for 26 days. At a rate of 0.8 mol H_2/mol of glucose, the evolution of H_2 occurred at an average rate of 120 ml/h/l in the medium. It was thought that the accumulation of acids such acetic, succinic, and lactic acids had an inhibitory effect, which caused the decrease in H_2 generation that was shown in the later phase.

5. Production of hydrogen by Aerobes

5.1 *Alcaligenes*

The aerobic H_2 producing bacteria can use H_2 and CO_2 as their sole source of energy and carbon, respectively. The organisms contain a soluble NAD-reducing hydrogenase (HD) and can grow heterotrophically (Probst and Schlegel,1977 ؛ Schneider and Schlegel,1977; Cordero et al., 2019) showed that *Alcaligenes eutrophus*, growing heterotrophically on gluconate or fructose, developed molecular H_2 from organic substrate under anaerobic conditions. *A. eutrophus* contains soluble

NAD-reducing HD, which reduces NAD directly with H_2 and disposes of excess reducing agent in the form of H_2 when grown under anaerobic conditions. Electrons that come from the breakdown of organic substrates do not enter the respiratory chain (Klibanov et al.,1982; Hards and Cook, 2017). The immobilized cells of *A. eutrophus* examined reversible reactions.

$$HCOOH \Leftrightarrow H_2 + CO_2$$

During the decomposition of formate, a higher concentration of formate (>0.5 M) inhibited H_2 production. Although the immobilized cells had good storage stability, no sustained formate lysis by the immobilized cells could be detected.

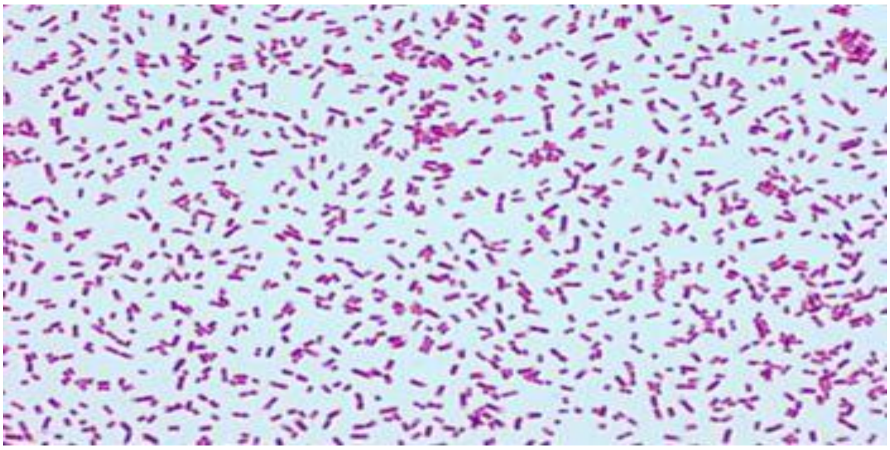

Fig. 10 Represent phenotypic examination of H_2 producing *Alcaligenes* bacteria (Adams and Moss, 2008)

5.2 *Bacillus*

The hydrogen-producing culture of *Bacillus licheniformis* (Kalia et al.,1994; Banerjee and Ghosh, 2020) from a mixed culture of H_2-producing bacteria from cattle manure. In the batch culture, *B. licheniformis* produces 13 l H_2/mol 3% (w/v) glucose in the medium (Kumar et al.,1995; Zhai et al., 2019). The cells were immobilized on brick dust and in calcium alginate beads. Alginate beads had an H_2 production efficiency of 16 L/mol glucose/day compared to 31 L/mol/day for cells immobilized on brick dust. The immobilized cells were stable in the continuous system for 60 days and an average conversion ratio of 1.5 mol H_2/mol glucose was achieved.

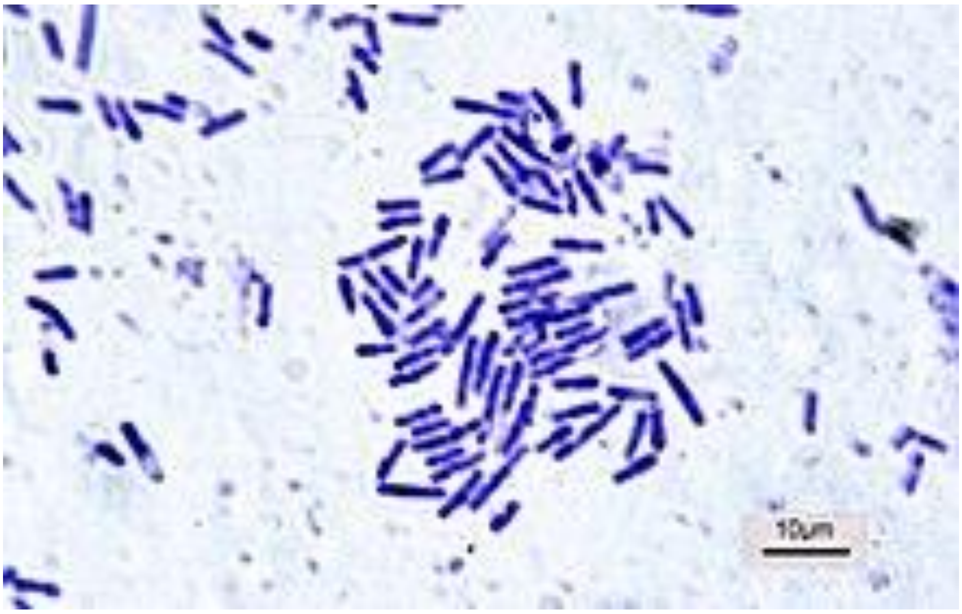

Fig. 11 represents phenotypic examination of *Bacillus* (Caulier et al., 2019)

6. Hydrogen Production by photosynthetic bacteria

The photosynthetic bacteria have a very diverse metabolic repertoire and have been recognized for many years to generate hydrogen when photofermentative growth. Here, recent advances in hydrogen production by these organisms are discussed and future directions are suggested. Photosynthetic bacteria that use certain organic wastes could be advantageous compared to other biological systems in the large-scale production of hydrogen along with high-quality dietary protein. One of the disadvantages of this system is its dependence on light, which requires the use of photobioreactors. This potentially significantly increases the cost of such a system. In another approach that avoids the use of light energy, the restricted oxidation phosphorylation driven microaerobic fermentation of organic acids to hydrogen, was demonstrated in principle. Further advances will likely require the use of metabolic engineering and more sophisticated process controls to achieve higher stoichiometries, approaches that could be applied to other, light-dependent hydrogen production processes in these organisms.

Research has been intensified to find new fuel sources to replace fossil hydrocarbons. Hydrogen is considered the ideal and pollutant-free fuel of the future (Benemann et al., 1974; Cano et al., 2015). Biological production of hydrogen has been observed in many microbial species (Gray et al., 1965; Puyol et al., 2017). Photosynthetic bacteria have long been studied for their ability to produce hydrogen from organic acids in a light-dependent reaction called photofermentation. Hydrogen is produced from substrates where this is normally difficult from a thermodynamic point of view (Hallenbeck, 2011; Neczaj and Grosser, 2019). This is possible because additional energy can be supplied through bacterial photosynthesis (Fig. 12).

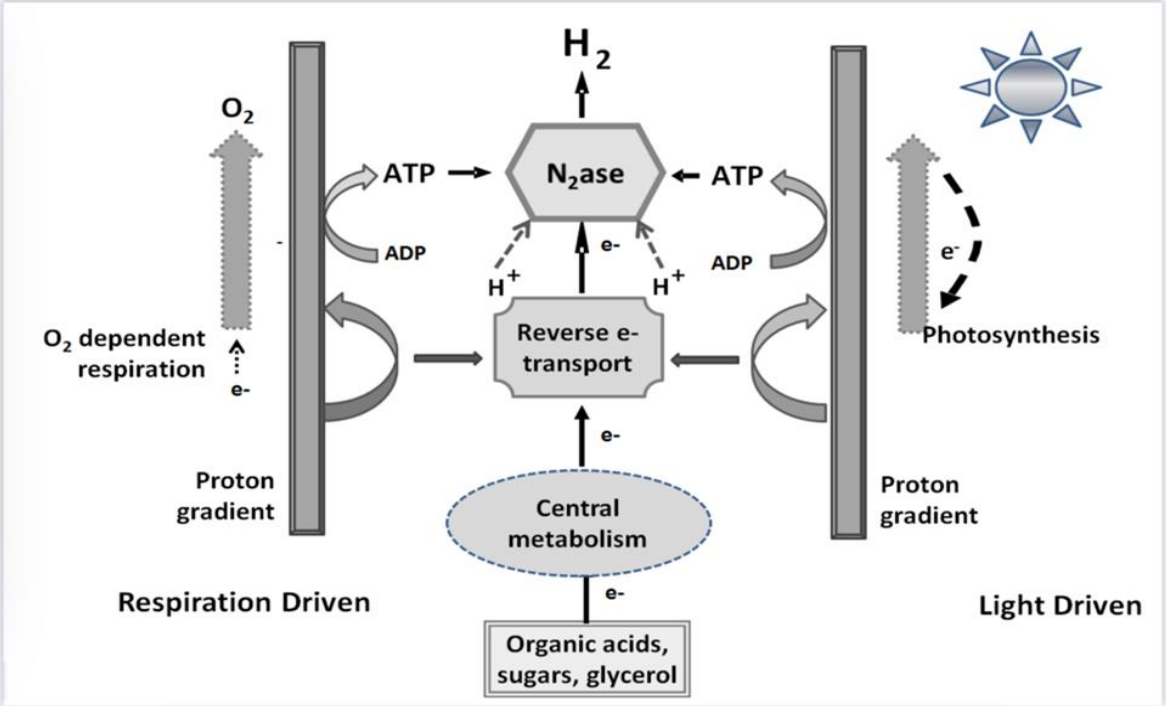

Fig 12. Integration of H_2 production by photosynthetic bacteria with cellular metabolism. A schematic of the interactions with various metabolisms necessary for nitrogenase activity in the photosynthetic bacterium *Rhodobacter capsulatus*. ATP is supplied either by oxidative phosphorylation during microaerobic respiration (left) or by cyclic photophosphorylation (right). High energy electrons are generated from central

metabolism through reverse electron flow driven by proton gradients created during microaerobic respiration (left) or cyclic photosynthesis (right) (Chookaew et al., 2015).

For large-scale production, photosynthetic organisms that can harness solar energy offer several advantages over heterotrophs. Light-dependent production of hydrogen by photosynthetic bacteria was first observed in cultures of *Rhodospirillum rubrum* (Gest et al., 1949; Grechanik et al., 2020). Purple sulfur-free bacteria produce hydrogen and CO_2 through light-dependent decomposition of several organic compounds (Gest et al., 1962; Ormerod et al., 1961; Kushkevych et al., 2024).

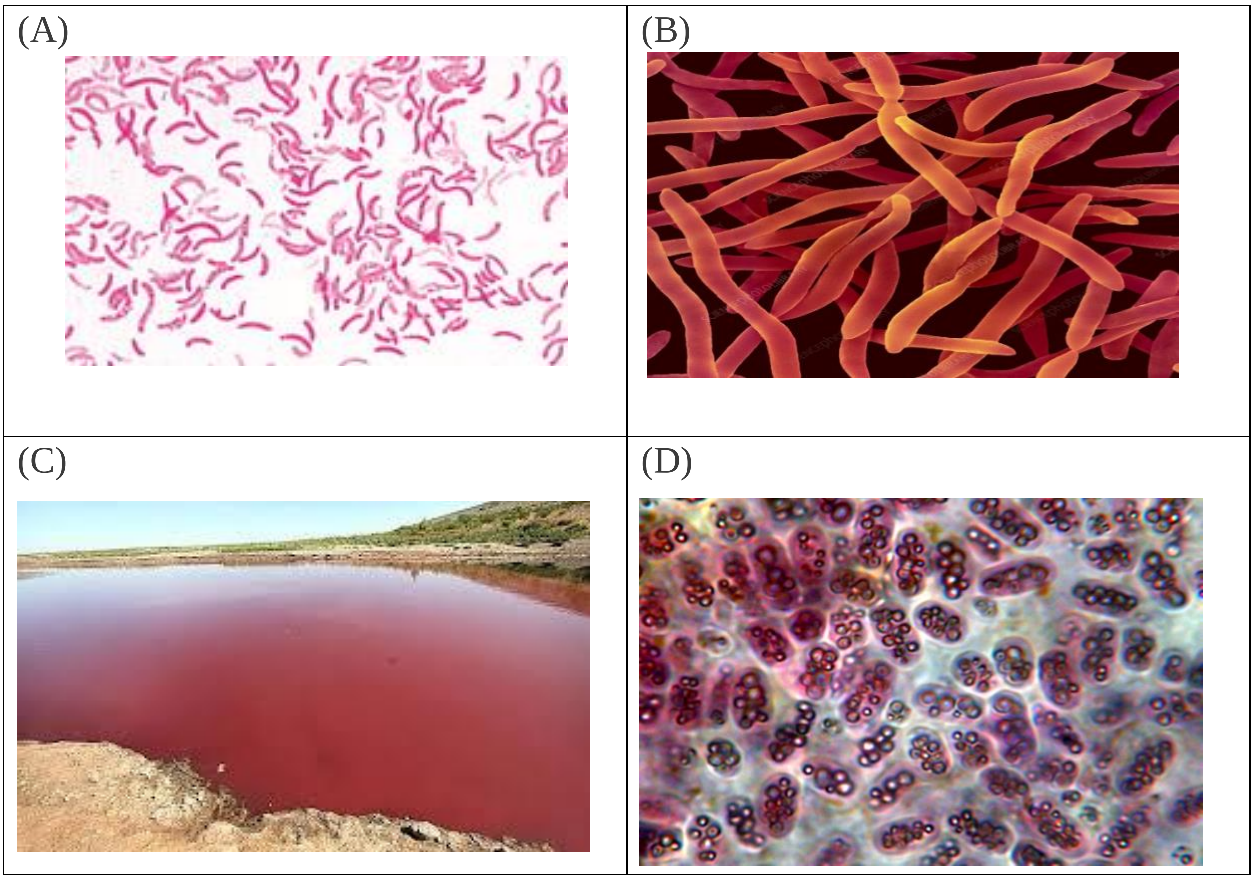

Fig. 13 (A, B) represents phenotypic examination of *Rhodospirillum rubrum* and (C, D) Purple sulfur-free bacteria produce hydrogen and CO_2 through light-dependent decomposition of several organic compounds (Narancic et al., 2018).

Since hydrogen evolution is inhibited by ammonium salts and molecular nitrogen in both photosynthetic bacteria and blue-green algae, Nitrogenase catalyzes the generation of hydrogen in both types of organisms. In addition to hydrogen, algae also produce oxygen. Before hydrogen can be used as fuel, it must be separated from oxygen and possibly other gases in an energy-intensive process.

However, in photosynthetic bacteria, the only gas produced besides hydrogen is CO_2. This is a great advantage because the gas released can be reused without further processing. Algae can use water as a hydrogen donor, whereas purple non-sulfur bacteria require an organic substrate. High yields of hydrogen can be achieved, for example with lactic acid as a hydrogen donor (Hillmer et al., 1977; Wei et al., 2020).
There are also wastes containing lactic acid that are present in large quantities. The mutual relationship between algae and three bacteria a group at the University of Córdoba’s study shows the highest hydrogen production obtained so far by this type of consortium (see Fig. 14)

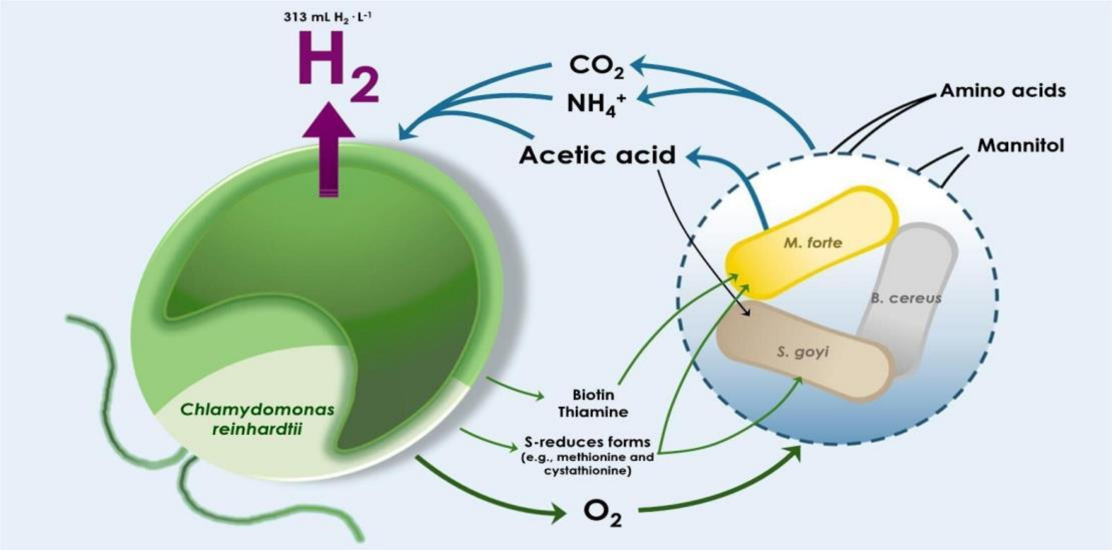

Fig. 14. A consortium of algae and bacteria boosts the production of green hydrogen and biomass while cleaning water (Fakhimi et al., 2019)

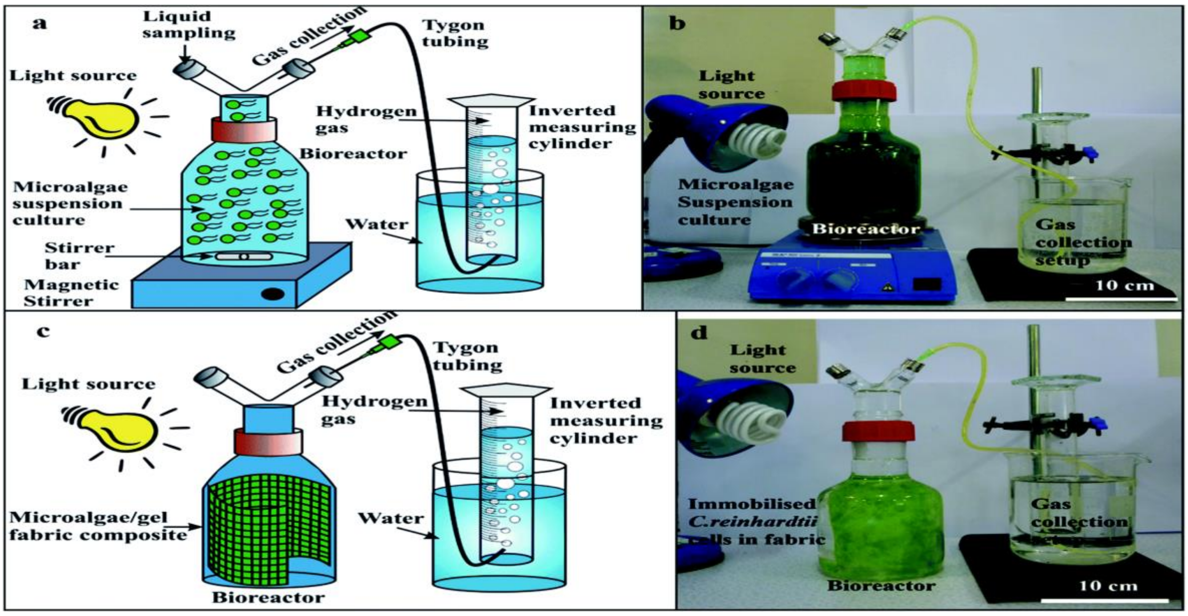

Fig. 15 Artificial leaf device for hydrogen generation from immobilised *Chlamydomonas reinhardtii* microalgae (Das et al., 2015)

6.1 Basic Mechanisms

Hydrogen normally evolved by aid of nitrogenase enzyme, which is normally active in N_2 reduction and reduces protons to hydrogen (Masepohl and Hallenbeck 2010; Hammes, 2015). This reaction is ATP dependent and therefore energetically demanding. Normally, this demand can be met by the action of bacterial photosynthesis, where ATP is produced through the light-controlled generation of a proton gradient. This proton gradient is also used to create the low potential electrons required for proton reduction through a process of reverse electron flow. The synthesis of this enzyme is repressed under N-rich conditions (Masepohl and Hallenbeck, 2010; Zhang et al., 2020), so substrates usually need to be N-poor unless special permissive mutants are used (Adessi et al,. 2012; Seefeldt et al., 2020). Knowledge about the metabolic pathways involved can be used through metabolic engineering in experiments to increase hydrogen yield (Hallenbeck et al,. 2012; El-Dalatony et al., 2020). Fundamentally, it appears that blocking pathways redirecting NADH, CO_2 fixation and polyhydroxybutyrate (PHB) synthesis should make more reducing agents available for nitrogenase and its reduction of protons to hydrogen.

6.2 Methods for improving hydrogen production by photosynthetic bacteria

6.2.1 Photofermentations

As mentioned previously, most studies on H_2 production by photosynthetic bacteria involved the use of organic acids, lactate, acetate and succinate as substrates (Han et al., 2013, Kobayashi et al., 2011, McKinlay et al., 2014). As already mentioned, these reactions only occur in the absence of ammonium, except in special mutant strains. While the metabolic advantage to the cell is not immediately apparent and might even appear contradictory considering how energy- intensive it is to repair carbon dioxide in the presence of an easily assimilable solid carbon source, various lines of evidence suggest that proton reduction to hydrogen under these conditions is necessary to achieve this to maintain cellular redox balance (Gordon and McKinlay, 2014). Recent results suggest that the formation of soluble microbial products (SMPs) is an additional factor limiting yields and that this can be overcome by adding a small amount of ethanol, an effect that is not currently fully understood (Kim et al., 2014). A reduction in SMP production would most likely contribute to an increase in hydrogen production, as this study showed, but a metabolic intervention that brings this about is not obvious since SMPs produced through various types of fermentation are a complex mixture of proteins, amino acids and nucleic acids appear to be organic acids, products of energy metabolism and cellular structural components (Kunacheva and Stucky, 2014)

6.2.2 Single and Two-Stage Systems

Various systems have been studied that use photosynthetic bacteria in conjunction with fermentative bacteria to drive the near-complete conversion of sugar to hydrogen. In principle, 12 moles of H_2 can be obtained from one mole of glucose (equation (1)).

$$(1)\ C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2$$

However, a maximum of 4 moles of H_2 can be produced through dark fermentation alone, which corresponds to a yield of only 33%, while at the same time organic acids such as acetate and lactate are produced as by-products. Many of these substrates are products of other microbial fermentations, suggesting that photofermentative hydrogen production could be used to produce additional hydrogen following or alongside dark hydrogen fermentation (Hallenbeck, 2011; Keskin et al., 2011; Lo et al., 2011; oledo et al., 2018).

Photosynthetic bacteria can be used to convert these organic acids into additional hydrogen, either in a single combined process or in a two-step process. Although these systems are elegant in principle, in practice they are difficult to use and optimize. In combined systems, the very different culture requirements of the two different organisms are problematic, and in systems with two stages, the waste from the dark fermentation stage requires significant treatment and handling, both costly with inefficient processes, to get it to a state where it becomes a suitable, non-inhibiting substrate for the photosynthetic bacteria.

Originally, dark fermentation and photofermentation were coupled as two sequential stages to achieve complete extraction of available electrons for H_2 production, which should theoretically yield 12 moles of H_2 per mole of glucose (Equation (1)). For example, one study used a hyperthermophile, *Caldicellulosiruptor saccharolyticus*, to ferment molasses in a dark stage, followed using several different photosynthetic bacteria to produce hydrogen in a light-controlled process using organic acids produced in the previous stage (Ozgur et al., 2010; Pandit et al., 2014), more details are shown in (Table 5).

This approach is essentially of low efficiency considering the high energy input and potentially complex design of a bioreactor suitable for the two very different processes. Substantially similar results were observed using integrated systems to convert potato starch to hydrogen, achieving overall yields close to 5.6 (Laurinavichene et al., 2012; Laurinavichene et al., 2010; Venkata et al., 2016), or with a system consisting of *Clostridium butyricum* and *Rhodopseudomonas palustris* that converts sucrose to hydrogen (Lo et al., 2010; Ramkumar et al., 2021) Finally, a two-stage system was proposed for the conversion of glycerol, which is a waste of biodiesel production, into hydrogen, but the molar yields were quite low (Table 5) (Chookaew et al., 2015). In fact, a simple one-step process appears to be very effective, allowing an essentially stoichiometric conversion of glycerol to hydrogen (see below Table 5). Recently, a significant improvement in yields for a two-stage system to 9.4 mol H_2 per mol glucose was demonstrated by using capnophilic fermentation of glucose to lactate and hydrogen by *Thermotoga neapolitana* (Table 5) (Dipasqual et al., 2015). This is essentially possible because increased CO_2 partial pressure shifts the fermentation products toward more lactate at the expense of acetate, thus creating a more productive substrate for the photosynthetic bacterium without affecting the hydrogen yield of *T. neapolitana*. In an alternative approach, which is relatively new as it has been little studied, the direct photofermentation of sugars to hydrogen is being developed.

Although organic acids are preferred for photosynthetic bacteria, early reports of these organisms noted that some could grow on different sugars. However, the conversion of these substrates, which may be quite common in various waste streams (Keskin et al., 2011; Koyama et al., 2016) or as energy crops, into hydrogen has been little investigated to date (Abo-Hashesh et al, 2011, Cai and Wang, 2014).

An initial study showed that the direct conversion of glucose to hydrogen was possible using the photosynthetic bacterium *Rhodobacter capsulatus* JP91 alone and an H_2 yield of 3 moles of H_2 per mole of glucose was achieved (Abo-Hashesh et al, 2011; Balachandar et al., 2020). Later work using the response surface method or continuous culture operation further improved the hydrogen yields to 5.5 and 9 mol

H_2 per mol glucose, respectively (Ghosh et al., 2012, Abo-Hashesh et al., 2013) (Table 5). In a more practical demonstration of a possible one-step process, the conversion of suitably diluted waste from sugar refining (beet and sugar beet molasses) was shown (Keskin and Hallenbeck, 2012; Ghimire et al., 2015). Similar yields (7.07) were also found for a mutant strain of *Rhodovulum sulfidophilum* (Cai and Wang, 2014). Further increases in rates and yields can be sought through the use of metabolic engineering. Related research has shown that glycerol, a common waste product of the biodiesel industry, can be quantitatively converted into hydrogen through photofermentation, achieving a yield of 6.9 mol H_2 per mol of crude glycerol, which is 96% of the theoretical amount (Ghosh et al., 2012; oledo et al., 2018). (Table 5), as shown in equation (2).

$$(2)\ C_3H_8O_3 + 3H_2O \leftrightarrow 3CO_2 + 7H_2$$

Current results also show that *Rhodopseudomonas palustris* CGA009 can be effectively used for the conversion of ethanol to hydrogen in a biological reforming reaction. A yield of 2 mol H_2 per mol ethanol was achieved, which is comparable to a chemical steam reforming process (Liu et al., 2015)

Table 4. Recent studies on single stage and two-stage H_2 production

Microorganism		Substrate	Yield (mol H_2/mol hexose or glycerol)	Major advantages/ disadvantages	Reference
Single-stage	*Rhodobacter capsulatus* JP91	Glucose	3	Simpler operation; suitable for metabolism study; substrates limited to a few sugars and some organic acids	(Abo-Hashesh et al., 2011)
	Rhodobacter capsulatus JP91	Glucose	5.5		(Ghosh et al.,2012)
	Rhodovulum sulfidophilum P5	Glucose	7.07		(Cai and Wang, 2014)
	Rhodobacter capsulatus JP91	Glucose	9		(Abo-Hashesh et al., 2013)
	Rhodobacter capsulatus JP91	Sucrose	7		(Keskin and Hallenbeck, 2012)
	Rhodopseudomonas palustris CGA009	Glycerol	6.69		(Ghosh et al., 2012)
Two-stage	*Thermotoga neapolitana DSM 4359T* and *Rhodopseudomonas palustris 42OL*	Glucose	9.4	Broad substrate range; addition operational cost from treatment of fermentation effluents of first stage	(Dispasquale et al., 2015)
	Microbial consortium and mixture of Rhodobacter capsulatus B10 and *Rhodobacter sphaeroides N7*	Starch	5.3		(Laurinavichene et al., 2012)
	Caldicellulosiruptor saccharolyticus and *Rhodobacter capsulatus YO3*	Beet molasses	6.8		(Ozgur et al., 2010)
	Microbial consortium and *Rhodobacter capsulatus B10*	Potato	5.6		(Laurinavichene et al., 2010)
	Clostridium butyricum CGS55 and *Rhodopseudomonas palustris WP3-5*	Sucrose	5.8		(Lo et al., 2010)
	Klebsiella sp.TR17 and Rhodopseudomonas palustris TN1	glycerol	0.8		Chookaew et al., 2015)

7. Hydrogen Production by Cyanobacteria

Because of the non-renewable fossil fuels will eventually run out, and there has been extremely substantial environmental contamination brought on using fossil fuels. Therefore, it's imperative to locate renewable and environmentally acceptable alternative energy sources (Das and Veziroglu, 2001; Das and Veziroglu, 2008). Hydrogen is considered as a renewable and clean energy source since it may be created by variable methods. Furthermore, hydrogen is highly environmentally benign since it only releases water when burned as fuel or used to generate energy (Hawkes et al., 2002; Kapdan and Kargi 2006; Hassan et al., 2023).

Hydrogen, as an alternative energy source, holds great promise and has gained an increase of global interest in the past few years. The biological technique is recognized as being less energy-intensive among the many hydrogen production procedures because it may be done at room temperature and pressure (Nishio and Nakashimada, 2004; Kraemer and Bagley, 2007; Li et al., 2024). Photosynthesis and fermentation are the two primary methods of producing hydrogen through biological means, though photosynthetic machinery of microorganisms which convert solar energy into hydrogen in a process that is theoretically flawless. However, it is challenging to put into practice because of the low light utilization efficiency and challenges in building reactors for hydrogen generation (Hawkes et al., 2007; Das and Veziroglu, 2008).

The benefits of fermentative hydrogen production include its easy operation and quick hydrogen production rate on the one hand. It can also produce hydrogen by fermentative means using a variety of organic wastes as a substrate on the other hand (see Fig .16). Thus, fermentation-based hydrogen production is more practical and therefore more extensively employed than photosynthetic hydrogen synthesis. Furthermore, it is crucial to use fermentative hydrogen production to create hydrogen from organic waste production, since it can produce both extremely clean energy and cleanse organic pollutants. As a result, fermentative hydrogen production has drawn more attention lately (Li, and Fang, 2007; Mohanakrishna and Pengadeth, 2024).

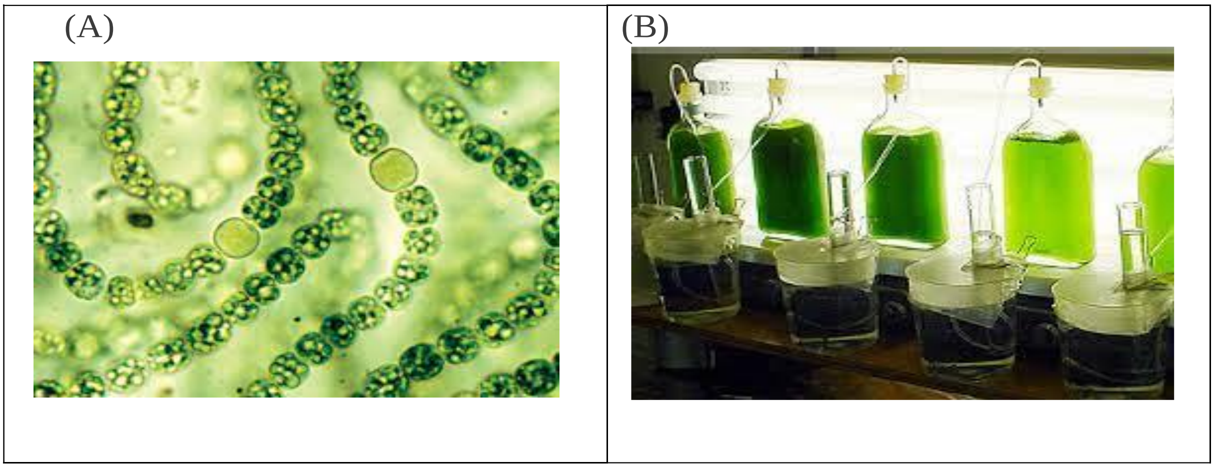

Fig. 16. (A) Phenotypic micrograph of photosynthetic cyanobacteria and (B)fermentative hydrogen production by cyanobacteria broth culture.

Fermentation-based hydrogen production is highly prevalent under anoxic environments. Electrons are created during the degradation of organic substrates by bacteria, and these electrons must be eliminated to preserve electrical neutrality. Protons can serve as an electron acceptor in anoxic settings to create molecular hydrogen (Das and Veziroglu, 2008; Akaniro et al, 2024)

Bacteria that create hydrogen can manufacture hydrogen from a variety of substrates, when fermentative hydrogen generation uses glucose as a model substrate. First, it is transformed into pyruvate by bacteria that produce hydrogen, using the glycolytic route to produce nicotinamide adenine dinucleotide (NADH) in its reduced state, that further transformed by pyruvate–ferredoxin oxidoreductase and hydrogenase into acetylcoenzyme A (acetyl-CoA), carbon dioxide, and hydrogen. Additionally, pyruvate can be transformed into formate and acetyl-CoA, which can then be easily transformed into carbon dioxide and hydrogen. Finally, acetyl-CoA is transformed into a number of soluble metabolites, including ethanol, butyrate, and acetate (Hawkes et al, 2007; Li, and Fang, 2007; Jain et al, 2024).

Furthermore, the process of producing hydrogen through fermentative means is highly intricate and dependent on numerous variables, including pH, temperature, nitrogen, phosphate, metal ions, substrate, inoculum, and reactor type. Furthermore, in the past few years, research conducted all over the world have reported on the influence of these parameters on the formation of fermentative hydrogen (Li and Fang, 2007; Das and Veziroglu, 2008; Jain et al., 2024).

Similar to green algae, cyanobacteria have garnered significant interest from researchers studying biophotolysis as a means of producing hydrogen. Water in biological systems breaks down into oxygen and molecular hydrogen (H_2) through a process called biophotolysis that occurs in the presence of light. Oxygendependent photosynthesis is a feature of photosynthetic microorganisms, including microalgae and cyanobacteria

Chlorophyll pigments in the thylakoid membrane of photosynthetic organisms absorb light and create molecular oxygen during photosynthesis. Then, with the aid of ATP and NADPH, carbon dioxide is transformed into triose phosphate through enzymatic processes. The physiological and morphological diversity of cyanobacteria allows them to produce hydrogen gas (H_2) through both direct and indirect biophotolysis(Brentner et al., 2010; Kossalbayev et al.,2020). (see Fig. 17).

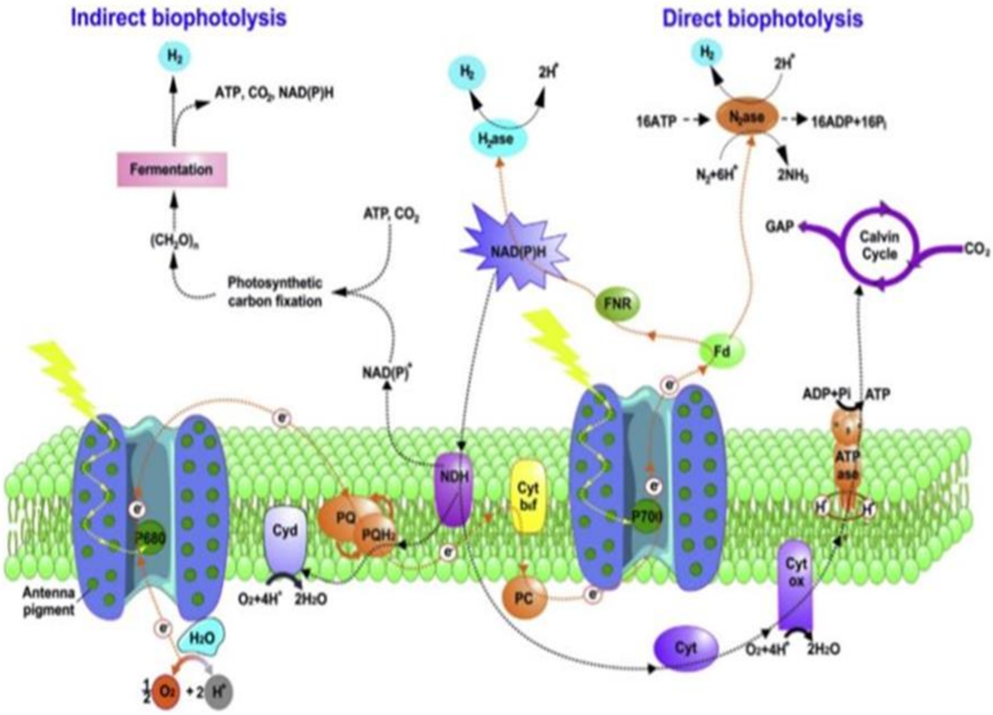

Fig. 17. Direct and indirect biophotolysis processes by photosynthetic microorganisms towards hydrogen production (Kossalbayev et al., 2020).

Water cleavage and further ferredoxin reduction are utilized in indirect biophotolysis, a sort of direct procedure, to fix carbon dioxide. The reduced carbohydrate molecule that results from this process can be employed to stimulate the release of hydrogen in a different reaction. This two-stage process divides the evolution of oxygen and hydrogen into distinct stages in space and time to prevent oxygen inhibition (Kossalbayev et al., 2020). Because different cyanogenases and hydrogenases are possessed by cyanobacteria, therefore, different species have different metabolic pathways to produce hydrogen (Khetkorn et al., 2012; Kufryk, 2013; Kossalbayev et al., 2020).

Both hydrogenase and nitrogenase can be synthesized by some cyanobacteria. Nitrogenase's action causes heterocystic cyanobacteria to release hydrogen, which is known to be the most promising form of hydrogen release because oxygen-producing photosynthesis and oxygen-sensitive proton reduction reactions are separated in space. However, since heterocyst formation is known to be accompanied by a massive energy consumption, an energy-efficient method of producing biohydrogen is the production of hydrogen based on cyanobacteria that do not form heterocysts. The majority of research, nevertheless, focuses on the nitrogenase-dependent cyanobacteria that produce hydrogen using heterocysts. Insufficient data exists regarding the utilization of nitrogenase and hydrogenase enzymes in the production of hydrogen by non-heterocytostic cyanobacteria strains. Three strains of cyanobacteria that do not form heterocysts were studied*:* *Desertifilum* sp. IPPAS B-1220*, Synechococcus* sp. *I12,* and *Phormidium corium* B-26. The goal was to identify more productive strains of phototrophic microorganisms that are actively producing hydrogen (Kossalbayev et al., 2020).

It was discovered that the strains *Synechococcus* sp, I12 and *Phormidium corium* B-26 lacked nitrogen-fixing activity during an initial investigation of nitrogenase activity, The nitrogenase and hydrogenase of the strain *Desertifilum* sp, IPPAS B-1220 are present. The four strains are all of interest when looking for potential hydrogen producers that don't require a lot of energy, similar to nitrogen-fixing heterocystic cultures (Ikeuchi and Tabata, 2001; Carrieri et al., 2008; Kossalbayev et al., 2020).

8. Major Factors influencing hydrogen production

There are many factors that increase the process of hydrogen production such as pH, temperature, substrates and other micro elements

8.1. Temperature

One of the most significant variables influencing the activities of bacteria that produce hydrogen, and the fermentative production of hydrogen is temperature. It has been shown that raising the temperature within a certain range may enhance the capacity of bacteria that produce hydrogen during the fermentative hydrogen production process, yet it could diminish if temperatures rise to even higher levels (Wang and Wan,2008).; de Gois Araújo Tavares et al, 2023).

Data in Table 5 outlines a number of studies looking into how temperature affects the production of fermentative hydrogen, although the ideal temperature for the generation of fermentative hydrogen was not always the same, it often fell between the mesophilic (about 37 °C) and thermophilic (about 55 °C) (Li and Fang, 2007).

Table 5. The effect of temperature fermentative hydrogen production

Inoculum	Substrates	Reactor type	Temperature (°C)		Optimal index (value)	References
			Range studied	Optimal		
Ethanoligenens harbinense YUAN-3	Glucose	Batch	20–44	37	Maximum hydrogen yield (1.34 mol/mol glucose)	(Xing, et al., 2008)
Anaerobic sludge	Glucose	Batch	25–55	40	Maximum hydrogen yield (275.1 mL/g glucose)	(Wang and Wan,2008)
Anaerobic sludge	Glucose	Batch	33–41	41	Maximum hydrogen yield (1.67 mol/mol glucose)	(Mu et al., 2006)
Anaerobic sludge	Sucrose	Batch	25–45	35.1	Maximum hydrogen yield (3.7 mol/mol sucrose)	Wang et al.,2005)
Anaerobic sludge	Sucrose	Batch	25–45	35.5	Maximum hydrogen yield (252 mL/g sucrose)	Mu et al.,2006)
Anaerobic digester sludge	Rice slurry	Batch	37–55	37	Maximum hydrogen yield (346 mL/g carbohydrate)	(Fang et al.,2006)
Municipal sewage sludge	Sucrose	Continuous	30–45	40	Maximum hydrogen yield (3.88 mol/mol sucrose)	(Lee et al., 2006)
Thermoanaerobacterium thermosaccharolyticum PSU-2	Sucrose	Batch	40–80	60	Maximum hydrogen yield (2.53 mol/mol hexose)	(Sompong et al., 2008)
Municipal sewage sludge	Starch	Batch	37–55	55	Maximum hydrogen yield (1.44 mmol/g starch)	(Lee et al., 2008)
Municipal sewage sludge	Xylose	Continuous	30–55	50	Maximum hydrogen yield (1.4 mol/mol xylose)	(Lin et al., 2008)
Cow dung	Cow dung	Batch	3 7–75	60	Maximum hydrogen yield (743 mL/kg cow dung)	(Yokoyama et al., 2007)
Cow waste slurry	Cow waste slurry	Batch	37–85	60	Maximum hydrogen yield (392 mL/L slurry)	(Yokoyama et al., 2007)
Anaerobic digester sludge	Organic waste	Semi-continuouss	37–55	55	Maximum hydrogen yield (360 mL/ g VS)	(Valdez-Vazquez et al., 2005)

8.2. pH

Because pH may have an impact on both the hydrogenase activity and the metabolic route, it is a significant component that controls the activities of hydrogen-producing bacteria and the fermentative hydrogen production process, it has been shown that raising pH within a certain range may help hydrogen-producing bacteria create more hydrogen during the fermentative hydrogen generation process, but raising pH to much higher levels may actually reduce hydrogen production. Just the impact of starting pH on fermentative hydrogen production was examined in these investigations because the majority were batch mode experiments without pH control. A number of research examining the impact of starting pH on batch mode fermentative hydrogen production are compiled in Table 6 (Khanal et al., 2004; Ananthi et al., 2024).

Table 6 illustrates the degree of dispute on the ideal starting pH for fermentative hydrogen production, as an illustration, Khanal et al. (2024) recorded the ideal starting pH for fermentative hydrogen production was at pH value of 4.5 however, the value stated by Lee and colleagues was 9.0 (Lee et al., 2002). Furthermore, throughout the examination into how the initial pH affected the formation of fermentative hydrogen, sucrose was the most frequently utilized substrate. It is therefore advised to look into how the initial pH affects the formation of fermentative hydrogen utilizing organic waste as the substrate (Mu et al., 2006).

Table 6. The effect of pH on fermentative hydrogen production

Inoculum	Substrates	initial pH		Optimal index (value)	References
		Range studied	Optimal		
Compost	Sucrose	4.5–6.5	4.5	Maximum hydrogen yield (214 mL/g COD)	(Khanal et al., 2004)
Anaerobic sludge	Starch	5.0–7.0	5.0	Maximum hydrogen yield (1.1 mol/mol hexose)	(Lin et al.,2008)
Clostridium butyricum CGS5	Sucrose	5.0–6.5	5.5	Maximum hydrogen yield (2.78 mol/mol sucrose)	(Chen et al., 2005)
Waste activated sludge	Food wastewater	4.0–8.0	6.0	Maximum hydrogen yield (47.1 mmol/g COD)	(Wu and Lin,2004).
Anaerobic sludge	Starch	4.0–9.0	6.0	Maximum hydrogen yield (92 mL/g starch)	(Zhang et al., 2003)
Thermoanaerobacterium thermosaccharolyticum PSU-2	Sucrose	4.0–8.5	6.2	Maximum hydrogen yield (2.53 mol/mol hexose)	(Sompong et al.,2008)
Municipal sewage sludge	Xylose	5.0–9.5	6.5	Maximum hydrogen yield (2.25 mol/mol xylose)	(Lin and Cheng 2006)
Municipal sewage sludge	Xylose	5.0–8.5	6.5	Maximum hydrogen yield (1.3 mol/mol xylose)	(Lin et al., 2006)
Cow dung compost	Cornstalk wastes	4.0–9.0	7.0	Maximum hydrogen yield (149.69 mL/TVS)	(Zhang et al.,2007)
Cow dung sludge	Cellulose	5.5–9.0	7.5	Maximum hydrogen yield (2.8 mmol/g cellulose)	(Lin and Hung, 2008).
Municipal sewage sludge	Sucrose	5.5–8.5	7.5	Maximum hydrogen yield (2.46 mol/mol sucrose)	(Wang et al., 2006)
Anaerobic granular sludge	Glucose	3.88–8.12	7.5	Maximum hydrogen yield (1.46 mol/mol glucose)	(Davila-Vazquez et al., 2008)
Cracked cereals	Starch	4.0–9.0	8.0	Maximum hydrogen yield (120 mL/g starch)	Liu and Shen,2004).
Anaerobic digester sludge	Sucrose	3.0–12.0	9.0	Maximum hydrogen yield (126.9 mL/g sucrose)	(Lee et al., 2002)

8.3. Nitrogen and Phosphate

Since proteins, nucleic acids, and enzymes are highly dependent on nitrogen for growth, nitrogen is also essential for the production of hydrogen by bacteria, it is among the most important nutrients required for the development of bacteria that produce hydrogen. As a result, the development of hydrogen-producing bacteria and the creation of fermentative hydrogen benefit from an adequate degree of nitrogen input (Bisaillon et al., 2006; Kamshybayeva et al., 2023)

The studies looking into how nitrogen content affects fermentative hydrogen production are compiled in Table 7.

Table 7. The effect of nitrogen concentration on fermentative hydrogen production

Inoculum	Substrates	initial pH		Optimal index (value)	References
		Range studied	Optimal		
Escherichia coli	Glucose	0–0.2 g N/L	0.01 g N/L	Maximum hydrogen yield (1.7 mol/mol glucose)	(Bisaillon et al., 2006)
Dewatered and thickened sludge	Glucose	0.5-10 g N/L	7 g N/L	Maximum hydrogen production (150 mL)	(Salerno et al., 2006)
Grass compost	Food wastes	0–0.6 g N/L	0.4 g N/L	Maximum hydrogen yield (77 mL/g TVS)	(Lay et al., 2005)
Cracked cereals	Starch	0.1–2 g N/L	1g N/L	Maximum hydrogen yield (146 mL/g starch)	(Liu and Shen, 2004).
Compost	Glucose	2–8% yeast extract	4% yeast extract	Maximum hydrogen production (70 mmol)	(Morimoto et al., 2004)
Enterobacter aerogenes HO-39	Glucose	0–5% polyptoton	2% polyptoton	Maximum hydrogen production (58 mL)	(Yokoi et al.,1995)

8.4 Metal ion

Fermentative hydrogen synthesis requires a trace quantity of metal ions, even if in higher concentrations they may limit the activity of hydrogen-producing bacteria (Li and Fang, 2007; Gu et al., 2020). A summary of many studies examining the impact of metal ion concentration on fermentative hydrogen generation can be found in Table 8. Given that hydrogenase requires Fe^{2+} to function. Table 8 demonstrates that Fe^{2+} was the metal ion that was studied the most for fermentative hydrogen generation (Wang and Wan, 2008).

Therefore, it is advised to conduct additional research on the impact of metal ion concentrations other than Fe^{2+} concentration on fermentative hydrogen production. Furthermore, there is some debate on the ideal Fe^{2+} concentration for fermentative hydrogen production. For instance, (Liu and Shen.,2004) found that 10 mg/L was the ideal Fe^{2+}concentration for fermentative hydrogen generation But, Zhang et al.'s result indicated 589.5 mg/L (Zhang et al., 2005).

The discrepancy between this research regarding the inoculum, substrate, and Fe^{2+} concentration range examined may have been the cause of this disagreement, Table 8 demonstrates that the most commonly utilized substrates in the study of the impact of metal ions on fermentative hydrogen generation were glucose and sucrose.

Consequently, utilizing organic waste as a substrate, research is being done on how nitrogen concentration affects the formation of fermentative hydrogen. Furthermore, the majority of the reviewed research examining the impact of metal ion concentration on fermentative hydrogen production were carried out in batch mode, as indicated by Table 8, and it is advised that more studies be carried out in continuous mode (Li and Fang, 2007; García-Depraect et al., 2023). The toxicity of heavy metals on the fermentation process that produces hydrogen was also examined in a number of research. According to Li and Fang's report, for instance, the following order represents the relative toxicity of six electroplating metals to fermentative hydrogen production: Pb > Ni–Zn > Cr > Cd > Cu (Li and Fang, 2007). Whereas Lin and Shei (2008) found that three heavy metals' proportional toxicity to the fermentation of hydrogen generation was in the in the subsequent order: Zn > Cu > Cr.

Table 8. The effect of metal ion concentrations on fermentative hydrogen production

Inoculum	Substrates	Reactor type	Mental ion	Concentration (mg/L)		Optimal index (value)	References
				Range studied	Optimal		
Cracked cereals	Starch	Batch	Fe^{2+}	1.2–100	10	Maximum hydrogen yield (140 mL/g starch)	(Liu and Shen,2004)
Anaerobic sludge	Starch	Batch	Fe^{2+}	0–1473.7	55.3	Maximum hydrogen yield (296.2 mL/g starch)	Yang and Shen, 2006)
Grass compost	Food wastes	Batch	Fe^{2+}	0–250	132	Maximum hydrogen yield (77 mL/g TVS)	(Lay et al., 2005)
Anaerobic sludge	Palm oil mill effluent	Batch	Fe^{2+}	2–400	257	Maximum hydrogen yield (6.33 L/L substrate)	(Sompong et al., 2008)
Digested sludge	Glucose	Batch	Fe^{2+}	0–1500	350	Maximum hydrogen yield (311.2 mL/g glucose)	(Wang and Wan,2008).
Anaerobic sludge	Starch	Batch	Fe^{2+}	0–1763.8	352.8	Maximum hydrogen yield (131.9 mL/g sucrose)	(Lee et al., 2001)
Cracked cereals	Sucrose	Batch	Fe^{2+}	0–1842.1	589.5	Maximum hydrogen yield (2.73 mol/mol sucrose)	(Zhang et al., 2005)
Anaerobic sludge	Glucose	Batch	Cu^{2+}	0–400	400	Maximum hydrogen yield (1.74 mol/mol glucose)	(Zheng and Yu,2004).
Anaerobic sludge	Glucose	Batch	Zn^{2+}	0–500	250	Maximum hydrogen yield (1.73 mol/mol glucose)	(Zheng and Yu,2004).
Hydrogen-producing bacterial B49	Glucose	Batch	Mg^{2+}	1.2–23.6	23.6	Maximum hydrogen yield (2360.5 mL/L culture)	(Wang et al.,2007)
Digested sludge	Glucose	Batch	Ni^{2+}	0–50	0.1	Maximum hydrogen yield (296.1 mL/g glucose)	(Wang, and Wan, 2008).
Digested sludge	Starch	Continuous	Ca^{2+}	0–300	150	Maximum hydrogen yield (3.6 mol/mol sucrose)	(Chang and Lin,2006)
Municipal sewage sludge	Starch	Continuous	Ca^{2+}	0–27.2	27.2	Maximum hydrogen yield (2.19 mol/mol sucrose)	(Lee et al., 2004)

8.5. Effects of microelements

Certain trace elements must be provided in order to ensure that a bacterium can carry out the metabolic pathways necessary for the creation of hydrogen efficiently. Because many enzymes require metal ions as co-factors, such as Na^{2+}, Mg^{2+}, Zn^{2+}, and Fe^{2+}, they can have an impact on how microbes produce hydrogen through metabolism. Important constituents of the main fermentative enzyme hydrogenase include iron and nickel. Hexokinase and pyruvate kinase, two essential enzymes in the glycolytic cycle, are activated by magnesium (Caserta et al.,2015; Rodionova et al., 2017)

8.6. Effect of Substrates

The effectiveness of fermentative hydrogen production can be significantly impacted by the kind of substrate that is utilized. Simple sugars, which are extensively employed as substrates in the fermentation process to produce hydrogen, include glucose, maltose, and xylose. These sugars are easily biodegradable. Where, acid hydrolysis or enzymatic hydrolysis can convert starch into glucose and maltose, given that most microbes are unable to directly digest cellulose. For example, low hydrogen yields are obtained when cellulose is used as a feedstock. But using trash that is high in carbohydrates from the food or agriculture industries, the cost of producing hydrogen can be greatly decreased by using wastewater from industries like sugar manufacture, starch processing, and brewing.

The majority of research has focused on pure bacterial cultures that create hydrogen using six-carbon carbohydrates like glucose serving as frequent substrates. Five-carbon sugars like xylose can be also used by a few strains that produce hydrogen, although the yields of hydrogen produced are not very high. As an illustration, the newly isolated *Clostridium* sp. YM1, only 0.82 mol H_2 mol^{-1} could be produced from xylose sugar, but 1.32 mol H_2 mol^{-1} sugar from glucose (Yang et al., 2014)

a lactate dehydrogenase-deficient *Thermoanaerobacterium* strain's hydrogen yield from glucose and xylose was compared, and it was discovered to be 2.71 and 1.45 mol H_2 mol^{-1} sugar, respectively, correspondingly. However, because of its high cost and limited availability brought on by the food crisis, pure glucose is not a suitable substrate for the synthesis of hydrogen,

For the creation of economically viable hydrogen on a big scale, renewable feedstock like organic waste shows greater promise. To illustrate, for every tone of starch production requires 5–8 m^3 of cassava trash. This wastewater is a good substrate for producing hydrogen because it is high in BOD and COD (>10 g L^{-1}) and rich in carbohydrates, organic waste. Besides, producing hydrogen at yields that are either higher than or comparable to those of pure substrates. Thus, the benefits of hydrogen production from zero-cost wastewater include cleaning the wastewater and generating clean energy at the same time without sacrificing efficiency. But a greater diversity of Hydrogen Producing Bacteria (HPB) is necessary due to the complexity of organic waste (Li et al., 2010).

9. Mixed Culture Technology.

Pure cultures are constrained in their ability to metabolize complicated substrates; in contrast, mixed cultures, also known as co-cultures, offer a variety of benefits, including low stress tolerance, high yield and productivity, and a diversity of metabolic and genetic pools. More significantly, mixed cultures are typically chosen for industrial applications because of their practicality and simplicity of control in non-sterile situations, which significantly reduces processing expenses (Sivagurunathan et al., 2016; Kleerebezem and van., 2007). Natural consortia, artificial consortia, and synthetic consortia are the three broad categories into which mixed culture technology can be subdivided based on the mixed inoculums utilized.

9.1 Mixed Culture of Nature Consortia

Numerous habitats, including soil, cow dung, municipal solid waste, activated sludge, and compost, are home to hydrogen producing bacteria (HPBs) (Wang et al., 2010; Wang et al., 2015; Yasin et al., 2013; Ren et al., 2010). In general, each of these sources (summarized in Table 6) can be utilized as an inoculum for the generation of hydrogen. But the nature consortia also include a range of non-hydrogen-producing microorganisms that might potentially compete with or impede the activity of HPB in addition to HPB itself.

Lactate-producing bacteria, for instance, can prevent HPB by competing for substrates or by releasing bacteriocins. Other microorganisms that compete with HPB for nutrients include those that reduce nitrate, iron, or sulphate. More significantly, H_2-consuming bacteria like homoacetogens and methanogens, which can convert hydrogen into acetate or CH_4, can also be found in nature consortia. These bacteria can result in a low hydrogen yield (Yasin et al., 2013; saady 2013; Wong et al., 2014; Singh and Wahid., 2015).

Enrichment for the favorable HPB requires pre-treating the inoculum in order to maximize hydrogen production and yield. The pre-treatment approach often rests on the capacity of HPB, such *Clostridium* sp. to generate spores, which enable them to withstand severe environments more readily than non-spore forming bacteria (like methanogens) (Wong et al., 2014; Song et al., 2012).

Under the right circumstances, HPB endospores can germinate following pre-treatment. A few of the many approaches that have been tried for inoculum pre-treatment because they have been well studied (Song et al., 2012; Bundhoo et al., 2015). Pre-therapies can be broadly categorized into physical treatments including heat shock, freezing, thawing, UV radiation as well as microwave radiation.

Table 9. Hydrogen production by mixed culture of nature consortia.

Inoculum	Substrate	Hydrogen yield	HPRa) [mmol H_2 L^{-1} h^{-1}]	Reference
Activated sludge	Glucose	1.93 mol H_2 mol−1 glucose	3.89b)	(Guo et al., 2013)
Activated sludge	Alkaline pretreated-sludge	15.6 mL g^{-1} volatile suspended solids	—	(Wang et al., 2018)
Activated sludge	Food waste	82.3 mL g^{-1} volatile solids	0.66 b)	(Nathao et al., 2013)
H_2-producing sludge	Food waste hydrolysate	85.6 mL g^{-1} food waste	15.80 b)	(Han et al., 2015)
Anaerobic digester sludge	Coffee drink wastewater	1.78 mol H_2 mol^{-1} glucose	123.21b)	(Jung et al., 2011)
Sewage sludge	Glucose	2.0 mol H_2 mol^{-1} glucose	4.33 c)	(Sivagurunathan et al., 2014)
Thickened sludge	Sludge /ryegrass	60 mL g^{-1} volatile solids	1.30 b)	(Yang and Wang., 2017)
Auto fermentation from synthetic wastewater	Sucrose	2.0 mol H_2 mol−1 glucose	5.29 b) , 2.11c)	(Penteado et al., 2013)

a) HPR, hydrogen production rate; b) maximum HPR; c) average HPR.

Table 10. Studies of dark fermentation with mixed culture of artificial consortia.

Inoculum	Substrate	Hydrogen yield	HPRa) [mmol H2 L^{-1} h−1]	Reference
Enterobacter aerogenes and *Clostridium butyricum*	Crude glycerol and Tween-80	32.1 mmol H_2 L−1 of medium	—	(Pachapur et al., 2016)
Enterobacter aerogenes and *C. butyricum*	Crude glycerol	1.5 mmol H_2 mol^{-1} glycerol	0.36 c)	(Pachapur et al., 2015)
Escherichia coli and Enterobacter aerogenes	Organic fraction of municipal solid waste	2.12 L H_2 L^{-1} substrate	7.90 b)	(Sharma and Melkania., 2017)
Clostridium thermocellum and *C. thermosaccharolyticum*	Sweet sorghum stalks	5.1 mmol H_2 g^{-1} substrate	—	(Islam et al., 2018)
Caldicellulosiruptor saccharolyticus and *C. kristjanssonii*	Glucose	3.7 mol H_2 mol^{-1} glucose	11.6 b)	(Zeidan et al., 2010)
Clostridium butyricum and Enterobacter aerogenes	Crude glycerol and apple pomace hydrolysate	26.07 mmol H_2 L^{-1} of medium	—	(Pachapur et al., 2015)
Bacillus cereus A1 and *Brevumdimonas naejangsanensis* B1	Corn starch	1.94 mol H_2 mol^{-1} glucose	0.95b)	(Bao et al., 2013)
Bacillus cereus A1 and *Brevumdimonas naejangsanensis* B1	Cassava starch	1.72 mol H_2 mol^{-1} glucose	1.25c)	(Wang et al., 2017)
Bacillus cereus A1 and *Brevumdimonas naejangsanensis* B1	Corn starch wastewaters	1.88 mol H2 mol−1 glucose	7.41b)	(Wang et al., 2016)

a) HPR, hydrogen production rate; b) maximum HPR; c) average HPR.

Also, chemical pre-treatments using substances like iodopropane, acid, alkali, and chloroform are used. Because heat shock is an easy, affordable, and efficient pre-treatment technique, it is most frequently employed to enrich spore-forming HPB such *Clostridium* sp. and *Bacillus* sp. Lactic acid bacteria, for instance, will be inhibited for 30 minutes at temperatures between 50 and 90 °C. (Noilke et al., 2002; Chang et al., 2011).

After 30 minutes of heating pre-treated waste activated sludge at 95 °C, the hydrogen output rose from 0.38 to 0.9 mol H_2 mol^{-1} glucose. Nevertheless, some research has revealed concerns that heat shock treatment may inhibit certain nonsporulating HPB, such as *Enterobacter* spp., and reduce hydrogen yield while partially suppressing some spore-forming H_2-consuming bacteria, such as *Clostridium aceticum* and *Clostridium thermoautotrophicum* (Bakonyi et al., 2014; Wang et al., 2011). Thus, in order to maximize hydrogen yield, each shock parameter needs to be independently tuned.

9.2. Mixed Culture of Artificial Consortia

Numerous researchers have concentrated on artificially enriching hydrogen producing bacteria (HPB) by combining various pure bacterial cultures as the presence of inhibitory and H_2-consuming bacteria limits the mixed culture of natural consortia (Wang et al., 2020). Compared to natural consortium mixed cultures, this artificial microbial mixed culture is more stable and has a higher hydrogen yield since it often has a fixed composition and well-characterized species (Wang et al., 2020). For instance, from activated sludge, Bao et al. (2012) recovered two HPB strains: *Bacillus cereus* A1 and *Brevumdimonas naejangsanensis B1*

Wang et al., (2020) showed that, with comparatively high yields, a mixed culture of the two strains could directly produce hydrogen from raw cassava starch, unhydrolyzed maize starch, and even wastewaters made from corn starch (Bao et al., 2012; Wang et al., 2017; Wang et al., 2016). Furthermore, Liu et al. (2012) created an artificial consortium for the fermentation of cornstalk waste by utilizing *Clostridium thermocellum* and *Clostridium thermosaccharolyticum* (Wang et al., 2020). When compared to the pure culture, they discovered that the mixed culture's hydrogen output was increased by 94.1% (Wang et al., 2020). However, building a stable and effective artificial consortium with three or more bacteria is extremely challenging because to the uneven growth rates of different bacteria and the imbalance between metabolite production and consumption (Weibel et al., 2008; Wang et al., 2020). To produce hydrogen from glycerol, Maru et al. (2013) for instance, created an artificial consortium made up of three strains: *Enterobacter* sp H_1, *Enterobacter* sp H_2, and *Citrobacter freundii* H_3 (Wang et al., 2020). The strains were mixed at a ratio of 1:1:1. In contrast to the individual strains, the mixed culture produced significantly less hydrogen and failed to demonstrate any synergistic effects (Wang et al., 2020).

9.3. Mixed Culture of Synthetic Consortia

Mixed culture of synthetic consortia is an emerging field of study due to the rapid advancement of genetic engineering and synthetic biology. Synthetic consortia, in contrast to artificial consortia, are rationally created, engineered, and built in order to provide the consortia with more specialized and effective functions. Research on synthetic consortia has been conducted in various domains, including bio electrochemical systems (Liu et al., 2017) manufacturing of bioethanol (Zuroff et al., 2013) and bioremediation of the environment (Chen et al., 2014)

Despite the fact that genetic engineering of individual strains that produce hydrogen has been thoroughly examined and reviewed (Maeda et al., 2012; Kanai et al., 2015). The production of hydrogen by synthetic consortia has been documented in a small number of investigations. A similar study described the development of a dual-organism system for hydrogen production using genetic engineering (Waks and Silver, 2009).

To do away with format consumption, this required deleting two of *Saccharomyces cerevisiae*'s format dehydrogenases, With the integration of an *Escherichia coli* PFL pathway to create a format-overproducing strain that has a 4.5-fold greater format content than the wild-type strain. Following that, *E. coli* produced 2.7 times as much hydrogen in the formate-rich wasted media as it did in the standard medium when it was employed for subsequent hydrogen generation. To be clear, this was not a mixed culture; rather, it was a two-step process, and there are still procedures to complete before creating a synthetic consortium (Waks and Silver, 2009).

A thorough grasp of cell-cell interactions in natural settings is necessary to construct a synthetic consortium. One of the main challenges impeding the creation of artificial consortia for hydrogen generation has been this Lately, Wang et al., (2019)

carried out a thorough analysis of a consortium that produces artificial hydrogen and includes *Bacillus cereus* A1. also known as *Brevundimonas naejangsanensis* B1, According to their findings, strains A1 and B1 generated glucoamylase and α-amylase, respectively, which worked in tandem to hydrolyze starch, in order to sustain strain B1's cell growth and hydrogen production, strain A1 converted starch to lactate, to increase the production of hydrogen, strain B1 gave back formate by acting as an electron shuttle to strain A1 (Fig. 18), This work improved our knowledge of intricate microbial relationships within a consortium that produces hydrogen and could be useful in the creation of more intricate artificial consortia. (Wang et al., 2019)

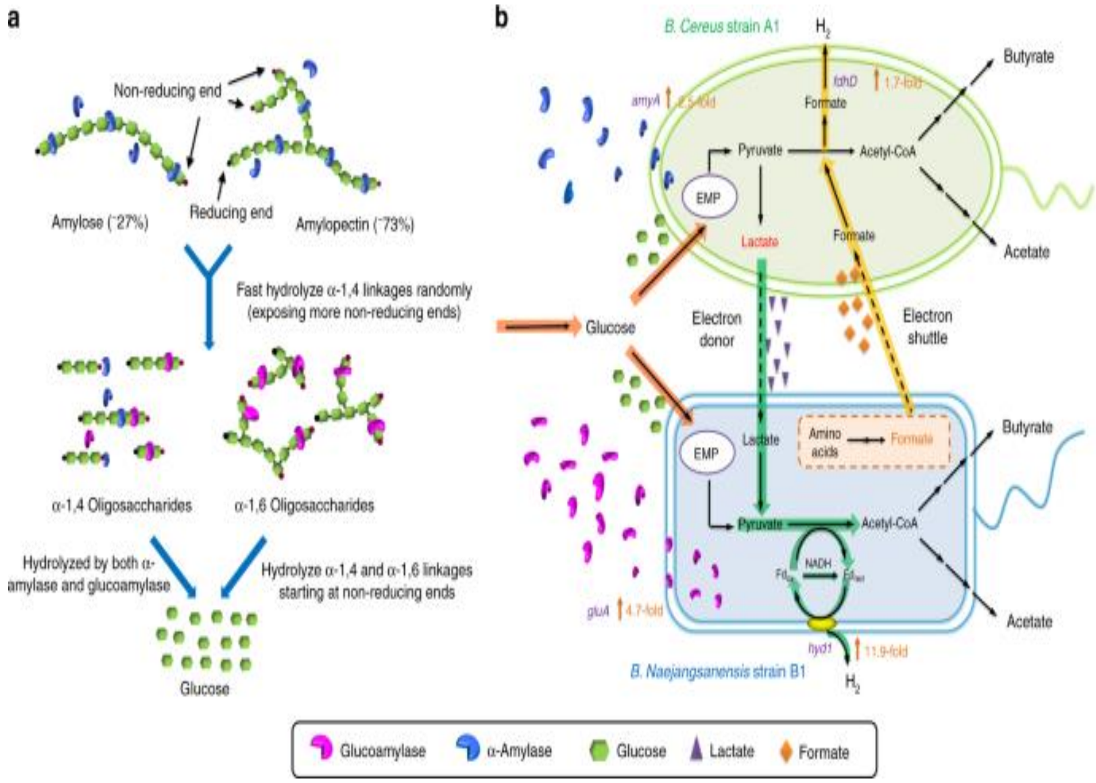

Fig. 18. Metabolite-based mutualism reported in a two-species microbial consortium enhances hydrogen production (Wang et al., 2019)

9.4 Cell Immobilization of Mixed Culture

Cell immobilization technology offers substantial advantages over suspended systems, especially in continuous processes, but many studies have concentrated on employing suspended-cell systems for dark fermentation. Immobilized-cell systems help prevent biomass washout issues, which frequently arise in continuous processes with low retention durations or high dilution rates. Additionally, cell immobilization can raise the biomass content, and carriers can be employed repeatedly while still producing hydrogen with high efficiency.

In addition, immobilized cells increase the tolerance to environmental perturbation and reduce the possibility of cell contamination (Singh et al., 2013; Singh and Wahid, 2015; Willaert, 2018). The immobilization technology can be applied to enhance mixed cultures' hydrogen generation. For example, hydrogen was produced from palm oil mill effluent using an immobilized mixed culture technique. The immobilized cell culture produced more hydrogen than the suspended cell culture, according to the study's findings (0.589 L H_2/[L POME h] and 0.348 L H_2/[L POME h], respectively)(Singh et al., 2013).

Additionally, in order to produce hydrogen, immobilized mixed culture of *Brevumdimonas naejangsanensis* and *Bacillus cereus* were assessed for multi-cycle use and hydrogen production, using three distinct carriers: maize stalk, activated carbon, and polyester fiber (Ma et al. 2017). They discovered that, with an average hydrogen output of 1.50 mol H_2 mol^{-1} glucose, maize stalk performed the best out of the three carriers and could be utilized again for ten batches. Using this co-immobilization system in a two-step continuous process, Wang et al. (2018) discovered that, for 18 days without a startup time, the production of hydrogen in two distinct reactors rose linearly.

References

Abe, J. O., Popoola, A. P. I., Ajenifuja, E., & Popoola, O. M. (2019). Hydrogen energy, economy and storage: Review and recommendation. International journal of hydrogen energy, 44(29), 15072-15086.

Abo-Hashesh, M., Desaunay, N., Hallenbeck, P.C. 2013. High yield single stage conversion of glucose to hydrogen by photofermentation with continuous cultures of Rhodobacter capsulatus JP91. Bioresource Technology, 128, 513-517.

Adams MR, Moss MO. 2008. Food Microbiology. Ed ke-3. The Royal Society of Chemistry: RSC Publishing.

Adams, M. W. W., The structure and mechanism of iron-hydrogenases, Biochim. Biophys. Acta, 1020, 115, 1990.

Adessi, A., McKinlay, J.B., Harwood, C.S., De Philippis, R. 2012. A Rhodopseudomonas palustris nifA* mutant produces H-2 from NH4+-containing vegetable wastes. International Journal of Hydrogen Energy, 37(21), 15893-15900.

Agyekum, E. B., Nutakor, C., Agwa, A. M., & Kamel, S. (2022). A critical review of renewable hydrogen production methods: factors affecting their scale-up and its role in future energy generation. Membranes, 12(2), 173.

Akaniro, I. R., Oladipo, A. A., & Onwujekwe, E. C. (2024). Metabolic engineering approaches for scale-up of fermentative biohydrogen production–A review. *International Journal of Hydrogen Energy*, *52*, 240-264.

Akhlaghi, N., & Najafpour-Darzi, G. (2020). A comprehensive review on biological hydrogen production. International Journal of Hydrogen Energy, 45(43), 22492-22512.

Al Lawati, A. (2018). Molecular-genetic analysis of the hyf gene-cluster encoding the Hydrogenase-4 system of E. coli; role in hydrogen production (Doctoral dissertation, University of Reading).

Allen, M. S. (2014). Drives and limits to feed intake in ruminants. Animal Production Science, 54(10), 1513-1524.

AlSayed, A., Fergala, A., & Eldyasti, A. (2018). Sustainable biogas mitigation and value-added resources recovery using methanotrophs intergrated into wastewater treatment plants. *Reviews in Environmental Science and Bio/Technology*, *17*, 351-393.

Ananthi, V., Bora, A., Ramesh, U., Yuvakkumar, R., Raja, K., Ponnuchamy, K., ... & Arun, A. (2024). A review on the technologies for sustainable biohydrogen production. Process Safety and Environmental Protection.

Aydin, M. I., Karaca, A. E., Qureshy, A. M., & Dincer, I. (2021). A comparative review on clean hydrogen production from wastewaters. Journal of Environmental Management, 279, 111793.

Azwar, M. Y., Hussain, M. A., & Abdul-Wahab, A. K. (2014). Development of biohydrogen production by photobiological, fermentation and electrochemical processes: a review. Renewable and Sustainable Energy Reviews, 31, 158-173.

Bakonyi, P., Borza, B., Orlovits, K., Simon, V., Nemestóthy, N., & Bélafi-Bakó, K. (2014). Fermentative hydrogen production by conventionally and unconventionally heat pretreated seed cultures: a comparative assessment. International journal of hydrogen energy, 39(11), 5589-5596.

Balachandar G, Varanasi JL, Singh V et al (2020) Biological hydrogen production via dark fermentation: a holistic approach from lab-scale to pilot-scale. Int J Hydrogen Energy 45:5202– 5215.

Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R., & Wolfe, R. (1979). Methanogens: reevaluation of a unique biological group. *Microbiological reviews*, *43*(2), 260-296.

Bao, M. D., Su, H. J., & Tan, T. W. (2013). Dark fermentative bio-hydrogen production: Effects of substrate pre-treatment and addition of metal ions or L-cysteine. Fuel, 112, 38-44.

Bao, M., Su, H., & Tan, T. (2012). Biohydrogen production by dark fermentation of starch using mixed bacterial cultures of Bacillus sp and Brevumdimonas sp. Energy & fuels, 26(9), 5872-5878.

Barber, J. (2016). Photosystem II: the water splitting enzyme of photosynthesis and the origin of oxygen in our atmosphere. Quarterly Reviews of Biophysics, 49, e14.

Basak, N., & Das, D. (2007). The prospect of purple non-sulfur (PNS) photosynthetic bacteria for hydrogen production: the present state of the art. World Journal of Microbiology and Biotechnology, 23, 31-42.

Basak, P. (2020). Study of the Role of Biologically-Relevant, Labile Nickel Pools in the Maturation of Nickel-Dependent Enzymes

Bedford, A., Beckett, L., Harthan, L., Wang, C., Jiang, N., Schramm, H., ... & White, R. R. (2020). Ruminal volatile fatty acid absorption is affected by elevated ambient temperature. Scientific Reports, 10(1), 13092.

Benemann, J. R., and N. M. Weare. 1974. Hydrogen evolution by nitrogen-fixing Anabaena cylindrica cul- tures.Science184:174-175.

Benterbusch, U., & Husar, J. (2013). International Energy Agency. Getting It Right Strategic Agenda for Reforms in Mexico: Strategic Agenda for Reforms in Mexico, 223

Bisaillon, A., Turcot, J., & Hallenbeck, P. C. (2006). The effect of nutrient limitation on hydrogen production by batch cultures of Escherichia coli. International Journal of Hydrogen Energy, 31(11), 1504-1508.

Blackwood, A. C., Neish, A. C., & Ledingham, G. A. (1956). Dissimilation of glucose at controlled pH values by pigmented and non-pigmented strains of Escherichia coli. *Journal of bacteriology*, *72*(4), 497-499.

Blasco-Gómez, R., Batlle-Vilanova, P., Villano, M., Balaguer, M. D., Colprim, J., & Puig, S. (2017). On the edge of research and technological application: a critical review of electromethanogenesis. International journal of molecular sciences, 18(4), 874.

Bohnenkamp, A. C., Wijffels, R. H., Kengen, S. W., & Weusthuis, R. A. (2021). Co-production of hydrogen and ethyl acetate in Escherichia coli. *Biotechnology for Biofuels*, *14*, 1-12.

Boichenko, V. A., Greenbaum, E. L. I. A. S., & Seibert, M. I. C. H. A. E. L. (2004). Hydrogen production by photosynthetic microorganisms. Photoconversion of solar energy, molecular to global photosynthesis, 2, 397-45

Brentner, L. B., Peccia, J., & Zimmerman, J. B. (2010). Challenges in developing biohydrogen as a sustainable energy source: implications for a research agenda. Environmental science & technology, 44(7), 2243-2254.

Buccioni, A., Cappucci, A., & Mele, M. (2015). Methane emission from enteric fermentation: methanogenesis and fermentation. Climate Change Impact on Livestock: Adaptation and Mitigation, 171-186.

Bundhoo, M. Z., Mohee, R., & Hassan, M. A. (2015). Effects of pre-treatment technologies on dark fermentative biohydrogen production: a review. Journal of environmental management, 157, 20-48.

Cai, J and Wang, G. 2014. Hydrogen production from glucose by a mutant strain of Rhodovulum sulfidophilum P5 in single stage photo-fermentation. International Journal of Hydrogen Energy, 39, 20979-20986.

Cammack, K. M., Austin, K. J., Lamberson, W. R., Conant, G. C., & Cunningham, H. C. (2018). Ruminnat nutrition symposium: Tiny but mighty: the role of the rumen microbes in livestock production. Journal of animal science, 96(2), 752-770.

Carrieri, D., Ananyev, G., Costas, A. M. G., Bryant, D. A., & Dismukes, G. C. (2008). Renewable hydrogen production by cyanobacteria: nickel requirements for optimal hydrogenase activity. International Journal of Hydrogen Energy, 33(8), 2014-2022.

Carrieri, D., Ananyev, G., Costas, A. M. G., Bryant, D. A., & Dismukes, G. C. (2008). Renewable hydrogen production by cyanobacteria: nickel requirements for optimal hydrogenase activity. International Journal of Hydrogen Energy, 33(8), 2014-2022.

Caserta, G., Roy, S., Atta, M., Artero, V., & Fontecave, M. (2015). Artificial hydrogenases: biohybrid and supramolecular systems for catalytic hydrogen production or uptake. Current Opinion in Chemical Biology, 25, 36-47.

Caulier, S., Nannan, C., Gillis, A., Licciardi, F., Bragard, C. & Mahillon, J. (2019). Overview of the Antimicrobial Compounds Produced by Members of the Bacillus subtilis Group. Frontiers in Microbiology. 10:302.

Chandrasekhar, K., Kumar, S., Lee, B. D., & Kim, S. H. (2020). Waste based hydrogen production for circular bioeconomy: Current status and future directions. Bioresource technology, 302, 122920

Chandrasekhar, K., Lee, Y. J., & Lee, D. W. (2015). Biohydrogen production: strategies to improve process efficiency through microbial routes. International journal of molecular sciences, 16(4), 8266-8293.

Chandrasekhar, K., Lee, Y. J., & Lee, D. W. (2015). Biohydrogen production: strategies to improve process efficiency through microbial routes. International journal of molecular sciences, 16(4), 8266-8293.

Chang, F. Y., & Lin, C. Y. (2006). Calcium effect on fermentative hydrogen production in an anaerobic up-flow sludge blanket system. Water science and technology, 54(9), 105-112.

Chang, F. Y., & Lin, C. Y. (2006). Calcium effect on fermentative hydrogen production in an anaerobic up-flow sludge blanket system. Water science and technology, 54(9), 105-112.

Chang, S., Li, J. Z., & Liu, F. (2011). Evaluation of different pretreatment methods for preparing hydrogen-producing seed inocula from waste activated sludge. Renewable Energy, 36(5), 1517-1522.

Chen, W. M., Tseng, Z. J., Lee, K. S., & Chang, J. S. (2005). Fermentative hydrogen production with Clostridium butyricum CGS5 isolated from anaerobic sewage sludge. International journal of hydrogen energy, 30(10), 1063-1070.

Chen, W. M., Tseng, Z. J., Lee, K. S., & Chang, J. S. (2005). Fermentative hydrogen production with Clostridium butyricum CGS5 isolated from anaerobic sewage sludge. International journal of hydrogen energy, 30(10), 1063-1070.

Chen, Y., Li, C., Zhou, Z., Wen, J., You, X., Mao, Y., ... & Jia, X. (2014). Enhanced biodegradation of alkane hydrocarbons and crude oil by mixed strains and bacterial community analysis. Applied biochemistry and biotechnology, 172, 3433-3447.

Chih, Y. K., Chen, W. H., & Tran, K. Q. (2020). Hydrogen production from methanol partial oxidation through the catalyst prepared using torrefaction liquid products. Fuel, 279, 118419.

Chookaew T, O-Thong S, Prasertsan P. Biohydrogen production from crude glycerol by two stage of dark and photofermentation. Int J Hydrogen Energy 2015;40:7433e8.

Chookaew, T., O-Thong, S., Prasertsan, P. 2015. Biohydrogen production from crude glycerol by two stage of dark and photofermentation. International Journal of Hydrogen Energy, 40,7433-7438.

Cole, J. A., & Wimpenny, J. W. T. (1966). The inter-relationships of low redox potential cytochrome c552 and hydrogenase in facultative anaerobes. *Biochimica et Biophysica Acta (BBA)-Enzymology and Biological Oxidation*, *128*(3), 419-425.

Cordero, P.R.F. et al. (2019) Atmospheric carbon monoxide oxidation is a widespread mechanism supporting microbial survival. ISME J. 13, 2868–2881

Cyphert, J. M., Trempus, C. S., & Garantziotis, S. (2015). Size matters: molecular weight specificity of hyaluronan effects in cell biology. *International journal of cell biology*, *2015*(1), 563818.

D. Puyol, D.J. Batstone, T. Hülsen, S. Astals, M. Peces, J.O. Krömer, Resource recovery from wastewater by biological technologies: Opportunities, challenges, and prospects, Front. Microbiol. 7 (2017)

da Silva Veras, T., Mozer, T. S., & da Silva César, A. (2017). Hydrogen: trends, production and characterization of the main process worldwide. International journal of hydrogen energy, 42(4), 2018-2033.

da Silva Veras, T., Mozer, T. S., & da Silva César, A. (2017). Hydrogen: trends, production and characterization of the main process worldwide. International journal of hydrogen energy, 42(4), 2018-2033.

Das, A. A., Esfahani, M. M., Velev, O. D., Pamme, N., & Paunov, V. N. (2015). Artificial leaf device for hydrogen generation from immobilised C. reinhardtii microalgae. Journal of Materials Chemistry A, 3(41), 20698-20707.

Das, D., & Veziroğlu, T. N. (2001). Hydrogen production by biological processes: a survey of literature. International journal of hydrogen energy, 26(1), 13-28.

Das, D., & Veziroglu, T. N. (2008). Advances in biological hydrogen production processes. International journal of hydrogen energy, 33(21), 6046-6057.

Das, D., & Veziroglu, T. N. (2008). Advances in biological hydrogen production processes. *International journal of hydrogen energy*, *33*(21), 6046-6057.

Davila-Vazquez, G., Alatriste-Mondragón, F., de León-Rodríguez, A., & Razo-Flores, E. (2008). Fermentative hydrogen production in batch experiments using lactose, cheese whey and glucose: influence of initial substrate concentration and pH. international journal of hydrogen energy, 33(19), 4989-4997.

Davila-Vazquez, G., Alatriste-Mondragón, F., de León-Rodríguez, A., & Razo-Flores, E. (2008). Fermentative hydrogen production in batch experiments using lactose, cheese whey and glucose: influence of initial substrate concentration and pH. international journal of hydrogen energy, 33(19), 4989-4997.

Dawood, F., Anda, M., & Shafiullah, G. M. (2020). Hydrogen production for energy: An overview. International Journal of Hydrogen Energy, 45(7), 3847-3869.

de Gois Araújo Tavares, T., Peiter, F. S., Chaves, T. C., Gois, G. N. S. B., & de Amorim, E. L. C. (2023). Effect of thermophilic temperatures on hydrogen and ethanol production in anaerobic fluidized bed reactor from cassava wastewater. Brazilian Journal of Chemical Engineering, 40(1), 115-127.

De Sales, C. A. V. B., Maya, D. M. Y., Lora, E. E. S., Jaén, R. L., Reyes, A. M. M., González, A. M., ... & Martínez, J. D. (2017). Experimental study on biomass (eucalyptus spp.) gasification in a two-stage downdraft reactor by using mixtures of air, saturated steam and oxygen as gasifying agents. Energy conversion and management, 145, 314-323.

Dey, S., Das, P. K., & Dey, A. (2013). Mononuclear iron hydrogenase. *Coordination Chemistry Reviews, 257*(1), 42-63.

Dincer, I., & Acar, C. (2015). Review and evaluation of hydrogen production methods for better sustainability. International journal of hydrogen energy, 40(34), 11094-11111.

Dipasquale, L., Adessi, A., d'Ippolito, G., Rossi, F., Fontana, A., De Philippis, R. 2015. Introducing capnophilic lactic fermentation in a combined dark-photo fermentation process: a route to unparalleled H2 yields. Appl Microbiol Biotechnol, 99, 1001-1010. [20] Abo-Hashesh, M., Ghosh, D., Tourigny, A., Taous, A., Hallenbeck, P.C. 2011. Single stage photofermentative hydrogen production from glucose: An attractive alternative to two stage photofermentation or co-culture approaches. International Journal of Hydrogen Energy, 36(21), 13889-13895.

Doğan, E. M. (2016). Understanding carbon metabolism in hydrogen production by PNS bacteria (Master's thesis, Middle East Technical University).

E. Neczaj, A. Grosser, Biogas production by thermal hydrolysis and ther- mophilic anaerobic digestion of waste-activated sludge, Industr. Municipal Sludge: Emerg. Concerns Scope Resour. Recover. (2019) 741–781.

Edwards, J. E., McEwan, N. R., Travis, A. J., & John Wallace, R. (2004). 16S rDNA library-based analysis of ruminal bacterial diversity. *Antonie Van Leeuwenhoek*, *86*, 263-281.

Egorov, A. M., Avilova, T. V., Dikov, M. M., POPOV, V. O., Rodionov, Y. V., & Berezin, I. V. (1979). NAD-Dependent Formate Dehydrogenase from Methylotrophic Bacterium, Strain 1: Purification and Characterization. *European Journal of Biochemistry*, *99*(3), 569-576.

El-Dalatony, M. M., Zheng, Y., Ji, M. K., Li, X., & Salama, E. S. (2020). Metabolic pathways for microalgal biohydrogen production: Current progress and future prospectives. Bioresource Technology, 318, 124253.

Fang, H. H., Li, C., & Zhang, T. (2006). Acidophilic biohydrogen production from rice slurry. *International Journal of Hydrogen Energy*, *31*(6), 683-692.

Fiala, G., & Stetter, K. O. (1986). Pyrococcus furiosus sp. nov. represents a novel genus of marine heterotrophic archaebacteria growing optimally at 100 C. *Archives of Microbiology*, *145*, 56-61.

Garcia, J. L., Patel, B. K., & Ollivier, B. (2000). Taxonomic, phylogenetic, and ecological diversity of methanogenic Archaea. *Anaerobe*, *6*(4), 205-226.

García-Depraect, O., Mirzazada, I., Martínez-Mendoza, L. J., Regueira-Marcos, L., & Munoz, R. (2023). Biotic and abiotic insights into the storage of food waste and its effect on biohydrogen and methane production potential. Journal of Water Process Engineering, 53, 103840.

Gerber, P. J., Benjamin, H., & Makkar, H. P. (2013). Mitigation of Greenhouse Gas Emission in Livestock Production. Food And Agriculture Organization of the united nations.

Gest, H. ,and M. D. Kamen. 1949. Photoproductionnof molecular hydrogen by Rhodospirillum rubrum. Science 109:558-559.

Gest, H., J. G. Ormerod, and K. S. Ormerod. 1962. Photometabolism of Rhodospirillum rubrum: light de pendent dissimilation of organic compounds to carbon dioxide and molecular hydrogen by an anaerobic citric acid cycle. Arch. Biochem. Biophys. 97:21-33.

Ghiasian, M. (2019). Biophotolysis-based hydrogen production by cyanobacteria. Prospects of renewable bioprocessing in future energy systems, 161-184.

GhimireA,FrunzoL,PirozziFetal(2015)Areviewondark fermentative biohydrogen production from organic biomass: pro- cess parameters and use of by-products. Appl Energy 144:73–95.

Ghosh, D., Sobro, I.F., Hallenbeck, P.C. 2012. Optimization of the hydrogen yield from single-stage photofermentation of glucose by Rhodobacter capsulatus JP91 using response surface methodology. Bioresource Technology, 123, 199-206.

Ghosh, D., Sobro, I.F., Hallenbeck, P.C. 2012. Stoichiometric conversion of biodiesel derived crude glycerol to hydrogen: Response surface methodology study of the effects of light intensity and crude glycerol and glutamate concentration. Bioresource Technology, 106, 154-160.

Gogotov, I. N., Netrusov, A. I., & Kondrat'eva, E. N. (1975). Hydrogenase activity of the methylotroph, Pseudomonas methylica. *Mikrobiologiia*, *44*(5), 779-783.

Gokfliz, P., I. Karapinar, Int. *J*. Hydrogen nergy 2017, 42, 2553

Gordon, G.C., McKinlay, J.B. 2014. Calvin cycle mutants of photoheterotrophic purple nonsulfur bacteria fail to grow due to an electron imbalance rather than toxic metabolite accumulation. Journal of Bacteriology, 196(6), 1231-1237.

Graham, J. E., Niks, D., Zane, G. M., Gui, Q., Hom, K., Hille, R., ... & Raman, C. S. (2022). How a formate dehydrogenase responds to oxygen: Unexpected O2 insensitivity of an enzyme harboring tungstopterin, selenocysteine, and [4Fe–4S] clusters. *ACS Catalysis*, *12*(16), 10449-10471.

Gray,C.T.,and H. Gest. 1965. Biological formation of molecular hydrogen. Science 148:186-192.

Gu, S., Kelly, B., & Xiu, D. (2020). Empirical asset pricing via machine learning. The Review of Financial Studies, 33(5), 2223-2273.

Guerrero-Cruz, S., Vaksmaa, A., Horn, M. A., Niemann, H., Pijuan, M., & Ho, A. (2021). Methanotrophs: discoveries, environmental relevance, and a perspective on current and future applications. Frontiers in microbiology, 12, 678057.

Guo, W. Q., Ding, J., Cao, G. L., Chen, C., Zhou, X. J., & Ren, N. Q. (2013). Accelerated startup of hydrogen production expanded granular sludge bed with l-Cysteine supplementation. Energy, 60, 94-98.

Haddock, B. A., & Jones, C. W. (1977). Bacterial respiration. *Bacteriological reviews*, *41*(1), 47-99.

Hafez, H., Nakhla, G., El Naggar, H., Ibrahim, G., & Elnashaie, S. S. (2014). Biological Hydrogen Production: Light-Driven Processes. *Handbook of hydrogen energy*, 321.

Hallenbeck, P. C. (2012). Hydrogen production by cyanobacteria. Microbial technologies in advanced biofuels production, 15-28.

Hallenbeck, P. C., & Liu, Y. (2016). Recent advances in hydrogen production by photosynthetic bacteria. International Journal of Hydrogen Energy, 41(7), 4446-4454.

Hallenbeck, P.C. 2011. Microbial paths to renewable hydrogen production. Biofuels 2, 285-302.

Hallenbeck, P.C., Abo-Hashesh, M., Ghosh, D. 2012. Strategies for improving biological hydrogen production. Bioresource Technology, 110, 1-9.

Hammes, P. S., & Jager, J. A. (1990). Net photosynthetic rate of potato at high temperature. *Potato Research, 33*(4), 515–520.

Hammes-Schiffer S Proton-Coupled Electron Transfer: Moving Together and Charging Forward. *J. Am. Chem. Soc* 2015*,* 137*,* 8860–8871.

Han, H., Jia, Q., Liu, B., Yang, H., Shen, J. 2013. Fermentative hydrogen production from acetate using Rhodobacter sphaeroides RV. International Journal of Hydrogen Energy, 38(25), 10773-10778.

Han, H., Jia, Q., Liu, B., Yang, H., Shen, J. 2013. Optimization of photosynthetic hydrogen production from acetate by Rhodobacter sphaeroides RV. International Journal of Hydrogen Energy, 38(29), 12886-12890.

Han, W., Liu, D. N., Shi, Y. W., Tang, J. H., Li, Y. F., & Ren, N. Q. (2015). Biohydrogen production from food waste hydrolysate using continuous mixed immobilized sludge reactors. Bioresource technology, 180, 54-58.

Hards K, Cook GM. 2018. Targeting bacterial energetics to produce new antimicrobials. Drug Resist Updat **36**:1–12. doi: 10.1016/j.drup.2017.11.001.

Hasnaoui, S., Pauss, A., Abdi, N., Grib, H., & Mameri, N. (2020). Enhancement of bio-hydrogen generation by spirulina via an electrochemical photo-bioreactor (EPBR). International Journal of Hydrogen Energy, 45(11), 6231-6242.

Hassan, Q., Abdulateef, A. M., Hafedh, S. A., Al-samari, A., Abdulateef, J., Sameen, A. Z., ... & Jaszczur, M. (2023). Renewable energy-to-green hydrogen: A review of main resources routes, processes and evaluation. *International Journal of Hydrogen Energy*, *48*(46), 17383-17408.

Hatfield, J. L., & Prueger, J. H. (2015). Temperature extremes: Effect on plant growth and development. *Weather and Climate Extremes, 10*, 4–10.

Hawkes, F. R., Dinsdale, R., Hawkes, D. L., & Hussy, I. (2002). Sustainable fermentative hydrogen production: challenges for process optimisation. *International journal of hydrogen energy*, *27*(11-12), 1339-1347.

Hawkes, F. R., Hussy, I., Kyazze, G., Dinsdale, R., & Hawkes, D. L. (2007). Continuous dark fermentative hydrogen production by mesophilic microflora: principles and progress. *International journal of hydrogen energy*, *32*(2), 172-184.

Hiflmer, P. and H.Gest 1977. H2 metabolism in the photosynthetic bacterium Rhodopseudomonas capsu- lata: H2 production by growing cultures. J. Bacteriol. 129:724-731.

Hoggard, M., Vesty, A., Wong, G., Montgomery, J. M., Fourie, C., Douglas, R. G., & Taylor, M. W. (2018). Characterizing the human mycobiota: a comparison of small subunit rRNA, ITS1, ITS2, and large subunit rRNA genomic targets. *Frontiers in microbiology*, *9*, 2208.

Holmes, D. E., & Smith, J. A. (2016). Biologically produced methane as a renewable energy source. Advances in applied microbiology, 97, 1-61.

Hu, B., Li, Y., Zhu, S., Zhang, H., Jing, Y., Jiang, D., ... & Zhang, Z. (2020). Evaluation of biohydrogen yield potential and electron balance in the photo-fermentation process with different initial pH from starch agricultural leftover. Bioresource Technology, 305, 122900.

Ignatov, I. (2016). Distribution of Molecules of ZEOLITH detox and ZEOLITH Creme in Water as Factor for Health. Russian Journal of Biological Research, 4-18.

Ikeuchi, M., & Tabata, S. (2001). Synechocystis sp. PCC 6803—a useful tool in the study of the genetics of cyanobacteria. Photosynthesis research, 70, 73-83.

Ikeuchi, M., & Tabata, S. (2001). Synechocystis sp. PCC 6803—a useful tool in the study of the genetics of cyanobacteria. Photosynthesis research, 70, 73-83.

Intergovernmental Panel on Climate Change. (2014). *Climate change 2014: Synthesis report. Contribution of working groups I, II, and III to the fifth assessment report of the Intergovernmental Panel on Climate Change* (R. K. Pachauri & L. A. Meyer, Eds.). Geneva: IPCC.

Ishikawa, M., Yamamura, S., Takamura, Y., Sode, K., Tamiya, E., & Tomiyama, M. (2006). Development of a compact high-density microbial hydrogen reactor for portable bio-fuel cell system. International Journal of Hydrogen Energy, 31(11), 1484-1489.

Islam, M. S., Guo, C., & Liu, C. Z. (2018). Enhanced hydrogen and volatile fatty acid production from sweet sorghum stalks by two-steps dark fermentation with dilute acid treatment in between. International Journal of Hydrogen Energy, 43(2), 659-666.

Jablonski, L. M., Wang, X., & Curtis, P. S. (2002). Plant reproduction under elevated CO2 conditions: a meta-analysis of reports on 79 crop and wild species. New Phytologist, 156(1), 9-26

Jain, R., Panwar, N. L., Jain, S. K., Gupta, T., Agarwal, C., & Meena, S. S. (2024). Bio-hydrogen production through dark fermentation: an overview. Biomass Conversion and Biorefinery, 14(12), 12699-12724.

Jiménez-Llanos, J., Ramírez-Carmona, M., Rendón-Castrillón, L., & Ocampo-López, C. (2020). Sustainable biohydrogen production by Chlorella sp. microalgae: A review. International Journal of Hydrogen Energy, 45(15), 8310-8328.

Johnson, K. A., & Johnson, D. E. (1995). Methane emissions from cattle. *Journal of animal science, 73*(8), 2483-2492.

Jung, K. W., Kim, D. H., & Shin, H. S. (2011). A simple method to reduce the start-up period in a H2-producing UASB reactor. International journal of hydrogen energy, 36(2), 1466-1473.

Kalia, V. C., Jain, S. R., Kumar, A., and Joshi, A. P., Fermentation of biowaste to H2 by Bacillus licheniformis, World J. Microbiol. Biotechnol., 10, 224, 1994.

Kamshybayeva, G. K., Kossalbayev, B. D., Sadvakasova, A. K., Bauenova, M. O., Zayadan, B. K., Bozieva, A. M., ... & Allakhverdiev, S. I. (2023). Screening and optimisation of hydrogen production by newly isolated nitrogen-fixing cyanobacterial strains. International Journal of Hydrogen Energy, 48(44), 16649-16662.

Kanai, T., Imanaka, H., Nakajima, A., Uwamori, K., Omori, Y., Fukui, T., ... & Imanaka, T. (2005). Continuous hydrogen production by the hyperthermophilic archaeon, Thermococcus kodakaraensis KOD1. Journal of biotechnology, 116(3), 271-282.

Kanai, T., Simons, J. R., Tsukamoto, R., Nakajima, A., Omori, Y., Matsuoka, R., ... & Atomi, H. (2015). Overproduction of the membrane-bound [NiFe]-

hydrogenase in Thermococcus kodakarensis and its effect on hydrogen production. Frontiers in microbiology, 6, 847.

Kapdan, I. K., & Kargi, F. (2006). Bio-hydrogen production from waste materials. *Enzyme and microbial technology*, *38*(5), 569-582.

Kawamura, S. U. G. I. O., O'Neil, J. G., & Wilkinson, J. F. (1983). Hydrogen production by methylotrophs under anaerobic conditions. *J. Ferment. Technol.;(Japan)*, *61*(2)

Keskin, T., Abo-Hashesh, M., Hallenbeck, P.C. 2011. Photofermentative hydrogen production from wastes. Bioresource Technology, 102, 8557-8568.

Keskin, T., Hallenbeck, P.C. 2012. Hydrogen production from sugar industry wastes using single-stage photofermentation. Bioresource Technology, 112, 131-136.

Khanal, S. K., Chen, W. H., Li, L., & Sung, S. (2004). Biological hydrogen production: effects of pH and intermediate products. *International journal of hydrogen energy*, *29*(11), 1123-1131.

Khanal, S. K., Chen, W. H., Li, L., & Sung, S. (2004). Biological hydrogen production: effects of pH and intermediate products. International journal of hydrogen energy, 29(11), 1123-1131.

Khetkorn, W., Lindblad, P., & Incharoensakdi, A. (2012). Inactivation of uptake hydrogenase leads to enhanced and sustained hydrogen production with high nitrogenase activity under high light exposure in the cyanobacterium Anabaena siamensis TISTR 8012. Journal of Biological Engineering, 6, 1-11.

Khetkorn, W., Lindblad, P., & Incharoensakdi, A. (2012). Inactivation of uptake hydrogenase leads to enhanced and sustained hydrogen production with high nitrogenase activity under high light exposure in the cyanobacterium Anabaena siamensis TISTR 8012. Journal of Biological Engineering, 6, 1-11.

Kim, D. H., & Kim, M. S. (2011). Hydrogenases for biological hydrogen production. Bioresource technology, 102(18), 8423-8431.

Kim, D.-H., Lee J.-H., Kang, S., Hallenbeck, P.C., Kim, E.-J., Lee, J.K. and Kim, M.-S. Enhanced photo-fermentative H2 production using Rhodobacter sphaeroides by ethanol addition and analysis of soluble microbial products. Biotechnology for Biofuels 2014, 7:79 doi:10.1186/1754-6834-7-79

Kim, M., Morrison, M., & Yu, Z. (2011). Status of the phylogenetic diversity census of ruminal microbiomes. *FEMS microbiology ecology*, *76*(1), 49-63.

Kim, Y. U., & Lee, B. W. (2019). Differential mechanisms of potato yield loss induced by high day and night temperatures during tuber initiation and bulking: Photosynthesis and tuber growth. *Frontiers in Plant Science, 10*, 300. https://doi.org/10.3389/fpls.2019.00300

Kimball, B. A. (1983). Carbon dioxide and agricultural yield: An assemblage and analysis of 770 prior observations. WCL Rep., 14, 71

Kleerebezem, R., & van Loosdrecht, M. C. (2007). Mixed culture biotechnology for bioenergy production. Current opinion in biotechnology, 18(3), 207-212.

Klibanov, A. M., Alberti, B. N., and Zale, S. E., Enzymatic synthesis of formic acid from hydrogen and carbon dioxide and production of hydrogen from formic acid, Biotechnol. Bio- eng., 24, 25, 1982.

Kobayashi, J., Yoshimune, K., Komoriya, T., Kohno, H. 2011. Efficient hydrogen production from acetate through isolated Rhodobacter sphaeroides. Journal of Bioscience and Bioengineering, 112(6), 602-605.

Kondusamy, D., & Kalamdhad, A. S. (2014). Pre-treatment and anaerobic digestion of food waste for high rate methane production–A review. Journal of Environmental Chemical Engineering, 2(3), 1821-1830.

Kossalbayev, B. D., Tomo, T., Zayadan, B. K., Sadvakasova, A. K., Bolatkhan, K., Alwasel, S., & Allakhverdiev, S. I. (2020). Determination of the potential of cyanobacterial strains for hydrogen production. *International Journal of Hydrogen Energy*, *45*(4), 2627-2639.

Koyama MH, Araújo Júnior MM, Zaiat M, Ferraz Júnior ADN (2016) Kinetics of thermophilic acidogenesis of typical Brazil- ian sugarcane vinasse. Energy 116:1097–1103.

Kraemer, J. T., & Bagley, D. M. (2007). Improving the yield from fermentative hydrogen production. *Biotechnology letters*, *29*, 685-695.

Krishnan, S., Kamyab, H., Nasrullah, M., Wahid, Z. A., Yadav, K. K., Reungsang, A., & Chaiprapat, S. (2023). Recent advances in process improvement of dark fermentative hydrogen production through metabolic engineering strategies. *Fuel*, *343*, 127980

Kufryk, G. (2013). Advances in utilizing cyanobacteria for hydrogen production. Advances in Microbiology, 2013.

Kufryk, G. (2013). Advances in utilizing cyanobacteria for hydrogen production. Advances in Microbiology, 2013.

Kumar, G., Sivagurunathan, P., Pugazhendhi, A., Thi, N. B. D., Zhen, G., Chandrasekhar, K., & Kadier, A. (2017). A comprehensive overview on light independent fermentative hydrogen production from wastewater feedstock and possible integrative options. Energy Conversion and Management, 141, 390-402

Kumar, N., & Das, D. (2000). Enhancement of hydrogen production by Enterobacter cloacae IIT-BT 08. Process Biochemistry, 35(6), 589-593.

Kumar,A.,Jain,S.R.,Sharma,C.B.,Joshi, A. P., and Kalia, V. C., Increased H2 production by immobilized microorganisms, World J. Microbiol. Biotechnol., 11, 156,1995.

Kumer, G., A. Mudhoo, P. Sivagurunathan, D. Nagarajan, A. Ghimire, C.-H, Lay, C.-Y.Lin, D.-J. Lee, J.-S. Chang, Bioresour. Technol. 2016, 219, 725.

Kunacheva, C., Stuckey, D.C. 2014. Analytical methods for soluble microbial products (SMP) and extracellular polymers (ECP) in wastewater treatment systems: A review. Water Research. 61:1-18

Kushkevych, I., Procházka, V., Vítězová, M., Dordević, D., Abd El-Salam, M., & Rittmann, S. K. M. (2024). Anoxygenic photosynthesis with emphasis on green sulfur bacteria and a perspective for hydrogen sulfide detoxification of anoxic environments. Frontiers in Microbiology, 15, 1417714.

L. Wei, B. Fan, J. Yi, T. Xie, K. Liu, W. Ma, Biotechnol. Biofuels 2020, 13, 64

Laurinavichene, T.V., Belokopytov, B. F., Laurinavichius, K.S., Tekucheva, D.N., Seibert, M., Tsygankou, A.A. 2010. Towards the integration of dark- and photofermentative waste treatment. 3. Potato as substrate for sequential dark fermentation and light driven H2 production. International Journal of Hydrogen Energy, 35, 8536- 8543.

Laurinavichene, T.V., Belokopytov, B.F., Laurinavichius, K.S., Khusnutdinova, A.N., Seibert, M., Tsygankov, A.A. 2012. Towards the integration of dark- and photofermentative waste treatment. 4. Repeated batch sequential dark- and photofermentation using starch as substrate. International Journal of Hydrogen Energy, 37, 8800-8810.

Lay, J. J., Fan, K. S., Hwang, J. I., Chang, J. I., & Hsu, P. C. (2005). Factors affecting hydrogen production from food wastes by Clostridium-rich composts. Journal of Environmental Engineering, 131(4), 595-602.

Lee, K. S., Hsu, Y. F., Lo, Y. C., Lin, P. J., Lin, C. Y., & Chang, J. S. (2008). Exploring optimal environmental factors for fermentative hydrogen production from starch using mixed anaerobic microflora. International Journal of Hydrogen Energy, 33(5), 1565-1572.

Lee, K. S., Lin, P. J., & Chang, J. S. (2006). Temperature effects on biohydrogen production in a granular sludge bed induced by activated carbon carriers. *International Journal of Hydrogen Energy*, *31*(4), 465-472.

Lee, K. S., Lin, P. J., Fangchiang, K., & Chang, J. S. (2007). Continuous hydrogen production by anaerobic mixed microflora using a hollow-fiber microfiltration membrane bioreactor. International journal of hydrogen energy, 32(8), 950-957.

Lee, K. S., Lo, Y. S., Lo, Y. C., Lin, P. J., & Chang, J. S. (2004). Operation strategies for biohydrogen production with a high-rate anaerobic granular sludge bed bioreactor. Enzyme and Microbial Technology, 35(6-7), 605-612.

Lee, Y. H., Sang, W. G., Baek, J. K., Kim, J. H., Shin, P., Seo, M. C., & Cho, J. I. (2020). The effect of concurrent elevation in CO2 and temperature on the growth, photosynthesis, and yield of potato crops. PloS one, 15(10), e0241081

Lee, Y. J., Miyahara, T., & Noike, T. (2001). Effect of iron concentration on hydrogen fermentation. Bioresource Technology, 80(3), 227-231.

Lee, Y. J., Miyahara, T., & Noike, T. (2002). Effect of pH on microbial hydrogen fermentation. Journal of Chemical Technology & Biotechnology, 77(6), 694-698.

Lee, Y. J., Miyahara, T., & Noike, T. (2002). Effect of pH on microbial hydrogen fermentation. Journal of Chemical Technology & Biotechnology, 77(6), 694-698.

Li, C., & Fang, H. H. (2007). Fermentative hydrogen production from wastewater and solid wastes by mixed cultures. Critical reviews in environmental science and technology, 37(1), 1-39.

Li, C., & Fang, H. H. (2007). Inhibition of heavy metals on fermentative hydrogen production by granular sludge. Chemosphere, 67(4), 668-673.

Li, Q., & Liu, C. Z. (2012). Co-culture of Clostridium thermocellum and Clostridium thermosaccharolyticum for enhancing hydrogen production via thermophilic fermentation of cornstalk waste. International Journal of Hydrogen Energy, 37(14), 10648-10654.

Li, S., Tabatabaei, M., Li, F., & Ho, S. H. (2024). A review of green biohydrogen production using anoxygenic photosynthetic bacteria for hydrogen economy: challenges and opportunities. *International Journal of Hydrogen Energy*, *54*, 218-238.

Li, X., Wang, Y. H., Zhang, S. L., Chu, J., Zhang, M., Huang, M. Z., & Zhuang, Y. P. (2009). Enhancement of phototrophic hydrogen production by Rhodobacter sphaeroides ZX-5 using a novel strategy—shaking and extra-light supplementation approach. International journal of hydrogen energy, 34(24), 9677-9685.

Lin, C. Y., & Cheng, C. H. (2006). Fermentative hydrogen production from xylose using anaerobic mixed microflora. International Journal of Hydrogen Energy, 31(7), 832-840.

Lin, C. Y., & Cheng, C. H. (2006). Fermentative hydrogen production from xylose using anaerobic mixed microflora. International Journal of Hydrogen Energy, 31(7), 832-840.

Lin, C. Y., & Hung, W. C. (2008). Enhancement of fermentative hydrogen/ethanol production from cellulose using mixed anaerobic cultures. International Journal of Hydrogen Energy, 33(14), 3660-3667.

Lin, C. Y., & Hung, W. C. (2008). Enhancement of fermentative hydrogen/ethanol production from cellulose using mixed anaerobic cultures. International Journal of Hydrogen Energy, 33(14), 3660-3667.

Lin, C. Y., & Shei, S. H. (2008). Heavy metal effects on fermentative hydrogen production using natural mixed microflora. International Journal of Hydrogen Energy, 33(2), 587-593.

Lin, C. Y., Chang, C. C., & Hung, C. H. (2008). Fermentative hydrogen production from starch using natural mixed cultures. International journal of hydrogen energy, 33(10), 2445-2453.

Lin, C. Y., Hung, C. H., Chen, C. H., Chung, W. T., & Cheng, L. H. (2006). Effects of initial cultivation pH on fermentative hydrogen production from xylose using natural mixed cultures. Process biochemistry, 41(6), 1383-1390.

Lin, C. Y., Hung, C. H., Chen, C. H., Chung, W. T., & Cheng, L. H. (2006). Effects of initial cultivation pH on fermentative hydrogen production from xylose using natural mixed cultures. Process biochemistry, 41(6), 1383-1390.

Lin, C. Y., Wu, C. C., & Hung, C. H. (2008). Temperature effects on fermentative hydrogen production from xylose using mixed anaerobic cultures. *International Journal of Hydrogen Energy*, *33*(1), 43-50.

Lin, Chiu-Yue, and Shi-Heu Shei. "Heavy metal effects on fermentative hydrogen production using natural mixed microflora." *International Journal of Hydrogen Energy* 33.2 (2008): 587-593.

Liu, G., & Shen, J. (2004). Effects of culture and medium conditions on hydrogen production from starch using anaerobic bacteria. Journal of bioscience and bioengineering, 98(4), 251-256.

Liu, G., & Shen, J. (2004). Effects of culture and medium conditions on hydrogen production from starch using anaerobic bacteria. Journal of bioscience and bioengineering, 98(4), 251-256.

Liu, G., & Shen, J. (2004). Effects of culture and medium conditions on hydrogen production from starch using anaerobic bacteria. Journal of bioscience and bioengineering, 98(4), 251-256.

Liu, H., Grot, S., & Logan, B. E. (2005). Electrochemically assisted microbial production of hydrogen from acetate. Environmental science & technology, 39(11), 4317-4320

Liu, S., Waqas, M. A., Wang, S. H., Xiong, X. Y., & Wan, Y. F. (2017). Effects of increased levels of atmospheric CO2 and high temperatures on rice growth and quality. PloS one, 12(11), e0187724

Liu, W., Sun, L., Li, Z., Fujii, M., Geng, Y., Dong, L., & Fujita, T. (2020). Trends and future challenges in hydrogen production and storage research. Environmental science and pollution research, 27, 31092-31104

Liu, Y., Ding, M., Ling, W., Yang, Y., Zhou, X., Li, B. Z., ... & Yuan, Y. J. (2017). A three-species microbial consortium for power generation. Energy & Environmental Science, 10(7), 1600-1609.

Liu, Y., Dipankar, G., Hallenbeck, P.C. 2015. Biological reforming of ethanol to H2 by Rhodopseudomonas palustris CGA009. Bioresource Technology, 176, 189-195.

Lo, Y., Chen, C., Lee, C., Chang, J. 2010 Sequential dark-photo fermentation and autotrophic microbial growth for high yield and CO2 free biohydrogen production. International Journal of Hydrogen Energy, 35, 10944-10953.

Lo, Y.-C., Chen, C.-Y., Lee, C.-M., Chang, J.-S. 2011. Photo fermentative hydrogen production using dominant components (acetate, lactate, and butyrate) in dark fermentation effluents. International Journal of Hydrogen Energy, 36(21), 14059-14068.

Lu, L., & Ren, Z. J. (2016). Microbial electrolysis cells for waste biorefinery: a state of the art review. Bioresource technology, 215, 254-264.

Łukajtis, R., Hołowacz, I., Kucharska, K., Glinka, M., Rybarczyk, P., Przyjazny, A., & Kamiński, M. (2018). Hydrogen production from biomass using dark fermentation. Renewable and Sustainable Energy Reviews, 91, 665-694.

Ma, Z. Li, C., Su, H. (2017). Renewable Energy, 105, 458.

Maeda, T., Sanchez-Torres, V., & Wood, T. K. (2012). Hydrogen production by recombinant Escherichia coli strains. Microbial Biotechnology, 5(2), 214-225.

Mai, X., & Adams, M. W. (1994). Indolepyruvate ferredoxin oxidoreductase from the hyperthermophilic archaeon Pyrococcus furiosus. A new enzyme involved in peptide fermentation. *Journal of Biological Chemistry*, *269*(24), 16726-16732.

Makowski, D., Marajo-Petitzon, E., Durand, J. L., & Ben-Ari, T. (2020). Quantitative synthesis of temperature, CO2, rainfall, and adaptation effects on global crop yields. European Journal of Agronomy, 115, 126041.

Mancusi, E., Bareschino, P., Brachi, P., Coppola, A., Ruoppolo, G., Urciuolo, M., & Pepe, F. (2021). Feasibility of an integrated biomass-based CLC combustion and a renewable-energy-based methanol production systems. Renewable Energy, 179, 29-36.

Manish, S., & Banerjee, R. (2008). Comparison of biohydrogen production processes. International Journal of Hydrogen Energy, 33(1), 279-286.

Maru, B. T., Constanti, M., Stchigel, A. M., Medina, F., & Sueiras, J. E. (2013). Biohydrogen production by dark fermentation of glycerol using Enterobacter and Citrobacter Sp. Biotechnology Progress, 29(1), 31-38.

Masepohl, B., Hallenbeck, P.C. 2010. Nitrogen and Molybdenum Control of Nitrogen Fixation in the Phototrophic Bacterium Rhodobacter capsulatus. Recent Advances in Phototrophic Prokaryotes, 675, 49-70.

Maxin, G., Rulquin, H., & Glasser, F. (2011). Response of milk fat concentration and yield to nutrient supply in dairy cows. Animal, 5(8), 1299-1310.

McCann, J. C., Wickersham, T. A., & Loor, J. J. (2014). High-throughput methods redefine the rumen microbiome and its relationship with nutrition and metabolism. Bioinformatics and biology insights, 8, BBI-S15389.

McKinlay, J. B., Oda, Y., Rühl, M., Posto, A. L., Sauer, U., and Harwood, C. S. (2014). Non-growing Rhodopseudomonas palustris Increases the Hydrogen Gas Yield from Acetate by Shifting from the Glyoxylate Shunt to the Tricarboxylic Acid Cycle. J. Biol. Chem. 289 (4), 1960–1970.

McSweeney, C., & Mackie, R. (2012). Micro-organisms and ruminant digestion: state of knowledge, trends and future prospects.

McTernan, P. M. (2015). *Genetic engineering and purification of the hydrogenases from the hyperthermophilic archaeon Pyrococcus furiosus* (Doctoral dissertation, University of Georgia).

Metcalfe, G. D. (2022). *On-line analysis of bacterial metabolism by modern spectroscopic laser techniques* (Doctoral dissertation, University of Sheffield).

Mohan, S. V., Chiranjeevi, P., Chandrasekhar, K., Babu, P. S., & Sarkar, O. (2019). Acidogenic biohydrogen production from wastewater. In Biohydrogen (pp. 279-320). Elsevier.

Mohanakrishna, G., & Pengadeth, D. (2024). Mixed culture biotechnology and its versatility in dark fermentative hydrogen production. *Bioresource technology*, 130286.

Morimoto, M., Atsuko, M., Atif, A. A. Y., Ngan, M. A., Fakhru'l-Razi, A., Iyuke, S. E., & Bakir, A. M. (2004). Biological production of hydrogen from glucose by natural anaerobic microflora. International Journal of Hydrogen Energy, 29(7), 709-713.

Mosin, O., & Ignatov, I. (2014). Methylotrophic Microorganisms. Metabolism and Physiology. *Journal of Health, Medicine and Nursing, 7,* 75-100.

Mu, Y., Yu, H. Q., & Wang, Y. (2006). The role of pH in the fermentative H2 production from an acidogenic granule-based reactor. Chemosphere, 64(3), 350-358.

Mu, Y., Zheng, X. J., Yu, H. Q., & Zhu, R. F. (2006). Biological hydrogen production by anaerobic sludge at various temperatures. *International Journal of Hydrogen Energy*, *31*(6), 780-785.

Myer, P. R., Wells, J. E., Smith, T. P., Kuehn, L. A., & Freetly, H. C. (2015). Microbial community profiles of the colon from steers differing in feed efficiency. Springerplus, 4, 1-13.

N. Fakhimi, A. Dubini, O. Tavakoli, D. González-Ballester, Bioresour. Technol. 2019, 289, 121648.

Nagarajan, D., Lee, D. J., Kondo, A., & Chang, J. S. (2017). Recent insights into biohydrogen production by microalgae–From biophotolysis to dark fermentation. Bioresource technology, 227, 373-387.

Nandi, R., & Sengupta, S. (1996). Involvement of anaerobic reductases in the spontaneous lysis of formate by immobilized cells of Escherichia coli. *Enzyme and microbial technology*, *19*(1), 20-25.

Nandi, R., Bhattacharyya, P. K., Bhaduri, A. N., & Sengupta, S. (1992). Synthesis and lysis of formate by immobilized cells of Escherichia coli. *Biotechnology and bioengineering, 39*(7), 775-780.

Narancic, T., Scollica, E., Cagney, G. & O'Connor, K. E. Three novel proteins co-localise with polyhydroxybutyrate (PHB) granules in Rhodospirillum rubrum S1. Microbiology (Read., Engl.) 164, 625–634 (2018).

NASA. (2019). *Global climate change: Vital signs of the planet*. Retrieved January 2020, from http://climate.nasa.gov/vital-signs/carbon-dioxide

Nathao, C., Sirisukpoka, U., & Pisutpaisal, N. (2013). Production of hydrogen and methane by one and two stage fermentation of food waste. International journal of hydrogen energy, 38(35), 15764-15769.

Navarre, R., & Pavek, M. J. (2014). Potato utilization and markets. In *The potato: Botany, production and uses* (pp. 29–34). CABI.

Ni, M., Leung, D. Y., Leung, M. K., & Sumathy, K. J. F. P. T. (2006). An overview of hydrogen production from biomass. Fuel processing technology, 87(5), 461-472.

Nishio, N., & Nakashimada, Y. (2004). High rate production of hydrogen/methane from various substrates and wastes. *Recent Progress of Biochemical and Biomedical Engineering in Japan I: -/-*, 63-87.

Noike, T., Takabatake, H., Mizuno, O., & Ohba, M. (2002). Inhibition of hydrogen fermentation of organic wastes by lactic acid bacteria. International journal of hydrogen energy, 27(11-12), 1367-1371.

Ntaikou, I., Gavala, H. N., Kornaros, M., & Lyberatos, G. (2008). Hydrogen production from sugars and sweet sorghum biomass using Ruminococcus albus. International Journal of Hydrogen Energy, 33(4), 1153-1163.

Oh, Y. K., Kim, H. J., Park, S., Kim, M. S., & Ryu, D. D. (2008). Metabolic-flux analysis of hydrogen production pathway in Citrobacter amalonaticus Y19. International journal of hydrogen energy, 33(5), 1471-1482.

O'Hara, J., Gray, C. T., Puig, J., & Pichinoty, F. (1967). Defects in formate hydrogenlyase in nitrate-negative mutants of Escherichia coli. *Biochemical and Biophysical Research Communications*, *28*(6), 951-957.

oledo-AlarcónJetal(2018)Basicsofbio-hydrogenproduction by dark fermentation. In: Liao Q, Chang Js, Herrmann C, Xia A (eds) Bioreactors for microbial biomass and energy conversion. Green energy and technology. Springer, Singapore.

Oledo-AlarcónJetal(2018)Basicsofbio-hydrogenproduction by dark fermentation. In: Liao Q, Chang Js, Herrmann C, Xia A (eds) Bioreactors for microbial biomass and energy conversion. Green energy and technology. Springer, Singapore.

Ordal, E. J., & Halvorson, H. O. (1939). A comparison of hydrogen production from sugars and formic acid by normal and variant strains of Escherichia coli. *Journal of Bacteriology, 38*(2), 199-220.

Ormerod, J.G.,K.S.Ormerod,andH.Geut 1961. Light dependent utilization of organic compounds and photoproduction of molecular hydrogen by photosynthetic bacteria; relationship with nitrogen metabolism. Arch. Biochem. Biophys. 94:449-463

Owens, F. N., & Basalan, M. (2016). Ruminal fermentation. Rumenology, 63-102.

Ozgur, E., Mars, A.E., Peksel, B., Louwerse, A., Yucel, M., Gunduz, U., Claassen, P.A.M., Eroglu, I. 2010. Biohydrogen production from beet molasses by sequential dark and photofermentation. International Journal of Hydrogen Energy, 35, 511-517.

Pachapur, V. L., Sarma, S. J., Brar, S. K., Bihan, Y. L., Buelna, G., & Verma, M. (2017). Hydrogen production from biodiesel industry waste by using a co-culture of Enterobacter aerogenes and Clostridium butyricum. *Biofuels, 8*(6), 651-662.

Pachapur, V. L., Sarma, S. J., Brar, S. K., Le Bihan, Y., Buelna, G., & Verma, M. (2016). Surfactant mediated enhanced glycerol uptake and hydrogen production from biodiesel waste using co-culture of Enterobacter aerogenes and Clostridium butyricum. Renewable Energy, 95, 542-551.

Pachapur, V. L., Sarma, S. J., Brar, S. K., Le Bihan, Y., Buelna, G., & Soccol, C. R. (2015). Evidence of metabolic shift on hydrogen, ethanol and 1, 3-

propanediol production from crude glycerol by nitrogen sparging under micro-aerobic conditions using co-culture of Enterobacter aerogenes and Clostridium butyricum. international journal of hydrogen energy, 40(28), 8669-8676.

Pachapur, V. L., Sarma, S. J., Brar, S. K., Le Bihan, Y., Buelna, G., & Verma, M. (2015). Biohydrogen production by co-fermentation of crude glycerol and apple pomace hydrolysate using co-culture of Enterobacter aerogenes and Clostridium butyricum. Bioresource technology, 193, 297-306.

Pan, C. M., Fan, Y. T., Xing, Y., Hou, H. W., & Zhang, M. L. (2008). Statistical optimization of process parameters on biohydrogen production from glucose by Clostridium sp. Fanp2. Bioresource technology, 99(8), 3146-3154.

PanditS, Balachandar G, Das D (2014) Improved energy recovery from dark fermented cane molasses using microbial fuel cells. Front Chem Sci Eng 8:43–54.

Papone T, Paungbut M, Leesing R. Producing of microbial oil by mixed culture of microalgae and ole- aginous yeast using sugarcane molasses as carbon substrate. JOCET. 2016;4:253–256.

Paramesh, K., Reddy, N. L., Shankar, M. V., & Chandrasekhar, T. (2018). Enhancement of biological hydrogen production using green alga Chlorococcum minutum. International Journal of Hydrogen Energy, 43(8), 3957-3966.

Park, J. Y., Kim, B. N., Kim, Y. H., & Min, J. (2018). Whole-genome sequence of purple non-sulfur bacteria, Rhodobacter sphaeroides strain MBTLJ-8 with improved CO2 reduction capacity. Journal of biotechnology, 288, 9-14.

Parkinson, B., Balcombe, P., Speirs, J. F., Hawkes, A. D., & Hellgardt, K. (2019). Levelized cost of CO 2 mitigation from hydrogen production routes. Energy & environmental science, 12(1), 19-40

Peck Jr, H. D., & Gest, H. (1957). Formic dehydrogenase and the hydrogenlyase enzyme complex in coli-aerogenes bacteria. *Journal of Bacteriology*, *73*(6), 706-721.

Peiro, C. (2021). *Engineering and compartmentalisation of a synthetic methanol utilisation pathway in E. coli* (Doctoral dissertation, INSA de Toulouse).

Penteado, E. D., Lazaro, C. Z., Sakamoto, I. K., & Zaiat, M. (2013). Influence of seed sludge and pretreatment method on hydrogen production in packed-bed anaerobic reactors. International Journal of Hydrogen Energy, 38(14), 6137-6145.

Pinske, C., Jaroschinsky, M., Linek, S., Kelly, C. L., Sargent, F., & Sawers, R. G. (2015). Physiology and bioenergetics of [NiFe]-hydrogenase 2-catalyzed H2-consuming and H2-producing reactions in Escherichia coli. *Journal of bacteriology*, *197*(2), 296-306.

Podesta, J. J., Gutierrez-Navarro, A. M., Estrella, C. N., & Esteso, M. A. (1997). Electrochemical measurement of trace concentrations of biological hydrogen produced by Enterobacteriaceae. Research in microbiology, 148(1), 87-93.

Probst, I. and Schlegel, H. G., Respiratory components and oxidase activities in Alcaligen- eseutrophus, Biochim. Biophys. Acta, 440, 412, 1977.

Qi, C., Yan, X., Wang, Y., Ning, Y., Yu, X., Hou, Y., ... & Yu, J. (2022). Flammability limits of combustible gases at elevated temperatures and pressures: recent advances and future perspectives. Energy & Fuels, 36(21), 12896-12916.

R. Cano, S.I. Pérez-Elvira, F. Fdz-Polanco, Energy feasibility study of sludge pretreatments: A review, Appl. Energy 149 (2015) 176–185.

Ramkumar N, Anupama PD, Nayak T, Subudhi S (2021) Scale up of biohydrogen production by a pure strain; Clostridium butyricum TM-9A at regulated pH under decreased partial pres- sure. Renew Energy 170:1178–1185.

Redwood, M. D., Paterson-Beedle, M., & Macaskie, L. E. (2009). Integrating dark and light bio-hydrogen production strategies: towards the hydrogen economy. Reviews in Environmental Science and Bio/Technology, 8, 149-185.

Ren, N. Q., Xu, J. F., Gao, L. F., Xin, L., Qiu, J., & Su, D. X. (2010). Fermentative bio-hydrogen production from cellulose by cow dung compost enriched cultures. International journal of hydrogen energy, 35(7), 2742-2746.

Ren, T., Patel, M. K., & Blok, K. (2008). Steam cracking and methane to olefins: Energy use, CO2 emissions and production costs. *Energy*, *33*(5), 817-833.

Rodionova, M. V., Poudyal, R. S., Tiwari, I., Voloshin, R. A., Zharmukhamedov, S. K., Nam, H. G., ... & Allakhverdiev, S. I. (2017). Biofuel production: challenges and opportunities. International Journal of Hydrogen Energy, 42(12), 8450-8461.

Roger, M., Reed, T. C., & Sargent, F. (2021). Harnessing Escherichia coli for bio-based production of formate under pressurized H2 and CO2 gases. *Applied and Environmental Microbiology*, *87*(21), e00299-21.

Rosenbaum, F. P., & Müller, V. (2021). Energy conservation under extreme energy limitation: the role of cytochromes and quinones in acetogenic bacteria. Extremophiles, 25(5), 413-424

Rousset, M., & Liebgott, P. P. (2014). Engineering hydrogenases for H2 production: bolts and goals. In Microbial BioEnergy: hydrogen production (pp. 43-77). Dordrecht: Springer Netherlands.

Ruiz-Herrera, J., & Alvarez, A. (1972). A physiological study of formate dehydrogenase, formate oxidase and hydrogenlyase from Escherichia coli K-12. *Antonie van Leeuwenhoek*, *38*, 479-491.

S. Banerjee, U. Ghosh, Isolation and identification of a strain for the production of biosurfactant, J. Maharaja Sayajirao Univ. Baroda 54 (2020) 165–171.

Saady, N. M. C. (2013). Homoacetogenesis during hydrogen production by mixed cultures dark fermentation: unresolved challenge. International Journal of Hydrogen Energy, 38(30), 13172-13191.

Saifuddin, N., & Priatharsini, P. (2016). Developments in bio-hydrogen production from algae: a review. Res J Appl Sci Eng Technol, 12(9), 968-982.

Salerno, M. B., Park, W., Zuo, Y., & Logan, B. E. (2006). Inhibition of biohydrogen production by ammonia. *Water research*, *40*(6), 1167-1172.

Sapountzaki, E., Rova, U., Christakopoulos, P., & Antonopoulou, I. (2023). Renewable hydrogen production and storage via enzymatic interconversion of CO2 and formate with electrochemical cofactor regeneration. *ChemSusChem*, *16*(17), e202202312.

Schneider,K.and Schlegel,H.G.,Localiza- tion and stability of hydrogenases from aero- bic hydrogen bacteria, Arch. Microbiol., 112, 229, 1977.

Schubert, C., & Unden, G. (2023). Fumarate, a central electron acceptor for Enterobacteriaceae beyond fumarate respiration and energy conservation. In *Advances in Microbial Physiology* (Vol. 82, pp. 267-299). Academic Pre Ss.

Seefeldt LC; Yang Z-Y; Lukoyanov DA; Harris DF; Dean DR; Raugei S; Hoffman BM Reduction of Substrates by Nitrogenases. Chem. Rev 2020, 120, 5082–5106.

Sekar, N., & Ramasamy, R. P. (2015). Recent advances in photosynthetic energy conversion. Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 22, 19-33.

Shah, Y. T. (2017). Chemical energy from natural and synthetic gas. CRC Press.

Sharma, P., & Melkania, U. (2017). Biosurfactant-enhanced hydrogen production from organic fraction of municipal solid waste using co-culture of E. coli and Enterobacter aerogenes. Bioresource Technology, 243, 566-572.

Simon, K. M. R., Lee, H. S., Lim, J. K., Kim, T. W., Lee, J. H., & Kang, S. G. (2015). One-carbon substrate-based biohydrogen production: microbes, mechanism, and productivity. *Biotechnology advances*, *33*(1), 165-177.

Singh, L., & Wahid, Z. A. (2015). Methods for enhancing bio-hydrogen production from biological process: a review. Journal of Industrial and Engineering Chemistry, 21, 70-80.

Singh, L., Z. A. Wahid,*J*. Ind. Eng. Chem. 2015, 21, 70.

Singh, R., Altaee, A., & Gautam, S. (2020). Nanomaterials in the advancement of hydrogen energy storage. Heliyon, 6(7)

Singh,L., M. F. Siddiqui, A. Ahmad, M. H. A. Rahim, M. Sakinah, Z. A. Wahid, *J*. Ind. Eng. Chem. 2013, 19, 659.

Sivagurunathan, P., Kumar, G., Bakonyi, P., Kim, S. H., Kobayashi, T., Xu, K. Q., ... & Bélafi-Bakó, K. (2016). A critical review on issues and overcoming strategies for the enhancement of dark fermentative hydrogen production in continuous systems. International Journal of Hydrogen Energy, 41(6), 3820-3836.

Sivagurunathan, P., Sen, B., & Lin, C. Y. (2014). Batch fermentative hydrogen production by enriched mixed culture: combination strategy and their microbial composition. Journal of bioscience and bioengineering, 117(2), 222-228.

Sivaramakrishnan, R., Shanmugam, S., Sekar, M., Mathimani, T., Incharoensakdi, A., Kim, S. H., ... & Pugazhendhi, A. (2021). Insights on biological hydrogen production routes and potential microorganisms for high hydrogen yield. Fuel, 291, 120136

Sompong, O., Prasertsan, P., Intrasungkha, N., Dhamwichukorn, S., & Birkeland, N. K. (2008). Optimization of simultaneous thermophilic fermentative hydrogen production and COD reduction from palm oil mill effluent by Thermoanaerobacterium-rich sludge. International Journal of Hydrogen Energy, 33(4), 1221-1231.

Sompong, O., Prasertsan, P., Karakashev, D., & Angelidaki, I. (2008). Thermophilic fermentative hydrogen production by the newly isolated Thermoanaerobacterium thermosaccharolyticum PSU-2. International journal of hydrogen energy, 33(4), 1204-1214.

Song, Z. X., Dai, Y., Fan, Q. L., Li, X. H., Fan, Y. T., & Hou, H. W. (2012). Effects of pretreatment method of natural bacteria source on microbial community and bio-hydrogen production by dark fermentation. International journal of hydrogen energy, 37(7), 5631-5636.

Srirangan, K., Pyne, M. E., & Chou, C. P. (2011). Biochemical and genetic engineering strategies to enhance hydrogen production in photosynthetic algae and cyanobacteria. Bioresource technology, 102(18), 8589-8604.

Stephenson, M., & Stickland, L. H. (1932). Hydrogenlyases: Bacterial enzymes liberating molecular hydrogen. *Biochemical Journal*, *26*(3), 712.

Stickland, L. H. (1929). The bacterial decomposition of formic acid. *Biochemical Journal*, *23*(6), 1187.

Strong, P. J., Xie, S., & Clarke, W. P. (2015). Methane as a resource: can the methanotrophs add value?. Environmental science & technology, 49(7), 4001-4018

Suman, S. (2018). Hybrid nuclear-renewable energy systems: A review. Journal of Cleaner Production, 181, 166-177

Tang, R., Niu, S., Zhang, G., Chen, G., Haroon, M., Yang, Q., & et al. (2018). Physiology and growth responses of potato cultivars to heat stress. *Botany, 96*(10), 897–912.

Tanisho, S., Kamiya, N., & Wakao, N. (1989). Hydrogen evolution of Enterobacter aerogenes depending on culture pH: mechanism of hydrogen evolution from NADH by means of membrane-bound hydrogenase. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *973*(1), 1-6.

TANISHO, S., WAKAO, N., & KOSAKO, Y. (1983). Biological hydrogen production by Enterobacter aerogenes. *Journal of chemical engineering of Japan*, *16*(6), 529-530.

Tarhan, C., & Çil, M. A. (2021). A study on hydrogen, the clean energy of the future: Hydrogen storage methods. Journal of Energy Storage, 40, 102676.

Tashie-Lewis, B. C., & Nnabuife, S. G. (2021). Hydrogen production, distribution, storage and power conversion in a hydrogen economy-a technology review. Chemical Engineering Journal Advances, 8, 100172.

Tishkov, V. I., Pometun, A. A., & Savin, S. S. (2023). Formate dehydrogenase: from NAD (P) H regeneration to targeting pathogen biofilms, composing highly efficient hybrid biocatalysts and atmospheric CO2 fixation. *Moscow University Chemistry Bulletin*, *78*(4), 151-169.

Turcot, J., Bisaillon, A., & Hallenbeck, P. C. (2008). Hydrogen production by continuous cultures of Escherchia coli under different nutrient regimes. International Journal of Hydrogen Energy, 33(5), 1465-1470.

Ueno, Y., Haruta, S., Ishii, M., & Igarashi, Y. (2001). Characterization of a microorganism isolated from the effluent of hydrogen fermentation by microflora. Journal of bioscience and bioengineering, 92(4), 397-400.

Ungerfeld, E. M. (2020). Metabolic hydrogen flows in rumen fermentation: principles and possibilities of interventions. Frontiers in Microbiology, 11, 589.

V. Grechanik, A. Romanova, I. Naydov, A. Tsygankov, Photosynth. Res. 2020, 143, 275.

Valdez-Vazquez I, Rı´os-Leal E, Esparza-Garcı´a F, Cecchi F, Poggi-Varaldo HM. Semi-continuous solid substrate anaerobic reactors for H_2 production from organic waste: mesophilic versus thermophilic regime. Int J Hydrogen Energy 2005;30:1383–91.

Van Niel, E. W. J., Budde, M. A. W., De Haas, G. G., Van der Wal, F. J., Claassen, P. A. M., & Stams, A. J. M. (2002). Distinctive properties of high hydrogen producing extreme thermophiles, *Caldicellulosiruptor saccharolyticus* and *Thermotoga elfii*. International Journal of Hydrogen Energy, 27(11-12), 1391-1398.

Venkata Mohan S, Nikhil GN, Chiranjeevi P et al (2016) Waste biorefinery models towards sustainable circular bioeconomy: critical review and future perspectives. Bioresour Technol 215:2– 12.

Vision, V. N. S. E. S. (2023). Towards 100% renewable energy by 2050.

Waks, Z., & Silver, P. A. (2009). Engineering a synthetic dual-organism system for hydrogen production. Applied and Environmental Microbiology, 75(7), 1867-1875.

Wang, C. H., Lin, P. J., & Chang, J. S. (2006). Fermentative conversion of sucrose and pineapple waste into hydrogen gas in phosphate-buffered culture seeded with municipal sewage sludge. Process Biochemistry, 41(6), 1353-1358.

Wang, C. H., Lin, P. J., & Chang, J. S. (2006). Fermentative conversion of sucrose and pineapple waste into hydrogen gas in phosphate-buffered culture seeded with municipal sewage sludge. Process Biochemistry, 41(6), 1353-1358.

Wang, D., Duan, Y., Yang, Q., Liu, Y., Ni, B. J., Wang, Q., ... & Yuan, Z. (2018). Free ammonia enhances dark fermentative hydrogen production from waste activated sludge. Water research, 133, 272-281.

Wang, D., Zeng, G., Chen, Y., & Li, X. (2015). Effect of polyhydroxyalkanoates on dark fermentative hydrogen production from waste activated sludge. Water research, 73, 311-322.

Wang, G., Mu, Y., & Yu, H. Q. (2005). Response surface analysis to evaluate the influence of pH, temperature and substrate concentration on the acidogenesis of sucrose-rich wastewater. *Biochemical engineering journal*, *23*(2), 175-184.

Wang, J., & Wan, W. (2008). Effect of Fe2+ concentration on fermentative hydrogen production by mixed cultures. International Journal of Hydrogen Energy, 33(4), 1215-1220.

Wang, J., & Wan, W. (2008). Effect of Fe2+ concentration on fermentative hydrogen production by mixed cultures. International Journal of Hydrogen Energy, 33(4), 1215-1220.

Wang, J., & Wan, W. (2008). Effect of temperature on fermentative hydrogen production by mixed cultures. International Journal of hydrogen energy, 33(20), 5392-5397.

Wang, J., & Wan, W. (2008). Influence of Ni^{2+} concentration on biohydrogen production. Bioresource technology, 99(18), 8864-8868.

Wang, J., & Wan, W. (2009). Factors influencing fermentative hydrogen production: a review. International journal of hydrogen energy, 34(2), 799-811.

Wang, J., & Yin, Y. (2017). Principle and application of different pretreatment methods for enriching hydrogen-producing bacteria from mixed cultures. *International Journal of Hydrogen Energy*, *42*(8), 4804-4823.

Wang, J., & Yin, Y. (2021). Clostridium species for fermentative hydrogen production: an overview. *International Journal of Hydrogen Energy*, 46(70),

Wang, J., Yin, Y., Wang, J., & Yin, Y. (2017). Microbiology and enzymology. *Biohydrogen Production from Organic Wastes*, 19-67.

Wang, S., Ma, Z., Zhang, T., Bao, M., & Su, H. (2017). Optimization and modeling of biohydrogen production by mixed bacterial cultures from raw cassava starch. Frontiers of Chemical Science and Engineering, 11, 100-106.

Wang, S., Ma, Z., Zhang, T., Bao, M., & Su, H. (2017). Optimization and modeling of biohydrogen production by mixed bacterial cultures from raw cassava starch. Front Chem Sci Eng 11: 100–106.

Wang, S., Tang, H., Peng, F., Yu, X., Su, H., Xu, P., & Tan, T. (2019). Metabolite-based mutualism enhances hydrogen production in a two-species microbial consortium. Communications biology, 2(1), 82.

Wang, S., Zhang, T., & Su, H. (2016). Enhanced hydrogen production from corn starch wastewater as nitrogen source by mixed cultures. Renewable energy, 96, 1135-1141

Wang, S., Zhang, T., & Su, H. (2016). Enhanced hydrogen production from corn starch wastewater as nitrogen source by mixed cultures. Renewable energy, 96, 1135-1141.

Wang, S., Zhang, T., Bao, M., Su, H., & Xu, P. (2020). Microbial production of hydrogen by mixed culture technologies: a review. Biotechnology journal, 15(1), 1900297.

Wang, S., Zhang, T., Bao, M., Su, H., & Xu, P. (2020). Microbial production of hydrogen by mixed culture technologies: a review. Biotechnology journal, 15(1), 1900297

Wang, X. J., Ren, N. Q., Xiang, W. S., & Guo, W. Q. (2007). Influence of gaseous end-products inhibition and nutrient limitations on the growth and hydrogen production by hydrogen-producing fermentative bacterial B49. International journal of hydrogen energy, 32(6), 748-754.

Wang, Y. Y., Ai, P., Hu, C. X., & Zhang, Y. L. (2011). Effects of various pretreatment methods of anaerobic mixed microflora on biohydrogen production and the fermentation pathway of glucose. International Journal of Hydrogen Energy, 36(1), 390-396.

Wang, Y., Wang, H., Feng, X., Wang, X., & Huang, J. (2010). Biohydrogen production from cornstalk wastes by anaerobic fermentation with activated sludge. International journal of hydrogen energy, 35(7), 3092-3099.

Weibel, D. B. (2008). Building communities one bacterium at a time. Proceedings of the National Academy of Sciences, 105(47), 18075-18076

Weimer, P. J. (2015). Redundancy, resilience, and host specificity of the ruminal microbiota: implications for engineering improved ruminal fermentations. Frontiers in microbiology, 6, 296.

Whittenbury, R., Phillips, K. C., & Wilkinson, J. F. (1970). Enrichment, isolation and some properties of methane-utilizing bacteria. *Microbiology*, *61*(2), 205-218.

Willaert, R. G. (2018). Cell immobilization and its applications in biotechnology: current trends and future prospects. In *Fermentation Microbiology and Biotechnology, Foutrh Edition* (pp. 323-358). CRC press.

Wong, Y. M., Wu, T. Y., & Juan, J. C. (2014). A review of sustainable hydrogen production using seed sludge via dark fermentation. Renewable and Sustainable Energy Reviews, 34, 471-482.

Worku, A. K., Ayele, D. W., Deepak, D. B., Gebreyohannes, A. Y., Agegnehu, S. D., & Kolhe, M. L. (2024). Recent advances and challenges of hydrogen production technologies via renewable energy sources. Advanced Energy and Sustainability Research, 5(5), 2300273.

Wu, J. H., & Lin, C. Y. (2004). Biohydrogen production by mesophilic fermentation of food wastewater. Water science and technology, 49(5-6), 223-228.

Wu, J. H., & Lin, C. Y. (2004). Biohydrogen production by mesophilic fermentation of food wastewater. Water science and technology, 49(5-6), 223-228.

Xing, D., Ren, N., Wang, A., Li, Q., Feng, Y., & Ma, F. (2008). Continuous hydrogen production of auto-aggregative Ethanoligenens harbinense YUAN-3 under non-sterile condition. *International Journal of Hydrogen Energy*, *33*(5), 1489-1495.

Xu, H., Li, D., Huang, D., Zhou, W., Yang, L., Li, Z., & Zhen, F. (2018). 8 Microbial Hydrogen Production. *Microbial Energy Conversion*, 385.

Y. Zhai, Z. Shao, M. Cai, L. Zheng, G. Li, Z. Yu, J. Zhang, Cyclo(l-Pro–l-Leu) of Pseudomonas putida MCCC 1A00316 isolated from antarctic soil: identification and characterization of activity against meloidogyne incognita, Molecules 24 (2019) 768

Yang, G., & Wang, J. (2017). Co-fermentation of sewage sludge with ryegrass for enhancing hydrogen production: performance evaluation and kinetic analysis. Bioresource Technology, 243, 1027-1036.

Yang, G., Wang, J., & Shen, Y. (2019). Antibiotic fermentation residue for biohydrogen production using different pretreated cultures: Performance evaluation and microbial community analysis. Bioresource technology, 292, 122012.

Yang, G., Yin, Y., & Wang, J. (2019). Microbial community diversity during fermentative hydrogen production inoculating various pretreated cultures. International Journal of Hydrogen Energy, 44(26), 13147-13156

Yang, H., & Shen, J. (2006). Effect of ferrous iron concentration on anaerobic bio-hydrogen production from soluble starch. International journal of hydrogen energy, 31(15), 2137-2146.

Yang, H., Zhang, J., Wang, X., Jiangtao, F., Yan, W., & Liejin, G. (2014). A newly isolated Rhodobacter sphaeroides HY01 with high hydrogen production performance. International journal of hydrogen energy, 39(19), 10051-10060.

Yang, J., Sudik, A., Wolverton, C., & Siegel, D. J. (2010). High capacity hydrogen storage materials: attributes for automotive applications and techniques for materials discovery. Chemical Society Reviews, 39(2), 656-675.

Yasin, N. H. M., Mumtaz, T., & Hassan, M. A. (2013). Food waste and food processing waste for biohydrogen production: a review. Journal of environmental management, 130, 375-385.

Yin, Y., & Wang, J. (2016). Changes in microbial community during biohydrogen production using gamma irradiated sludge as inoculum. *Bioresource Technology*, *200*, 217-222.

Yokoi, H., Ohkawara, T., Hirose, J., Hayashi, S., & Takasaki, Y. (1995). Characteristics of hydrogen production by aciduric Enterobacter aerogenes strain HO-39. Journal of Fermentation and Bioengineering, 80(6), 571-574.

Yokoyama, H., Waki, M., Moriya, N., Yasuda, T., Tanaka, Y., & Haga, K. (2007). Effect of fermentation temperature on hydrogen production from cow waste slurry by using anaerobic microflora within the slurry. *Applied microbiology and biotechnology*, *74*, 474-483.

Yokoyama, H., Waki, M., Ogino, A., Ohmori, H., & Tanaka, Y. (2007). Hydrogen fermentation properties of undiluted cow dung. Journal of bioscience and bioengineering, 104(1), 82-85.

Yubi, Y., Jun, L., Haiyang, N., & Xiuyun, Z. (2019). Collaborative influence of elevated CO2 concentration and high temperature on potato biomass accumulation and characteristics. Open Chemistry, 17(1), 728-737

Zanon, T., Komainda, M., Ammer, S., Isselstein, J., & Gauly, M. (2022). Diverse feed, diverse benefits-the multiple roles of feed diversity at pasture on ruminant livestock production-a review. J. Veterinary Sci. Anim. Husbandry, 10(1), 1-20.

Zeidan, A. A., Rådström, P., & van Niel, E. W. (2010). Stable coexistence of two Caldicellulosiruptor species in a de novo constructed hydrogen-producing co-culture. Microbial cell factories, 9, 1-13.

Zhang T, Liu H, Fang HHP. Biohydrogen production from starch in wastewater under thermophilic condition. J Environ Manage 2003;69:149–56.

Zhang, B., Zhang, S. X., Yao, R., Wu, Y. H., & Qiu, J. S. (2021). Progress and prospects of hydrogen production: Opportunities and challenges. Journal of Electronic Science and Technology, 19(2), 100080.

Zhang, M. L., Fan, Y. T., Xing, Y., Pan, C. M., Zhang, G. S., & Lay, J. J. (2007). Enhanced biohydrogen production from cornstalk wastes with acidification pretreatment by mixed anaerobic cultures. Biomass and Bioenergy, 31(4), 250-254.

Zhang, M. L., Fan, Y. T., Xing, Y., Pan, C. M., Zhang, G. S., & Lay, J. J. (2007). Enhanced biohydrogen production from cornstalk wastes with acidification pretreatment by mixed anaerobic cultures. Biomass and Bioenergy, 31(4), 250-254.

Zhang, T., Liu, H., & Fang, H. H. (2003). Biohydrogen production from starch in wastewater under thermophilic condition. Journal of environmental management, 69(2), 149-156.

Zhang, Y., Liu, G., & Shen, J. (2005). Hydrogen production in batch culture of mixed bacteria with sucrose under different iron concentrations. International Journal of Hydrogen Energy, 30(8), 855-860.

Zhao, N., Liang, D., Meng, S., & Li, X. (2020). Bibliometric and content analysis on emerging technologies of hydrogen production using microbial electrolysis cells. International Journal of Hydrogen Energy, 45(58), 33310-33324.

Zheng, X. J., & Yu, H. Q. (2004). Biological hydrogen production by enriched anaerobic cultures in the presence of copper and zinc. Journal of Environmental Science and Health, Part A, 39(1), 89-101.

Zuroff, T. R., Xiques, S. B., & Curtis, W. R. (2013). Consortia-mediated bioprocessing of cellulose to ethanol with a symbiotic Clostridium phytofermentans/yeast co-culture. Biotechnology for biofuels, 6, 1-12.