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ORIGINAL ARTICLE

Enhancing biohydrogen production from mono-substrates and co-substrates using a novel bacterial strains

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Abstract

The staggering increase in pollution associated with a sharp tightening in global energy demand is a major concern for organic substances. Renewable biofuel production through simultaneous waste reduction is a sustainable approach to meet this energy demand. This study co-fermentation of dairy whey and SCB was performed using mixed and pure bacterial cultures of *Salmonella bongori*, *Escherichia coli*, and *Shewanella oneidensis* by dark fermentation process for hydrogen production. The maximum H_2 production was 202.7 ± 5.5 H_2/mL/L, 237.3 ± 6.0 H_2/mL/L, and 198 ± 9.9 H_2/mL/L obtained in fermentation reactions containing dairy whey, solid and liquid hydrolysis of pretreated sugarcane bagasse as mono-substrates. The H_2 production was greater in co-substrate by 347.3 ± 18.5 H_2/mL/L under optimized conditions (pH 7.0, temperature 37 °C, substrate concentration 30:50 g/L) than expected in mono-substrate conditions, which confirms that co-fermentation of different substrates enhances the H_2 potential. Fermentation medium during bio-H2 production under GC analysis has stated that using mixed cultures in dark fermentation favored acetic acid and butyric acid. Co-substrate degradation produces ethyl alcohol, benzoic acid, propionic acid, and butanol as metabolic by-products. The difference in the treated and untreated substrate and carbon enrichment in the substrates was evaluated by FT-IR analysis. The present study justifies that rather than the usage of mono-substrate for bio-H_2 production, the co-substrate provided highly stable H_2 production by mixed bacterial cultures. Fabricate the homemade single-chamber microbial fuel cell to generate electricity.

Keywords Biohydrogen · Co-substrate · Mono-substrate · Microbial fuel cell · Dark fermentation · Co-culture

Introduction

Bioenergy is renewable energy derived from sources that cannot be depleted or replenished within the lifetime of a human (Owusu and Asumadu-Sarkodie 2016; Joseph 2019). Bioenergy can be derived from different types of sources, including wood residues, agricultural residues, and livestock residues (Scarlat et al. 2011; Prakash Kumar Sarangi et al. 2022). Limiting demand for imported fuels in low-income countries and diversifying national energy resources are potential benefits (Shrestha and Shakya 2012; Aditiya and Aziz 2021). Bio-H_2 can produce electricity at a minimal cost in underdeveloped countries, here modern conversion technologies and processes are applied appropriately (Nath and Das 2011). Bio-H_2 (hydrogen) is an alternative energy and sustainable power source in the current situation (Bharathiraja et al. 2014; Kadier et al. 2022). Bio-H_2 is a clean and ecologically friendly fuel with high energy consumption and yield (Liu et al. 2019). It is produced using a variety of ways, including dark fermentation, photo fermentation, and bio-photolysis (Dinamarca and Bakke 2011; Kumar et al. 2019). The dark fermentation technique is a developing trend in which H_2 is produced naturally (Sinha and Pandey 2011). Dark fermentation is the conversion of organic matter into various organic and inorganic products (Bastidas-Oyanedel et al. 2015; Qyyum et al. 2022). In the absence of sunlight, dark fermentation uses microbial resources to produce hydrogen from waste biomass (Oztekin et al. 1993; Bolatkhan et al. 2019). A variety of microbes such as *Enterobacter*, *Bacillus*, and *Clostridium* are involved in bio-H_2 production (Davila-Vazquez et al. 2009; Khan et al. 2021). In photofermentation, hydrogen is produced by the decomposition of organic matter with the help of nitrogenase, which is produced by photosynthetic bacteria (Basak and Das 2007;

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Hitam and Jalil 2020). A variety of photosynthetic bacteria, including *R. capsulatus*, *R. sphaeroides*, *R. sulfidophilum, R. rubrum*, and *R. palustris* (Nath and Das 2011; Li et al. 2022).

Dark fermentation is an intricate process and is highly affected by many parameters. These parameters include nutritional requirements, substrate concentration, substrate specificity, reactor configuration, temperature, pH, hydraulic retention time (HRT), and organic loading rate (Logan et al. 2002; Wu et al. 2003; Lin and Lay 2004; Bernal et al. 2021; Nurhayat and Bilgin 2022). In heterogeneous hydrogen-producing reactors, pH changes might significantly alter populations of different species because pH inhibits bacterial growth (Horiuchi et al. 1999; Audu et al. 2021; Varghese et al. 2022). Microbial metabolism is highly affected by pH variations that lie within the growth range of microorganisms (Dijkstra et al. 2012; Jayachandran and Basak 2022). This possibility such substrate Corn stalks, wheat stalks and Sugarcane bagasse degradation efficiency, utilization of carbon and energy sources, synthesis of protein and discharge of the metabolic end products from cells (Pant et al. 2010; Prathiba et al. 2022). Much research has been conducted on the effects of pH on H_2 production from carbohydrate-rich wastes (Mizuno et al. 2000; Liu et al. 2020). Maximum substrate utilization rates by methanogens are affected by temperature (Rebac et al. 1997; Dalby et al. 2021). Biomass concentration has a significant impact on the spread of fermentation products, as biomass utilization rates can be a major problem by biomass concentration (Gil et al. 2003; Nath and Das 2011). Pretreatment approaches based on acid, alkaline, enzymatic, and combination treatment are universally acceptable and the easiest means of producing bio-H_2 (Xing et al. 2006; Johnstone et al. 2010). Without pretreatment, direct conversion yields very little H_2; however with pretreatment, the complex structure of cellulosic biomass breaks down and yields a very high H_2 generation efficiency (Fujita et al. 2016; Galbe and Zacchi 2012). The lignin–carbohydrate complexes in sugarcane bagasse (SCB) are destroyed or disturbed during acid hydrolysis, making the cellulose freely available to enzymes and microorganisms (Karimi et al. 2014; Shukla et al. 2023). Acid treatment of SCB with H_2SO_4 at different concentrations (0.25–7.0% volume) is a commonly used approach since it is environmentally beneficial (Abubakar et al. 2022).

Bio-H_2 production from different wastewater is used as substrates using the pure and mixed culture of bacteria (Kyazze et al. 2005). Pure culture systems produce a lower diversity of biomass, making metabolic shifts easy to detect (Elsharnouby et al. 2013; Amin et al. 2022). In addition, studies with pure cultures can provide valuable insights into the conditions that promote hydrogen production (Hawkes et al. 2002; Cao et al. 2022). The hydrogen-producing pure culture can be genetically modified to block, inactivate, and shut down undesirable metabolic pathways. The advantages of pure culture systems include their minimal inoculum steps, technical feasibility, and ease of use despite lower yields compared to co-cultures (Hallenbeck and Ghosh 2009; Mahata and Das 2022). It is economically beneficial to support and maintain anaerobic conditions for hydrogen-producing bacteria to eliminate unwanted compounds (Juan et al. 2015; Lui et al. 2020). In the fermentation process, co-culture catalysis improves the conversion of complex sugars and degraded organic substances into hydrogen and provides a wide range of pH. According to most studies, economics and technology were important factors that interrelate. Co-cultures combine strict and facultative anaerobes in the first category. Strict anaerobic bacteria are very sensitive to oxygen, and hydrogen-producing bacteria can block oxygen. Another way facultative anaerobes consume O_2 in a medium eliminates the need for an expensive reducing agent, making it simple to achieve anaerobic conditions. Enhanced hydrogen production through batch fermentation process under anaerobic conditions using co-cultures of *Enterobacter* sp. (facultative anaerobes) and *Clostridium* sp. (strict anaerobes) was performed to achieve stable hydrogen production and high yield was observed (Nath and Das 2011; Kamilah et al. 2018).

Nevertheless, the use of different substrates for bio-H_2 production is a more feasible and sustainable alternative. An important novelty of this work is that it is the first report to investigate the production of H_2 from whey and sugarcane bagasse as co-substrates using a novel bacterial strain *E.coli*, *S. bongori*, and *S. oneidensis*. In this study, the bio-H_2 potential of industrial waste and lignocellulosic substrates was investigated by dark fermentation using pure and co-cultures of efficient bio-H_2-producing bacteria. Dairy whey effluents and solid and liquid hydrolysates from sugarcane bagasse are rich in carbohydrates and bacterial nutrients, and therefore the most suitable substrates for bio-H_2 production by dark fermentation. This work involves the use of an MFC by facultative anaerobic bacteria with co-substrate.

Materials and methods

Culture collection, subculture, and pure culture maintenance

Bio-H_2-producing facultative anaerobic bacteria viz., *E. coli*, *S. bongori*, *S. oneidensis* of different anaerobic sludge collected from in and around Dindigul district, Tamil Nadu in India, which were previously isolated in our laboratories were utilized for the present study (Mumtha et al. 2022a). The three selected bacterial strains were cultured on the

nutrient agar at pH 7 and 37 °C for 24 h and maintained in the refrigerator at 4 °C for further study.

Substrate collection and processing

Dairy whey was collected from the SPS Dairy and Foods Ltd, Nadunayagapatti, Dindigul District, Tamil Nadu, India. One liter of samples was collected in sterile containers and transported immediately to the lab and stored at 4 °C for further processing. Sugarcane bagasse was collected from the Rajshree Sugarcane Industry and Chemical limited near Vaigai dam, Theni District, Tamil Nadu, India. The collected sugarcane bagasse was once soaked in distilled water and dried at 40 °C for 24 h in a hot air oven. The dried substrates were milled to a size less than 0.5 mm and stored in a closed container at room temperature.

Substrate pretreatment

Dairy whey of about 200 mL was heat treated at 100 °C for 55 min in a hot air oven (Venkata Mohan et al. 2008). Sugarcane bagasse of 10 g was pretreated by acid hydrolysis treatment with 0.5% (v/v) H_2SO_4 (hydrochloric acid) and autoclaved for 120 min at 121 °C (Reddy et al. 2017). After cooling, the substrate was strained through Whatman filter paper under a vacuum. Both the liquid and solid hydrolyzed residues of sugarcane bagasse collected were adjusted to pH 7 by 1N of NaOH and stored at room temperature in an airtight container for further study.

Substrate characterization before and after pretreatment

Before and after pretreatment of substrates such as dairy whey and sugarcane bagasse substrates, the physiochemical characteristics including pH, TS (total solids), COD (chemical oxygen demand), VSS (volatile suspended solids), TSS (total suspended solid), VDS (volatile dissolved solids), phosphates, sodium, nitrogen, calcium, cellulose, hemicellulose, lignin, ash, and moisture content were tested. The COD of the substrates was analyzed by the APHA method (Federation 2012). Carbohydrate content was determined using the sulfuric phenol method (Nielsen et al. 2010) and reducing sugars were quantified by the DNS method (Hu et al. 2008). Protein was determined according to the standard Lowry method (Waterborg 2009) with a UV–Vis spectrophotometer (Thermo scientific Genesys UV–Visible Spectrophotometer) at 660 nm using Bovine Serum Albumin (BSA) as standard. FTIR analysis, to determine functional group properties of a treated and untreated sample of dairy whey and solid and liquid hydrolysate of sugarcane bagasse (LHS) analyzed by FT-IR 460 plus in the range of 4000–500 cm^{-1} wavenumber (Fourier Transform Infrared Spectrometer, Model (JASCO FA/IR-4700). The SEM and EDX were used to determine the chemical identification and element concentration of untreated and pretreated SCB. Zhang and Shen (2006) employed scanning electron microscopy (SEM) to visualize the morphological characteristics of the substrates at different magnifications. Energy dispersive X-ray spectrometer (Bruker-EDX) which provides 30 kV, and X-ray absorption spectroscopy create a difference in energy which results in peak formation.

Factors' effects on microbial bio-H_2 production

To optimize the factors affecting pure- and co-culture-based microbial bio-H_2 production, batch fermentation was performed in a 150 mL glass bottle with 75 mL of working volume. Different operational factors responsible for bio-H_2 production, viz., different pH (6, 6.5, 7, 7.5, 8, and 8.5), temperatures (28 °C, 37 °C, 42 °C, and 50 °C), mono-substrate concentrations (10, 30, 50 and 70 g/L), and co-substrate concentrations (10:5, 30:15, 50:25 and 70:30 g/L), effects on microbial bio-H_2 production were optimized. Dairy whey and sugarcane bagasse were supplemented as a carbon source in a fermentative medium (3 g/L NH_4HCO_3, 0.125 g/L KH_2PO_4, 0.015 g/L $MnSO_4$.$6H_2O$ and 0.200 g/L $MgCl_2.6H_2O$, trace element 0.005 g/L $CuSO_4.5H_2O$, 0.002 g/L $CoCl_2.5H_2O$, 5.37 g/L $NaHCO_3$ and 0.025 g/L $FeSO_4.{}_7H_2O$,) for pure and co-culture microbial fermentation process (Mumtha et al. 2022). Each serum bottle containing fermentation medium was inoculated with 1% of the selected bacterial culture under aseptic conditions. The inoculated amber bottles were blocked with a soft cork and sealed with an aluminium led using the squeezing forceps after the N_2 and CO_2 gas had been fixed for 5 min. The serum bottles were kept in an Orbital Shaking Incubator (Remi Instrument) at 150 rpm for 62 h. H_2 gas production was analyzed by GC-TCD (Gas Chromatography with a Thermal Conductivity Detector Shimadzu GC 2014).

Analytical methods for bio-H_2 production for factor optimization

During factor optimization for bio-H_2 production, the culture growth was monitored by UV–Vis spectrophotometer (Thermo scientific Genesys UV–Visible Spectrophotometer), OD at 660 nm after 64 h. Protein was determined by the Lowry method (Waterborg 2009) using UV–Vis spectrophotometer at 660 nm. Reducing sugars were quantified by DNS assay, and carbohydrate content was determined using the sulfuric phenol method. VFAs (volatile fatty acids) were analyzed by (GC-FID /Shimadzu GC 2014) and the samples were centrifuged at 5000 rpm at 5 °C. The collected supernatant was filtered through a 0.2 mm membrane filter (2% H_3PO_4 80/100 mesh and capillary column coated with

10% PEG-20 M). Detector, injection port, and programmed column temperatures were 130–175 °C, 220 °C, and 240 °C, respectively. Nitrogen gas with a flow rate of 20 mL min^{-1} was used as carrier gas. The gasses H_2 and CO_2 were analyzed by gas chromatography (Shimadzu GC 2014) using a thermal conductivity detector (GC-TCD). Propak Q tube (80/100 mesh) was employed as the packing material and nitrogen gas acted as the carrier gas with an oven, injection port, and detector in the temperature range of 150 °C, 100 °C, and 80 °C. A volume of approximately 1 ml of bio-H_2 was manually injected.

Maximum H_2 production rates were estimated from cumulative H_2 production using the modified Gompertz equation.

$$H_{(t)} = P.\exp\left(-\exp\frac{R_m.e(\lambda - t) + 1}{P}\right)$$

where, cumulative H_2 production (mL) H (t), H_2 production potential (mL) P, maximum H_2 production rate (mL/h) Rm, $e = 2.71828$, lag-phase (h) and t time (h).

Results and discussion

Physicochemical characteristics of substrates

Physicochemical characteristics of substrates employed for bio-H_2 production were investigated. In dairy whey (DW), pH was found to be alkaline (9.4 ± 0.3), this may be due to the use of detergent for washing, and sugarcane bagasse (SCB) showed an acidic pH of 5.6 ± 0.1, consistent with the data published by Webber III et al. (2017). Dairy whey is rich in organic content and useful for the production of bio-H_2 which can be converted to electrical energy (Murugan et al. 2021). COD tests determine the amount of O_2 required for oxidizing organic substances with a strong chemical oxidant. The chemical oxygen demand (COD) of DW (908 ± 0.7 g/L) was higher than the value obtained for the SCB (1008 ± 2.9 g/L), respectively. Meanwhile, nitrogen content was measured by total nitrogen (TN) and soluble protein. The total nitrogen (TN) of the substrate was 0.73 ± 0.06 g/L which is less in DW compared to the TN acquired for the SCB. Furthermore, the SCB soluble protein value was higher than the DW value. Kumari and Das (2017) reported an ash and moisture content of SCB of 2.0 ± 0.7 g/L and 6.7 ± 0.5 g/L, respectively, comparable to the ash and moisture content values of SCB in the present study. VSS (volatile suspended solids) and SS (suspended solids) were estimated to determine wastewater strength and treatment ability. In the present research, TDS (total dissolved solid) and TSS (total suspended solid) of dairy whey were found to be 145 ± 0.3 g/L and 2.2 ± 0.02 g/L, respectively. For rice husk, pine wood, and palm fruit bunch, the maximum sugar concentrations were obtained at the severity factors of 1.96, 1.74, and 1.76, respectively (Gonzales et al. 2016). For the SCB, the values of TS (Total solid) and VS (volatile solid) were 50.6 ± 0.2 g/L and 97.21 ± 1.2 g/L, respectively, which correspond to the values reported by Talha et al. (2016). Trace elements and micronutrients were also analyzed in the DW and SCB. Table 1 shows two of the samples had high Mg content of 0.12 ± 0.001 g/L and 41 ± 0.01 g/L. It has also been found that soluble inorganic salts, such as calcium (Ca) and sodium (Na) soluble in the liquid product, were higher in the DW sample (48.9 ± 0.09 and 3.2 ± 0.02 g/L). Only calcium showed a higher concentration in the DW (48.9 ± 0.09 g/L) compared to the SCB sample.

In particular, most of the inorganic elements in DW are higher than that of SCB. The trace elements such as iron (Fe) were found to be higher in DW (3.00 ± 0.07 g/L), while Fe in SCB had a value of 0.45 ± 0.05 g/L. Iron limitation somehow inhibited the formation of ethanol. It is possible that the alcohol dehydrogenases involved are iron dependent (Zhang and Shen 2006). Trace elements and macronutrients play an essential role in biological H_2 production although they are required in minimal amounts by some co-enzymes and enzymes for their action (Zhang et al. 2013). The results indicate that DW and SCB have higher nitrogen content while SCB has higher carbon content, so their complementary properties can provide appropriate co-degradation in carbon and optimal.

Functional group characteristics of treated and untreated substrates by FT-IR analysis

To quantify the functional group present in the main component in an untreated and treated dairy whey, FT-IR spectroscopy was performed (Fig. 1). The strong and broad peak intensity attained at 3326 cm^{-1}, attributed to O–H stretching either by the presence of hydroxyl group or carbohydrates that indicate the presence of lipids, proteins, and carbohydrates. (Ekka and Mierin 2022) similarly observed similar kinds of peaks viz., a strong and broad peak within the range of 3450–3285 cm^{-1}, associated with carbohydrates, proteins, and lipids. The peaks that belong to carbohydrates and proteins in dairy whey after heat and ultrasonication treatment for 55 min achieved a high intensity rather than untreated dairy whey. The strong peak at 2504 cm^{-1} is assigned to the carbon dioxide O=C=O stretching group. The CO_2 was soluble, as a result peak intensity disappears after heat treatment. The O=C=O stretching group at 669 and 2600 cm^{-1} could be due to the presence of CO_2 bands (Tan and Lebron 2012). After pretreatment, the strong peak in the peptide C=O bond on protein-relevant peaks was observed at 1633 cm^{-1}. The peak at this wavelength indicates whether

Table 1 Physiochemical characteristics of various substrates of the present study compared with an earlier study

S. No.	Parameter	Value of present study		Values of earlier study report		References
		DW	SCB	DW	SCB	Hassan and Zohri (2019), Oztekin et al. (1993), Zhang et al. (2013)
1	pH	9.4 ± 0.3	5.6 + 0.1	9.5	5.1	Ahmed et al. (2022)
2	Chemical oxygen demand (COD)	908 ± 0.7	1008 ± 0.9	48–1026	–	Pandey and Wang (2019), Talha et al. (2016)
3	Total solids (TS)	2.7 ± 0.04	50.6 ± 0.2	2.805	51.3	Emerald et al. (2012), Queiroz et al. (2020)
4	Total dissolved solids (TDS)	145 ± 0.3	ND	130–8469	–	Miito et al. (2021)
5	Total suspended solids (TSS)	2.2 ± 0.02	ND	2.5	–	Jihen et al. (2015), Kumari et al. (2019), Patil et al. (2016)
6	Volatile solids	43.8 ± 0.5	97.21 ± 1.2	20.5–1079	97.91	Chakraborty et al. (2018), Mehrotra et al. (2016)
7	Calcium	48.9 ± 0.09	0.63 ± 0.05	55–115	0.7–2.37	Anukam et al. (2016), Kumari et al. (2019), Mehrotra et al. (2016)
8	Nitrogen	0.73 ± 0.06	1.2 ± 0.03	1–180	0.29–1.72	Anukam et al. (2016), Halder et al. (2020)
9	Carbon	34.6 ± 0.7	40.1 ± 0.23	301.71	47.62	Kumari et al. (2019), Patil et al. (2016)
10	Protein	3.4 ± 0.6	3.8 ± 0.1	3.85	2–46	Kumari et al. (2019), Patil et al. (2016)
11	Total sugar	1.8 ± 0.9	2.6 ± 0.4	1.68	1–50	Ethaib et al. (2017)
12	Cellulose	ND	40.1 ± 0.03	–	40.79	Ethaib et al. (2017)
13	Hemicellulose	ND	20.6 ± 0.3	–	22.32	Kumari et al. (2019)
14	Lignin	ND	16.3 ± 0.4	–	17.4	Kumari et al. (2019)
15	Moisture content	ND	6.7 ± 0.5	–	6.83	Kumari et al. (2019)
16	Ash content	ND	2.01 ± 0.7	–	0.41–2.09	Mehrotra et al. (2016), Morán et al. (2008)
17	Sodium	3.2 ± 0.02	1.3 ± 0.06	60–810	0.97	Mehrotra et al. (2016)
18	Phosphorus	52.3 ± 0.8	21.2 ± 0.2	9–210	–	Morán et al. (2008)
19	Iron	3.00 ± 0.07	0.45 ± 0.05	3	–	Morán et al. (2008)
20	Magnesium	0.12 ± 0.001	0.41 ± 0.01	0.08–70	–	Hassan and Zohri (2019), Oztekin et al. (1993), Zhang et al. (2013)

Mean ± S.E/all the parameters are in mg/L^{-1}except pH

DW dairy whey, *SCB* sugarcane bagasse

the peak decreased or increased before and after pretreatment about protein degradation, and it confirms the presence of protein in dairy effluent (Tang et al. 2017). The band at 602 cm^{-1} is assigned to the C=C bending alkene group that disappears after heat treatment.

The chemical structure of biomass and acid hydrolysis was determined using FT-IR analysis. In this study, the FT-IR spectra of the untreated sugar cane bagasse (SCB), and solid and liquid hydrolysis-treated SCB show a wide change in their functional groups (Fig. 1). The band assigned at 4000–2995 cm^{-1} was determined as cellulose (Morán et al. 2008). The peak at 3427 cm^{-1} represents the OH group and indicates crystalline cellulose presence in both treated and untreated samples. In liquid hydrolysis, the peak that appears at 3305 cm^{-1} corresponds to the hydroxyl (OH) group, indicating that the cellulose is solubilized after acid hydrolysis treatment. The absorption at 2920 and 2911 cm^{-1} was related to –CH stretching of methyl and methylene group that shows a great difference in their peak intensity for solid and liquid hydrolysis. In both solid hydrolysis and liquid hydrolysis, the peak intensity of 1623 cm^{-1} was assigned to C–H bond deformations, and aromatic ring vibration was associated with the lignin. The intense peak observed at 1558 cm-1 is assigned to the stretching of carbonyl groups (1728 cm^{-1}) and vibration of aromatic rings (1600,1635,1510 cm^{-1}); all of these contain lignin compounds (Moretti et al. 2014). The bands at 1462 cm^{-1} and 1375 cm^{-1} correspond to the symmetric CH_2 bending (Cao and Tan 2004). Treated and untreated SCB shows a similar peak intensity at 1299 cm^{-1} assigned to be C–O ether group. Solid hydrolysis indicates that the peak intensity recorded at 1162 cm^{-1} corresponds to the vibration of the ring C–O–C in hemicellulose (Ju et al. 2011). The acid hydrolysis treatment affected the cellulose and hemicellulose solubility, the peak intensity at 1025 cm^{-1} was associated with C–O stretching. The stronger band at 897 cm^{-1} was observed in acid hydrolysis treatment, which is present in the cellulose II or amorphous cellulose form, characterized by the C–O–C stretching at the β-1,4-glycosidic linkage. A lignocellulose

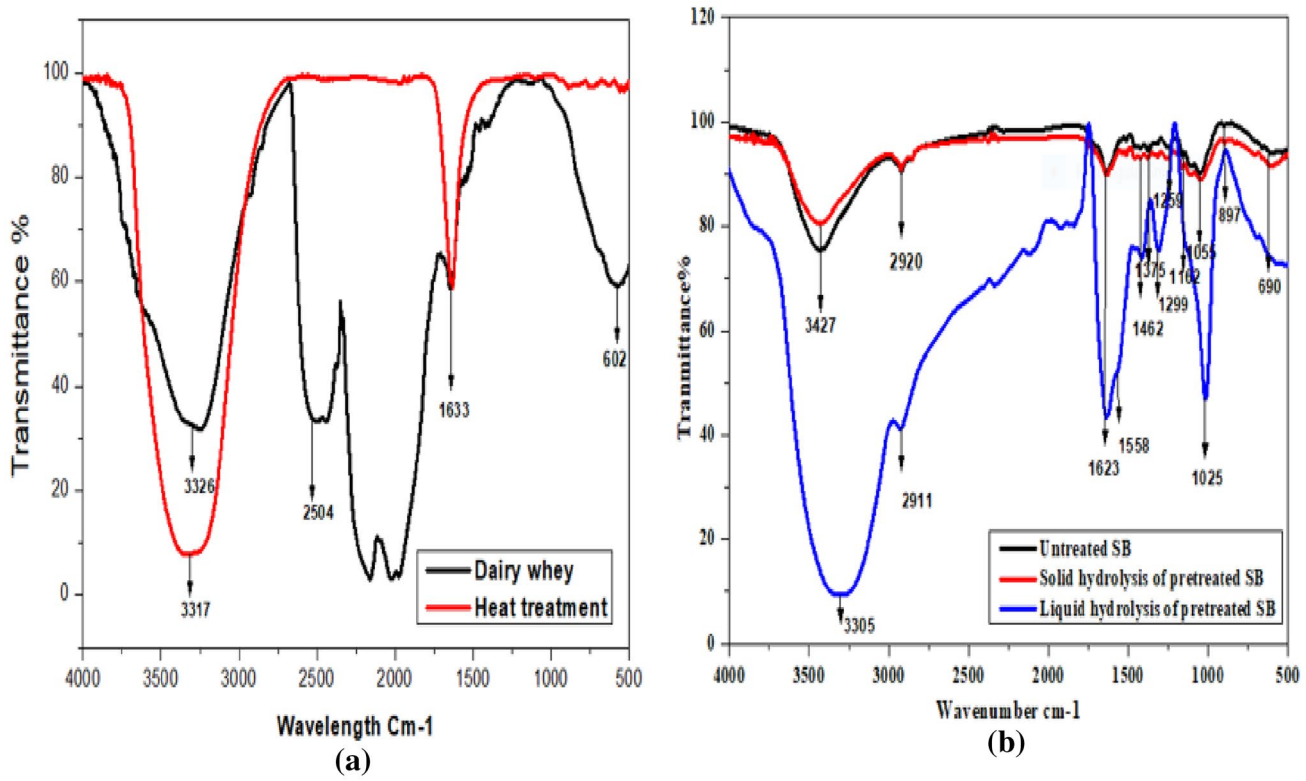

Fig. 1 Functional group characteristics by FT-IR analysis. **a** Treated and untreated dairy whey, **b** treated and untreated sugarcane bagasse

matrix is confirmed by the presence of these bands in the SCB, due to its content of cellulose, hemicellulose, and lignin (Naik et al. 2010).

SEM and EDAX analysis

SEM study of treated and untreated sugarcane bagasse showed that pretreatment caused physical changes to the biomass. Significant morphological differences were observed between the native SCB and acid hydrolysispretreated SCB. The untreated sugarcane bagasse has a smooth and continuous surface, whereas 120 min of acid hydrolysis pretreated SCB showed that some fibers detached, indicating the presence of SCB cellulose, hemicellulose, and lignin components. Acid hydrolysis treatment at 120 min, which partially removed lignin and hemicellulose, showed unstructured SCB formation. Elemental analyses were performed simultaneously on SCB. The EDX results indicated that untreated SCB contained carbon (30%), oxygen (13.71%), and nitrogen (0.5%) as dominant compositions. Since sulfuric acid can be employed in the hydrolysis process, the sample contains sulfur following pretreatment with acid hydrolysis. This interpretation agrees with the information provided by Laluce et al. (2019). Carbon (69.42%), oxygen (36.06%), and nitrogen (3.12%) percentages were increased during acid hydrolysis pretreatment of SCB.

Heat treatment of dairy whey changes the biological, chemical, and physical properties (Fig. 2). Heat-treated dairy whey exhibits greater reactions due to an increase in reducing sugars (lactose is broken down into simple sugars like glucose and galactose) (Harju et al. 2012; Chen 2023). Acid hydrolysis treatment converts complex carbohydrates into a substrate for fermentation. (Karimi-Maleh et al. 2023). Hydrogen production can be enhanced by efficient pretreatment methods to produce more simple sugars (Wu et al. 2003). To promote hydrogen production from substrates, the optimal treatment method is essential for an inexpensive and environmentally friendly strategy (Sillero et al. 2023). Pretreatment using thermochemistry and enzymes has some limitations, such as disposal problems, and energy-consuming and time-consuming processes; therefore, unconventional methods such as microwaves, ionizing irradiation, electroporation, IR radiation, ultrasound, etc., need to be further investigated (Bhurat et al. 2023).

Effect of various factors on microbial bio-H_2 production

The variation in H_2 production differs based on the microbial strains, substance concentrations, pH, and temperature (Zhang et al. 2003; Xiao et al. 2013; Li et al. 2020). In most cases, the optimal pH for hydrogen production lies between

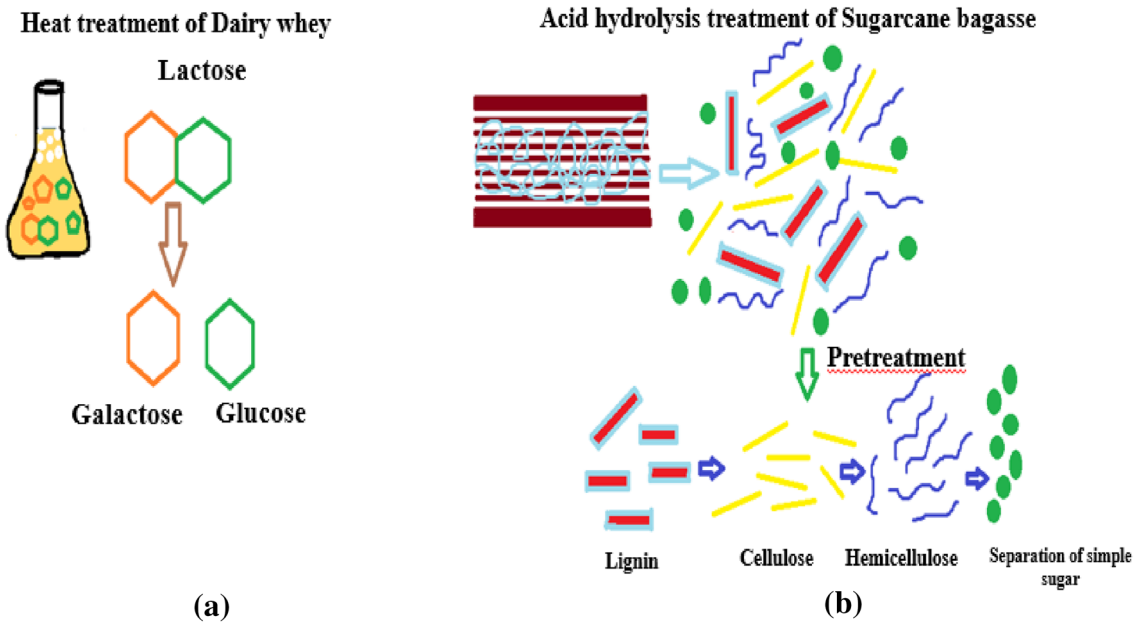

Fig. 2 Catalysis reaction of treated biomass. **a** Heat treatment of dairy whey lactose and **b** acid hydrolysis treatment of sugarcane bagasse

6.0 and 8.0, and the fermentative pathway is supported in slightly acidic conditions to achieve the best bacterial growth (Sinha and Pandey 2011; Alexandropoulou et al. 2020). Numerous studies have reported that the optimum temperature for H_2 production depends on the bacteria and substrate (Wang and Wan 2008a). Substrate concentration increases H_2 production by providing enough organics for microorganisms and promoting bacterial growth (Lay and Chang 2012). Hydrogen production was inhibited by further increasing the substrate concentration, as less H_2 was collected, and similar studies were reported (Leo et al. 2008; Pascualone et al. 2019). The optimum co-digestion ratio of carbohydrates and proteins achieved the maximum hydrogen yield, production rate, and process stability (Xue et al. 2019). In this study, pH, temperature, and substrate concentration played a significant role in optimizing the process.

Effect of pH

The critical factor, the pH of the fermentative medium, was one of the most important parameters that affects the production of bio-H_2. The extreme pH range inhibits the activity of Fe-hydrogenase which leads to a reduction in hydrogen production (Ahmed et al. 2022; Husna et al. 2022). The optimal pH of the maximal bio-H_2 production depends on the microbial activity and substrates used for fermentation. The impact of pH on bacterial growth and bio-H_2 production from various substrates including dairy whey (DW), solid and liquid hydrolysis of pretreated sugarcane bagasse (SH/LH-PSCB), and co-substrate (DW and SH-PSCB) by pure culture *E. coli*, *S. bongori*, *S. oneidensis* and co-culture of these strains was studied by testing at pH of 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5. Bio-H_2-producing bacteria utilize substrate carbon and protein as a major nutrient source for their growth and H_2 yield (Jarunglumlert et al. 2018; Hassan and Zohri 2019; Karimi-Maleh et al. 2023). Hence, to research the impact of pH on bio-H_2 production, changes in carbohydrate, protein, and reducing sugar contents present in the substrates were analyzed and cumulative H_2 productions were recorded, as shown in Figs. 3 and 4.

S. bongori and *S. oneidensis* utilize substrate carbon, protein, and reducing sugar effectively except *E. coli* that produces a maximum H_2 at pH 7.0. The strain *E. coli* utilizes carbon and protein effectively at pH 7.5 but shows an increase in reducing sugars (Fig. 4) due to the liberation of simple sugars on digestion of complex carbon of substrate. This leads to less H_2 yield from dairy whey at pH 7.5 by *E. coli* (Fig. 4), due to the effect of metabolite products formed during the digestion of carbon and protein at a high rate. SCB is a lignocellulose material rich in hemicellulose and cellulose that can be hydrolyzed and fermented by microbes (Fangkum and Reungsang 2011; Bu et al. 2021). *S. bongori* at pH 7.5 shows high substrate utilization whereas other strains utilize highly at pH 7.0 with high yield. The substrate LH-PSCB utilization widely differs with the pH of the fermentation medium. *E. coli* and *S. bongori* utilize maximum protein at pH 7.5 and reducing sugar at pH 7.0, while, carbon utilization by *E. coli* was high at pH 6.5 whereas *S. bongori* shows maximum utilization at pH 7.0. *S. oneidensis* utilizes carbon, reducing sugar, and protein at a high rate at pH 7.5.

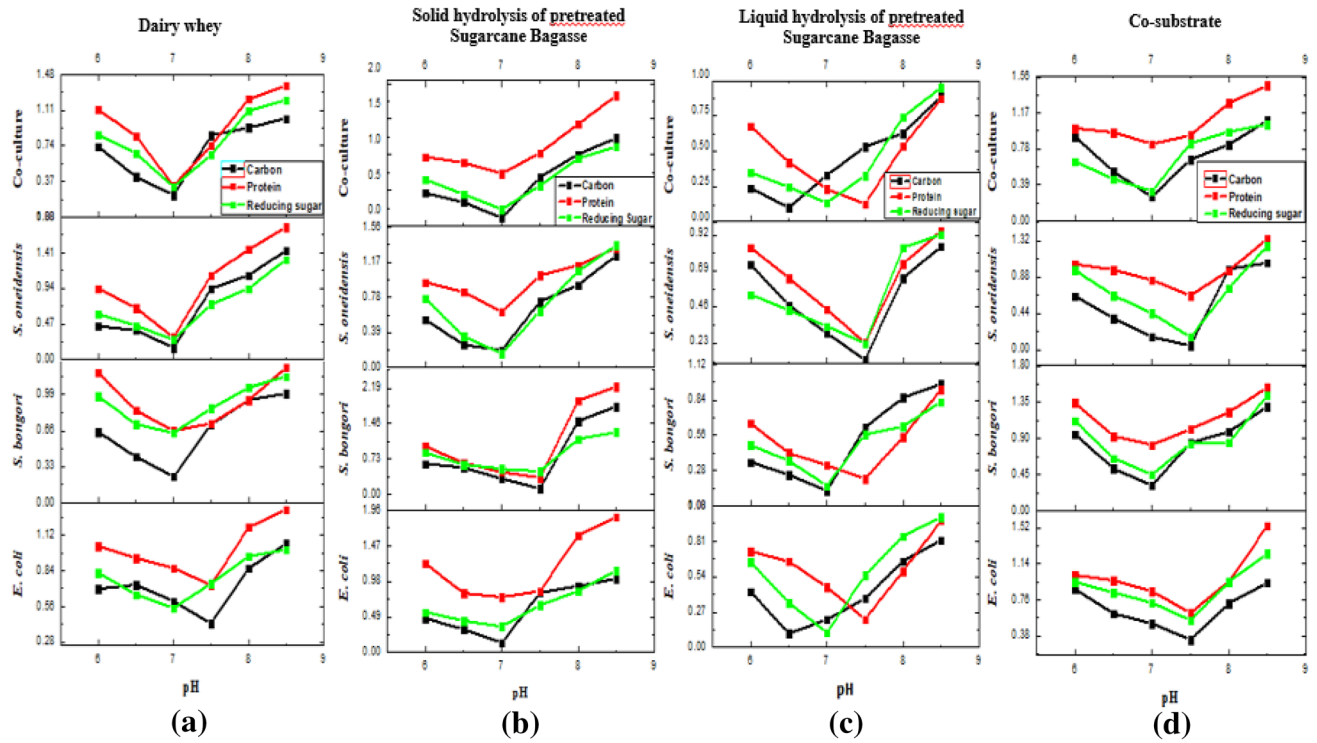

Fig. 3 Bio-H_2 production in pure and co-culture systems was tested at different pH for carbon, protein, and sugar utilized on various substrates: **a** dairy whey, **b** soil hydrolysis of pretreated sugarcane bagasse, **c** liquid hydrolysis of pretreated sugarcane bagasse, **d** co-substrate

In the case of co-culture, the carbon, reducing sugar, and protein utilization vary in the pH range of 6.5, 7.0, and 7.5. *E. coli* and *S. oneidensis* utilize maximum co-substrate at pH 7.5 while *S. bongori* and co-culture show maximum utilization at pH 7.0. At pH 7.0, the microbial biomass was at a high rate with an increase in substrate consumption (Ghimire et al. 2015; Rao and Basak 2021).

Performing bio-H_2 production from various substrates with pure culture at different pH shows that *E. coli*, *S. bongori,* and *S. oneidensis* reached a maximum H_2 production of 108.5 ± 4.81 H_2/mL/L, 88.33 ± 2.1 H_2/mL/L, and 142 ± 2.4 H_2/mL/L, respectively, at pH 7.0 for dairy whey (Fig. 4a). Saratale et al. (2018) used a pure culture of *E. coli* to produce bio-H_2 from wet SCB biomass with an average production rate of 1000 pL H_2 CFU1 day. The solid hydrolysate of acid-pretreated sugarcane bagasse generated bio-H_2 under batch fermentation with a pure culture of *E. coli* and *S. oneidensis*, and the H_2 production at pH 7.0 was 135.5 ± 1.7 H_2/mL/L and 159.1 ± 1.5 H_2/mL/L, respectively. The maximum production of bio-H_2 from SH-PSCB was achieved by *S. bongori*, 178.3 ± 2.5 H_2/mL/L at pH 7.5 with high substrate utilization. Ramprakash and Muthukumar (2018) studied bio-H_2 production from 1.5% acid-hydrolyzed-treated rice mill wastewater using co-culture and pure culture of *Citrobacter freundii* and *Enterobacter aerogenes*, the maximum H_2 content of 1.61 mol/mol sugar yield was achieved in mixed culture. In this study, H_2 production from liquid hydrolysate of acid-pretreated SCB was maximum at pH 7.5 with a pure culture of *E. coli*, *S. bongori,* and *S. oneidensis*—144.2 ± 2.3 H_2/mL/L, 145.1 ± 1 H_2/mL/L and 155.4 ± 2.7 H_2/mL/L respectively. Co-substrate of DW and SH-PSCB revealed that the generation of H_2 by a pure culture of *E. coli and S. bongori* was 169.4 ± 1.6 H_2/mL/L and 149.6 ± 2.2 H_2/mL/L and was maximum at pH 7.0. *S. oneidensis* H_2 production was 158.1 ± 0.6 H_2/mL/L from co-substrate at pH 7.5 (Fig. 5a). In an anaerobic baffled reactor, dark fermentation (DF) is used to generate biohydrogen from brewery effluents. Operating parameters included temperature, pH, and retention time. The maximum HPR was obtained at 18.16 mL/h, and the lowest HPR was 0.94 mL/h for 35 °C at pH 5–2.5, respectively (Mutsvene et al. 2023).

The pure and co-culture of bacterial strains obtained high-efficient H_2 yield at pH 7.0 while using potential substrate dairy whey and solid hydrolysis of pretreated sugarcane bagasse as a co-substrate in the batch fermentation. Since *S. bongori* and *E. coli* effectively utilize dairy whey and solid hydrolysis of pretreated sugarcane bagasse for H_2 production, batch fermentation with co-substrate by co-culture at different pH 7 shows maximum H_2 yield of 1.9 $H_2/gVs_{(removal)}$ rather than the mono-substrate (Fig. 4b). (Karimi-Maleh et al. 2023) (author reported that *Pseudomonas resinovorans* were used to produce polyhydroxyalkanoate (PHA)

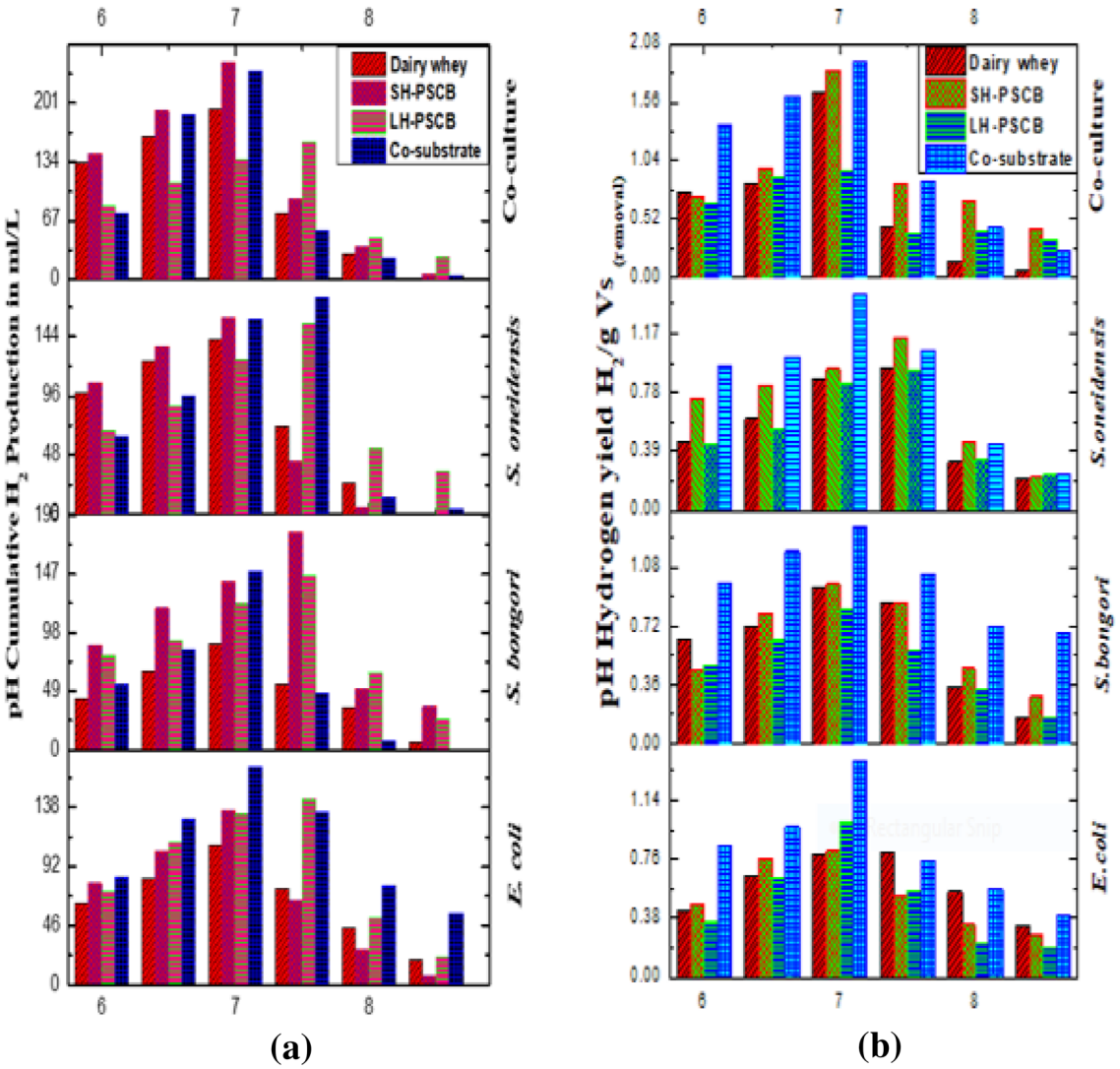

Fig. 4 Effect of biohydrogen production from various substrates by pure and co-culture at different pH: **a** cumulative H_2 production and **b** H_2 yield

from spent coffee grounds (SCGs). A *Clostridium butyricum* DSM10702 batch experiment was conducted to produce biohydrogen from oil-extracted SCGs (OESCGs). The maximum H_2 yield was obtained at 2.37 H_2 yield (mol-H_2/mol-consumed or added sugar). Liu et al. (2011) and Cai et al. (2021) also reported that the maximum HPR by *Clostridium sp*. was obtained at an optimal pH range of 6.0–8.2. Balakrishnan et al. (2022) and Mohammadi and Mohammadi (2022) investigated the H_2 production from sucrose and glucose at different pH 5.5–8.0, while Sinbuathong and Sillapacharoenkul (2021) reported that pH 7.0 was suitable for H_2 production from starch factory waste water. The present study found that the H_2 production from dairy whey and pretreated sugarcane bagasse yielded maximum H_2 at pH 7.0 and pH 7.5.

Bio-H_2 production from DW with co-culture fermentation compared to the pure culture at pH 7.0 yielded the highest cumulative bio-H_2 production of 194.3 ± 1.7 H_2/mL/L. Bio-H_2 production with alkaline pretreated sugarcane bagasse (SCB) using co-culture of *Clostridium thermocellum* and *Thermoanaerobacterium aotearoense* produces maximum bio-H_2 of 48.02 ± 2.37 mmol/L (Cheng and Zhu 2016). Generation of bio-H_2 from the solid hydrolysate of acid-pretreated SCB by co-culture of *E. coli, S. bongori, and S. oneidensis* produces the greatest cumulative H_2 production of 187 ± 1.6 H_2/mL/L at pH 7. Fermentation by co-culture yields an H_2 of 161.5 ± 1.9 H_2/mL/L from LH-PSCB at pH 7.5. Co-substrate fermentation with co-culture yields a maximum H_2 production of 206 ± 1.5 H_2/mL/L at pH 7 (Fig. 4a).

Dursun and Gülşen (2021) reported that these bacterial strains *Enterobacteria*, *Salmonella bongori*, *Erwinia*

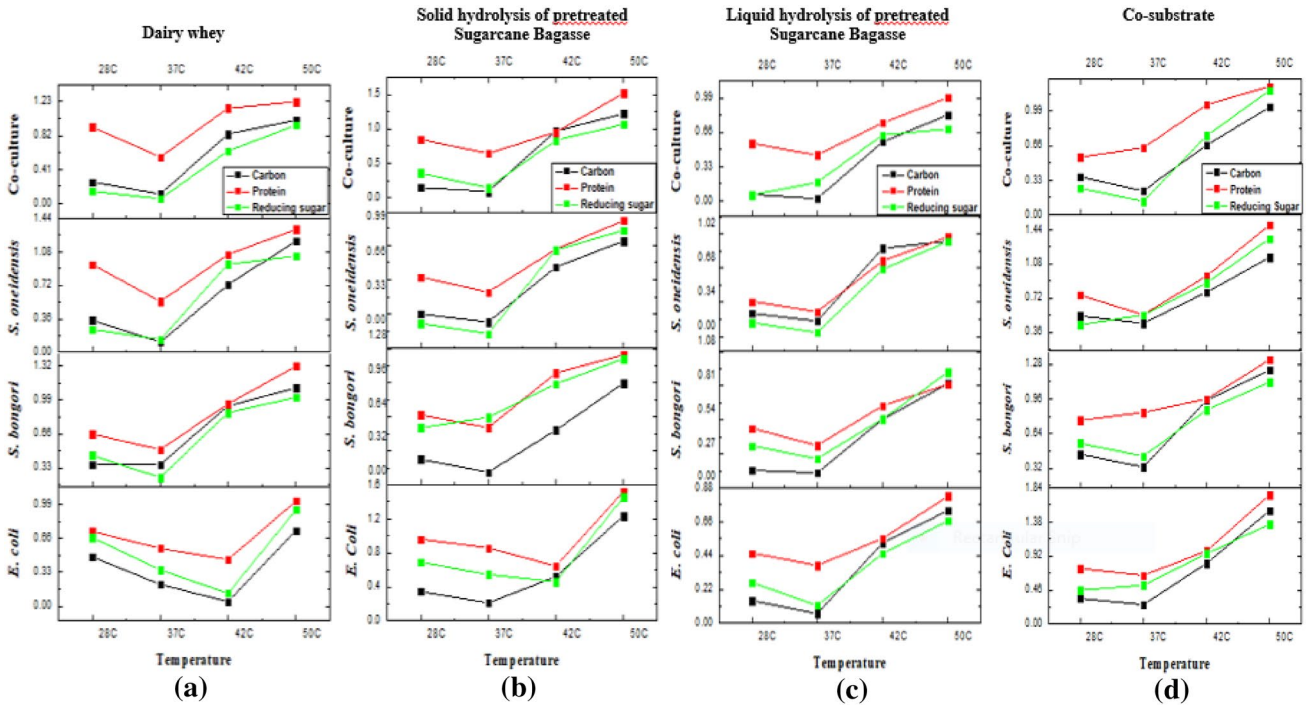

Fig. 5 Bio-H_2 production in pure and co-culture systems was tested at different temperatures for carbon, protein and sugar utilized on various substrates: **a** dairy whey, **b** soil hydrolysis of pretreated sugarcane bagasse, **c** liquid hydrolysis of pretreated sugarcane bagasse, **d** co-substrate

amylovora, *Brenneria goodwinii*, *Sulfurospirillum* sp., *Thiofractor thiocaminus*, and *Hydrogenimonas thermophile* in completely fluidized bed reactor and stirred tank reactor produce maximum H_2 of 25.3 and 11.1 mL/day at pH 5. Fermentation medium with low pH inhibits methanogens and improves H_2 gas production. The optimal pH range for *E. coli* growth was between pH 6 and 7. *E. coli* H_2 production is high at pH 5.5–6, and further increases in pH may decrease H_2 production. H_2 production by *E. coli* is increased at pH 5.5–6 whereas a further increase in pH can reduce H_2 production (Fang and Liu 2002; Zhang 2021). *Salmonella* survives well in a pH range of pH 3.8 to 9.5 with an optimal pH range of 6.5–7.5 at 37 °C; however, growth was recorded between 2 and 4 °C and at 54 °C (Kotzekidou 2013; Ryan et al. 2017). The optimal pH of 6.8–7.0 was closely related to the growing conditions of *Shewanella* sp. (Kim et al. 2002; Biffinger et al. 2007).

Effect of temperature

Temperature significantly affected the bio-H_2-producing microorganisms and their H_2 production rates. Dark fermentation for bio-H_2 production could occur at various temperatures which range from thermophilic (40–65 °C), mesophilic (25–40 °C), and hyperthermophilic (65–80 °C) (Levin et al. 2004; Toledo-Cervantes et al. 2020). In the current study, bio-H_2 production was tested at a wide range of temperatures including 28 ℃, 37 ℃, 42 ℃, and 50 ℃ for various bacterial strains, such as *E. coli*, *S. bongori*, *S. oneidensis*, and Co-culture. Hence, to research the impact of temperature on bio-H_2 production, changes in carbon, protein, and reducing sugar were analyzed and calculated results of cumulative H_2 productions were recorded as shown in Figs. 5 and 6.

Three selected bio-H_2-producing strains such as *S. bongori*, *E. coli*, and *S. oneidensis* highly consume substrate carbon, protein, and reducing sugar, at 37 ℃ with maximum H_2 production. *S. bongori* at 50 °C degrades protein and carbon at low rates and produces less H_2 from dairy due to the metabolites formed during degradation (Fig. 5). A batch experiment was conducted under mesophilic conditions, and all the trial tests had reduced volatile solids and total solids, and substrate utilization increased (Sillero et al. 2023). At optimal temperature, the growth of *E. coli* and the production of H_2 were at a maximum rate. Above 42 °C, the growth of *E. coli* becomes slower and its production decreases due to the sudden decrease in enzyme activity in the cell (Bakonyi et al. 2012). *S. bongori* and *S. oneidensis* at a temperature of 37 °C show high utilization of SCB, whereas other strains utilize highly at a temperature of 28 °C with a higher HY. LH-PSCB substrate utilization varies widely depending on the temperature of the fermentation medium. *S. oneidensis* utilizes maximum protein at 37 °C and reducing sugar at 28 °C, carbon utilization by *E. coli* was high at 37 °C whereas *S. oneidensis* shows maximum utilization at

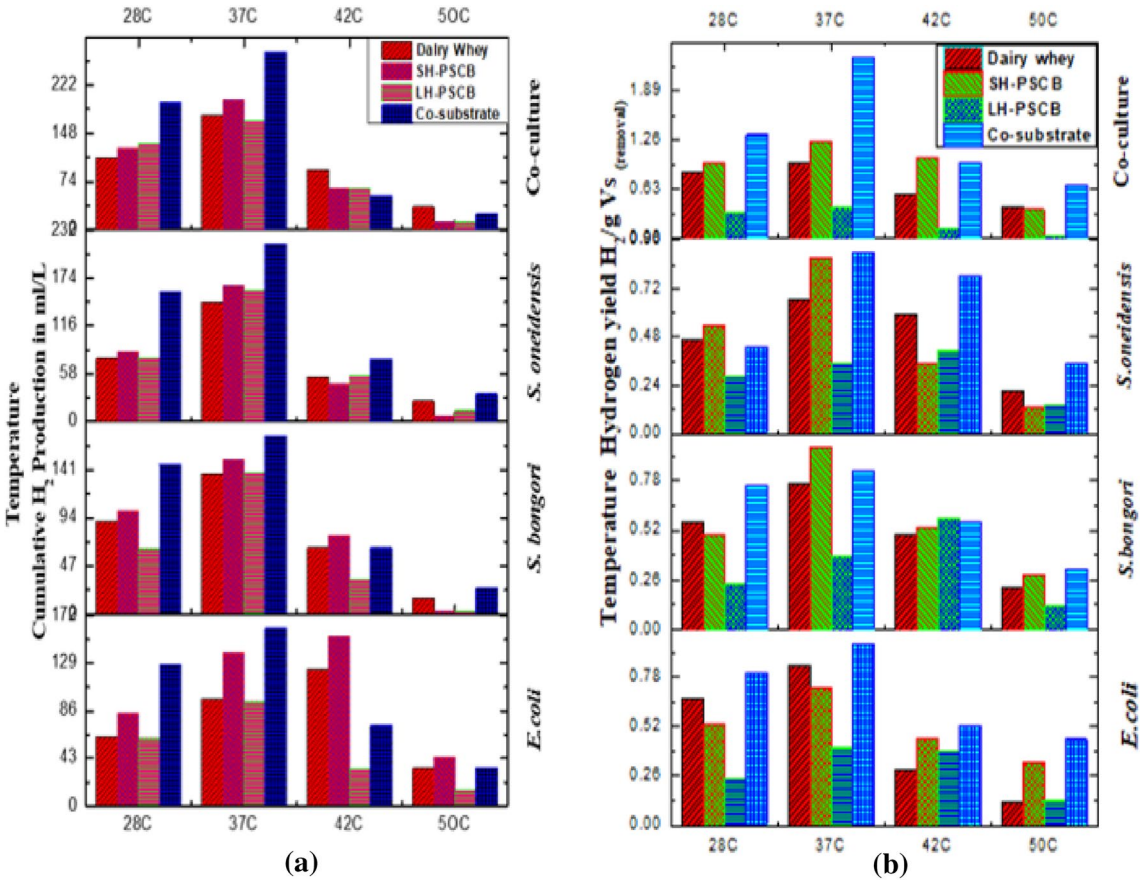

Fig. 6 Effect of biohydrogen production from various substrates by pure and co-culture at different temperatures: **a** cumulative H_2 production and **b** H_2 yield

37 °C. In the case of co-culture, the carbon, reducing sugar, and protein utilization vary at a temperature range of 28 °C, 37 °C, and 42 °C. Co-culture shows maximum utilization at a temperature of 37 °C while *E. coli* utilizes minimum co-substrate at a temperature of 42 °C (Fig. 5).

Based on the bio-H_2 production activity from DW by the pure culture at different temperatures, it is observed that *E. coli*, *S. bongori*, and *S. oneidensis* reached a maximum H_2 production of 96.7 ± 1.4 H_2/mL/L, 136.7 ± 1.4 H_2/mL/L, and 145.0 ± 0.7 H_2/mL/L, respectively, at 37 ℃ in pH 7.0 (Fig. 7a). Mthethwa et al. (2018) achieved maximum hydrogen production by DF (dark fermentation) of lactate f9 wastewater at pH 7.5 and 45 °C with mixed and pure cultures of *Clostridium* sp. and *E. coli*. Bio-H_2 production from DW with co-culture fermentation compared to pure culture yield the highest cumulative H_2 production of 155.7 ± 2.6 H_2/mL/L at 37 ℃. The solid hydrolysate of acid-pretreated sugarcane bagasse generated H_2 under batch fermentation with a pure culture of *E. coli*, *S. bongori*, and *S. oneidensis*—H_2 production at 37 ℃ was 138.7 ± 1.0 H_2/mL/L, 148.7 ± 1.3 H_2/mL/L, and 164 ± 1.7 H_2/mL/L, respectively. The minimum production of bio-H_2 from SH-PSCB by co-cultures was 150 ± 9.9 H_2/mL/L 37 ℃. The maximum bio-H_2 production from liquid hydrolysate of acid-pretreated SCB with pure culture *E. coli*, *S. bongori* and *S. oneidensis* was found to be 94 ± 2.7 H_2/mL/L, 139 ± 0.9 H_2/mL/L, and 158.7 ± 1.3 H_2/mL/L, respectively (Fig. 7a). Genetically modified *E. coli* yields 0.63 mol H_2/mol formate under optimal conditions for pH 6.5, which was 1.5 times more than the wild type (Bakonyi et al. 2012). *E. coli* poses a complex of enzymes hydrogenase (Hyd-3), formate dehydrogenase (FDH-H), and numerous electron transfer mediators. Formate is the only precursor for H_2 production in *E. coli*, formate is converted to CO_2 and H_2 by the enzyme complex FHL (formate hydrogen lyase) (Sawers 2005; Yoshida et al. 2007; Mathews and Wang 2009; Taifor et al. 2017). Fermentation by co-cultures yields an H_2 production of 166 ± 2.7 H_2/mL/L from LH-PSCB at 37 ℃. Bio-H_2 production from co-substrate consisting of DW and SH-PSCB with pure culture revealed that the production of hydrogen by *E. coli* and *S. bongori* was 161 ± 4.8 H_2/mL/L and 174.1 ± 2.7 H_2/mL/L, maximum at the temperature 37 ℃. *S. oneidensis* H_2 production was 215.3 ± 8.3 H_2/mL/L from co-substrate at 37 ℃. Co-substrate fermentation with co-cultures yielded

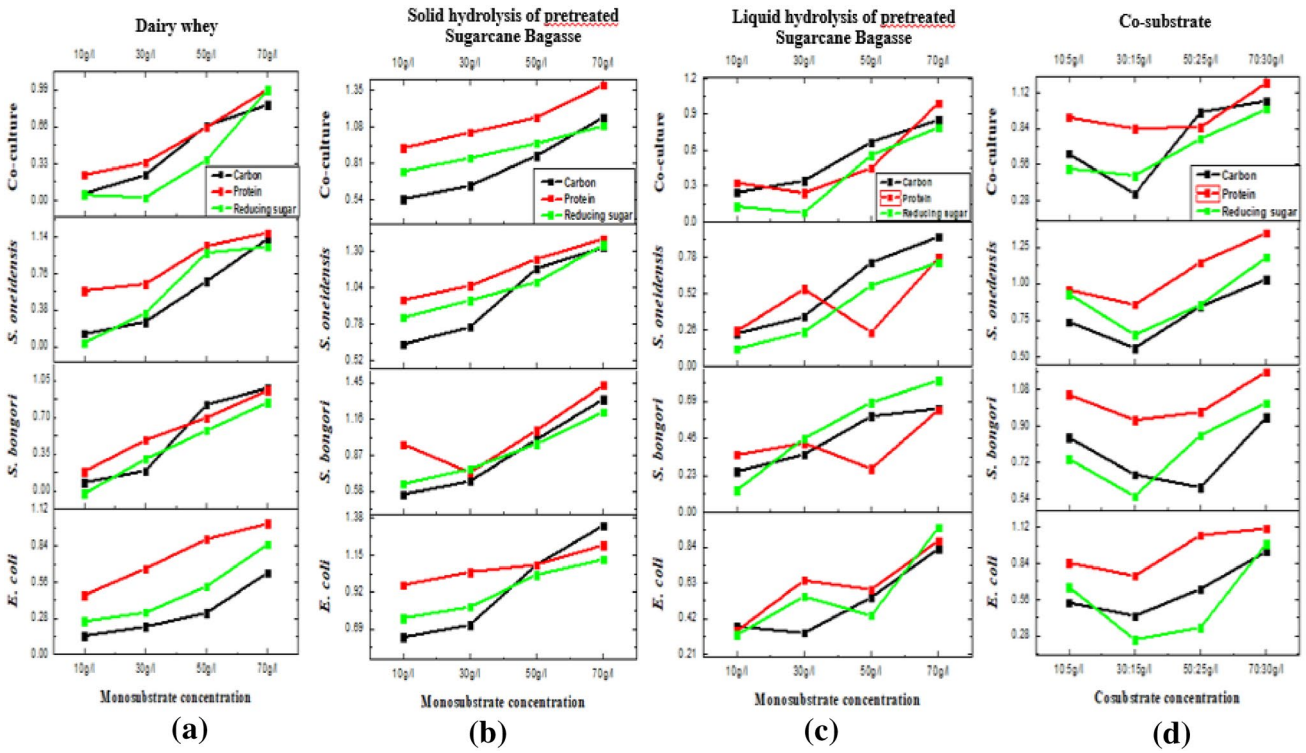

Fig. 7 Bio-H_2 production in pure and co-culture systems was tested at different substrate concentrations for carbon, protein, and sugar utilized on various substrates: **a** dairy whey, **b** soil hydrolysis of pretreated sugarcane bagasse, **c** liquid hydrolysis of pretreated sugarcane bagasse, **d** co-substrate

a maximum production of H_2 of 230.7 ± 2.7 H_2/mL/L at the temperature of 37 ℃ (Fig. 6a). Current research utilized magnetite to enhance the bioactivity of H_2-producing bacteria during DF. A maximum bio-H_2 production was obtained of 73.59 ml/gVS (Gökçek et al. 2023).

The pure and co-culture of bacterial strains obtained high-efficient hydrogen yield at pH 7.0 while using potential substrate dairy whey and solid hydrolysis of pretreated sugarcane bagasse as a co-substrate in the batch fermentation. Since *S. bongori* and *E. coli* effectively utilize dairy whey and solid hydrolysis of pretreated sugarcane bagasse for hydrogen production, batch fermentation with co-substrate by co-culture at different temperatures shows a maximum yield of 2.32 $H_2/gVs_{(removal)}$ at 37 ℃ rather than the mono-substrate (Fig. 7b). Lakshmidevi and Muthukumar (2010) investigated the impact of significant profiles on the performance of the process, such as enzyme dosage, substrate concentration, and acid concentration. Biohydrogen production from pretreated using pure culture at a maximum specific HY of 126 ml/g of VSS day (Lakshmidevi and Muthukumar 2023).

Mono- and co-substrate concentration

Optimizing the organic load has a crucial impact on anaerobic bio-H_2 production (Brindhadevi et al. 2021). Several researchers have revealed that the ideal substrate fixation is expected to assume an essential function in bio-H_2 production (Hawkes et al. 2002; Oceguera-Contreras et al. 2019; Ferraren-De Cagalitan and Abundo 2021; Nagarajan et al. 2021). Besides the characteristics of the substrate, substrate concentration significantly affects microbial activity and metabolic pathways in dark fermentation (Wang and Yin 2018). Bio-H_2 production was tested at different substrate concentrations, including 10, 30, 50, and 70 g/L from various substrates such as dairy whey (DW), solid and liquid hydrolysis of pretreated sugarcane bagasse (SH/LH-PSCB). Co-substrates (DW and SH-PSCB) were mixed in a ratio of 10:5 g/L, 30:15 g/L, 50:35 g/L, and 70:30 g/L and tested for bio-H_2 production. The DW is rich in carbohydrates and can be converted into high energy and gasses like H_2 and methane. In the dairy processing industry, carbon is mainly present in the form of fat, protein, and carbohydrates, which are suitable substrates for bio-H_2 production through the process of dark fermentation.

Wu and Zhou 2012 stated that the H_2 yield was maximum at 10 g/L of substrate concentration, but when it exceeds or drops

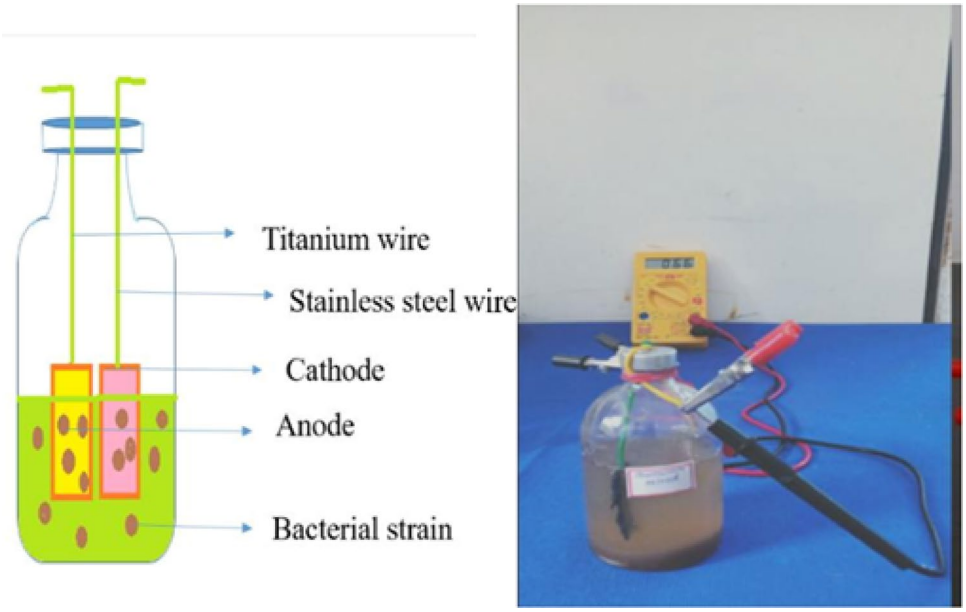

Fig. 8 Model of single-chamber microbial fuel cell

from 10 g/L, H_2 production decreases. The high substrate concentration compared with the optimal concentration leads the microorganisms to overproduce the inhibitors, mainly volatile fatty acids and ethanol, which reduce the H_2 yield (Eker and Sarp 2017). Wang and Wan (2008b) reported that ethanol, propionate, acetate, and butyrate affected H_2 yield in the range of about 0–100 mmol/L. The inhibitory effect increases with the higher metabolite concentration. Undissociated soluble metabolites permeated the cell membrane of H_2-producing microorganisms and disrupt the intracellular physiological balances (Bundhoo and Mohee 2016). The formation of organic acids (e.g., propionate, acetate, lactate, and butyrate) and a decrease in pH disrupt the dynamic equilibrium of NAD^+/NADH reducing the metabolic reaction of fermenting microbes. High substrate concentrations also lead to high H_2 partial pressures in the fermentation medium, leading to low H_2 production (Hawkes et al. 2002). Low substrate concentrations reduce biomass (volatile suspended solids) hydrogen content and influence the activity of fermentative bacteria due to the lack of enough carbon sources. Hence, the substrate concentration needs to be optimized for maximum H_2 production. The H_2 production rate was increased with increasing organic matter loading rate but decreased rapidly when the biomass concentration exceeded the optimized condition (Lee et al. 2014).

In this study, *S. bongori*, *E. coli*, and *S. oneidensis* show high depletion in the substrate carbon, protein, and reducing sugar at substrate concentration of 30 g/L of dairy whey but generated an excellent H_2 production. *E. coli* and co-culture at a substrate concentration of 30 g/L show high utilization in SCB, while bio-H_2 production by *S. bongori* shows a maximum utilization at 10 g/L substrate concentration. The amount of substrate utilized was significantly affected by the LH-PSCB at different substrate concentrations. *S. oneidensis* utilizes maximum protein, carbon, and, reducing sugar at a substrate concentration of 10 g/L, carbon utilization by *E. coli* was high at a substrate concentration of 50 g/L, whereas *S. bongori* shows low utilization at a substrate concentration of 70 g/L. In LH-PSCB co-culture, carbon, reducing sugar, and protein were consumed less at substrate concentrations of 10 g/L, 30 g/L, 50 g/L, and 70 g/L. Reducing sugar utilization by *E. coli* was high at a substrate concentration of 30:15 g/L whereas *S. oneidensis* shows low utilization at a substrate concentration of 50:25 g/L (Fig. 7).

Performing bio-H_2 production from DW with pure culture at different temperatures shows that *E. coli S. bongori*, and *S. oneidensis* reached a maximum H_2 production of 122.7 ± 1.7 H_2/mL/L, 54.4 ± 2.4 H_2/mL/L, and 195.7 ± 1 H_2/mL/L, respectively, at substrate concentration of 50 g/L. Bio-H_2 production from dairy whey with co-culture fermentation, compared to the pure culture, at pH 7.0 was highest, yielding a cumulative H_2 production of 202.7 ± 5.5 H_2/mL/L. The solid hydrolysate of acid-pretreated sugarcane bagasse under batch fermentation with pure culture *E. coli and S. oneidensis* yielded a maximum H_2 production of 123.7 ± 2.8 H_2/mL/L and 137.3 ± 1.6 H_2/mL/L, respectively. The maximum production of H_2 from SH-PSCB by *S. bongori* was 148 ± 0.9 ml/L at a substrate concentration of 30 g/L. Generation of bio-H_2 from the solid hydrolysate of acid-pretreated sugarcane bagasse by co-culture of *E. coli*, *S. bongori*, and *S. oneidensis* produces the highest cumulative H_2 production of 237.3 ± 6.0 H_2/mL/L at substrate concentration 30 g/L (Fig. 8a). Lu et al. (2018) achieved the highest H_2 range of 51.39% at a biomass concentration of 30 g/L. H_2 production rate (100.16 mol/$m3^{-d}$) was estimated to be high at 10–30 g/L of substrate concentration and decreases with an increasing substrate concentration of 30 g/L (Lu et al. 2019). The pure and co-culture of bacterial strains

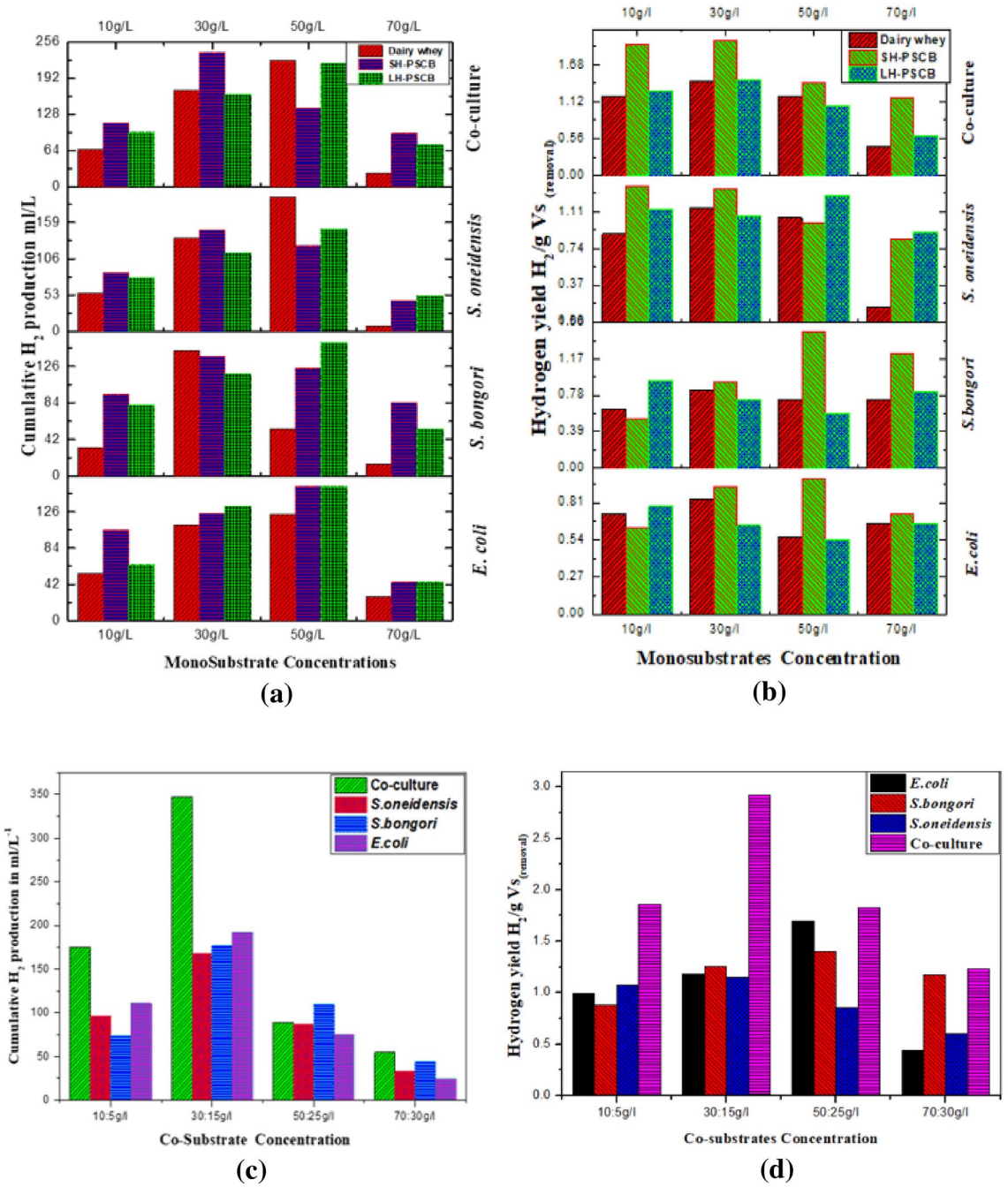

Fig. 9 Effect of biohydrogen production from various substrates by pure and co-culture at different substrate concentrations: **a** mono-substrates cumulative H_2 production, **b** mono-substrate H_2 yield, **c** co-substrates cumulative H_2

obtained high-efficient H_2 yield at pH 7.0 while using potential substrate dairy whey and solid hydrolysis of pretreated sugarcane bagasse as a co-substrate in the batch fermentation. Since *S. bongori* and *E. coli* effectively utilize dairy whey and solid hydrolysis of pretreated sugarcane bagasse for hydrogen production, batch fermentation with co-substrate by co-culture at different substrate concentrations shows a maximum H_2 yield of 2.92 H_2/g$Vs_{(removal)}$ at 30:15 g/L rather than the mono-substrate. For both acidic and alkaline hydrolysates, co-digesting the pretreated SMS with cattle manure enhances hydrogen and methane production (Vasilakis et al. 2023).

H_2 production from liquid hydrolysate of acid-pretreated sugarcane bagasse with a pure culture of *E. coli*, *S. bongori*, and *S. oneidensis* (154.4 ± 2.2 H_2/mL/L, 153.5 ± 1.3 H_2/mL/L, and 149.9 ± 3.4 H_2/mL/L) was maximum at substrate concentration of 50 g/L. Fermentation by co-culture yields

an H_2 of 198 ± 9.9 H_2/mL/L from LH-PSCB at a substrate concentration of 50 g/L. H_2 production from co-substrate consisting of DW and SH-PSCB with pure culture revealed that the production of H_2 by *S. bongori* and *S. oneidensis* 177.3 ± 1.6 H_2/mL/L and 168 ± 0.9 H_2/mL/L was maximum at 30:15 g/L. *E. coli* yields an H_2 of 192.7 ± 1.8 H_2/mL/L from co-substrate at 30:15 g/L. Co-substrate fermentation with co-culture yields a maximum H_2 of 347.3 ± 18.5 H_2/mL/L at a substrate concentration of 30:15 g/L (Fig. 9). The bio-H_2 was produced using GAS (granulated anaerobic sludge) and RH (rice husk) as substrate, along with a hydrochar to degrade the dye rhodamine B (RhB). Maximum hydrogen yield was obtained at 5.37 mL g RH^{-1} and 0.179 ml H_2 h^{-1} g RH^{-1} (Silvestri et al. 2023).

Bio-H_2 production has found that the use of co-cultures for DF has a significant limitation: besides HPB (hydrogen-producing bacteria), it can also consist of various microbes like HCB (hydrogen-consuming bacteria) and microbes that compete with HPB with the substrate (Saady 2013). The HPB produced various end products like ethyl alcohol, benzoic acid, propionic acid, butanol, butyric acid, acetic acid, lactic (Table 2). VFA like acetate, lactate, and propionate are formed as intermediates by-products during anaerobic H_2 production**.** An increase in H_2 production was arrested if propionic acid and lactic acid are formed as intermediates (Song et al. 2011; Popall et al. 2022). If the end product is ethanol or butyric acid, there will be no excess NADH to convert to H_2 (Song et al. 2011; Chen et al. 2022). The high partial pressure of H_2 can lead to a chemical change, which can cause the formation of ethanol, lactate, butanol, and acetone at the expense of H_2. Organic acids found that the micro-addition of propionic acid increased biological H_2 production, while the micro-addition of butyric, ethanol, and acetic acid decreased bio-H_2 production (Xia et al. 2015). In dark fermentation, obligate or facultative anaerobes utilize a different type of organic waste to generate bio-H_2 at a higher rate than other fermentation processes. During anaerobic fermentation, organic substrates are broken down into simpler elements, producing bio-H_2 along

Table 2 Optimization studies of dark fermentation biohydrogen production from waste biomass

S. No.	Substrate	Hydrogen produing bacteria	Optimization of operation condition other studies	VFA	Cumulative H_2 production H_2/mL/L/ or HPR l^{-1}/h^{-1}	References
1	Rice starch wastewater	*Enterobacter aerogenes* MTCC 2822 and *Clostridium acetobutylicum* MTCC 11274	Substrate conc: 4.0 g/L Temperature: 37 °C pH: 6.5	acetate, butyrate, propionate and lactate	1.13 L H_2/L media	Jayachandran and Basak (2022)
2	Glucose	*Clostridium beijerinckii*	Substrate conc: 3 g/L Temperature: 30 °C pH: 6.1	Butyrate, formate and ethanol	71 ml H_2/(h L)	Skonieczny and Yargeau (2009)
3	Galactose and Glucose	*Clostridium butyricum* and *Lactobacillus casei*	Substrate conc:0.2–5.7 g/L Temperature: 35 °C pH:7.0	NA	NA	Park et al. (2018)
4	Beverage wastewater	Mixed culture	Substrate conc: 20 g/L Temperature: 37 °C pH:5.5	Acetate, butyrate and ethanol	2367 ml H_2/L	Sivagurunathan and Lin (2020)
5	Sugarcane bagasse hydrolysate	*Clostridium butyricum*	Substrate conc: 20 -COD g/L Temperature: 37 °C pH:5.5	Acetic acid, butyric acid, propionic acid, ethanol and butanol	1611 ml H_2/L/day	Pattra et al. (2008)
6	Dairy wastewater	*Rhodobacter sphaeroides*	Substrate conc: 60 v/v Temperature: 28 °C pH:7	lactic acid	1.97 l^{-1}/h^{-1}	Seifert et al. (2010)
7	Dairy whey and Sugarcane bagasse	*E.coli* *S.bongori* *S.oneidensis*	Substrateconc: 30:15 g/L Temperature: 37 °C pH:7	Ethyl alcohol, Benzoic acid, Propionic acid, Butanol, Butyric acid, Acetic acid, Lactic acid and Acetyl	347.3 ± 18.5 H_2/ml/L	In this study

NA not available, *Conc* concentration, *VFA* Volatile fatty acid, *HPR* Hydrogen production rate and *COD* Chemical oxygen demand

with acetic acid, lactic acid, propionic acid, fertilizers, and ethanol as high-value chemical intermediates**.** In the production of bio-H_2 from glucose, pyruvate is the major anaerobic metabolite. Pyruvate is converted to acetyl-CoA by one of the enzymes, namely formate lyase or ferredoxin oxidoreductase (Siriwongrungson et al. 2007).

MFC biohydrogen generation system

The major challenges of using biohydrogen are transport, storage, and electrical conversion. In this case, we can combine the biohydrogen-generating system with the fuel cell system to generate electricity. This study used biohydrogen produced from anaerobic fermentation to generate electricity using a homemade microbial fuel cell. In the literature, however, there are few references to direct fuel cell electricity generation based on biohydrogen (Flores et al. 2023).

The microbial fuel cell achieved a voltage of 0.68 ± 0.04 V per cell 24 h. As a result of the experiment, anaerobically fermented biohydrogen gas was found to be a suitable fuel for fuel cells. This study has the advantage of focusing on the relationship between co-substrates energy recovery and electricity generation. The commercial application of biohydrogen associated with fuel cells will be more.

Conclusion

In this study, the potentiality of bio-H_2 production through the co-substrate process of DW with sugarcane bagasse was tested. Results showed that co-culture was more effective at enhancing bio-H_2 production than pure culture. SCB was treated with acid using a hydrolysis time of 1 h at 121 °C and H_2SO_4 concentration 0.2% (v/v) and dairy whey after heat treatment in a hot air oven at 100 ℃ for 45 min to inactive the non-spore-forming bacteria. pH, temperature, and substrate concentration were shown to be important factors for stability in bio-H_2 production. Different strains of hydrogen-producing bacteria were isolated and operated under optimal conditions. In SCB, the highest cumulative H_2 production of 237.3 ± 6.0 H_2/mL/L was obtained at 37 ℃ for pH 7, 50 g/L in co-culture. The optimal DW + SCB mixing ratio can facilitate bio-H_2 production during 62 h of co-fermentation with co-culture that yield maximum hydrogen of 347.3 ± 18.5 H_2/mL/L and 2.92 $H_2/gVs_{(removal)}$ at 30:15 g/L at 37 ℃. The metabolic profile in GC was correlated with the co-culture assimilated in the fermentation medium to produce acetic acid and butyric acid during bio-H_2 production. Instead of using mon-substrate for bio-H_2 production, the present study concludes that co-culture produces more stable hydrogen from co-substrates, i.e., co-fermentation produces biohydrogen. There is a need for future analyses to advance this technology to the commercial field while implementing eco-friendly and economical approaches.

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Declarations

Conflict of interest All authors of this paper reveal that they do not have any significant conflicts of interest.

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Informed consent Not applicable.

References

Abubakar A, Peter R, Ahmad IG et al (2022) Dilute sulphuric acid pretreatment for efficient production of bioethanol from sugarcane bagasse using *Saccharomyces cerevisiae*. J Biotechnol 1:56–65. https://doi.org/10.36108/jbt/2202.10.0150

Aditiya HB, Aziz M (2021) Prospect of hydrogen energy in Asia-Pacific: a perspective review on techno-socio-economy nexus. Int J Hydrogen Energy 46:35027–35056. https://doi.org/10.1016/j.ijhydene.2021.08.070

Ahmed SF, Mofijur M, Islam N et al (2022) Insights into the development of microbial fuel cells for generating biohydrogen, bioelectricity, and treating wastewater. Energy. https://doi.org/10.1016/j.energy.2022.124163

Alexandropoulou M, Antonopoulou G, Trably E et al (2020) Continuous biohydrogen production from a food industry waste: influence of operational parameters and microbial community analysis to cite this version. J Clean Prod. https://doi.org/10.1016/j.jclepro.2017.11.078

Amin M, Shah HH, Fareed AG et al (2022) Hydrogen production through renewable and non-renewable energy processes and their impact on climate change. Int J Hydrogen Energy 47:33112–33134. https://doi.org/10.1016/j.ijhydene.2022.07.172

Anukam A, Mamphweli S, Reddy P, Meyer E, Okoh O (2016) Pre-processing of sugarcane bagasse for gasification in a downdraft biomass gasifier system: a comprehensive review. Renew Sustain Energy Rev 66:775–801. https://doi.org/10.1016/j.rser.2016.08.046

Audu JO, Ibrahim N, Ibrahim Z et al (2021) Optimization of the operational parameters for mesophilic biohydrogen production from palm oil mill effluent using enriched mixed culture. Biomass Convers Biorefinery. https://doi.org/10.1007/s13399-021-01488-9

Bakonyi P, Nemestóthy N, Bélafi-Bakó K (2012) Comparative study of various *E. coli* strains for biohydrogen production applying response surface methodology. Sci World J. https://doi.org/10.1100/2012/819793

Balakrishnan D, Manmai N, Ponnambalam S et al (2022) Optimized model of fermentable sugar production from Napier grass for biohydrogen generation via dark fermentation. Int J Hydrogen Energy. https://doi.org/10.1016/j.ijhydene.2022.12.011

Basak N, Das D (2007) The prospect of purple non-sulfur (PNS) photosynthetic bacteria for hydrogen production: the present state of the art. World J Microbiol Biotechnol 23:31–42. https://doi.org/10.1007/s11274-006-9190-9

Bastidas-Oyanedel JR, Bonk F, Thomsen MH, Schmidt JE (2015) Dark fermentation biorefinery in the present and future (bio)chemical industry. Rev Environ Sci Biotechnol 14:473–498. https://doi.org/10.1007/s11157-015-9369-3

Bernal AP, de Menezes CA, Silva EL (2021) A new side-looking at the dark fermentation of sugarcane vinasse: improving the carboxylates production in mesophilic EGSB by selection of the hydraulic retention time and substrate concentration. Int J Hydrogen Energy 46:12758–12770. https://doi.org/10.1016/j.ijhydene.2021.01.161

Bharathiraja B, Yogendran D, Ranjith Kumar R et al (2014) Biofuels from sewage sludge—a review. Int J ChemTech Res 6:4417–4427

Bhurat KS, Banerjee T, Bobde PV, Sureshchandra Bhurat S (2023) Effect of chemical, physical, and biological pre-treatment of food wastes on bio-hydrogen production by dark anaerobic fermentation under mesophilic conditions. Energy Sources A Recovery Util Environ Eff 45:1017–1029. https://doi.org/10.1080/15567036.2023.2174615

Biffinger JC, Pietron J, Ray R et al (2007) A biofilm enhanced miniature microbial fuel cell using *Shewanella oneidensis* DSP10 and oxygen reduction cathodes. Biosens Bioelectron 22:1672–1679. https://doi.org/10.1016/j.bios.2006.07.027

Bolatkhan K, Kossalbayev BD, Zayadan BK et al (2019) Hydrogen production from phototrophic microorganisms: reality and perspectives. Int J Hydrogen Energy 44:5799–5811. https://doi.org/10.1016/j.ijhydene.2019.01.092

Brindhadevi K, Shanmuganathan R, Pugazhendhi A et al (2021) Biohydrogen production using horizontal and vertical continuous stirred tank reactor—a numerical optimization. Int J Hydrogen Energy 46:11305–11312. https://doi.org/10.1016/j.ijhydene.2020.06.155

Bu J, Wang Y, Deng M, Zhu M (2021) Bioresource technology enhanced enzymatic hydrolysis and hydrogen production of sugarcane bagasse pretreated by peroxyformic acid. Bioresour Technol 326:124751. https://doi.org/10.1016/j.biortech.2021.124751

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

Cai J, Wang R, Wu Q et al (2021) Characterization of a hydrogen-producing bacterium *Clostridium* sp. 5A–1. Int J Green Energy 18:624–633. https://doi.org/10.1080/15435075.2021.1875469

Cao Y, Tan H (2004) Structural characterization of cellulose with enzymatic treatment. J Mol Struct 705:189–193. https://doi.org/10.1016/j.molstruc.2004.07.010

Cao Y, Liu H, Liu W et al (2022) Debottlenecking the biological hydrogen production pathway of dark fermentation: insight into the impact of strain improvement. Microb Cell Fact 21:1–16. https://doi.org/10.1186/s12934-022-01893-3

Chakraborty S, Hosen MI, Ahmed M, Shekhar HU (2018) Onco-multi-OMICS approach: a new frontier in cancer research. BioMed Res Int. https://doi.org/10.1155/2018/9836256

Chen Y (2023) SWORD—South West Open Research Deposit The use of membrane filtration to increase native whey protein in infant formula, and effects on bioaccessibility and bioactivity infant formula, and effects on bioaccessibility and bioactivity

Chen H, Wu J, Huang R et al (2022) Effects of temperature and total solid content on biohydrogen production from dark fermentation of rice straw: performance and microbial community characteristics. Chemosphere 286:131655. https://doi.org/10.1016/j.chemosphere.2021.131655

Cheng JR, Zhu MJ (2016) Biohydrogen production from pretreated lignocellulose by *Clostridium thermocellum*. Biotechnol Bioprocess Eng 21:87–94. https://doi.org/10.1007/s12257-015-0642-7

Dalby FR, Hafner SD, Petersen SO et al (2021) Understanding methane emission from stored animal manure: a review to guide model development. J Environ Qual 50:817–835. https://doi.org/10.1002/jeq2.20252

Davila-Vazquez G, Cota-Navarro CB, Rosales-Colunga LM et al (2009) Continuous biohydrogen production using cheese whey: improving the hydrogen production rate. Int J Hydrogen Energy 34:4296–4304. https://doi.org/10.1016/j.ijhydene.2009.02.063

de Leo A, Davila-vazquez G, Alatriste-mondrago F (2008) Fermentative hydrogen production in batch experiments using lactose, cheese whey and glucose: influence of initial substrate concentration and pH. Int J Hydrogen Energy 33:4989–4997. https://doi.org/10.1016/j.ijhydene.2008.06.065

de Moretti MMS, Bocchini-Martins DA et al (2014) Pretreatment of sugarcane bagasse with microwaves irradiation and its effects on the structure and on enzymatic hydrolysis. Appl Energy 122:189–195. https://doi.org/10.1016/j.apenergy.2014.02.020

Dijkstra J, Ellis JL, Kebreab E et al (2012) Ruminal pH regulation and nutritional consequences of low pH. Anim Feed Sci Technol 172:22–33. https://doi.org/10.1016/j.anifeedsci.2011.12.005

Dinamarca C, Bakke R (2011) Process parameters affecting the sustainability of fermentative hydrogen production: a short-review. Int J Energy Environ 2:1076–1078

Dursun N, Gülşen H (2021) Evaluation of industrial waste black cumin (*Nigella Sativa*) for biohydrogen production without pretreatment. Environ Dev Sustain. https://doi.org/10.1007/s10668-021-01939-3

Eker S, Sarp M (2017) Hydrogen gas production from waste paper by dark fermentation: effects of initial substrate and biomass concentrations. Int J Hydrogen Energy 42:2562–2568. https://doi.org/10.1016/j.ijhydene.2016.04.020

Ekka B, Mierin I (2022) Quantification of different fatty acids in raw dairy wastewater. Clean Eng Technol 7:1–6. https://doi.org/10.1016/j.clet.2022.100430

Elsharnouby O, Hafez H, Nakhla G, El Naggar MH (2013) A critical literature review on biohydrogen production by pure cultures. Int J Hydrogen Energy 38:4945–4966. https://doi.org/10.1016/j.ijhydene.2013.02.032

Emerald NC, Lillycrop KA, Garratt E, Sheppard A, McLean C, Burdge G, Cooper C (2012) Evaluation of methylation status of the eNOS promoter at birth in relation to childhood bone mineral content. Calcif Tissue Int 90:120–127. https://doi.org/10.1007/s00223-011-9554-5

Ethaib S, Omar R, Mustapa Kamal SM, Awang Biak DR, Syam S, Harun MY (2017) Microwave-assisted pretreatment of sago palm bark. J Wood Chem Technol 37(1):26–42. https://doi.org/10.1080/02773813.2016.1224249

Fang HHP, Liu H (2002) Effect of pH on hydrogen production from glucose by a mixed culture. Bioresour Technol 82:87–93

Fangkum A, Reungsang A (2011) Biohydrogen production from sugarcane bagasse hydrolysate by elephant dung: effects of initial pH and substrate concentration. Int J Hydrogen Energy 36:8687–8696. https://doi.org/10.1016/j.ijhydene.2010.05.119

Federation WE (2012) APHA, AWWA, WEF. "Standard methods for examination of water and wastewater." An Hidrol Méd 5:185–186. https://doi.org/10.5209/rev_ANHM.2012.v5.n2.40440

Ferraren-De Cagalitan DDT, Abundo MLS (2021) A review of biohydrogen production technology for application towards hydrogen fuel cells. Renew Sustain Energy Rev 151:111413. https://doi.org/10.1016/j.rser.2021.111413

Flores SR, Asis ER, Caramutti JD et al (2023) An analysis of global trends from 1990 to 2022 of microbial fuel cells: a bibliometric analysis. Sustainability 15(4):3651

Fujita T, Nakao E, Takeuchi M et al (2016) Characterization of starch-accumulating duckweeds, *Wolffia globosa,* as renewable carbon source for bioethanol production. Biocatal Agric Biotechnol 6:123–127. https://doi.org/10.1016/j.bcab.2016.03.006

Galbe M, Zacchi G (2012) Pretreatment: the key to efficient utilization of lignocellulosic materials. Biomass Bioenerg 46:70–78. https://doi.org/10.1016/j.biombioe.2012.03.026

Ghimire A, Frunzo L, Pirozzi F et al (2015) A review on dark fermentative biohydrogen production from organic biomass: process parameters and use of by-products. Appl Energy 144:73–95. https://doi.org/10.1016/j.apenergy.2015.01.045

Gil GC, Chang IS, Kim BH et al (2003) Operational parameters affecting the performance of a mediator-less microbial fuel cell. Biosens Bioelectron 18:327–334. https://doi.org/10.1016/S0956-5663(02)00110-0

Gökçek ÖB, Baş F, Muratçobanoğlu H, Demirel S (2023) Investigation of the effects of magnetite addition on biohydrogen production from apple pulp waste. Fuel. https://doi.org/10.1016/j.fuel.2023.127475

Gonzales RR, Sivagurunathan P, Kim SH (2016) Effect of severity on dilute acid pretreatment of lignocellulosic biomass and the following hydrogen fermentation. Int J Hydrogen Energy 41:21678–21684. https://doi.org/10.1016/j.ijhydene.2016.06.198

Hallenbeck PC, Ghosh D (2009) Advances in fermentative biohydrogen production: the way forward ? Trends Biotechnol 27:287–297. https://doi.org/10.1016/j.tibtech.2009.02.004

Harju M, Kallioinen H, Tossavainen O (2012) Lactose hydrolysis and other conversions in dairy products: technological aspects. Int Dairy J 22:104–109. https://doi.org/10.1016/j.idairyj.2011.09.011

Hassan S, Zohri AA (2019) Electricity generation from sugarcane molasses using microbial fuel cell technologies. Energy. https://doi.org/10.1016/j.energy.2019.04.087

Hawkes FR, Dinsdale R, Hawkes DL, Hussy I (2002) Sustainable fermentative hydrogen production: challenges for process optimisation. Int J Hydrogen Energy 27:1339–1347. https://doi.org/10.1016/S0360-3199(02)00090-3

Hitam CNC, Jalil AA (2020) A review on biohydrogen production through photo-fermentation of lignocellulosic biomass. Biomass Convers Biorefinery. https://doi.org/10.1007/s13399-020-01140-y

Horiuchi JI, Shimizu T, Kanno T, Kobayashi M (1999) Dynamic behavior in response to pH shift during anaerobic acidogenesis with a chemostat culture. Biotechnol Tech 13:155–157

Hu R, Lin L, Liu T et al (2008) Reducing sugar content in hemicellulose hydrolysate by DNS method: a revisit. J Biobased Mater Bioenergy 2:156–161. https://doi.org/10.1166/jbmb.2008.306

Husna A, Aziz A, Sakinah N et al (2022) ScienceDirect synergistic enhancement of biohydrogen production by supplementing with green synthesized magnetic iron nanoparticles using thermophilic mixed bacteria culture. Int J Hydrogen Energy. https://doi.org/10.1016/j.ijhydene.2022.09.105

Jarunglumlert T, Prommuak C, Putmai N, Pavasant P (2018) Scaling-up bio-hydrogen production from food waste: feasibilities and challenges. Int J Hydrogen Energy 43:634–648. https://doi.org/10.1016/j.ijhydene.2017.10.013

Jayachandran V, Basak N (2022) Optimization of dark fermentative biohydrogen production from rice starch by *Enterobacter aerogenes* MTCC 2822 and *Clostridium acetobutylicum* MTCC 11274. Bioprocess Biosyst Eng. https://doi.org/10.1007/s00449-022-02838-1

Jihen BA, Boutheina BS, Haifa Z, Besma D, Mohamed Ali B, Saloua A (2016) Prognostic factors of advanced stage non-small-cell lung cancer

Johnstone N, Haščič I, Popp D (2010) Renewable energy policies and technological innovation: evidence based on patent counts. Environ Resour Econ 45:133–155. https://doi.org/10.1007/s10640-009-9309-1

Joseph TE (2019) Investigating renewable energy potentials in solving energy crisis in Niger delta riverine communities Nigeria. Int J Electr Comput 7:905–915

Ju YH, Huynh LH, Kasim NS et al (2011) Analysis of soluble and insoluble fractions of alkali and subcritical water treated sugarcane bagasse. Carbohyd Polym 83:591–599. https://doi.org/10.1016/j.carbpol.2010.08.022

Juan ET, Fernando ER, Renaud P et al (2015) Biohydrogen production by dark fermentation: scaling-up and technologies integration for a sustainable system. Rev Environ Sci Bio/technol. https://doi.org/10.1007/s11157-015-9383-5

Kadier A, Wang J, Chandrasekhar K et al (2022) Performance optimization of microbial electrolysis cell (MEC) for palm oil mill effluent (POME) wastewater treatment and sustainable Bio-H2 production using response surface methodology (RSM). Int J Hydrogen Energy 47:15464–15479. https://doi.org/10.1016/j.ijhydene.2021.09.259

Kamilah N, Jalil A, Asli UA et al (2018) Biohydrogen production from pineapple biomass residue using immobilized co-culture of clostridium sporogenes and enterobacter aerogenes. J Energy Resour Technol 01:51–57

Karimi M, Jenkins B, Stroeve P (2014) Ultrasound irradiation in the production of ethanol from biomass. Renew Sustain Energy Rev 40:400–421. https://doi.org/10.1016/j.rser.2014.07.151

Karimi-Maleh H, Orooji Y, Karimi F et al (2023) Two-stage biohydrogen and polyhydroxyalkanoate production: upcycling of spent coffee grounds. J Inst Eng (india) Ser E. https://doi.org/10.48048/tis.2023.6462. (**In Press**)

Khan I, Anburajan P, Kumar G et al (2021) Comparative effect of silver nanoparticles (AgNPs) derived from actinomycetes and henna on biohydrogen production by *Clostridium beijerinckii* (KTCC1737). Int J Energy Res 45:17269–17278. https://doi.org/10.1002/er.6076

Kim HJ, Park HS, Hyun MS et al (2002) A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. Enzyme Microb Technol 30:145–152. https://doi.org/10.1016/S0141-0229(01)00478-1

Kotzekidou P (2013) Survey of *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* O157: H7 in raw ingredients and ready-to-eat products by commercial real-time PCR kits. Food Microbiol 35:86–91. https://doi.org/10.1016/j.fm.2013.03.007

Kumar G, Mathimani T, Rene ER, Pugazhendhi A (2019) Application of nanotechnology in dark fermentation for enhanced biohydrogen production using inorganic nanoparticles. Int J Hydrogen Energy 44:13106–13113. https://doi.org/10.1016/j.ijhydene.2019.03.131

Kumari S, Das D (2017) Improvement of biohydrogen production using acidogenic culture. Int J Hydrogen Energy 42:4083–4094. https://doi.org/10.1016/j.ijhydene.2016.09.021

Kumari T, Das G, Sharma A, Gangwar RK (2019) Design approach for dual element hybrid MIMO antenna arrangement for wideband applications. Int J RF Microw Computer-Aided Eng 29(1):e21486. https://doi.org/10.1002/mmce.21486

Kyazze G, Dinsdale R, Premier GC et al (2005) Influence of substrate concentration on the stability and yield of continuous biohydrogen production. Biotechnol Bioeng. https://doi.org/10.1002/bit.20802

Lakshmidevi R, Muthukumar K (2010) Enzymatic saccharification and fermentation of paper and pulp industry effluent for biohydrogen production. Int J Hydrogen Energy 35:3389–3400. https://doi.org/10.1016/j.ijhydene.2009.12.165

Lakshmidevi R, Muthukumar K (2023) Biohydrogen production from enzymatically digested cotton stalks using *Citrobacter freundii*. J Inst Eng (india) Ser E. https://doi.org/10.1007/s40034-022-00265-1

Laluce C, Roldan IU, Pecoraro E et al (2019) Effects of pretreatment applied to sugarcane bagasse on composition and morphology of cellulosic fractions. Biomass Bioenerg 126:231–238. https://doi.org/10.1016/j.biombioe.2019.03.002

Lay C, Chang J (2012) fermentative hydrogen production from wastewaters: a review and prognosis. Int J Hydrogen Energy. https://doi.org/10.1016/j.ijhydene.2012.02.072

Lee DY, Xu KQ, Kobayashi T et al (2014) Effect of organic loading rate on continuous hydrogen production from food waste in submerged anaerobic membrane bioreactor. Int J Hydrogen Energy 39:16863–16871. https://doi.org/10.1016/j.ijhydene.2014.08.022

Levin DB, Pitt L, Love M (2004) Biohydrogen production: prospects and limitations to practical application. Int J Hydrogen Energy 29:173–185. https://doi.org/10.1016/S0360-3199(03)00094-6

Li L, Kong Z, Qin Y et al (2020) Temperature-phased anaerobic codigestion of food waste and paper waste with and without recirculation: biogas production and microbial structure. Sci Total Environ 724:1–9. https://doi.org/10.1016/j.scitotenv.2020.138168

Li S, Tabatabaei M, Li F, Ho SH (2022) A review of green biohydrogen production using anoxygenic photosynthetic bacteria for hydrogen economy: challenges and opportunities. Int J Hydrogen Energy. https://doi.org/10.1016/j.ijhydene.2022.11.014

Lin CY, Lay CH (2004) Carbon/nitrogen-ratio effect on fermentative hydrogen production by mixed microflora. Int J Hydrogen Energy 29:41–45. https://doi.org/10.1016/S0360-3199(03)00083-1

Liu IC, Whang LM, Ren WJ, Lin PY (2011) The effect of pH on the production of biohydrogen by clostridia: thermodynamic and metabolic considerations. Int J Hydrogen Energy 36(1):439–449. https://doi.org/10.1016/j.ijhydene.2010.10.045

Liu J, Zhou W, Fan S et al (2019) Cell degeneration and performance decline of immobilized *Clostridium acetobutylicum* on bagasse during hydrogen and butanol production by repeated cycle fermentation. Int J Hydrogen Energy 44:26204–26212. https://doi.org/10.1016/j.ijhydene.2019.08.102

Liu Y, Min J, Feng X et al (2020) A review of biohydrogen productions from lignocellulosic precursor via dark fermentation: perspective on hydrolysate composition and electron-equivalent balance. Energies. https://doi.org/10.3390/en13102451

Logan BE, Oh SE, Kim IS, Van Ginkel S (2002) Biological hydrogen production measured in batch anaerobic respirometers. Environ Sci Technol 36:2530–2535. https://doi.org/10.1021/es015783i

Lu C, Zhang Z, Zhou X et al (2018) Effect of substrate concentration on hydrogen production by photo-fermentation in the pilot-scale baffled bioreactor. Biores Technol 247:1173–1176. https://doi.org/10.1016/j.biortech.2017.07.122

Lu C, Wang Y, Lee DJ et al (2019) Biohydrogen production in pilot-scale fermenter: effects of hydraulic retention time and substrate concentration. J Clean Prod 229:751–760. https://doi.org/10.1016/j.jclepro.2019.04.233

Lui J, Chen W, Tsang DCW, You S (2020) A critical review on the principles, applications, and challenges of waste-to-hydrogen technologies. Renew Sustain Energy Rev 134:110365. https://doi.org/10.1016/j.rser.2020.110365

Mahata C, Das D (2022) Current status and prospects of biohydrogen production process. Springer Nature Singapore

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

Mehrotra R, Devuyst O, Davies SJ, Johnson DW (2016) The current state of peritoneal dialysis. J Am Soc Nephrol JASN 27(11):3238. https://doi.org/10.1681/ASN.2016010112

Miito GJ, Ndegwa P, Alege FP, Coulibaly SS, Davis R, Harrison J (2021) A vermifilter system for reducing nutrients and organic-strength of dairy wastewater. Environ Technol Innov 23:101648. https://doi.org/10.1016/j.eti.2021.101648

Mizuno O, Dinsdale R, Hawkes FR et al (2000) Enhancement of hydrogen production from glucose by nitrogen gas sparging. Biores Technol 73:59–65. https://doi.org/10.1016/S0960-8524(99)00130-3

Mohammadi M, Mohammadi P (2022) Developing single-substrate steady-state model for biohydrogen production in continuous anaerobic activated sludge–rotating biological contactor system: novel insights on the process. Int J Energy Res 46:2041–2050. https://doi.org/10.1002/er.7265

Morán JI, Alvarez VA, Cyras VP, Vázquez A (2008) Extraction of cellulose and preparation of nanocellulose from sisal fibers. Cellulose 15:149–159. https://doi.org/10.1007/s10570-007-9145-9

Mthethwa NP, Nasr M, Bux F, Kumari S (2018) Utilization of *Pistia stratiotes* (aquatic weed) for fermentative biohydrogen: electron-equivalent balance, stoichiometry, and cost estimation. Int J Hydrogen Energy 43:8243–8255. https://doi.org/10.1016/j.ijhydene.2018.03.099

Mumtha C, Subashri D, Mahalingam PU (2022) Biohydrogen producing facultative anaerobic bacteria from different anaerobic sludge. J Pure Appl Microbiol 16:1861–1872. https://doi.org/10.22207/JPAM.16.3.32

Murugan RS, Dinesh GH, Raja RK et al (2021) Dark fermentative biohydrogen production by *Acinetobacter junii*-AH4 utilizing various industry wastewaters. Int J Hydrogen Energy 46:11297–11304. https://doi.org/10.1016/j.ijhydene.2020.07.073

Mutsvene B, Chetty M, Kumari S, Bux F (2023) Biohydrogen production from brewery wastewater in an Anaerobic baffled reactor. A preliminary techno-economic evaluation. S Afr J Chem Eng 43:9–23. https://doi.org/10.1016/j.sajce.2022.09.012

Nagarajan D, Di Dong C, Chen CY et al (2021) Biohydrogen production from microalgae—major bottlenecks and future research perspectives. Biotechnol J. https://doi.org/10.1002/biot.202000124

Naik S, Goud VV, Rout PK et al (2010) Characterization of *Canadian biomass* for alternative renewable biofuel. Renew Energy 35:1624–1631. https://doi.org/10.1016/j.renene.2009.08.033

Nath K, Das D (2011) Modeling and optimization of fermentative hydrogen production. Biores Technol 102:8569–8581. https://doi.org/10.1016/j.biortech.2011.03.108

Nielsen SS (2010) Phenol–sulfuric acid method for total carbohydrates. Springer, pp 47–53

Nurhayat K, Bilgin M (2022) Biogas production from potato peel. Acta Biol Turcica 35(1):49–56

Oceguera-Contreras E, Aguilar-Juárez O, Oseguera-Galindo D et al (2019) Biohydrogen production by vermihumus-associated microorganisms using agro industrial wastes as substrate. Int J Hydrogen Energy 44:9856–9865. https://doi.org/10.1016/j.ijhydene.2018.10.236

Owusu PA, Asumadu-Sarkodie S (2016) A review of renewable energy sources, sustainability issues and climate change mitigation. Cogent Eng. https://doi.org/10.1080/23311916.2016.1167990

Oztekin R, Kapdan IK, Kargi F, Argun H (1993) Optimization of media composition for hydrogen gas production from hydrolyzed wheat starch by dark fermentation. Int J Hydrogen Energy 33:4083–4090. https://doi.org/10.1016/j.ijhydene.2008.05.052

Pandey A, Wang D (2019) A new framework for CNN-based speech enhancement in the time domain. IEEE/ACM Trans Audio Speech Lang Process 27(7):1179–1188

Pant D, Van Bogaert G, Diels L, Vanbroekhoven K (2010) A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Biores Technol 101:1533–1543. https://doi.org/10.1016/j.biortech.2009.10.017

Park JH, Kim DH, Kim SH et al (2018) Effect of substrate concentration on the competition between *Clostridium* and *Lactobacillus* during biohydrogen production. Int J Hydrogen Energy 43:11460–11469. https://doi.org/10.1016/j.ijhydene.2017.08.150

Pascualone MJ, Costa MBG, Dalmasso PR (2019) Fermentative biohydrogen production from a novel combination of vermicompost as inoculum and mild heat-pretreated fruit and vegetable waste. Biofuel Res J 23:1046–1053. https://doi.org/10.18331/BRJ2019.6.3.5

Patil SS, Shedbalkar UU, Truskewycz A, Chopade BA, Ball AS (2016) Nanoparticles for environmental clean-up: a review of potential risks and emerging solutions. Environ Technol Inno 5:10–21. https://doi.org/10.1016/j.eti.2015.11.001

Pattra S, Sangyoka S, Boonmee M, Reungsang A (2008) Bio-hydrogen production from the fermentation of sugarcane bagasse hydrolysate by *Clostridium butyricum*. Int J Hydrogen Energy 33:5256–5265. https://doi.org/10.1016/j.ijhydene.2008.05.008

Popall RM, Heussner A, Kerzenmacher S et al (2022) Screening for hyperthermophilic electrotrophs for the microbial electrosynthesis of organic compounds. Microorganisms 10:2249. https://doi.org/10.3390/microorganisms10112249

Prathiba S, Kumar PS, Vo DVN (2022) Recent advancements in microbial fuel cells: a review on its electron transfer mechanisms, microbial community, types of substrates and design for bio-electrochemical treatment. Chemosphere 286:131856. https://doi.org/10.1016/j.chemosphere.2021.131856

Queiroz MG, de Aquino MLA, Brito ADL, Medeiros CCM, da Silva Simões MO, Teixeira A, de Carvalho DF (2020) Hipertensão arterial no idoso-doença prevalente nesta população: uma revisão integrativa. Brazilian J Develop 6(4):22590–22598

Qyyum MA, Ihsanullah I, Ahmad R et al (2022) Biohydrogen production from real industrial wastewater: potential bioreactors, challenges in commercialization and future directions. Int J Hydrogen Energy 47:37154–37170. https://doi.org/10.1016/j.ijhydene.2022.01.195

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 (2021) Optimization and modelling of dark fermentative hydrogen production from cheese whey by *Enterobacter aerogenes* 2822. Int J Hydrogen Energy 46:1777–1800. https://doi.org/10.1016/j.ijhydene.2020.10.142

Rebac S, Van Lier JB, Janssen MGJ et al (1997) High-rate anaerobic treatment of malting waste water in a pilot-scale EGSB system under psychrophilic conditions. J Chem Technol Biotechnol 68:135–146. https://doi.org/10.1002/(SICI)1097-4660(199702)68:2%3c135::AID-JCTB583%3e3.0.CO;2-C

Reddy K, Nasr M, Kumari S et al (2017) Biohydrogen production from sugarcane bagasse hydrolysate: effects of pH, S/X, Fe2+, and magnetite nanoparticles. Environ Sci Pollut Res 24:8790–8804. https://doi.org/10.1007/s11356-017-8560-1

Ryan MP, O'Dwyer J, Adley CC (2017) Evaluation of the complex nomenclature of the clinically and veterinary significant pathogen salmonella. BioMed Res Int. https://doi.org/10.1155/2017/3782182

Saady NMC (2013) Homoacetogenesis during hydrogen production by mixed cultures dark fermentation: unresolved challenge. Int J Hydrogen Energy 38:13172–13191. https://doi.org/10.1016/j.ijhydene.2013.07.122

Sarangi PK, Subudhi S, Bhatia L et al (2022) Utilization of agricultural waste biomass and recycling towards circular bioeconomy. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-022-20669-1

Saratale GD, Saratale RG, Kim SH, Kumar G (2018) Screening and optimization of pretreatments in the preparation of sugarcane bagasse feedstock for biohydrogen production and process optimization. Int J Hydrogen Energy 43:11470–11483. https://doi.org/10.1016/j.ijhydene.2018.01.187

Sawers RG (2005) Formate and its role in hydrogen production in *Escherichia coli*. Biochem Soc Trans 33:42–46. https://doi.org/10.1042/BST0330042

Scarlat N, Blujdea V, Dallemand JF (2011) Assessment of the availability of agricultural and forest residues for bioenergy production in Romania. Biomass Bioenerg 35:1995–2005. https://doi.org/10.1016/j.biombioe.2011.01.057

Seifert K, Waligorska M, Laniecki M (2010) Hydrogen generation in photobiological process from dairy wastewater. Int J Hydrogen Energy 35:9624–9629. https://doi.org/10.1016/j.ijhydene.2010.07.015

Shrestha RM, Shakya SR (2012) Benefits of low carbon development in a developing country: case of Nepal. Energy Econ. https://doi.org/10.1016/j.eneco.2012.03.014

Shukla A, Kumar D, Girdhar M et al (2023) Strategies of pretreatment of feedstocks for optimized bioethanol production: distinct and integrated approaches. Biotechnol Biofuels Bioproducts. https://doi.org/10.1186/s13068-023-02295-2

Sillero L, Solera R, Perez M (2023) Effect of temperature on biohydrogen and biomethane production using a biochemical potential test with different mixtures of sewage sludge, vinasse and poultry manure. J Clean Prod 382:135237. https://doi.org/10.1016/j.jclepro.2022.135237

Silvestri S, Roman LMG, Alcaraz-González V, Carissimi E (2023) Rice husk to produce both bio-H2 via dark fermentation and photocatalytic hydrochar. Waste Biomass Valorization. https://doi.org/10.1007/s12649-023-02073-z. (**In Press**)

Sinbuathong N, Sillapacharoenkul B (2021) Dark fermentation of starch factory wastewater with acid- and base-treated mixed microorganisms for biohydrogen production. Int J Hydrogen Energy 46:16622–16630. https://doi.org/10.1016/j.ijhydene.2020.06.109

Sinha P, Pandey A (2011) An evaluative report and challenges for fermentative biohydrogen production. Int J Hydrogen Energy 36:7460–7478. https://doi.org/10.1016/j.ijhydene.2011.03.077

Siriwongrungson V, Zeng RJ, Angelidaki I (2007) Homoacetogenesis as the alternative pathway for H2 sink during thermophilic anaerobic degradation of butyrate under suppressed methanogenesis. Water Res 41:4204–4210. https://doi.org/10.1016/j.watres.2007.05.037

Sivagurunathan P, Lin CY (2020) Biohydrogen production from beverage wastewater using selectively enriched mixed culture. Waste Biomass Valorization 11:1049–1058. https://doi.org/10.1007/s12649-019-00606-z

Skonieczny MT, Yargeau V (2009) Biohydrogen production from wastewater by *Clostridium beijerinckii*: effect of pH and

substrate concentration. Int J Hydrogen Energy 34:3288–3294. https://doi.org/10.1016/j.ijhydene.2009.01.044
Song W, Cheng J, Zhao J et al (2011) Improvement of hydrogen production by over-expression of a hydrogen-promoting protein gene in *Enterobacter cloacae*. Int J Hydrogen Energy 36:6609–6615. https://doi.org/10.1016/j.ijhydene.2011.02.086
Taifor AF, Zakaria MR, Mohd Yusoff MZ et al (2017) Elucidating substrate utilization in biohydrogen production from palm oil mill effluent by *Escherichia coli*. Int J Hydrogen Energy 42:5812–5819. https://doi.org/10.1016/j.ijhydene.2016.11.188
Talha Z, Ding W, Mehryar E et al (2016) Alkaline pretreatment of sugarcane bagasse and filter mud codigested to improve biomethane production. Biomed Res Int. https://doi.org/10.1155/2016/8650597
Tan TL, Lebron GB (2012) Determination of carbon dioxide, carbon monoxide, and methane concentrations in cigarette smoke by Fourier transform infrared spectroscopy. J Chem Educ 89:383–386. https://doi.org/10.1021/ed200178s
Tang J, He J, Liu T, Xin X (2017) Removal of heavy metals with sequential sludge washing techniques using saponin: optimization conditions, kinetics, removal effectiveness, binding intensity, mobility and mechanism. RSC Adv 7:33385–33401. https://doi.org/10.1039/c7ra04284a
Toledo-Cervantes A, Villafán-Carranza F, Arreola-Vargas J et al (2020) Comparative evaluation of the mesophilic and thermophilic biohydrogen production at optimized conditions using tequila vinasses as substrate. Int J Hydrogen Energy 45:11000–11010. https://doi.org/10.1016/j.ijhydene.2020.02.051
Varghese VK, Poddar BJ, Shah MP et al (2022) A comprehensive review on current status and future perspectives of microbial volatile fatty acids production as platform chemicals. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2021.152500
Vasilakis G, Rigos E, Giannakis N et al (2023) Spent mushroom substrate hydrolysis and utilization as potential alternative feedstock for anaerobic co-digestion. Microorganisms. https://doi.org/10.3390/microorganisms11020532
Venkata Mohan S, Lalit Babu V, Sarma PN (2008) Effect of various pretreatment methods on anaerobic mixed microflora to enhance biohydrogen production utilizing dairy wastewater as substrate. Biores Technol 99:59–67. https://doi.org/10.1016/j.biortech.2006.12.004
Wang J, Wan W (2008a) Factors influencing fermentative hydrogen production: a review. Int J Hydrogen Energy. https://doi.org/10.1016/j.ijhydene.2008.11.015
Wang J, Wan W (2008b) The effect of substrate concentration on biohydrogen production by using kinetic models. Sci China Ser B Chem 51:1110–1117. https://doi.org/10.1007/s11426-008-0104-6
Wang J, Yin Y (2018) Fermentative hydrogen production using pretreated microalgal biomass as feedstock. Microb Cell Fact 17:1–16. https://doi.org/10.1186/s12934-018-0871-5
Waterborg JH (2009) The lowry method for protein quantitation. Humana Press, pp 7–10
Webber CL III, White PM Jr, Spaunhorst DJ, Petrie EC (2017) Impact of sugarcane bagasse ash as an amendment on the physical properties, nutrient content and seedling growth of a certified organic greenhouse growing media. J Agric Sci 9:1. https://doi.org/10.5539/jas.v9n7p1
Wu F, Zhou S (2012) Anaerobic biohydrogen fermentation from paper mill sludge. J Energy Eng 138:1–6. https://doi.org/10.1061/(asce)ey.1943-7897.0000054
Wu SY, Lin CN, Chang JS (2003) Hydrogen production with immobilized sewage sludge in three-phase fluidized-bed bioreactors. Biotechnol Prog 19:828–832. https://doi.org/10.1021/bp0201354
Xia A, Cheng J, Ding L et al (2015) Substrate consumption and hydrogen production via co-fermentation of monomers derived from carbohydrates and proteins in biomass wastes. Appl Energy 139:9–16. https://doi.org/10.1016/j.apenergy.2014.11.016
Xiao L, Deng Z, Fung KY, Ng KM (2013) Biohydrogen generation from anaerobic digestion of food waste. Int J Hydrogen Energy 38:13907–13913. https://doi.org/10.1016/j.ijhydene.2013.08.072
Xing D, Ren N, Li Q et al (2006) *Ethanoligenens harbinense* gen. nov., sp. nov., isolated from molasses wastewater. Int J Syst Evol Microbiol 56:755–760. https://doi.org/10.1099/ijs.0.63926-0
Xue S, Zhao N, Song J, Wang X (2019) Interactive effects of chemical composition of food waste during anaerobic co-digestion under thermophilic temperature. Sustainability 11(10):2933
Yoshida A, Nishimura T, Kawaguchi H et al (2007) Efficient induction of formate hydrogen lyase of aerobically grown *Escherichia coli* in a three-step biohydrogen production process. Appl Microbiol Biotechnol 74:754–760. https://doi.org/10.1007/s00253-006-0721-y
Zhang G (2021) Hydrogen production during the dark fermentation of glycerol. University of Windsor
Zhang Y, Shen J (2006) Effect of temperature and iron concentration on the growth and hydrogen production of mixed bacteria. Int J Hydrogen Energy 31:441–446. https://doi.org/10.1016/j.ijhydene.2005.05.006
Zhang T, Liu H, Fang HHP (2003) Biohydrogen production from starch in wastewater under thermophilic condition. J Environ Manag 69:149–156. https://doi.org/10.1016/S0301-4797(03)00141-5
Zhang C, Su H, Tan T (2013) Batch and semi-continuous anaerobic digestion of food waste in a dual solid-liquid system. Biores Technol 145:10–16. https://doi.org/10.1016/j.biortech.2013.03.030