Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-024-35668-7

REVIEW ARTICLE

Recent advances in dark fermentative hydrogen production from vegetable waste: role of inoculum, consolidated bioprocessing, and machine learning

Devu Pengadeth[1] · **Nitai Basak**[1] · **Luca Bernabò**[2] · **Alessandra Adessi**[2]

Received: 13 December 2023 / Accepted: 24 November 2024

Abstract

Waste-centred-bioenergy generation have been garnering interest over the years due to environmental impact presented by fossil fuels. Waste generation is an unavoidable consequence of urbanization and population growth. Sustainable waste management techniques that are long term and environmentally benign are required to achieve sustainable development. Energy recovery from waste biomass via dark fermentative hydrogen production is a sustainable approach to waste management. Vegetable waste is generated in plenty over the food supply chain and being a rich source of carbon and other nutrients it has been studied for production of biohydrogen. This review aims to offer a comprehensive overview on the potential of vegetable waste as a feedstock for dark fermentative biohydrogen production. The hydrogen output from dark fermentative process is lower and additional strategies are required to improve the production. This review addresses the challenges generally encountered during dark fermentative hydrogen production using vegetable waste and the importance of methods such as bioaugmentation and application of extremophiles for process enhancement. The role of machine learning in the field of biohydrogen production is briefly discussed. The application of dark fermentative effluents for secondary valuable product generation and its contribution to the biohydrogen biorefinery is discussed as well.

Keywords Bioaugmentation · Biohydrogen · Extremophiles · Inhibitors · Pretreatment · Single-pot bioconversion

Abbreviations

ANFIS	Adaptive Neuro-Fuzzy Interference System
ANN	Adenosine triphosphate
ATP	Artificial neural network
CBP	Chemical oxygen demand
COD	Consolidated bioprocessing
Fd	Ferredoxin
FVW	Fruit and vegetable waste
GA	Genetic algorithm
GDOC	Groundnut deoiled cake
GHGs	Greenhouse gases
LAB	Lactic acid bacteria
MEC	Microbial electrolysis cell
MFC	Microbial fuel cell
MKLMA	Microbial kinetic with Levenberg–Marquardt
ML	Machine learning
MLPANN	Multilayer perception artificial neural network
NADP	Nicotinamide adenine dinucleotide phosphate
PHA	Polyhydroxyalkanoates
RF	Random forests
RSM	Response surface methodology
SVM	Support vector machines
VFA	Volatile fatty acids
VS	Volatile solids

Introduction

Waste generation specifically food waste is an inevitable consequence of urbanization and anthropogenic activities. More than US$1 trillion worth of food is wasted every year leading to economic and habitat loss due to food loss and conversion of natural ecosystems to agricultural land respectively. The waste biomass being generated has a lasting effect on the

---

Responsible Editor: Ta Yeong Wu

✉ Nitai Basak
basakn@nitj.ac.in

[1] Department of Biotechnology, Dr. B R Ambedkar National Institute of Technology Jalandhar, Jalandhar 144 008, India

[2] Department of Agriculture, Food, Environment and Forestry, University of Florence, Florence, Italy

environment with food waste contributing about 8 to 10% of greenhouse gas (GHG) emissions. The food waste generated contains up to 62% fruit and vegetable waste (by mass), and these waste materials are generally subject to either open dumping and landfilling or are used as animal feed (United Nations Environment Programme 2024). Although these practices make use of the waste to a certain level, the environmental impact associated with their management still remains. Landfilling generates large amounts of methane, a potent GHG with 28 times greater global warming potential than carbon dioxide over a 100-year period (IEA 2024). The anaerobic decay of the organic wastes in the landfill leads to methane generation and its release to environment. It has been estimated that up to 11% of anthropogenic CH_4 emissions occur from landfills (Singh et al. 2018; Kaza et al. 2018). Along with the landfill gas generation, the effluent generated from the decay can contaminate groundwater and could be a breeding point for potential disease-causing agents.

With the advent of waste-centred-circular economy approach, waste biomass is viewed as a nutrient-rich resource rather than a harmful byproduct. Waste generated in different sectors of the economy is utilized as a feedstock for the production of valuable products. This approach to waste remediation enables the establishment of sustainable waste management practices that are longer-term and eco-friendly. Vegetable waste has been studied for its potential as a feedstock for generation of diverse commercially important products such as biochar; bioactive compounds (pectin); bioenergy (biohydrogen, biomethane, and bioethanol); and biofertilizers. Biohydrogen, the carbon neutral and energy dense (143 GJ/tonne) bioenergy source, is produced from vegetable waste by dark and photo fermentative process with an anaerobic microorganism (Jayachandran et al. 2022). The gross calorific energy value of mixed vegetable waste (comprising of beetroot, broad beans, banana plantain, cabbage green beans onion, okra, potato, radish, and tomato) is 18.92 MJ/Kg (Prem Ananth Surendran and Shanmugam 2021), making it an efficient feedstock for biohydrogen production. In theory, the dark fermentative route can generate 20.83 mol H_2 from 1 kg COD (chemical oxygen demand) of vegetable waste with an energy output of 5906.97 kJ (1.6 kWh) via the acetic acid fermentation pathway (Dahiya et al. 2018). However, in reality, the yield is much lesser due to cell metabolic requirements and the distribution of energy for the generation of unwanted byproducts. Nevertheless, the biological route of hydrogen production has displayed promising results and is considered as a sustainable alternative to fossil fuels thereby enabling environmentally benign waste management and renewable energy generation.

Dark fermentative hydrogen production from vegetable waste have been widely studied over the years (Mohan et al. 2009; Mohanakrishna et al. 2010a). The research in this area have demonstrated the potential of vegetable waste as an efficient feedstock for hydrogen producers. However, the biohydrogen yield from vegetable waste is lower, and researchers have opted for numerous methods to improve it. The pretreatment of vegetable waste to improve the yield of simpler sugars is a common practice to enhance the hydrogen production. Methods such as chemical pretreatment namely acid and alkaline treatment, hydrothermal treatment, and enzymatic hydrolysis have been studied (Kumar and Mohan 2018). Although these processes help to improve the total carbohydrate concentration available for hydrogen producers, the majority of the pretreatment techniques generate byproducts that inhibit the growth of microorganisms (Bundhoo and Mohee 2016). An additional detoxification process would be required to remove the inhibitors that reduce the quantity of hydrogen produced rendering the overall process costly. The cost of operation, transportation of goods, and many more factors determine the successful commercial application of the fermentation process. The biomass-based microbial production of hydrogen cost is estimated to be INR 813/Kg**,** €2.5 to 5.5/Kg by the European Union, and the US Department of Energy (DOE) has put forth a target lesser than $2/Kg for hydrogen production with $8/kWh for storage (DST 2020; EU 2020; DOE 2018). The feedstock cost is a critical factor that impacts the overall production cost; the application of waste as a potential substrate could help reduce the overall cost. Therefore, the selection of cost-effective routes and strategies that would improve the hydrogen production is needed. Recently, bioaugmentation and application of mixed microbial cultures and extremophiles with diverse metabolic capabilities have demonstrated good results. Additionally, the integration of machine learning technology with biohydrogen production can help to better understand the critical process parameters.

This review explores the advantages and limitations associated with the use of vegetable waste as a feedstock for dark fermentative hydrogen production. The limitations associated with the pretreatment methods are briefly discussed and the application of strategies such as extremophiles, bioaugmentation, single-pot conversion, and mixed culture biotechnology for enhancement of hydrogen yield is extensively explored. The role of machine learning and its application to dark fermentation are discussed in the context of vegetable waste. Further, vegetable waste and its part in the waste-centred-biorefinery route for generation of valuable products are explored.

Dark fermentation: a brief introduction

Dark fermentative hydrogen production is a promising route to hydrogen generation due to its high production rate, low-cost nature, and ability to handle versatile substrates (Srivastava et al. 2021; Mohanakrishna et al. 2023). The theoretical yield of hydrogen through dark fermentation is dependent on the type of anaerobic microorganisms that are used (Mathews and Wang 2009). Depending upon the metabolic pathway and end product generated by the microorganisms, the hydrogen yield will change; with the acetate pathway generating 4 mol of H_2 and the butyrate pathway producing evolve 2 mol of H_2 per mol of glucose respectively (Eqs. (1) and (2)) (Ahmad et al. 2024). Obligate anaerobes such as *Clostridium acetobutylicum* utilize dark fermentation via glycolysis to breakdown glucose into pyruvate and NADH. Pyruvate formed is then oxidized into acetyl-CoA and CO_2 by pyruvate ferredoxin oxidoreductase. This step requires the reduction of a ferredoxin (Fd). Acetyl-CoA is converted to acetyl phosphate, which results in the formation of ATP and acetate. Hydrogen gas is generated by the activity of hydrogenase, which oxidizes the reduced ferredoxin to regenerate the oxidized ferredoxin (Tenca et al. 2011; Sevinç et al. 2012). Further, hydrogen is produced at very low partial pressures (<60 Pa) from the NADH formed during the earlier glycolysis. NADH is oxidized by the Fd reduction and an NADH-ferredoxin reductase (Mathews and Wang 2009). Dark fermentation in facultative anaerobes like *Enterobacter aerogenes* follows the oxidative conversion of pyruvate into formate and acetyl-CoA by the activity of pyruvate formate lyase. Hydrogen gas is formed by the conversion of formate into CO_2 and H_2 by hydrogenase enzymes. Acetyl-CoA is converted into acetyl phosphate resulting in the formation of ATP and acetate (Sevinç et al. 2012; Jayachandran et al. 2022).

Acetate pathway

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \;\; \Delta G^{\circ} = -126.0\,KJ/mol \tag{1}$$

Butyrate pathway

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \;\; \Delta G^{\circ} = -214.3\,KJ/mol \tag{2}$$

Vegetable waste for production of value-added-products

Vegetable waste is generated from different sectors of the food supply chain in the form of peels, stems, rotten residues, and damaged produce (Fig. 1). The waste generated contains high COD and carbohydrate content and can be used for the production of a wide variety of products of commercial value. Prem Ananth Surendran and Shanmugam (2021) reported that a mixed vegetable waste comprising of beetroot, broad beans, banana plantain, cabbage green beans onion, okra, potato, radish, and tomato waste contains 6.37% total solids. The organic solids are further classified as 82.81% volatile solids (VS) and 17.19% ash. The total soluble COD and moisture content of the waste was 1.58 g COD/g VS and 93.63% making it an efficient substrate for biohydrogen production. Vegetable waste generated can have simpler (easily biodegradable) and complex composition (lignocellulosic) based on the source of waste. Since waste generated can vary from time to time, characterization or composition analysis of the feedstock (physical, chemical, or biological) is important for the digestion process. Understanding the physio-chemical characteristics such as total fixed solids, ash content, volatile solids, pH, moisture content, and measure of cellulose, hemicellulose, nitrogen, starch, lignin, organic carbon, COD level before the fermentation proceeds is important for the overall digestion

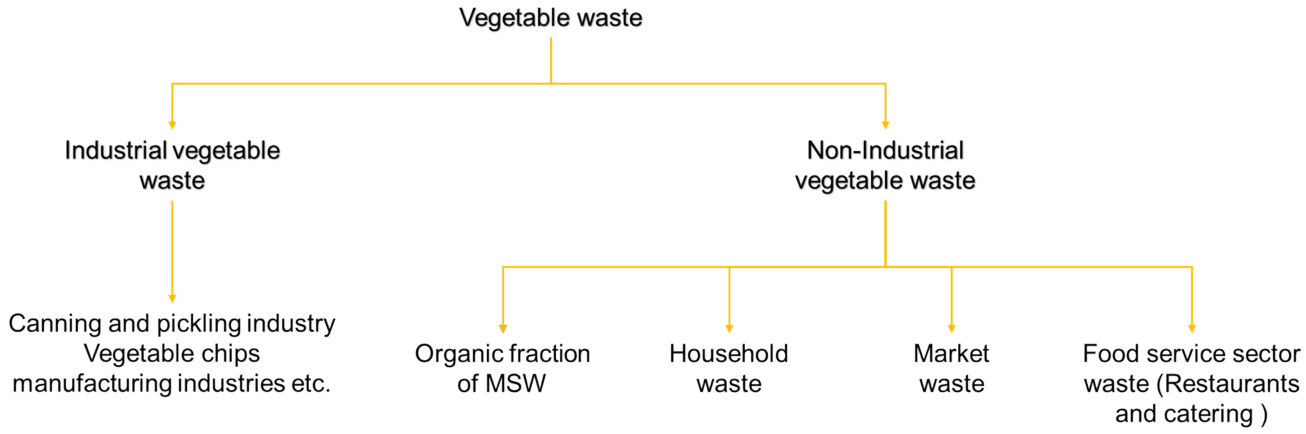

Fig. 1 Schematic representation of different sources of vegetable waste

process to be successful (Singh et al. 2012). These factors will greatly influence the metabolic pathways of the hydrogen producers and essentially the end-product of the fermentation. Variations in the initial pH, sugar content, and presence of nutrients can have a greater influence on the fermentation route, and in order for hydrogen production to be successful, a good grasp on these physio-chemical characteristics of the waste is essential. Hence, while working with vegetable waste, it is important to characterize the substrate before proceeding with the fermentation.

Limitations of feedstock pretreatment

The dark fermentative hydrogen production from vegetable waste has been extensively studied over the years (Panin et al. 2021; Martínez-Mendoza et al. 2022). The studies pertaining to vegetable waste have explored co-digestion with other feedstock, pretreatment of substrate, and nutrient supplementation to enhance the biohydrogen production. Feedstock pretreatment is commonly used to enhance the yield of simple sugars that in turn could be utilized by the hydrogen producers. The pretreatment methods are categorised into physical, mechanical, chemical, physio-chemical, and biological techniques. Briefly, the physical pre-treatment methods such as thermal and microwave pre-treatment treat the sample using different types of radiation such as gamma rays, microwaves, and electron beams (Rajesh Banu et al. 2020). These treatments enhance the liquefaction of the substrate by heat generation causing cell disruption and also breakage of the glycosidic linkages under standard pressure and temperature conditions. Mechanical pre-treatment mainly ultrasonication creates vacuum bubbles inside the substrate solutions due to pressure; these bubbles collapse resulting in the phenomenon called cavitation that sends vibrations across the solution causing cell disruption due to heat generation (Jiradechakorn et al. 2023). Chemical pre-treatment generally involves the hydrolysis of the substrate using concentrated or dilute acids (H_2SO_4 and HCl mainly) and alkalis (NaOH) (Rodríguez-Valderrama et al. 2020). Physio-chemical pre-treatments can be thermo-chemical, hot compressed water treatment, hydrothermal, and autoclave pre-treatment. Biological pre-treatments involve microbial and enzymatic treatment of the substrate to release the sugars to the solution for hydrogen producers to consume (Lo et al. 2009; Hitit et al. 2017). Microbial pretreatment can be carried out by either fungal or cellulolytic bacteria, while enzymes such as cellulases, hemicellulases, laccases, and xylanases are used for enzymatic treatment (Ni et al. 2006).

Among all these pre-treatment techniques, acid and alkaline hydrolysis are mostly used. In the majority of studies that dealt with food waste comprising of fruits, vegetables, and other organic wastes, acid and alkaline hydrolysis of the substrate have proven to be very efficient (Ramprakash and Muthukumar 2018). Acidic pre-treatments are carried out using hydrochloric acid (HCl), sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2), and acetic acid (CH_3COOH) (Hafid et al. 2015). Acid hydrolysis of lignocellulosic vegetable waste (stems, stalk, and peels) is performed to efficiently remove the hemicellulose by breaking ether bonds in lignin/phenolic-carbohydrate complexes, the lignocellulosic components will breakdown into low energy compounds causing an increase in solubilisation and hydrolysis rate (Hafid et al. 2015). Whereas alkaline treatment using sodium hydroxide (NaOH), calcium hydroxide ($Ca\ (OH)_2$), or ammonia is mostly used for lignin hydrolysis. The hydrolysis takes place by the cleavage of ester bonds in the lignin/phenolic-carbohydrate complexes followed by saponification of esters of the uronic bonds existing between hemicelluloses and lignin (Monlau et al. 2013). This causes the bulging of biomass, an increase in the internal surface area, and a reduction in the degree of polymerization. As a result, the lignin structure will be disrupted and can be separated from the raw material as a fraction rich in phenolic compounds. This increased pore size can facilitate the diffusion of hydrolytic enzymes (if combined with biological pre-treatment). Although acidic and alkaline pre-treatment improve hydrogen production by many folds, they become obsolete due to the neutralization of substrate required after the treatment and the by-products they generate. These pre-treatments of substrates also result in the formation of by-products like furan derivatives such as furfural and 5-hydroxymethylfurfural (5-HMF), phenolic compounds such as vanillin and syringaldehyde, and weak acids such as acetate (Bundhoo and Mohee 2016; Muñoz-Páez et al. 2019). These byproducts of pretreatments have often been observed to be toxic to the microbial growth, decrease the hydrogen productivity, and reduce the quantity of hydrogen produced rendering the process costly.

Strategies to improve hydrogen production from vegetable waste

Mixed microbial cultures

While considering dark fermentation from an economic point of view, optimum operational conditions are needed for the deployment of the biohydrogen process. Although pure strains have demonstrated substantial hydrogen production, for commercial application, pure cultures are not ideal. Vegetable waste generation vary as a result of geographical distribution, seasonal variations, and consumer demand. The pH of vegetable waste generally ranges between 4.0 and 4.8 (acidic range) (Table 1) and can be complex in nature. The lignocellulosic vegetable waste would require an initial hydrolysis treatment prior to fermentation. The accumulation of volatile fatty acids (VFA) upon fermentation can inhibit the the bacteria activity as well. Hence, the characteristics of the inoculum are a

Table 1 Dark fermentative hydrogen production from vegetable waste using mixed microbial inoculum

Feedstock	Composition of vegetable waste	Substrate characteristics	Inoculum	Comments	Hydrogen production	Reference
Composite vegetable waste	Tomato, potato, carrot, cabbage, brinjal, beetroot, okra, and coccinia residues	COD (with pulp): 57.6 g COD/L COD (without pulp): 52.0 g COD/L	Anaerobic mixed culture	Acetic acid (51.69 to 77.28%) and butyric acid were found in higher amounts	23.96 mmol/day	Mohan et al. 2009
FVW	Eggplant, carrot, tomato, cucumber, onion, radish, potato, capsicum, cabbage, pumpkin	COD: 111.5 ± 5.1 g/L Total carbohydrate: 78.9 ± 2.9 g/L pH: 4.6	Anaerobic digestate	Major organic acid was lactic acid, acetic acid, butyric acid, propionic acid and formic acid	49.5 NmL H_2/g VS_{fed}	Martínez-Mendoza et al. 2022
FVW	Radish, pepper, pumpkin, tomato, onion, potato, eggplant, carrot, cucumber, cabbage	COD: 9.5 g/L Total sugar: 0.818 g/L pH: 4.75 ± 0.1	Anaerobic mixed consortium	Acetic acid remained the dominant organic acid	10.23 mL $H_2/g_{vs\ added}$	
Fruit-vegetable and swine manure	Potato, Zucchini	TS: 133.0 ± 8.0 g/kg pH: 4.60 ± 0.10	Thermophilic inoculum	Acetic acid concentration (8.3 to 12.9 g acetic acid/kg	126 ± 22 mL H_2 g/ VS_{added}	Tenca et al. 2011
FVW	Radish, pepper, pumpkin, tomato, onion, potato, eggplant, carrot, cucumber, cabbage	—	Pre-treated inoculum	Acetic acid (13,023 mg/L) and isobutyric acid (7605 mg/L) were the major metabolites generated	23.53 NmL H_2 /g VS	
Vegetable waste	Leaf-shaped vegetable refuses and potato peels	TS%: 5.3 ± 0.1 VS%: 4.15 ± 0.07	Indigenous microflora	Mixed acid profile with acetate, lactate, butyrate etc	24 ± 2 L H_2/kg VS	Marone et al. 2014
Vegetable waste	Rotten potato, tomatoes, cabbage, carrot, brinjal, coccinia, okra, and beetroot	Soluble COD: 11.4 g/L Soluble reducing sugars: 2.8 g/L pH: 3.82	Anaerobic pre-treated inoculum	Acetic acid was dominant in the effluent	1.22 L H_2	Kumar and Mohan 2018
Vegetable waste + sewage	Unused/residual vegetables like tomato, potato, carrot, cabbage, brinjal, beet-root, okra, coccinia, etc	COD: 5.2 kg COD/m^3	Anaerobic mixed microflora	Acetic acid was dominate among other VFAs18.4	18.4 mmol H_2/day	Mohanakrishna et al. 2010a, b

*FVW fruit and vegetable waste

key factor that influence the fermentation process. A mixed microbial culture with a broad enzymatic pool and metabolic capabilities is required to adapt with the complex waste and dynamic environment. Mixed cultures primarily anaerobic sludge have demonstrated efficient waste degradation and biohydrogen production from vegetable waste. The presence of diverse microbial communities in the mixed culture offers efficient substrate utilization and ease in operational control and scale up (Mohanakrishna and Pengadeth 2024). However, the possibility of hydrogen consumption accompanied by methane and acetic acid generation by methanogenic and homoacetogenic bacteria is high. The unpredictable generation of unwanted byproduct in response to environmental changes is also plausible. This could be avoided by selective enrichment of microbial communities, but such techniques require additional energy input to the system.

Extremophiles for single pot bioconversion of substrate to hydrogen

The advancements in the field of biological hydrogen production have led to the transformation of biohydrogen production from a multi-vessel bioconversion process to a single-vessel approach termed consolidated bioprocessing (CBP). Although pre-treatment of vegetable waste helps to enhance the yield of hydrogen, the commercial scale hydrogen production is hindered greatly by the high cost of substrate pre-treatment followed by removal of inhibitory compounds of pre-treatment. Hence, to decrease the cost of production, the identification and utilization of suitable bacterium that can perform hydrolysis and biohydrogen production is needed. Thermophiles (60–75 °C) or hyperthermophiles (75–90 °C) that contain membrane-bound NADPH-dependent hydrogenase enzyme have shown to produce higher yields of hydrogen. Thermophilic hydrogen fermentations using thermophilic bacteria such as *Caldicellulosiruptor saccharolyticus* (Pawar et al. 2013) and *Thermotoga maritima* (Saidi et al. 2018) have shown promising results for hydrogen production in comparison to the mesophilic ones. Higher yields under these conditions are due to the inactivation of hydrogen consumers such as methanogens and sulphate reducing bacteria. Additionally, several thermophiles can produce hydrogen from both pentose and hexose sugars (Bibra et al. 2018).

Taking into account, the capability of thermophilic strains in converting complex wastes to simple sugars and producing hydrogen the single pot approach or CBP is more attractive in comparison with the multi-vessel dark fermentation (Fig. 2). In CBP enzyme production, feedstock saccharification, hydrolysis, and fermentation, all take place without the additional need of substrate pretreatment. This will not only reduce the use of costly pre-treatment methods but also offer the complete utilization of the substrate without waste generation. Bibra et al. (2018) in their study on biohydrogen production using thermophiles via single-pot bioconversion of prairie cordgrass demonstrated the use of a thermophilic

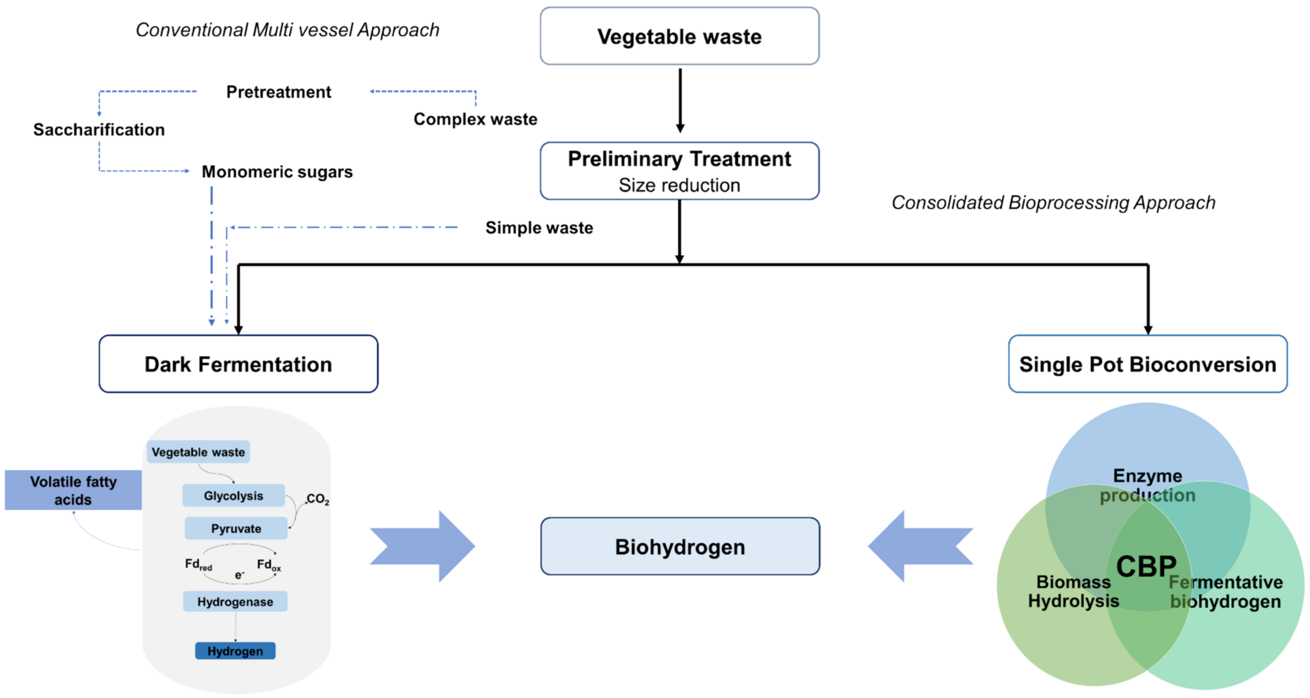

Fig. 2 Schematic comparison of dark fermentation and single pot conversion process for biohydrogen production

consortium for hydrogen production from lignocellulosic biomass. The thermophilic consortium utilized both glucose and xylose at 60 °C and produced 2.2 mmol H_2/g of prairie cordgrass. Hasyim et al. (2011) studied the feasibility of producing biohydrogen from sago starch in wastewater using a thermophilic mixed culture enriched from a hot spring. Thermophiles such as *T. saccharolyticum*, *T. thermosulfurigenes*, and uncultured *Thermoanaerobacterium* sp. were the predominant hydrogen producers in the mixed thermophilic culture, and a maximum hydrogen yield of 422 mL H_2/g starch was obtained. Pawar et al. (2013) demonstrated biohydrogen production from wheat straw hydrolysate using *Caldicellulosiruptor saccharolyticus* DSM 8903 and observed a maximum production rate of 5.2 L H_2/L/Day.

Therefore, the application of thermophilic bacterium or thermophilically derived enzymes that are thermostable for hydrogen production and pretreatment of vegetable waste can greatly contribute towards the commercial applicability of the fermentation process. Single-pot bioconversion or CBP is a promising area of research that can turn the tide in the fermentative biohydrogen production from lignocellulolytic-based waste. In-depth studies on the role metabolic pathway associated with the extremophiles can help to understand the microbial behaviour under specific conditions.

Indigenous microbial communities

Most of the studies on vegetable used an anaerobic inoculum that required a pre-treatment to inactivate the methanogens that consume hydrogen produced during fermentation. As aforementioned, substrate and inoculum pre-treatment require additional energy. Vegetable waste or any waste biomass in itself contains abundant indigenous microflora, which are capable of producing hydrogen. In nature, waste biomass self-decompose under room temperature if the required conditions are met (Marone et al. 2014). This principle allows natural selection to take place by controlling the reactor operating conditions. Biohydrogen production by self-fermentation without any substrate pre-treatment and inoculum but using the indigenous microbes within the substrate has been studied. Yokoyama et al. (2007) in the study on hydrogen production from a dairy cow waste slurry examined hydrogen production by batch culture using the microflora naturally existing within the slurry in a temperature range of 37 to 85 °C. They observed hydrogen producing moderate and extremophiles such as *Clostridium thermocellum* and *Caldanaerobacter subterraneus* in the slurry. If the suitable operating conditions are provided, the self-fermenting potential of waste biomasses can help to decrease the initial pretreatment cost associated with hydrogen production. In another study, Kim et al. (2011) demonstrated a similar simple method to naturally induce hydrogen production from food waste by controlling the operation temperature. They cultivated the food waste at a temperature range of 35 to 60 °C with a 5 °C interval without the addition of any inoculum or any pretreatment. Successful hydrogen production was achieved in the food waste incubated at a range of 50–60 °C with a hydrogen yield of 1.79 mol H_2/mol hexose added. Hydrogen producers such as *Clostridium* sp., *Acetanaerobacterium elongatum*, and *Caloramater indicus* were predominantly present in the fermentation. Marone et al. (2014) demonstrated the potential of vegetable waste as a substrate and source for microflora for hydrogen production in their study on dark fermentation on two different substrates made of leaf-shaped vegetable refuses (V) and leaf-shaped vegetable refuses plus potato peels (VP). From batch experiments under two different mesophilic anaerobic conditions (28 °C and 37 °C), they were able to isolate and identify different hydrogen producers such as those belonging to the family *Enterobacteriaceae* (γ-*proteobacteria*) and *Streptococcaceae* (*firmicutes*). Apart from this, they also found four different genera namely *Pectobacterium*, *Raoultella*, *Rahnella*, and *Lactococcus* that were not previously reported as HPB from glucose. A hydrogen yield of 1.6–2.2 mol H_2/mol glucose added was observed from low glucose concentration of 1 g/L.

From the literature overview (Shimizu et al. 2008; Kim et al. 2009), hydrogen production by self-fermentation is achieved without the addition of an external inoculum or by pre-treatment of substrate but by carefully controlling the important operating parameters such as pH and temperature to enhance the growth of hydrogen producers and suppressing the activity of hydrogen consumers. Apart from the dependency of hydrogen production on the operating pH and temperature, the microbial analysis especially by Kim et al. (2011) revealed the temperature-based vulnerability of lactic acid bacteria (LAB) and the role of high temperature in suppressing the same. LAB are present in most form of food products and owing to its unique metabolic characteristics and antibiotic functions they supress the growth of other microbes and impede hydrogen production. Hence by carefully monitoring the operating temperature and pH, self-fermentation of vegetable waste can help in reducing the pre-treatment requirements and can help to enrich the indigenous microbes within the substrate to enhance the hydrogen production.

Bioaugmentation

Enrichment of the indigenous microbial communities help to improve the hydrogen production and the application of bioaugmentation in this context is advantageous. Bioaugmentation is the practice of supplementing the existing inoculum with specific microbes to improve the substrate utilization efficiency, promote microbial growth towards hydrogen production, and reduce the initial lag phase (Mohan et al. 2007). In the study by Marone et al. (2012) on hydrogen production from vegetable waste, enhancement of biohydrogen production was achieved by bioaugmentation with indigenous microbial communities namely *Buttiauxella* sp. 4, *Rahnella* sp. 10, and *Raoultella* sp. 47. The individual strains enriched from vegetable waste was supplemented to the self-fermentative biohydrogen process. The study observed that all the individual bacterial inoculum promoted hydrogen yield and the production rate. Indigenous microbial communities *Buttiauxella* sp. 4, *Rahnella* sp. 10, and *Raoultella* sp. 47 demonstrated potential for hydrogen production from cellulolytic hydrolysates as well. Yang et al. (2016) observed a shift in the metabolic pathway of native microbial communities towards hydrogen production upon bioaugmentation of anaerobic granular sludge with *Hydrogenispora ethanolica* LX-B. The hydrogen production improved within a range of 2.3 to 3.5 mmol H_2/gVS. In another study by Mohan et al. (2007), the effect bioaugmentation on enhancing the hydrogen production in an anaerobic sequencing batch biofilm reactor (AnSBBR) was studied using a selectively enriched anaerobic mixed consortium. Bioaugmentation improved the reactor performance while the microbes persisted in the system until the end of the operation imparting specific functional characteristics to the native species. Villanueva-Galindo and Moreno-Andrade (2021) observed an improved hydrogen production of 84.5 mL H_2/g VS and decreased lag phase from food waste upon bioaugmentation of anaerobic sludge with *Bacillus subtilis* culture. Therefore, the application of bioaugmentation to dark fermentative process can help in enhancing the performance of the hydrogen producers. Synthetic consortium with individual microbes performing selective functions can be developed by studying the properties of the augmented inoculums.

Machine learning in biohydrogen production

When biohydrogen production from complex feedstock such as vegetable waste are studied, the control over the process is challenged by the diverse and variable nature of the feedstock. When mixed cultures are used as the biocatalyst, the variability increases and this would make it difficult to identify the operational parameters involved and the community dynamics existing within the system. Hence, mathematical models would fail to optimize the nonlinear and complex relationships that exist in dark fermentation. Modelling of biohydrogen is the cornerstone for scale up and optimization of the dynamic biohydrogen generation system (Wang et al. 2021a; Kumar Sharma et al. 2022). Machine learning (ML) based statistical tools such as Artificial neural networks (ANNs), Random forests (RF), Adaptive neuro-fuzzy interference system (ANFIS), Genetic algorithm (GA), and Support vector machines (SVMs) have shown great potential in hydrogen-related technologies over the past few years (Table 2). The diverse algorithms enable accurate prediction of the expected outcomes by pooling through the databases (Kazemi et al. 2020; Hosseinzadeh et al. 2020; Vendruscolo et al. 2020).

ML being a data-driven method is independent of the complex interconnections that exist in the mathematical models. Unlike the conventional methods where the stoichiometry and background mechanisms are considered, ML's entire approach is based on publicly accessible online data or old recording of the procedure and being a data-driven method; the algorithms can handle the complex multivariate data and predict non-linear connection (Kumar Sharma et al. 2022). Apart from this, it maps the non-linear input–output connections and does not necessarily require a general understanding of stoichiometry or background mechanisms. ML algorithm based biohydrogen prediction generally follows a trend. For a dark fermentative biohydrogen production from waste biomass for example vegetable waste, the initial inputs and the outputs of the fermentation are used to predict the performance, optimize, and predict the outcome of the process. Based on the objective of the process, the models (ANN, SVM, RF, and GA) with the help of the existing data available in literature will reduce the variables of the process and generate the expected of the fermentation. Wang et al. (2021a) compared and evaluated three different modelling techniques namely the multilayer perception artificial neural network (MLPANN), the response surface methodology (RSM), and the microbial kinetic with Levenberg–Marquardt algorithm (MKLMA) for dark fermentation. In the study, a robust paradigm was proposed for modelling the major metabolic intermediates during dark fermentation for biohydrogen production. From the study, it was conferred that with respect to the data, MKLMA model requires a longer computing time in comparison to the MLPANN model. MLPANN model being a soft computing approach has an added advantage of being faster in implementing the kinetic correlations between the key metabolic intermediates.

In another study by Wang et al. (2021b) on dark fermentation, optimization of the process was demonstrated by a novel hybrid approach that combined ANNs with RSM. The methodology applied helped in the analysis of critical operational parameters such as the carbon sources (from potato peel wastes and starchy wastes); pH; microbial load on hydrogen production; concentration of the intermediate metabolites (acetic acid, propionic acid etc.); and the metal cofactor Fe^0. Apart from this, the hybrid model demonstrated the generation of 106.2 cm^3/g hydrogen under the optimal operational conditions. Mahata et al. (2020) in their study on improvement of dark fermentative, hydrogen production from organic wastes using acidogenic mixed consortia used ML techniques such as ANN and SVM. The experimental results of biohydrogen production from starchy wastewater supplemented with groundnut de-oiled cake (GDOC) were analysed by RSM, ANN, and SVM. From their studies, they demonstrated that the SVM model a previously less explored model had similar prediction capability in comparison with RSM and ANN. Apart from that they combined the ML techniques with GA and particle swarm optimization (PSO) to estimate the optimal process parameters. The SVM-based model showed a 2.1-fold hydrogen production in comparison to the un-optimized process.

ML algorithms offer potential as a faster prediction tool for biohydrogen production by taking into account the non-linear interactions between the operational parameters. Models such as ANN that are simple and could imitate the structures of the biological neural networks will clarify the intricate connections that exist between numerous components of fermentation process and would prove useful in prediction of optimum operational conditions. The research in this field is at its nascent state and require in-depth studies to contribute towards optimization of process parameters. Additionally, research should be directed to studying the effect of inhibitors on fermentation along with development of the optimal process conditions for maximum yield of biohydrogen.

Table 2 Different machine learning models used in biohydrogen production

ML algorithms	Function	Application in biohydrogen production	Reference
Support vector machine (SVM)	Nonlinear mapping to develop a model to train or classify data accurately	Hydrogen yield prediction and process optimization	Kazemi et al. 2020; Mahata et al. 2020; Hosseinzadeh et al. 2022
Random forest (RF)	Decision tree construction followed by forest development using bootstrap samples from original data source	Asses the change in microbial community and correlate parameters with biogas production Models for hydrogen production prediction	Vendruscolo et al. 2020; Bagherzadeh et al. 2021; Pandey et al. 2023
Artificial neural network (ANN)	Computing tools called neuron control the environment and backpropagation method predict the outcome of the process by making use of a large data pool	Predict, control, and monitor H_2 production, and hydrogen production rate Predict and monitor VFA production kinetic	Nasr et al. 2013; El-Shafie 2014; Hosseinzadeh et al. 2020; Wang et al. 2021b
Genetic algorithm (GA)	Stochastic search technique to evaluate multiple options at once	Global optimization in biohydrogen production Investigate effect of operational parameters on fermentation	Prakasham et al. 2011; Wang et al. 2021b; Pandey et al. 2023

Limitations, future direction and biohydrogen-biorefinery

In spite of having a high hydrogen production rate (192 m^3 H_2/ m^3-d), dark fermentative biohydrogen production technology is not commercialised for bioenergy generation due to low hydrogen yield. The maximum hydrogen yield observed is around 2 mol H_2/ mol hexose because of thermodynamic limitations and it accounts to only 20% of substrate electrons. This would imply that 60–70% of electrons are trapped within the carboxylate end products and 10 to 20% electrons for cell growth (biomass associated and utilization-associated electron) (Lee et al. 2022). Hence, considering the dark fermentative process from an economic perspective, the practical implementation of the biohydrogen production units would be costly. Therefore, post-hydrogen production from the carboxylate would play a major role in commercializing dark fermentation technology. The integration of microbial electrolysis cell (MEC) with dark fermentation can help to counter these challenges. MECs being versatile technology produce hydrogen in the cathodic compartment by making use of the carboxylate fraction of dark effluent in the anodic compartment (Magdalena et al. 2023). The anode colonised by exoelectrogenic bacterial communities capable of carboxylate metabolism would help to generate hydrogen with a maximum production rate of 18 m^3 H_2/ m^3-d. Up to 6 mol H_2 mol H_2/mol hexose can be generated when MEC is combined with the dark fermentation process (Lee et al. 2022).

The VFA generated from the dark fermentation would serve as low-cost carbon source for microalgal cultivation and photo fermentative hydrogen production and as precursors for the production of secondary metabolites such as polyhydroxyalkanoates (PHA), and 1,3-propanediol (1,3-PD) (Kora et al. 2023) (Fig. 3). Dark fermentative hydrogen production, although has higher production rate, yields lower hydrogen molecules in comparison with the photo fermentative route of hydrogen production. The fermentative effluent serves as a simpler carbon source for photo fermentative bacteria such as the purple non-sulfur bacteria belonging to *Rhodobacteraceae* and *Rhodospirillaceae* family for hydrogen generation in the presence of light under anaerobic condition (Rao and Basak 2022; Sen et al. 2024). Although, photo-fermentative hydrogen production from VFAs have been explored, their dependency on sunlight can restrict their application. The residual carbon fraction of the effluent could also serve as the primary substrate for exoelectrogenic microbes in bioelectrochemical systems such as microbial fuel cells (MFC) bioelectricity production as well (Mohanakrishna et al. 2010b). Similarly, the effluent would be an efficient alternative to nutrient media for commercially relevant microalgal growth and biomass generation. Microalgal cultivation in the organic acid rich effluent contributes greatly towards the vision of microalgae-centred-waste-biorefinery. Microalgae, the photosynthetic microorganism, is considered a powerhouse of lipids, carbohydrates, proteins, and other nutrients and has potential applications in the biodiesel, nutraceutical, and bioenergy fields, and would enable energy recovery and effluent treatment simultaneously. Several secondary metabolite productions occur from the harvested algal biomass thereby setting off a chain of diverse product generation (Chiranjeevi and Venkata Mohan 2017). One of the most prominent routes to acidogenic effluent utilization is its application in generation of PHAs, the emerging alternative to petroleum-based plastics (Martinez et al. 2016; Tamang and Nogueira 2021; Lagoa-Costa et al. 2022). The microbial generation of these polyesters have been extensively studied and a PHA generation ranging between 30 and 70% have been observed from fermentative effluent by mixed and pure bacterial cultures namely *Bacillus tequilensis*, *Cupriavidus necator*, *Pseudomonas oleovorans*, *Alcaligenes eutrophus*, and many more (Venkateswar Reddy and Venkata Mohan 2012; Aremu et al. 2021; Khatami et al. 2022).

Hence, the integration of multiple unit operations can help in the profitability of the dark fermentative process. As aforementioned, the utilization of fermentative effluents, mixed microbial cultures, and extremophiles with diverse metabolic capabilities can improve the prospects of the process. However, further in-depth studies on the dynamics between the microbial communities and their

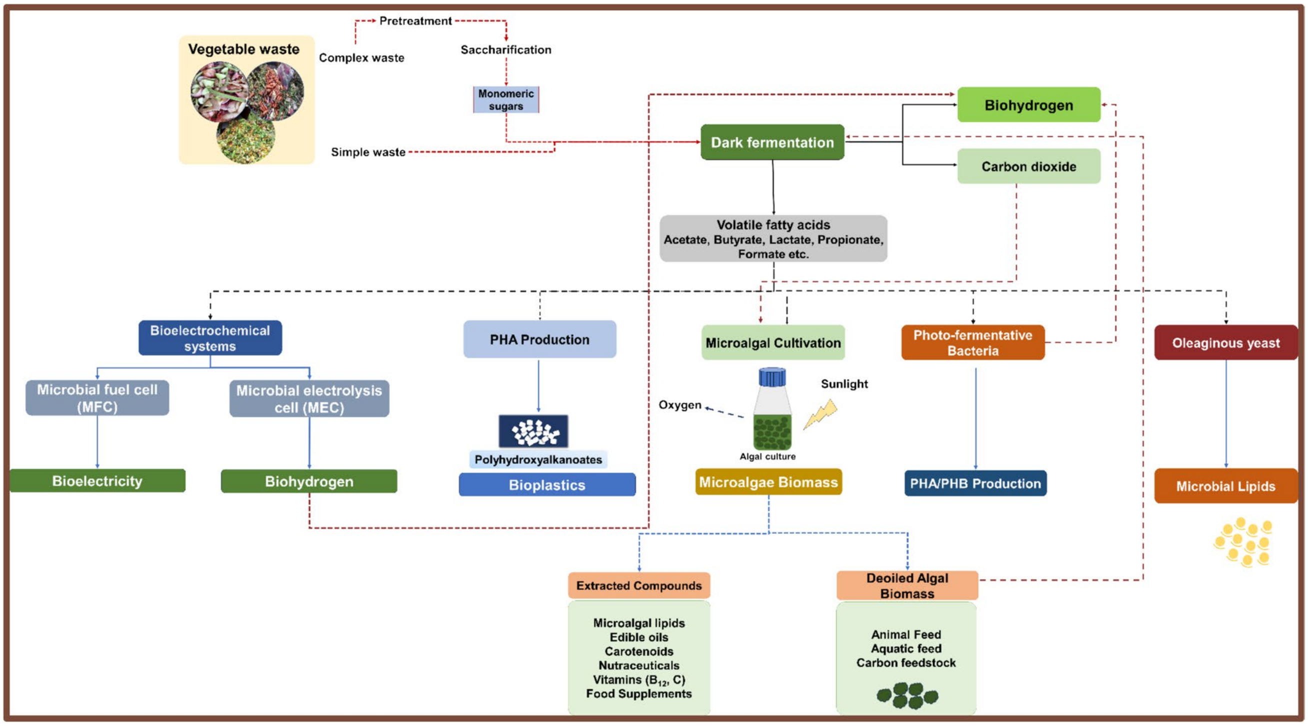

Fig. 3 Schematic diagram on biohydrogen biorefinery utilizing the acidogenic effluents from dark fermentation

influence on the end product generation is required. Although synergic interactions yield effective results, the presence of diverse bacterial communities could also induce feedstock related competition and antagonism as well (Mohanakrishna and Pengadeth 2024). With the recent advancements in synthetic biology, the keystone species in the mixed cultures can be identified and could be further modelled to direct their metabolism towards hydrogen production by metabolic pathway manipulation. The development of synthetic cultures with known hydrogen producers would offer a better understanding on the intricate pathways involved in byproduct generation. Further studies on extremophiles and their integration with genetic engineering can help to develop bacterial strains capable of carrying out substrate hydrolysis and hydrogen production simultaneously to avoid the expensive pretreatment methods (Byrne et al. 2021). The addition of machine learning tools in biohydrogen processes has paved the way for researchers in understanding the process parameters and their influence in hydrogen production; however, the studies on ML pertaining to vegetable waste is scarce in literature and needs further exploration. The studies on hydrogen production from vegetable waste are restricted to batch systems mostly. However, for commercial deployment of production units, continuous fermentation set-ups are required and research is needed in this direction.

Conclusion

Dark fermentative hydrogen production from vegetable waste is an attractive solution for waste management and sustainable energy generation. This review explored the recent strategies to improve hydrogen production from vegetable waste with emphasis on bioaugmentation and mixed cultures. The advantages of extremophiles for single-pot-conversion of feedstock to hydrogen was reviewed. One of the major setbacks in dark fermentation is the formation of unwanted byproducts, the application of these effluents for secondary value-added products would help to improve the development of full-scale fermentation set-ups. With process integration, green hydrogen generation and its implementation within the framework of circular bioeconomy could be achieved.

Acknowledgements The authors are thankful to the Ministry of Education (MoE), Govt of India for bestowing the first author with the post-graduation fellowship. The authors are extremely grateful to the facilities and support provided by the Department of Biotechnology, Dr. B R Ambedkar National Institute of Technology, Jalandhar, India. The paper is a result of the existing collaboration in the frame of Memorandum of Understanding (MoU) Of Cultural and Scientific Cooperation between the Dr. B. R. Ambedkar National Institute of Technology, Jalandhar, India and the University of Florence, Italy.

Author contribution Devu Pengadetha has contributed towards conceptualization and designing of the work, material preparation, data collection, analysis, and manuscript preparation. Nitai Basak has contributed towards conceptualization and designing of the work, data analysis, and manuscript preparation, revision of manuscript, and overall supervision of the project. Luca Bernabò has contributed for data analysis and revision of manuscript. Alessandra Adessi has contributed to the study's conception, design and revision of manuscript. All authors read and approved the final manuscript.

Data availability This is review paper; all the data are taken from the respective literature has been properly cited in the entire manuscript.

Declarations

Competing interests The authors declare no competing interests.

References

Ahmad A, Rambabu K, Hasan SW, Show PL, Banat F (2024) Biohydrogen production through dark fermentation: recent trends and advances in transition to a circular bioeconomy. Int J Hydrogen Energy 52:335–357. https://doi.org/10.1016/j.ijhydene.2023.05.161

Aremu MO, Ishola MM, Taherzadeh MJ (2021) Polyhydroxyalkanoates (PHAs) Production from volatile fatty acids (VFAs) from organic wastes by *Pseudomonas oleovorans*. Fermentation 7:287. https://doi.org/10.3390/fermentation7040287

Bagherzadeh F, Mehrani M-J, Basirifard M, Roostaei J (2021) Comparative study on total nitrogen prediction in wastewater treatment plant and effect of various feature selection methods on machine learning algorithms performance. J Water Process Eng 41:102033. https://doi.org/10.1016/j.jwpe.2021.102033

Bibra M, Kumar S, Wang J, Bhalla A, Salem DR, Sani RK (2018) Single pot bioconversion of prairie cordgrass into biohydrogen by thermophiles. Bioresour Technol 266:232–241. https://doi.org/10.1016/j.biortech.2018.06.046

Bundhoo MAZ, Mohee R (2016) Inhibition of dark fermentative biohydrogen production: a review. Int J Hydrogen Energy 41:6713–6733. https://doi.org/10.1016/j.ijhydene.2016.03.057

Byrne E, Björkmalm J, Bostick JP, Sreenivas K, Willquist K, Niel EdWJV (2021) Characterization and adaptation of *Caldicellulosiruptor strains* to higher sugar concentrations, targeting enhanced hydrogen production from lignocellulosic hydrolysates. Biotechnol Biofuels 14:210. https://doi.org/10.1186/s13068-021-02058-x

Chiranjeevi P, Venkata Mohan S (2017) Diverse acidogenic effluents as feedstock for microalgae cultivation: dual phase metabolic transition on biomass growth and lipid synthesis. Bioresour Technol 242:191–196. https://doi.org/10.1016/j.biortech.2017.04.059

Dahiya S, Kumar AN, Shanthi Sravan J, Chatterjee S, Sarkar O, Venkata Mohan S (2018) Food waste biorefinery: sustainable strategy for circular bioeconomy. Bioresour Technol 248:2–12. https://doi.org/10.1016/j.biortech.2017.07.176

DOE (2018) DOE hydrogen and fuel cell program. https://www.nrel.gov/docs/fy19osti/73353.pdf. Accessed 23 Aug 2024

DST (2020) Department of Science and Technology, India country status report on hydrogen and fuel cells. https://dst.gov.in/collation-indias-hydrogen-fuel-cells-research-status-launched. Accessed 21 Aug 2024

El-Shafie A (2014) Neural network nonlinear modeling for hydrogen production using anaerobic fermentation. Neural Comput Appl 24:539–547. https://doi.org/10.1007/s00521-012-1268-8

EU (2020) A hydrogen strategy for a climate-neutral Europe. Communication from the commission to the European parliament, the council, the European economic and social committee and the committee of the regions

Hafid HS, Rahman NA, Md Shah UK, Baharudin AS (2015) Enhanced fermentable sugar production from kitchen waste using various pretreatments. J Environ Manag 156:290–298. https://doi.org/10.1016/j.jenvman.2015.03.045

Hasyim R, Imai T, O-Thong S, Sulistyowati L (2011) Biohydrogen production from sago starch in wastewater using an enriched thermophilic mixed culture from hot spring. Int J Hydrogen Energy 36:14162–14171. https://doi.org/10.1016/j.ijhydene.2011.04.211

Hitit ZY, Lazaro CZ, Hallenbeck PC (2017) Single stage hydrogen production from cellulose through photo-fermentation by a coculture of *Cellulomonas fimi* and *Rhodopseudomonas palustris*. Int J Hydrogen Energy 42:6556–6566. https://doi.org/10.1016/j.ijhydene.2016.12.035

Hosseinzadeh A, Zhou JL, Altaee A, Baziar M, Li D (2020) Effective modelling of hydrogen and energy recovery in microbial electrolysis cell by artificial neural network and adaptive network-based fuzzy inference system. Bioresour Technol 316:123967. https://doi.org/10.1016/j.biortech.2020.123967

Hosseinzadeh A, Zhou JL, Altaee A, Li D (2022) Machine learning modeling and analysis of biohydrogen production from wastewater by dark fermentation process. Bioresour Technol 343:126111. https://doi.org/10.1016/j.biortech.2021.126111

IEA (2024) Global methane tracker 2024. IEA, Paris. https://www.iea.org/reports/global-methane-tracker-2024. Accessed 23 Aug 2024

Jayachandran V, Basak N, De Philippis R, Adessi A (2022) Novel strategies towards efficient molecular biohydrogen production by dark fermentative mechanism: present progress and future perspective. Bioprocess Biosyst Eng 45:1595–1624. https://doi.org/10.1007/s00449-022-02738-4

Jiradechakorn T, Chuetor S, Kirdponpattara S et al (2023) Performance of combined hydrochemo-mechanical pretreatment of rice straw for bioethanol production. Energy Rep 9:180–185. https://doi.org/10.1016/j.egyr.2023.08.081

Kaza S, Yao LC, Bhada-Tata P, Van Woerden F (2018) What a waste 2.0: a global snapshot of solid waste management to 2050. Urban Development; © Washington, DC: World Bank. http://hdl.handle.net/10986/30317. Accessed 18 Aug 2024

Kazemi P, Giralt J, Bengoa C, Steyer J-P (2020) Data-driven fault detection methods for detecting small-magnitude faults in anaerobic digestion process. Water Sci Technol 81:1740–1748. https://doi.org/10.2166/wst.2020.026

Khatami K, Perez-Zabaleta M, Cetecioglu Z (2022) Pure cultures for synthetic culture development: next level municipal waste treatment for polyhydroxyalkanoates production. J Environ Manag 305:114337. https://doi.org/10.1016/j.jenvman.2021.114337

Kim D-H, Kim S-H, Shin H-S (2009) Hydrogen fermentation of food waste without inoculum addition. Enzyme Microb Technol 45:181–187. https://doi.org/10.1016/j.enzmictec.2009.06.013

Kim D-H, Wu J, Jeong K-W, Kim MS, Shin HS (2011) Natural inducement of hydrogen from food waste by temperature control. Int J Hydrogen Energy 36:10666–10673. https://doi.org/10.1016/j.ijhydene.2011.05.153

Kora E, Patrinou V, Antonopoulou G, Ntaikou I, Tekerlekopoulou AG, Lyberatos G (2023) Dark fermentation of expired fruit juices for biohydrogen production followed by treatment and biotechnological exploitation of effluents towards bioplastics and microbial lipids. Biochem Eng J 195:108901. https://doi.org/10.1016/j.bej.2023.108901

Kumar AN, Mohan SV (2018) Acidogenic valorization of vegetable waste for short chain carboxylic acids and biohydrogen production: influence of pretreatment and pH. J Clean Prod 203:1055–1066. https://doi.org/10.1016/j.jclepro.2018.08.198

Lagoa-Costa B, Kennes C, Veiga MC (2022) Influence of feedstock mix ratio on microbial dynamics during acidogenic fermentation for polyhydroxyalkanoates production. J Environ Manag 303:114132. https://doi.org/10.1016/j.jenvman.2021.114132

Lee H-S, Xin W, Katakojwala R, Mohan SV, Tabish NMD (2022) Microbial electrolysis cells for the production of biohydrogen in dark fermentation–a review. Bioresour Technol 363:127934. https://doi.org/10.1016/j.biortech.2022.127934

Lo Y-C, Saratale GD, Chen W-M, Bai M-D, Chang J-S (2009) Isolation of cellulose-hydrolytic bacteria and applications of the cellulolytic enzymes for cellulosic biohydrogen production. Enzyme Microb Technol 44:417–425. https://doi.org/10.1016/j.enzmictec.2009.03.002

Magdalena JA, Pérez-Bernal MF, Bernet N, Trably E (2023) Sequential dark fermentation and microbial electrolysis cells for hydrogen production: volatile fatty acids influence and energy considerations. Bioresour Technol 374:128803. https://doi.org/10.1016/j.biortech.2023.128803

Mahata C, Ray S, Das D (2020) Optimization of dark fermentative hydrogen production from organic wastes using acidogenic mixed consortia. Energy Convers Manag 219:113047. https://doi.org/10.1016/j.enconman.2020.113047

Marone A, Massini G, Patriarca C, Signorini A, Varrone C, Izzo G (2012) Hydrogen production from vegetable waste by bioaugmentation of indigenous fermentative communities. Int J Hydrogen Energy 37:5612–5622. https://doi.org/10.1016/j.ijhydene.2011.12.159

Marone A, Izzo G, Mentuccia L, Massini G, Paganin P, Rosa S, Varrone C, Signorini A et al (2014) Vegetable waste as substrate and source of suitable microflora for bio-hydrogen production. Renew Energy 68:6–13. https://doi.org/10.1016/j.renene.2014.01.013

Martinez GA, Rebecchi S, Decorti D, Domingos JMB, Natolino A, Daniel DR, Bertin L, Caria DP, Faya F (2016) Towards multipurpose biorefinery platforms for the valorisation of red grape pomace: production of polyphenols, volatile fatty acids, polyhydroxyalkanoates and biogas. Green Chem 18:261–270. https://doi.org/10.1039/C5GC01558H

Martínez-Mendoza LJ, Lebrero R, Muñoz R, García-Depraect O (2022) Influence of key operational parameters on biohydrogen production from fruit and vegetable waste via lactate-driven dark fermentation. Bioresour Technol 364:128070. https://doi.org/10.1016/j.biortech.2022.128070

Mathews J, Wang G (2009) Metabolic pathway engineering for enhanced biohydrogen production. Int J Hydrogen Energy 34:7404–7416. https://doi.org/10.1016/j.ijhydene.2009.05.078

Mohan SV, Mohanakrishna G, Veer Raghavulu S, Sarma PN (2007) Enhancing biohydrogen production from chemical wastewater treatment in anaerobic sequencing batch biofilm reactor (AnSBBR) by bioaugmenting with selectively enriched kanamycin resistant anaerobic mixed consortia☆. Int J Hydrogen Energy 32:3284–3292. https://doi.org/10.1016/j.ijhydene.2007.04.043

Mohan SV, Mohanakrishna G, Goud RK, Sarma PN (2009) Acidogenic fermentation of vegetable based market waste to harness biohydrogen with simultaneous stabilization. Bioresour Technol 100:3061–3068. https://doi.org/10.1016/j.biortech.2008.12.059

Mohanakrishna G, Pengadeth D (2024) Mixed culture biotechnology and its versatility in dark fermentative hydrogen production. Bioresour Technol 394:130286. https://doi.org/10.1016/j.biortech.2023.130286

Mohanakrishna G, Kannaiah Goud R, Mohan SV, Sarma PN (2010a) Enhancing biohydrogen production through sewage supplementation of composite vegetable based market waste. Int J Hydrogen Energy 35:533–541. https://doi.org/10.1016/j.ijhydene.2009.11.002

Mohanakrishna G, Mohan SV, Sarma PN (2010b) Utilizing acid-rich effluents of fermentative hydrogen production process as substrate for harnessing bioelectricity: an integrative approach.

Int J Hydrogen Energy 35:3440–3449. https://doi.org/10.1016/j.ijhydene.2010.01.084

Mohanakrishna G, Sneha NP, Rafi SM, Sarkar O (2023) Dark fermentative hydrogen production: potential of food waste as future energy needs. Sci Total Environ 888:163801. https://doi.org/10.1016/j.scitotenv.2023.163801

Monlau F, Aemig Q, Trably E, Hamelin J, Steyer JP, Carrere H (2013) Specific inhibition of biohydrogen-producing *Clostridium sp.* after dilute-acid pretreatment of sunflower stalks. Int J Hydrogen Energy 38:12273–12282. https://doi.org/10.1016/j.ijhydene.2013.07.018

Muñoz-Páez KM, Alvarado-Michi EL, Buitrón G, Valdez-Vazquez I (2019) Distinct effects of furfural, hydroxymethylfurfural and its mixtures on dark fermentation hydrogen production and microbial structure of a mixed culture. Int J Hydrogen Energy 44:2289–2297. https://doi.org/10.1016/j.ijhydene.2018.04.139

Nasr N, Hafez H, El Naggar MH, Nakhla G (2013) Application of artificial neural networks for modeling of biohydrogen production. Int J Hydrogen Energy 38:3189–3195. https://doi.org/10.1016/j.ijhydene.2012.12.109

Ni M, Leung DYC, Leung MKH, Sumathy K (2006) An overview of hydrogen production from biomass. Fuel Process Technol 87:461–472. https://doi.org/10.1016/j.fuproc.2005.11.003

Pandey AK, Park J, Ko J, Joo HH, Raj T, Singh LK, Singh N, Kim SH (2023) Machine learning in fermentative biohydrogen production: advantages, challenges, and applications. Bioresour Technol 370:128502. https://doi.org/10.1016/j.biortech.2022.128502

Panin S, Setthapun W, Elizabeth Sinsuw AA, Sintuya H, Chu CY (2021) Biohydrogen and biogas production from mashed and powdered vegetable residues by an enriched microflora in dark fermentation. Int J Hydrogen Energy 46:14073–14082. https://doi.org/10.1016/j.ijhydene.2020.09.246

Pawar SS, Nkemka VN, Zeidan AA, Murto M, Niel EWJV (2013) Biohydrogen production from wheat straw hydrolysate using *Caldicellulosiruptor saccharolyticus* followed by biogas production in a two-step uncoupled process. Int J Hydrogen Energy 38:9121–9130. https://doi.org/10.1016/j.ijhydene.2013.05.075

Prakasham RS, Sathish T, Brahmaiah P (2011) Imperative role of neural networks coupled genetic algorithm on optimization of biohydrogen yield. Int J Hydrogen Energy 36:4332–4339. https://doi.org/10.1016/j.ijhydene.2011.01.031

Prem Ananth Surendran S, Shanmugam P (2021) Correlation between empirical formulae based stoichiometric and experimental methane potential and calorific energy values for vegetable solid wastes. Energy Rep 7:19–31. https://doi.org/10.1016/j.egyr.2020.10.071

Rajesh Banu J, Merrylin J, Mohamed Usman TM, Kannah RY, Gunasekaran M, Kim SH, Kumar G (2020) Impact of pretreatment on food waste for biohydrogen production: a review. Int J Hydrogen Energy 45:18211–18225. https://doi.org/10.1016/j.ijhydene.2019.09.176

Ramprakash B, Muthukumar K (2018) Influence of sulfuric acid concentration on biohydrogen production from rice mill wastewater using pure and coculture of Enterobacter aerogenes and *Citrobacter freundii*. Int J Hydrogen Energy 43:9254–9258. https://doi.org/10.1016/j.ijhydene.2018.03.198

Rao R, Basak N (2022) Sequential dark-photo batch fermentation and kinetic modelling for biohydrogen production using cheese whey as a feedstock. Appl Biochem Biotechnol 194:3930–3960. https://doi.org/10.1007/s12010-022-03958-w

Rodríguez-Valderrama S, Escamilla-Alvarado C, Magnin J-P, Rivas-Garcia P, Valdez-Vazquez I, Rios-Leal E (2020) Batch biohydrogen production from dilute acid hydrolyzates of fruits-and-vegetables wastes and corn stover as co-substrates. Biomass Bioenergy 140:105666. https://doi.org/10.1016/j.biombioe.2020.105666

Saidi R, Liebgott PP, Gannoun H, Gaida LB, Miladi B, Hamdi M, Bouallagui H, Auria R (2018) Biohydrogen production from hyperthermophilic anaerobic digestion of fruit and vegetable wastes in seawater: simplification of the culture medium of *Thermotoga maritima*. Waste Manag 71:474–484. https://doi.org/10.1016/j.wasman.2017.09.042

Sen AS, Rao R, Basak N (2024) CFD modelling of hydrodynamics and temperature distribution during photo fermentative biohydrogen generation from cheese whey effluent in cylindrical bioreactor. Int J Hydrogen Energy 60:740–755. https://doi.org/10.1016/j.ijhydene.2024.02.207

Sevinç P, Gündüz U, Eroglu I, Yücel M (2012) Kinetic analysis of photosynthetic growth, hydrogen production and dual substrate utilization by *Rhodobacter capsulatus*. Int J Hydrogen Energy 37:16430–16436. https://doi.org/10.1016/j.ijhydene.2012.02.176

Sharma AK, Ghodke PK, Goyal N, Nethaji S, Chen WH (2022) Machine learning technology in biohydrogen production from agriculture waste: recent advances and future perspectives. Bioresour Technol 364:128076. https://doi.org/10.1016/j.biortech.2022.128076

Shimizu S, Fujisawa A, Mizuno O, Kameda T, Yoshioka T (2008) Fermentative hydrogen production from food waste without inocula. In: AIP conference proceedings. American Institute of Physics 987(1):171–174. https://doi.org/10.1063/1.2896968

Singh A, Kuila A, Adak S, Bishai M, Banerjee R (2012) Utilization of vegetable wastes for bioenergy generation. Agric Res 1:213–222. https://doi.org/10.1007/s40003-012-0030-x

Singh CK, Kumar A, Roy SS (2018) Quantitative analysis of the methane gas emissions from municipal solid waste in India. Sci Rep 8:2913. https://doi.org/10.1038/s41598-018-21326-9

Srivastava N, Srivastava M, Abd-Allah EF, Singh R, Hashem A, Gupta VK (2021) Biohydrogen production using kitchen waste as the potential substrate: a sustainable approach. Chemosphere 271:129537. https://doi.org/10.1016/j.chemosphere.2021.129537

Tamang P, Nogueira R (2021) Valorisation of waste cooking oil using mixed culture into short- and medium-chain length polyhydroxyalkanoates: effect of concentration, temperature and ammonium. J Biotechnol 342:92–101. https://doi.org/10.1016/j.jbiotec.2021.10.006

Tenca A, Schievano A, Perazzolo F, Adani F, Oberti R (2011) Biohydrogen from thermophilic co-fermentation of swine manure with fruit and vegetable waste: maximizing stable production without pH control. Bioresour Technol 102:8582–8588. https://doi.org/10.1016/j.biortech.2011.03.102

United Nations Environment Programme (2024) Food waste index report 2024. Think eat save: tracking progress to halve global food waste. Nairobi. https://www.unep.org/resources/publication/food-waste-index-report-2024. Accessed 24 Aug 2024

Vendruscolo ECG, Mesa D, Rissi DV, Meyer BH, Pedrosa FdO, Souza EMd, Cruz LM (2020) Microbial communities network analysis of anaerobic reactors fed with bovine and swine slurry. Sci Total Environ 742:140314. https://doi.org/10.1016/j.scitotenv.2020.140314

Venkateswar Reddy M, Venkata Mohan S (2012) Influence of aerobic and anoxic microenvironments on polyhydroxyalkanoates (PHA) production from food waste and acidogenic effluents using aerobic consortia. Bioresour Technol 103:313–321. https://doi.org/10.1016/j.biortech.2011.09.040

Villanueva-Galindo E, Moreno-Andrade I (2021) Bioaugmentation on hydrogen production from food waste. Int J Hydrogen Energy 46:25985–25994. https://doi.org/10.1016/j.ijhydene.2020.11.092

Wang Y, Tang M, Ling J, Wang Y, Liu Y, Jin H, He J, Sun Y (2021a) Modeling biohydrogen production using different data driven approaches. Int J Hydrogen Energy 46:29822–29833. https://doi.org/10.1016/j.ijhydene.2021.06.122

Wang Y, Yang G, Sage V, Xu J, Sun G, He J, Sun Y (2021b) Optimization of dark fermentation for biohydrogen production using a hybrid artificial neural network (ANN) and response surface methodology (RSM) approach. Environ Prog Sustain Energy 40:e13485. https://doi.org/10.1002/ep.13485

Yang Z, Guo R, Shi X, He S, Wang L, Dai M, Qiu Y, Dang X (2016) Bioaugmentation of Hydrogenispora ethanolica LX-B affects hydrogen production through altering indigenous bacterial community structure. Bioresour Technol 211:319–326. https://doi.org/10.1016/j.biortech.2016.03.097

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. Appl Microbiol Biotechnol 74:474–483. https://doi.org/10.1007/s00253-006-0647-4