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Effects of Seed Sludge Pretreatment and Fermentation Temperature on Dark Fermentation Hydrogen Production: A Review Postprint

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Abstract

Anaerobic fermentation for hydrogen production not only treats organic waste but also yields clean energy, thereby achieving waste resource utilization. This review summarizes the development of seed sludge pretreatment methods for dark fermentation hydrogen production, discusses the effects of seed sludge pretreatment and fermentation temperature on hydrogen production, and proposes future research directions for this field.

Full Text

Preamble

A Review on Effects of Seed Sludge Pretreatment and Fermentation Temperature on Dark Fermentative Hydrogen Production

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Abstract

Anaerobic fermentative hydrogen production can not only treat organic wastes but also recover clean energy, thereby achieving waste resource utilization. This review examines the development of hydrogen-producing seed sludge pretreatment methods and the effects of seed sludge pretreatment and fermentation temperature on dark fermentative hydrogen production, with future research directions proposed for this field.

Keywords: Seed sludge pretreatment; Fermentation temperature; Dark fermentative hydrogen production

Introduction

As fossil fuel reserves gradually deplete, anaerobic fermentation of biomass resources (such as wastewater, livestock manure, and municipal solid waste) for hydrogen production has emerged as a key research focus for clean energy generation and waste resource utilization [1-5]. Anaerobic fermentative hydrogen production comprises two approaches: dark fermentation and photo-fermentation. Compared with photo-fermentation, dark fermentation offers advantages including no requirement for external light sources, simpler reactor configurations, and superior hydrogen production rates and yields. Seed sludge for dark fermentative hydrogen production can be sourced from diverse origins, including farmland soil, aerobic and digested sludge from municipal wastewater treatment plants, sediment from municipal sewer networks, and sludge from anaerobic bioreactors [6]. However, due to the wide variety of microorganisms present in seed sludge, abundant hydrogen-producing bacterial communities coexist with substantial populations of hydrogen-consuming bacteria (primarily methanogens and homoacetogens), which inevitably reduces the overall hydrogen production efficiency of the system.

Therefore, pretreatment of hydrogen-producing seed sludge is typically performed prior to inoculating dark fermentation reactors to selectively inhibit or eliminate hydrogen-consuming bacteria while enriching hydrogen-producing microbial communities, which is crucial for rapid startup and efficient operation of dark fermentative hydrogen production systems. Additionally, fermentation temperature represents a critical factor in enzymatic reactions and constitutes an important parameter influencing fermentative hydrogen production. Elevating fermentation temperature appropriately proves to be an effective strategy for enhancing hydrogen production, particularly when utilizing recalcitrant substrates [7]. Moreover, synergistic effects exist between seed sludge pretreatment methods and subsequent fermentation temperatures on hydrogen production efficiency.

This review addresses two key aspects: the development of seed sludge pretreatment methods for dark fermentative hydrogen production and the influence of fermentation temperature on hydrogen production efficiency. We discuss commonly employed seed sludge pretreatment approaches and the effects of fermentation temperature on anaerobic fermentative hydrogen production processes, while also providing perspectives on future research directions in this field.

1 Effects of Seed Sludge Pretreatment Methods on Dark Fermentative Hydrogen Production

The primary microorganisms responsible for dark fermentative hydrogen production belong to two genera: *Clostridium* and *Enterobacter*. *Clostridium* species are Gram-positive, obligate anaerobes capable of spore formation under extreme conditions, whereas *Enterobacter* species are Gram-negative facultative anaerobes. Based on these distinct microbial characteristics, common seed sludge pretreatment methods fall into two main categories: (1) Utilizing the spore-forming capability of *Clostridium* to eliminate hydrogen-consuming bacteria through harsh conditions such as heat, acid, or alkali treatment; and (2) Leveraging the facultative anaerobic nature of *Enterobacter* through aeration treatment to eradicate obligate anaerobic hydrogen-consuming bacteria, particularly methanogens. Additionally, hydrogen-consuming bacterial activity can be suppressed through the addition of inhibitors such as 2-bromoethanesulfonate (BESA), chloroform, and iodoethane. These inhibitors act as analogs of coenzyme M in the methanogenesis pathway, specifically interfering with the metabolic activities of methanogens and thereby inhibiting their activity.

Throughout the development of dark fermentative hydrogen production research, fermentation substrates have evolved from simple compounds like glucose to increasingly complex materials, pretreatment methods have diversified, and production scales have progressed from batch systems to pilot and demonstration scales. through summarize the optimal pretreatment conditions for hydrogen-producing seed sludge and the corresponding hydrogen production efficiencies under various substrate conditions.

1.1 Heat Treatment

Heat treatment represents the most prevalent seed sludge pretreatment method, particularly in early studies on dark fermentative hydrogen production, where the majority of reports employed this approach. summarizes the conditions for heat treatment of seed sludge and the corresponding optimal hydrogen production efficiencies. As shown in , heat treatment promotes dark fermentative hydrogen production across various substrates and reactor types, with the exception of sucrose. Simple substrates such as glucose achieve the highest hydrogen yields, ranging from 160–400 mL/g-VS, followed by food waste substrates with yields of 30–60 mL/g-VS. Less favorable hydrogen production is observed with rice straw, cassava ethanol stillage, and other substrates, yielding only 0.4–14 mL/g-VS. Heat treatment conditions comprise two parameters: temperature and duration. Hu et al. [8] investigated the effect of heat treatment duration on anaerobic fermentative hydrogen production using glucose as substrate and anaerobic granular sludge and anaerobic sludge as seed inocula, demonstrating that 30 min treatment was more conducive to hydrogen production than 10 min. Baghchehsaraee et al. [9] examined the impact of heat treatment temperature on anaerobic fermentative hydrogen production using glucose as substrate and anaerobic digested sludge and activated sludge as seed inocula, finding that a treatment temperature of 65°C yielded the highest hydrogen production compared to 80°C and 95°C. Conversely, Liu [14] reported optimal hydrogen production from food waste using anaerobic activated sludge at 80°C for 15 min after comparing various temperature-time combinations. These findings indicate that the optimal heat treatment temperature and duration vary depending on the seed sludge source.

1.2 Acid and Base Treatment

Acid or alkali treatment of hydrogen-producing seed sludge primarily exploits the extreme pH sensitivity of hydrogen-consuming bacteria such as methanogens, eliminating these organisms through pH adjustment. and summarize the optimal conditions and corresponding hydrogen yields for acid and base treatments, respectively. As shown in , sucrose as substrate achieves the highest hydrogen yield of 347 mL/g-VS, followed by glucose at 120-230 mL/g-VS, food waste at 50-80 mL/g-VS, and wastewater at merely 0.3 mL/g-VS. Liu [14] investigated anaerobic fermentative hydrogen production from food waste using anaerobic activated sludge, finding that acid pretreatment at pH 4 yielded the best hydrogen production after comparing various pH levels. Unlike heat treatment, acid pretreatment does not always enhance hydrogen production and may even exert inhibitory effects. Hu et al. [8] subjected anaerobic granular sludge and anaerobic sludge to acid treatment (pH 3, 24 h) for hydrogen production from glucose, revealing complete inhibition of hydrogen production with granular sludge and a 20% reduction in hydrogen yield with anaerobic sludge.

The effects of base treatment of seed sludge on anaerobic fermentative hydrogen production are presented in . Glucose as substrate achieves the highest hydrogen yield of 200-230 mL/g-VS, followed by sucrose at 150 mL/g-VS, while food waste substrates show poorer performance with yields of 30-60 mL/g-VS. Overall, base treatment of most seed sludges promotes dark fermentative hydrogen production. Liu [14] examined the effects of different base treatment pH levels on hydrogen production from food waste using anaerobic activated sludge, identifying pH 12 as optimal for hydrogen yield. Additionally, as shown in , when anaerobic digested sludge and aerobic activated sludge were treated with alkali (pH 12, 24 h) using glucose (10 g/L) as substrate, the degree of enhancement varied due to differences in seed sludge origin and inherent properties (anaerobic vs. aerobic). The promoting effect was more pronounced for aerobic activated sludge, whose hydrogen yield was 4.3 times that of the untreated control. These results underscore the need for further investigation into optimal pH levels and treatment durations for acid and base treatments, as seed sludge origin significantly influences both the optimal conditions and resulting hydrogen yields.

1.3 Aeration Treatment

summarizes the conditions for aeration pretreatment of seed sludge and the corresponding hydrogen yields. Overall, aeration pretreatment exhibits no inhibitory effects on dark fermentative hydrogen production and provides promoting effects in most cases. Wu et al. [18] investigated the effects of continuous versus repeated aeration on anaerobic fermentative hydrogen production, demonstrating that repeated aeration pretreatment of seed sludge yielded higher hydrogen production. As shown in , when food waste served as the fermentation substrate, insufficient aeration duration failed to promote hydrogen production, with hydrogen yields after 2 h of aeration being significantly lower than those observed after 7 d and 10 d of treatment. Therefore, both the aeration mode and duration must be comprehensively considered to obtain seed sludge with favorable hydrogen-producing activity.

1.4 Inhibitor Treatment

summarizes the conditions for various inhibitor treatments of seed sludge and the corresponding hydrogen yields. Inhibitors such as chloroform, BESA, and iodoethane primarily target methanogens. The type and dosage of inhibitors constitute key factors affecting hydrogen production. Li et al. [12] pretreated aerobic activated sludge with different inhibitors for hydrogen production from glucose, finding that chloroform enhanced hydrogen production while BESA exhibited inhibitory effects. Hu et al. [8] used chloroform as an inhibitor to pretreat anaerobic granular sludge and anaerobic sludge for hydrogen production from glucose, revealing that the optimal inhibitor dosage differed by a factor of five between the two seed sludge types.

1.5 Effects of Seed Sludge Pretreatment on Fermentation Types and Microbial Communities

Dark fermentative hydrogen production efficiency is closely associated with fermentation type. During anaerobic fermentation, hydrogen generation occurs primarily through four metabolic pathways: acetate pathway, butyrate pathway, ethanol pathway, and mixed acetate-butyrate pathway. Concurrently, metabolic reactions that either do not produce hydrogen or actively consume hydrogen may also occur, such as lactate pathway, propionate pathway, and hydrogen conversion to acetate pathway.

Previous studies have investigated the types of volatile fatty acids in the liquid phase and the corresponding hydrogen-producing fermentation patterns following seed sludge pretreatment. Baghchehsaraee et al. [9] analyzed volatile fatty acids and hydrogen-producing metabolic pathways using glucose as substrate with anaerobic digested sludge and activated sludge as seed inocula after heat pretreatment, concluding that butyrate and acetate were the primary volatile acid products under optimal hydrogen-producing conditions, with a mixed butyrate-acetate fermentation type. Ghimire et al. [16] similarly reported that butyrate and acetate were the dominant volatile acids at maximum hydrogen production efficiency when using tomato and pumpkin as substrates with anaerobic digested sludge pretreated with BESA inhibitor, also exhibiting a mixed butyrate-acetate fermentation pattern. Consistent conclusions were drawn by Bellucci et al. [11], Li et al. [17], and Wu et al. [18]. Regarding the effect of butyrate-to-acetate ratio on hydrogen production, Bellucci et al. [11] compared hydrogen yields with butyrate/acetate ratios following thermal, acid, and aeration pretreatments, finding that pretreatment did not significantly affect this ratio. In contrast, Wu et al. [18] observed different butyrate/acetate ratios under various seed sludge pretreatment conditions. Ghimire et al. [16] reported similar findings and demonstrated that higher butyrate/acetate ratios correlated with greater hydrogen yields, attributing this to the fact that increased acetate accumulation enhances homoacetogenic activity, leading to greater hydrogen consumption and reduced hydrogen production. Conversely, Bellucci et al. [11] reached the opposite conclusion, suggesting that higher butyrate/acetate ratios resulted in poorer hydrogen production; further microbial analysis revealed no homoacetogens in this process, and acetate-type fermentation exhibited higher hydrogen conversion efficiency compared to butyrate-type fermentation. Additionally, Li et al. [12] identified butyrate-type fermentation as optimal for hydrogen production, while Yuan et al. [15] reported ethanol-type fermentation as the most efficient metabolic pattern. These discrepancies underscore the necessity of comprehensively considering both the hydrogen-producing microbial species present in seed sludge and their metabolic pathways when investigating optimal hydrogen production conditions.

Seed sludge pretreatment significantly influences hydrogen-producing microbial species and community structure, thereby affecting dark fermentative hydrogen production efficiency. Several researchers have investigated the microbial aspects of dark fermentative hydrogen production. Baghchehsaraee et al. [9] examined the relationship between different heat treatment temperatures and hydrogen production, concluding through microbial phase analysis that elevated treatment temperatures reduced hydrogen yields by decreasing population diversity, which is detrimental to hydrogen production. In contrast, Bellucci et al. [11] found that thermal, acid, and aeration pretreatments did not significantly affect microbial population diversity but rather influenced hydrogen production by altering microbial community structure. Furthermore, Lamaison et al. [22] reported that environmental conditions such as pH also affect microbial metabolic pathways; for instance, *Clostridium* species can metabolize substrates to produce butanol and acetone. Currently, research on the microbial impacts of seed sludge pretreatment remains relatively limited. However, investigating changes in hydrogen-producing microbial communities and dominant populations under different pretreatment conditions is essential for understanding how seed sludge pretreatment affects hydrogen production efficiency. Therefore, future studies should further explore seed sludge origins, microbial categories, and microbial community analysis under various pretreatment conditions to systematically examine suitable pretreatment methods for specific microbial communities or the effects of particular pretreatment approaches on community composition and structure.

2 Effects of Fermentation Temperature on Dark Fermentative Hydrogen Production

Anaerobic fermentative hydrogen production is typically conducted under four temperature regimes: low temperature (10–20°C), mesophilic (30–40°C), thermophilic (50–60°C), and hyperthermophilic (80°C), with mesophilic conditions being most commonly employed. However, studies have shown that elevating fermentation temperature not only increases substrate solubility in the liquid phase, facilitating microbial metabolism and degradation [25], but also enhances enzymatic activity in seed sludge microorganisms, accelerating metabolic rates and promoting hydrogen production. Some researchers have also suggested that increased temperature reduces hydrogen solubility in solution [26], facilitating hydrogen release and increasing hydrogen yields. Consequently, numerous researchers have investigated hydrogen production at various fermentation temperatures to examine temperature effects, with relevant findings summarized in .

reveals that the optimal fermentation temperature for hydrogen production varies depending on substrate and seed sludge type. For instance, when using sedimentation tank sludge for dark fermentative hydrogen production from food waste, rice, and noodles, the optimal temperatures are 55°C, 37°C, and 55°C, respectively. Similarly, using aerobic dewatered sludge and sedimentation tank sludge for food waste fermentation yields optimal temperatures of 35°C and 55°C, respectively. This variation arises because temperature plays a crucial role in the growth and metabolism of microbial communities in seed sludge, with different hydrogen-producing microorganisms having distinct optimal temperature requirements. While most hydrogen-producing microbes are mesophilic with an optimal temperature of 37°C, secondary sedimentation tank sludge exhibits optimal hydrogen production at 55°C [38], and compost sludge at 60°C [39]. Therefore, investigating the dominant microbial communities and their optimal temperatures for hydrogen production from different substrates is essential for selecting appropriate fermentation temperatures to enhance hydrogen yields.

Sivagurunathan et al. [36] reported optimal hydrogen production from beverage industry wastewater using food waste compost at 45°C, with *Clostridium* as the dominant genus. Literature indicates that mesophilic hydrogen-producing bacteria mainly include *Clostridium*, *Enterobacter*, and *Bacillus* species, whereas thermophilic and hyperthermophilic conditions favor *Thermoanaerobacterium*, *Thermocellum*, and *Bacillus* species [40]. Gao et al. [41] found that hyperthermophilic fermentation reduces population diversity compared to mesophilic and thermophilic conditions.

Furthermore, hydrolysis is generally considered the rate-limiting step in anaerobic fermentation, and increasing fermentation temperature can accelerate organic matter hydrolysis. However, Cao et al. [28] and Arslan et al. [29] demonstrated through hydrogen production experiments at various temperatures that the rate-limiting step is not hydrolysis but rather acidification. Since pH varies across different fermentation temperatures and the optimal pH for dark fermentative hydrogen production ranges from 5.5–6.0 [42], excessively low or high pH values inhibit hydrogen production. Moreover, literature reports indicate that homoacetogens exhibit high activity under low pH conditions, converting hydrogen to methane and thereby decreasing hydrogen yields [43-44].

Therefore, while appropriately increasing temperature within the tolerable range of hydrogen-producing microorganisms promotes hydrogen production, indiscriminate temperature elevation not only drastically reduces microbial community diversity but also causes enzyme inactivation and even cell death. Additionally, higher fermentation temperatures increase energy consumption and operational costs. Consequently, both energy production and consumption must be comprehensively considered to achieve maximum hydrogen production efficiency in the most economical manner.

3 Combined Effects of Seed Sludge Pretreatment and Fermentation Temperature on Dark Fermentative Hydrogen Production

To comprehensively compare the effects of seed sludge pretreatment methods and fermentation temperature on anaerobic fermentative hydrogen production, several researchers have conducted experimental studies combining both factors. Luo et al. [19] investigated dark fermentative hydrogen production from cassava stillage under three conditions: mesophilic (37°C), mesophilic (37°C) + heat-pretreated seed sludge (90°C, 60 min), and thermophilic (60°C). Their results indicated that seed sludge pretreatment had no significant effect on hydrogen production, whereas elevated fermentation temperature inhibited homoacetogenic activity, thereby promoting hydrogen production. Liu et al. [45] examined anaerobic fermentative hydrogen production from a mixture of food waste and sludge under three conditions: mesophilic (37°C) + heat-pretreated seed sludge (100°C, 30 min), thermophilic (55°C) + heat-pretreated seed sludge (100°C, 30 min), and thermophilic (55°C) + aeration-pretreated seed sludge. They found that thermophilic fermentation following aeration pretreatment represented the optimal operating condition in terms of both hydrogen yield and methanogen inhibition. Further microbial analysis revealed that fermentation temperature exerted a greater influence on bacterial community structure than pretreatment method, whereas pretreatment approach had a more substantial impact on bacterial community diversity.

Due to variations in hydrogen-producing microbial categories, substrate types, and pretreatment conditions, existing research findings show discrepancies. Therefore, further systematic experimental studies examining substrate types, seed sludge categories, and pretreatment methods are needed to investigate the combined effects of these factors on anaerobic fermentative hydrogen production.

Given the limited microbial phase analysis in existing anaerobic fermentation studies, future research should systematically investigate the categories of hydrogen-producing microbial populations in inoculum sludge and the corresponding changes in microbial communities under different pretreatment conditions.

Since hydrogen-producing microorganisms have inherent temperature limitations, future hydrogen production experimental studies should focus on screening and inoculating highly efficient hydrogen-producing bacteria to enhance hydrogen generation. According to research [46], isolated hydrogen-producing bacteria such as strain S can convert ethanol to acetate and hydrogen, while *Syntrophomonas wolfei* can oxidatively decompose butyrate into acetate and hydrogen. Therefore, subsequent fermentative hydrogen production experiments should further screen for or appropriately inoculate highly efficient hydrogen-producing microbial consortia.

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Table 1 Effects of heat treatment of seed sludge on dark fermentative hydrogen production

No.	Substrate	Seed Sludge	Reactor Type	Treatment Conditions	H Yield	Effect	Comparison
7	Food waste 21.1 g-VS/L	Anaerobic granular sludge	Batch (35°C, 23 mL)	100°C, 30 min	160.0 mL/g-VS	Promoted	300× untreated
8		Batch (35°C, 23 mL)	100°C, 30 min	177.7 mL/g-VS	Promoted	1.11× untreated	
		Anaerobic digested sludge	Batch (37°C, 100 mL)	65°C, 30 min	408.8 mL/g-VS	Promoted	5.35× untreated
		Anaerobic digested sludge	Batch (37°C, 100 mL)	65°C, 30 min	284.4 mL/g-VS	Promoted	6.15× untreated
		Aerobic activated sludge	Batch (35°C, 100 mL)	100°C, 15 min	320.0 mL/g-VS	Promoted	4.50× untreated

No.	Substrate	Seed Sludge	Reactor Type	Treatment Conditions	H Yield	Effect	Comparison
		Anaerobic digested sludge	Batch (37°C, 100 mL)	90°C, 15 min	248.8 mL/g-VS	Promoted	1.17× untreated
		Aerobic activated sludge	Batch (35°C, 100 mL)	100°C, 30 min	160.0 mL/g-VS	Promoted	3.00× untreated
		Anaerobic granular sludge	Batch (35°C, 40 mL)	100°C, 20 min	291.0 mL/g-VS	Inhibited	0.50× untreated
	Food waste 25 g-VS/L	Anaerobic activated sludge	Batch (35°C, 200 mL)	80°C, 15 min	61.6 mL/g-VS		
			Batch (35°C, 200 mL)	70°C, 30 min	10.8 mL/g-VS	Promoted	10.80× untreated
9	Tomato & pumpkin 5.25 g-VS/L	Anaerobic digested sludge	Batch (35°C, 600 mL)	105°C, 240 min	44.9 mL/g-VS		
10	Rice & lettuce 20 g-VS/L	Anaerobic digested sludge	Batch (37°C, 400 mL)	100°C, 30 min	119.7 mL/g-VS	Promoted	5.20× untreated
11	Food waste & excess sludge 43.5 g-VS/L	Anaerobic digested sludge	Batch (55°C, 150 mL)	100°C, 30 min	26.8 mL/g-VS	Promoted	1.29× untreated
12	Cassava stillage 28 g/L	Anaerobic activated sludge	Batch (37°C, 200 mL)	90°C, 60 min	13.8 mL/g-VS	No significant effect	

No.	Substrate	Seed Sludge	Reactor Type	Treatment Conditions	H Yield	Effect	Comparison
13	Rice straw		Batch (35°C, 120 mL)	100°C, 60 min	14.22 mL/g-VS	Promoted	1.98× untreated
14	Emulsion wastewater 1.05 g/L	Anaerobic activated sludge	Batch (29°C, 200 mL)	100°C, 60 min	0.4 mL/g-VS	Promoted	6.67× untreated
15	Glucose 5 g/L	Aerobic activated sludge					
17	Cheese whey 17.6 g-VS/L	Anaerobic granular sludge	Anaerobic SBR (55°C, 450 mL)	80°C, 15 min	0.2 L/L.d	Promoted	5.00× untreated
			UASB (37°C, HRT=3 h, OLR=133 g-COD/L·d)	100°C, 50 min	13.0 L/L.d	Promoted	1.86× untreated
			UASB (35°C, HRT=8 h, OLR=48 g-COD/L·d)	100°C, 60 min	1.7 L/L.d	Promoted	1.79× untreated

Table 2 Effects of acid treatment of seed sludge on dark fermentative hydrogen production

No.	Substrate	Seed Sludge	Reactor Type	Treatment Conditions	H Yield	Effect	Comparison
5	Sucrose 10 g/L	Anaerobic granular sludge	Batch (35°C, 23 mL)	pH=3, 24 h		Inhibited	No gas
		Anaerobic sludge	Batch (35°C, 23 mL)	pH=3, 24 h	124.4 mL/g-VS	Inhibited	0.8× untreated
		Anaerobic digested sludge	Batch (35°C, 100 mL)	pH=3, 24 h	142.2 mL/g-VS	Promoted	2.0× untreated
		Anaerobic digested sludge	Batch (37°C, 100 mL)	pH=3, 24 h	213.3 mL/g-VS	No significant effect	
		Aerobic activated sludge	Batch (35°C, 100 mL)	pH=3, 24 h	231.1 mL/g-VS	Promoted	5.0× untreated
		Anaerobic digested sludge	Batch (35°C, 40 mL)	pH=3, 0.5 h	347.0 mL/g-VS	Inhibited	0.6× untreated
6	Food waste 21.1 g-VS/L	Aerobic activated sludge	Batch (35°C, 200 mL)	pH=4, 1/3 h	53.1 mL/g-VS		
7	Rice & lettuce 20 g-VS/L	Anaerobic digested sludge	Batch (37°C, 400 mL)	pH=3, 6 h		Inhibited	No gas
8	Food waste & excess sludge 43.5 g-VS/L	Anaerobic digested sludge	Batch (55°C, 150 mL)	pH=2, 24 h	81.3 mL/g-VS	Promoted	3.9× untreated
9	Emulsion wastewater 1.05 g/L	Anaerobic activated sludge	Batch (29°C, 200 mL)	pH=3, 24 h	0.3 mL/g-VS	Promoted	5.0× untreated

No.	Substrate	Seed Sludge	Reactor Type	Treatment Conditions	H Yield	Effect	Comparison
10	Glucose 5 g/L	Aerobic activated sludge	Anaerobic SBR (55°C, 450 mL)	pH=3, 24 h	0.2 L/L.d	Promoted	4.4× untreated

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Table 3 Effects of base treatment of seed sludge on dark fermentative hydrogen production

No.	Substrate	Seed Sludge	Reactor Type	Treatment Conditions	H Yield	Effect	Comparison
2	Glucose 10 g/L	Anaerobic digested sludge	Batch (35°C, 100 mL)	pH=10, 24 h		Promoted	2.8× untreated
		Aerobic activated sludge	Batch (35°C, 100 mL)	pH=10, 24 h		Promoted	4.3× untreated
3	Sucrose 10 g/L	Anaerobic digested sludge	Batch (35°C, 40 mL)	pH=10, 0.5 h		Inhibited	0.3× untreated
4	Food waste 21.1 g-VS/L	Aerobic activated sludge	Batch (35°C, 200 mL)	pH=12, 1/3 h		Promoted	1.4× untreated
5	Food waste & excess sludge 43.5 g-VS/L	Anaerobic digested sludge	Batch (55°C, 150 mL)	pH=12, 24 h			

Table 4 Effects of aeration treatment of seed sludge on dark fermentative hydrogen production

No.	Substrate	Seed Sludge	Reactor Type	Treatment Conditions	H Yield	Effect	Comparison
4	Sucrose 10 g/L	Anaerobic digested sludge	Batch (35°C, 100 mL)			Promoted	2.3× untreated
		Anaerobic digested sludge	Batch (37°C, 100 mL)			Promoted	1.3× untreated
		Aerobic activated sludge	Batch (35°C, 100 mL)			Promoted	2.0× untreated
		Anaerobic digested sludge	Batch (35°C, 40 mL)			No significant effect	
5	Rice & lettuce 20 g-VS/L	Anaerobic digested sludge	Batch (37°C, 400 mL)			No significant effect	
6	Tomato & pumpkin 5.25 g-VS/L	Anaerobic digested sludge	Batch (35°C, 600 mL)	0.5 h			
7	Food waste & excess sludge 43.5 g-VS/L	Anaerobic digested sludge	Batch (55°C, 150 mL)	Continuous 7 d		Promoted	3.6× untreated
			Batch (55°C, 150 mL)	Repeated 3 h, static 21 h		Promoted	4.2× untreated

Table 5 Effects of inhibitors on dark fermentative hydrogen production

No.	Substrate	Seed Sludge	Reactor Type	Inhibitor Content & Time	H Yield	Effect	Comparison
2	Glucose 10 g/L	Anaerobic granular sludge	Batch (35°C, 23 mL)	5%, 2.5%, 1%, 0.5%, 0.25%, 0.1%		Promoted	33.0× untreated
		Anaerobic sludge	Batch (35°C, 23 mL)	5%, 2.5%, 1%, 0.5%, 0.25%, 0.1%		Promoted	1.2× untreated
3	Glucose 10 g/L	Aerobic activated sludge	Batch (35°C, 100 mL)	2%, 24 h		Promoted	1.8× untreated
			Batch (35°C, 100 mL)	0.1%, 24 h		Promoted	1.7× untreated
4	Sucrose 10 g/L	Anaerobic digested sludge	Batch (35°C, 100 mL)	10 mM, 24 h		Inhibited	0.7× untreated
5	Tomato & pumpkin 5.25 g-VS/L	Anaerobic digested sludge	Batch (35°C, 40 mL)	10 mM, 30 min		No significant effect	
			Batch (35°C, 40 mL)	10 mM, 30 min		No significant effect	
6	Food waste & excess sludge 43.5 g-VS/L	Anaerobic digested sludge	Batch (35°C, 600 mL)	25 mM, 24 h		Promoted	2.5× untreated
7	Emulsion wastewater 1.05 g/L	Anaerobic activated sludge	Batch (55°C, 150 mL)	10 mM, 24 h		Promoted	18.3× untreated

No.	Substrate	Seed Sludge	Reactor Type	Inhibitor Content & Time	H Yield	Effect	Comparison
			Batch (29°C, 200 mL)	1 mM, 24 h			

Table 6 Effects of fermentation temperature on dark fermentative hydrogen production

No.	Substrate	Seed Sludge	Reactor Type	Temperature	H Yield
1	Glucose	Enterobacter aerogenes	Batch (150 mL)	25°C, 30°C, 35°C, 38°C	348.0 mL/g-VS
		Aerobic dewatered sludge		25°C, 35°C, 50°C	63.5 mL/g-VS
	Food waste	Sedimentation tank sludge	Batch (400 mL)	37°C, 55°C	21.57 mL/g-VS
	Rice	Sedimentation tank sludge	Batch (400 mL)	37°C, 55°C	32.76 mL/g-VS
	Noodles	Sedimentation tank sludge	Batch (400 mL)	37°C, 55°C	22.89 mL/g-VS
5	Brewery wastewater	Anaerobic activated sludge	Batch (200 mL)	25°C, 35°C, 45°C	237.6 mL/g-VS
6	Cassava ethanol wastewater	Anaerobic granular sludge	Batch (200 mL)	37°C, 60°C, 65°C, 70°C, 75°C, 80°C	70.0 mL/g-VS
7	Cassava stillage	Anaerobic activated sludge	Batch (200 mL)	37°C, 60°C	69.6 mL/g-VS
8	Food waste	Anaerobic activated sludge	Batch (200 mL)	25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C	37.0 mL/g-VS
9	Apple residue		Batch (400 mL)	20°C, 35°C, 50°C	17.5 mL/g-VS
10	Dairy processing waste	Anaerobic activated sludge	Induced bed reactor (60 L)	40°C, 60°C	72.3 mL/g-VS
11	Rice straw	Sedimentation tank sludge	CSTR (20 L)	37°C, 55°C	15.3 mL/g-VS

No.	Substrate	Seed Sludge	Reactor Type	Temperature	H Yield
12	Beverage industry wastewater		CSTR (2 L)	37°C, 45°C	13.6 L/L.d
13	Cellulose	Anaerobic digested sludge	CSTR (6 L)	37°C, 55°C, 80°C	295.2 mL/g-cellulose

Note: Figure translations are in progress. See original paper for figures.

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