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Biohydrogen From Anaerobic Bacteria And Fungi: Bio-Resources For Environmental Sustainability

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Abstract
Dark fermentation by anaerobic bacteria and fungi converts agricultural, food and forestry residues into hydrogen under mild conditions while treating the waste. This review summarises recent evidence on the organisms, enzymes, feedstocks, purification steps and end uses of biohydrogen. Clostridium and thermophilic Caldicellulosiruptor are the main producers, with yields capped near 4 mol H_2 mol^{-1} hexose. Anaerobic gut fungi (Neocallimastigomycota) release H_2 from untreated fibre through hydrogenosomes, while white-rot fungi remove lignin before bacterial fermentation. Wood-feeding termites are the most efficient natural hydrogen-cycling system known, turning over 9–33 m^3 H_2 per m^3 of hindgut each day, and are a source of hydrogen-producing isolates. Membranes and pressure swing adsorption (PSA) upgrade the H_2/CO_2 gas to fuel-cell grade, and two-stage hydrogen–methane systems recover more energy overall. Low yield and lack of pilot-scale data remain the main barriers.
Keywords: *Biohydrogen, Dark fermentation, Clostridium, Anaerobic fungi, Termite gut symbionts, Biohythane*

1. Introduction

Hydrogen can decarbonise sectors that are hard to electrify, yet almost all current supply comes from natural gas and coal, mainly for refining and ammonia synthesis (Griffiths et al., 2021). The largest climate gains come from using low-carbon hydrogen in steel, ammonia and biofuel production (Terlouw et al., 2025). Among production routes, dark fermentation is the fastest biological option: it needs no light and accepts sugars, food waste, manure and pretreated lignocellulose (Soares et al., 2020; Osman et al., 2022).

Three groups of organisms drive this process:

- **Anaerobic bacteria** – *Clostridium*, *Enterobacter* and thermophiles such as *Caldicellulosiruptor*.
- **Fungi** – anaerobic gut fungi that release H_2 from hydrogenosomes, and aerobic fungi that break down lignin.
- **Insect gut microbiota** – especially of wood-feeding termites, where H_2 is the central intermediate of wood digestion (Brune, 2014).

This review covers the biochemistry, producing organisms, insect systems, process design, gas purification and uses of biohydrogen.

2. Biochemistry Of Fermentative Hydrogen

In dark fermentation, glucose is oxidised to pyruvate and the surplus electrons are released as H_2 by hydrogenases. When all pyruvate is converted to acetate, the theoretical maximum is 4 mol H_2 per mol glucose (equation 1), the so-called Thauer limit; the butyrate route gives only 2 mol (equation 2) (Thauer et al., 1977).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (2)$$

Most fermentative H_2 is made by [FeFe]-hydrogenases, which are very active but oxygen-sensitive and occur widely in clostridia and anaerobic eukaryotes, whereas [NiFe]-hydrogenases more often take up H_2 (Morra, 2022). Electron-bifurcating [FeFe]-hydrogenases use NADH and reduced ferredoxin together; their reaction becomes thermodynamically unfavourable as H_2 partial pressure rises, which is why removing H_2 from the culture raises yield (Jay et al., 2020).

3. Hydrogen-Producing Anaerobic Bacteria

Clostridium species are the workhorses of dark fermentation, while extreme thermophiles give the highest yields per mole of sugar (Table 1).

- **Mesophilic clostridia** (*C. butyricum*, *C. beijerinckii*, *C. populeti*) form heat-resistant spores, so heat-treating an inoculum at about 100 °C enriches them and kills methanogens (Soares et al., 2020). Cellulolytic *C. populeti* FZ10 ferments corn stalk directly (Zhang et al., 2022b).
- **Facultative anaerobes** (*Enterobacter*, *Klebsiella*, *Bacillus*) consume residual oxygen and protect strict anaerobes in co-culture, but their formate route gives at most 2 mol H_2 mol^{-1} glucose.
- **Extreme thermophiles** (*Caldicellulosiruptor*, *Thermoanaerobacterium*) grow at 60–80 °C, resist contamination and degrade biomass without conventional pretreatment.

Table 1. Selected recent reports of bacterial hydrogen production

Organism	Substrate and conditions	Reported performance	Reference
Designed artificial consortium	Glucose	5.6 mol H_2 mol^{-1} glucose	Ergal et al. (2020)
Caldicellulosiruptor kronotskyensis	Sugars; continuous, immobilised cells	30 mmol H_2 L^{-1} h^{-1}; 2.95 mol H_2 mol^{-1} sugar	Vongkampang et al. (2023)
C. bescii (adapted)	Untreated cattle manure, 75 °C	161.3 mL H_2 g^{-1} VS	Tunca et al. (2023)
C. bescii JWCB018 (Δ*ldh*)	Barley straw	63% more H_2 than wild type	Cha et al. (2023)
Clostridium populeti FZ10	Microcrystalline cellulose; corn stalk	177.5 and 92.5 mL H_2 g^{-1}	Zhang et al. (2022b)

Yields improve when lactate formation is blocked genetically (Cha et al., 2023), soluble Fe^{2+} is supplied for hydrogenase assembly (Xiao et al., 2026), headspace H_2 is removed by sparging, which raised yield by 47% (Tunca et al., 2023), or cells are immobilised to prevent wash-out (Vongkampang et al., 2023).

4. Fungi In Biohydrogen Production

Fungi contribute in two ways: anaerobic gut fungi produce H_2 themselves, and aerobic fungi open lignocellulose for hydrogen-producing bacteria.

Anaerobic gut fungi

Neocallimastigomycota (*Neocallimastix*, *Piromyces*, *Anaeromyces*, *Pecoramyces*) live in herbivore guts and are among the strongest known degraders of raw plant fibre.

- **Enzymes.** The *Neocallimastix lanati* genome encodes 1,788 lignocellulolytic enzymes, 585 of them linked to the cellulosome (Wilken et al., 2021).
- **Hydrogenosomes.** These organelles release H_2, CO_2, formate and acetate; accumulated H_2 inhibits them, so H_2 removal is essential (Ma et al., 2022).
- **Direct H_2 from biomass.** In a stirred-tank reactor, *N. cameroonii* G341 grew best at 38.5–41.5 °C and pH 6.6–6.8; stirring raised H_2 output and pH control at 6.8 nearly doubled it (Stabel et al., 2022).
- **Partnerships.** H_2-consuming methanogens increase fungal fibre degradation and hydrogenosomal activity (Li et al., 2021), confirming that removing H_2 drives fungal productivity.

Fungal pretreatment

Aerobic fungi remove lignin without forming the furan and phenolic inhibitors produced by harsh chemical pretreatment (Basak et al., 2020). The edible fungus *Gymnopus contrarius* removed 22.4% of rice-straw lignin in 15 days and raised H_2 yield by 74%, with no need for added nutrients (Sheng et al., 2018). White-rot pretreatment of coal increased H_2 from anaerobic digestion 20.3-fold (Zhang et al., 2022a).

5. Insects As Models And Microbial Sources

No insect is farmed for hydrogen gas, but wood-feeding termites house the most efficient natural hydrogen-cycling system known and supply hydrogen-producing strains (Table 2; Figure 1).

- **The termite gut as a bioreactor.** Daily H_2 turnover reaches 9–33 m^3 H_2 per m^3 hindgut, equal to 22–26% of the insect's respiration; reductive acetogenesis consumes most of it and almost none escapes (Pester & Brune, 2007).

- **Protist hydrogenases.** The flagellate *Pseudotrichonympha grassii* of *Coptotermes formosanus* carries [FeFe]-hydrogenases that favour H_2 release even at high H_2 pressure (Inoue et al., 2007).
- **Removing consumers releases H_2.** Antibiotic-treated wood reduced gut bacteria and raised H_2 emission 6–7-fold (Cao et al., 2012), the same principle used in bioreactors.
- **Higher termites (Termitidae).** Lacking flagellates, they rely on bacteria; the *Nasutitermes* hindgut metagenome revealed diverse [FeFe]-hydrogenase genes (Warnecke et al., 2007).
- **Termite-derived isolates.** *Bacillus*, *Clostridium* and the cultivated fungus *Termitomyces* from *Odontotermes formosanus* were combined to mimic the gut for H_2 production (Mathew et al., 2013), and *Lacrimispora xylanolytica* Ter3 from termite guts produced gas with 41.5% H_2 (Yang et al., 2025).
- **Other insects.** Black soldier fly larvae fed fermented food waste showed gut enrichment of Clostridiaceae (Quan et al., 2023), but their microbes have not yet been tested for biohydrogen, an open research gap.

Termite symbionts depolymerise 74–99% of cellulose within hours, making the gut a model for biorefinery design and a reservoir for bioprospecting (Dar et al., 2024; Sero et al., 2025).

Table 2. Insect systems relevant to biohydrogen

Insect	Microbial partners	Relevance to H_2	Reference
Lower termites	Flagellates, acetogens	9–33 $m^3\ m^{-3}\ d^{-1}$ H_2 turnover	Pester & Brune (2007)
Coptotermes formosanus	*Pseudotrichonympha grassii*	H_2-evolving hydrogenases; 6–7× emission after antibiotics	Inoue et al. (2007); Cao et al. (2012)
Odontotermes formosanus	*Bacillus*, *Clostridium*, *Termitomyces*	Biomimetic H_2 co-culture	Mathew et al. (2013)
Termite gut and nest soil	*Lacrimispora xylanolytica* Ter3	41.5% H_2 in fermentation gas	Yang et al. (2025)
Hermetia illucens larvae	Clostridiaceae, *Enterococcus*	Potential source; untested	Quan et al. (2023)

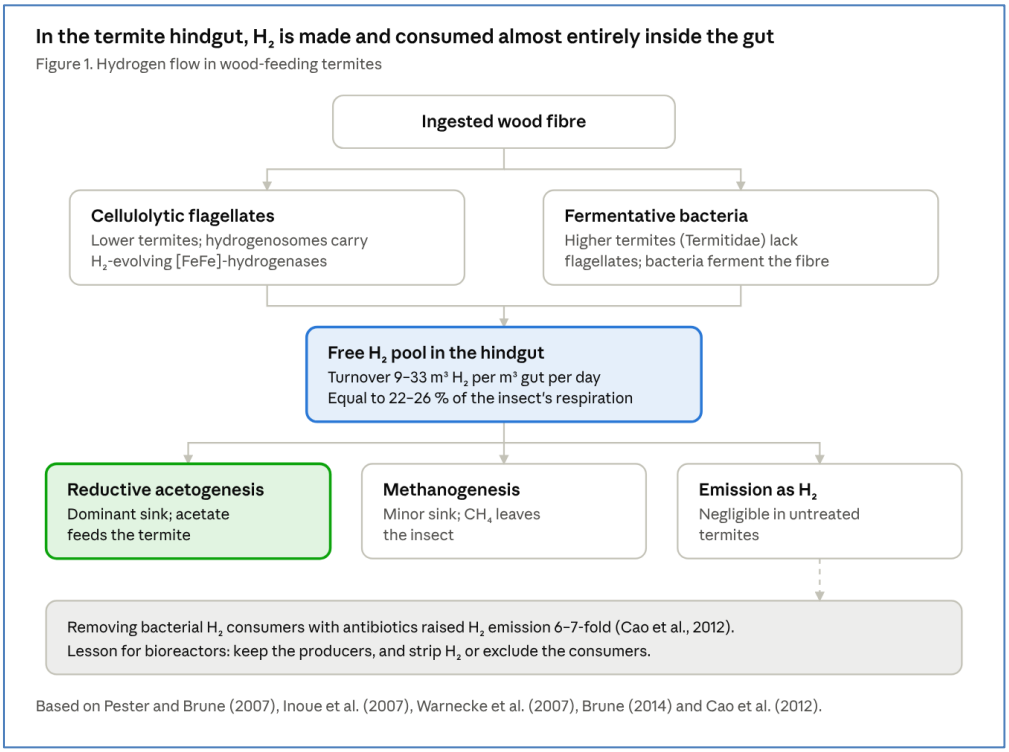


Figure 1. Hydrogen flow in the termite hindgut. Fibre-fermenting protists and bacteria release H_2 into a shared pool that acetogens consume almost as fast as it forms; only suppressing the consumers lets H_2 escape.

6. Feedstocks, Process Conditions And Two-Stage Systems

Sugar-rich wastes ferment readily, while abundant lignocellulosic residues (straw, bagasse, wood) need pretreatment; reported yields from these residues range from 12 to 7,019 mL H_2 L^{-1} (Soares et al., 2020). Dilute acid is the most common pretreatment but forms furfural, 5-hydroxymethylfurfural and phenolic inhibitors (Basak et al., 2020; Zhao et al., 2024). Typical operating ranges for the two stages are given in Table 3.

Table 3. Typical operating ranges for two-stage hydrogen–methane digestion (Soares et al., 2020; Abdur Rawoof et al., 2021)

Parameter	H_2 stage	CH_4 stage	Reason
pH	5.5–6.5	7.0–7.5	Low pH suppresses methanogens
Temperature	37 °C, or 55–80 °C for thermophiles	37 °C	Heat favours the acetate route
Retention time	1–3 d	10–15 d	Short HRT washes out methanogens
Inoculum	Heat-shocked, about 100 °C	Anaerobic sludge	Enriches spore-forming clostridia

Dark fermentation leaves most substrate energy in volatile fatty acids. Passing this effluent to a methanogenic reactor recovers it as methane, and the blended gas (biohythane, 10–30% H_2) cut NO_x emissions by 45–50% in vehicles compared with methane (Abdur Rawoof et al., 2021). Co-digesting sugar-industry wastes gave 193.6 mL H_2 L^{-1} d^{-1} and 422.0 mL CH_4 L^{-1} d^{-1}, a combined 5.17 kJ g^{-1} VS (Wongarmat et al., 2025). After about 50 years of laboratory study, however, two-stage digestion has not reached industrial scale (Holl et al., 2022). The integrated process chain is shown in Figure 2.

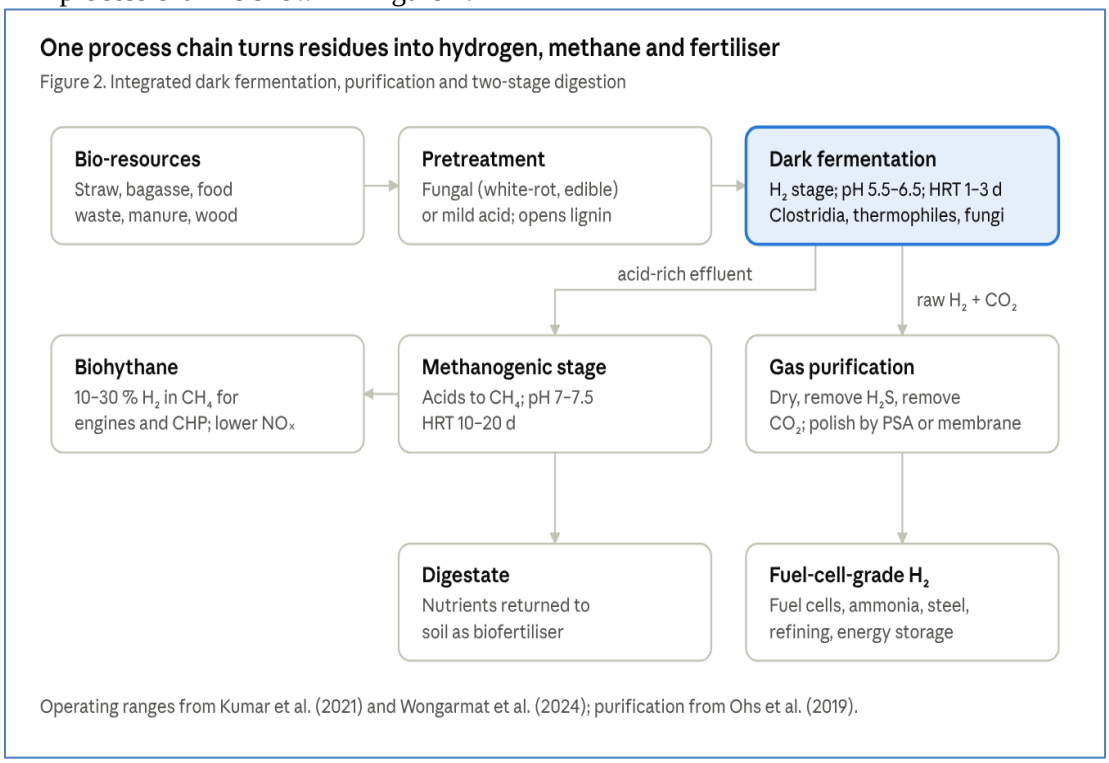


Figure 2. Integrated biohydrogen process chain. The H_2 stage sends its gas to purification and its acid-rich effluent to methanogens, so one feedstock yields hydrogen, methane and fertiliser.

7. Purification Of Fermentative Gas

Raw fermentation gas is mainly H_2 and CO_2, saturated with water and often carrying H_2S, which must be removed for fuel-cell, industrial or grid use (Rey et al., 2026). A typical train dries the gas, removes H_2S (iron-oxide or activated-carbon beds), removes bulk CO_2, and then polishes the H_2. The main options are compared in Table 4.

Table 4. Purification options for fermentative hydrogen

Technology	H_2 purity	Strengths	Limitations	Reference
Chemical absorption	Moderate (CO_2 removal only)	Mature, simple	Solvent regeneration energy	Abdul Muin et al. (2021)
Pressure swing adsorption	Up to 99.99+%	Highest purity; >90% recovery in multi-bed units	Needs compression	Luberti & Ahn (2022)
Membranes (polymeric, carbon, Pd)	Moderate to high	Compact, low energy; carbon sieves exceed 7,000 H_2/CO_2 selectivity	Purity–recovery trade-off	Iyer et al. (2025)
Hybrid membrane–PSA	Fuel-cell grade	0.60–1.86 € kg^{-1} H_2 for 75–25 mol% feeds	More complex design	Ohs et al. (2019)
Cryogenic	High	Handles large flows	Energy-intensive, costly	Abdul Muin et al. (2021)

Because purification cost rises steeply as H_2 concentration falls (Ohs et al., 2019), keeping the fermented off-gas rich in H_2 is as important as the separation step itself.

8. Uses Of Biohydrogen

Low-carbon hydrogen does most for the climate as an industrial feedstock; a global life-cycle study ranked steel-making, ammonia and biofuels highest and advised against its use for road transport and domestic heating (Terlouw et al., 2025).

- **Chemical feedstock** – ammonia and fertiliser, refining and methanol, the largest existing hydrogen markets (Griffiths et al., 2021).
- **Steel** – direct reduction of iron ore in place of coke.
- **Fuel cells** – proton-exchange membrane fuel cells for buses, fleets and back-up power; these need fully purified gas (Fan et al., 2021).
- **Biohythane** – H_2–CH_4 blends for engines and combined heat and power, requiring no separation (Abdur Rawoof et al., 2021).
- **Energy storage** – seasonal storage in salt caverns or porous rock (Osman et al., 2022).
- **Co-products** – an engineered *C. bescii* process was modelled to give 206 kg acetone and 25 kg H_2 per tonne of soybean hulls (Bing et al., 2022); digestate returns nutrients to soil.

9. Challenges And Future Prospects

Low yield, laboratory-scale evidence and purification cost keep biohydrogen from commercial use; scalability and feedstock variability remain the key bottlenecks (Binchebo et al., 2026).

- **Limitations:** yields well below 4 mol H_2 mol^{-1} hexose; competition from lactic acid bacteria, homoacetogens and methanogens; inhibitors from chemical pretreatment; dilute, CO_2-rich gas; and anaerobic fungi that are slow-growing and lack genetic tools (Wilken et al., 2021).
- **Directions:** designed consortia that exclude H_2 consumers (Ergal et al., 2020); termite-inspired reactors and bioprospecting of wood-feeding higher termites for new hydrogenases and strains (Sero et al., 2025); fungal bioreactors with continuous H_2 stripping (Stabel et al., 2022); coupling with microbial electrolysis cells to convert residual acids into more H_2 (Pandit et al., 2025); and pilot-scale trials with life-cycle assessment.

10. Conclusion

Anaerobic bacteria and fungi can convert low-value residues into hydrogen while treating waste, making them a real bio-resource for environmental sustainability. *Clostridium* and *Caldicellulosiruptor* are the most productive single organisms, and designed consortia have exceeded the Thauer limit in the laboratory. Anaerobic gut fungi produce H_2 directly from untreated fibre, while aerobic fungi offer low-inhibitor delignification. The termite hindgut is both the best natural model of efficient hydrogen cycling and a source of hydrogen-producing isolates. Membrane–PSA purification delivers fuel-cell-grade gas, and two-stage biohythane

systems maximise energy recovery. Continuous pilot-scale operation is now needed to show whether these laboratory gains hold at scale.

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