

Enhancing Biohydrogen Production from Straw: Synergistic with Coal Gangue and Freeze- Thaw Pretreatment

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Abstract

Hydrogen conversion efficiency is the primary factor limiting the large-scale production of hydrogen from straw fermentation. This study applied freeze-thaw pretreatment to improve straw bioavailability and combined it with coal gangue addition to enhance acid and hydrogen production, while evaluating environmental benefits. Comparison of hydrogen yields before and after pretreatment showed a 27.5% increase in hydrogen production from pretreated straws. After gangue addition, both hydrogen yield and the concentration of volatile fatty acids (VFAs) in the fermentation broth first increased and then decreased with decreasing gangue particle size and increasing dosage. The maximum hydrogen yield was achieved with 80-mesh gangue at a dosage of 30 g/L, resulting in a 31.5% increase over the control group. Additionally, the incorporation of coal gangue into anaerobic digestion systems reduced carbon dioxide emissions by 32.1%, demonstrating significant environmental benefits and contributing to the synergistic achievement of waste resource utilization and carbon emission reduction goals.

1 Introduction

Energy is fundamental to the advancement of human civilization and societal progress. However, with global energy consumption on the rise, non-renewable fossil fuels—including coal, oil, and natural gas—face increasing depletion. In this context, biomass energy, as the world's fourth-largest energy resource after coal, oil, and natural gas, presents a promising alternative due to its vast reserves and renewable nature. Crop straw represents its most prevalent material [1]. Straw energy conversion technologies are primarily categorized into three types: direct solid combustion, liquefaction, and gasification, which yield solid biofuels, liquid biofuels (e.g., ethanol), and gaseous biofuels (e.g., biogas and hydrogen), respectively[2].

Among these, hydrogen produced via anaerobic fermentation is especially notable. This approach combines the advantages of low cost and environmental sustainability with the dual outcomes of waste valorization and clean energy generation, making it a promising route for straw energy conversion[3]. However, the cellulose and hemicellulose structures in straw are tightly surrounded by lignin, creating a complex physicochemical barrier that significantly hinders direct microbial access and utilization. This results in slower hydrolysis rates and lower hydrogen production yields [4, 5].

Pre-treating straw to disrupt its dense structure is a critical step in enhancing hydrogen production efficiency during subsequent anaerobic fermentation [6, 7]. Among existing pretreatment methods, freeze-thaw pretreatment stands out as a physical approach, offering advantages such as simple operation, no requirement for chemical reagents, and the absence of inhibitory byproducts. This method is especially promising for regions with cold climates [8]. In Northeast China, for instance, leveraging the region's naturally low winter temperatures to freeze-thaw corn stover enables “zero-energy consumption” pretreatment, significantly reducing operational costs. [9] Li et al. pretreated corn stover under natural conditions at an average temperature of -5.5 to 2°C for 21 d, which resulted in a 40.5% increase in methane yield[10].

Beyond pretreatment, hydrogen yield can be further enhanced by adding exogenous additives [11]. Straw pretreatment improves hydrolysis efficiency, providing microorganisms with more readily accessible carbon sources. The addition of additives enhances electron transfer efficiency and promotes the metabolic activity of acid-producing bacteria and hydrogen-producing bacteria. Research indicates that adding appropriate amounts of inorganic minerals—such as natural ores rich in trace elements like iron, nickel, and cobalt—to fermentation systems can significantly stimulate the activity of relevant enzymes (e.g., ferredoxin, hydrogenase), optimize microbial community structure, and thereby enhance the hydrogen production process [12, 13]. Coal gangue is a solid waste product generated during coal mining and washing processes. Large-scale stockpiling not only occupies land but also poses environmental risks [14]. Coal gangue has a composition containing 2–10% Fe_2O_3 and essential trace elements (e.g., Cu, Zn, Ni). These elements are required for enzymatic synthesis and signal transduction in anaerobic fermentation, which makes coal gangue a promising exogenous additive for the process.

This study treated corn straw under the optimal pretreatment conditions identified in previous research [15]. The investigation analyzed changes in the concentration and microstructure of hydrolysis products before and after pretreatment to assess the impact of the freeze-thaw process on these properties and examined the mechanisms underlying pretreatment. Furthermore, the study examined the effects of adding coal gangue with varying particle sizes and concentrations on hydrogen production characteristics and system stability during the anaerobic fermentation of pretreated corn straw. Based on these experiments, the optimal coal gangue concentration and particle size for enhancing hydrogen yield were identified, and the environmental benefits of coal gangue addition were assessed by calculating the reduction in carbon dioxide emissions from hydrogen combustion. It holds significant environmental benefits and practical significance for promoting the resource utilization of agricultural waste (straw) and industrial solid waste (coal gangue).

2 Materials and methods

2.1 Material and inoculum

Corn straws were collected from a farmland in Liaozhong, Liaoning Province, China and stored at room temperature. Corn straws were air-dried, smashed by plant mill and grinded to $\leq 1\text{ mm}$ powders. The corn straws powders (CSP) were stored dryly until further use.

The hydrogen production strain *Clostridium guangxiense* G1^T (CICC 24070) was activated as the inoculum with PYG medium (pH 6.7), which contains (g/L) glucose 10, peptone 4, yeast powder 1 L cysteine 0.5, $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 14.33, KH_2PO_4 3.63, and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.37 [16].

The coal gangue was sourced from a coal yard in Shenyang. It was then ground in a ball mill, sieved through 10, 20, 40, 80, and 100 mesh screens, dried at 105 °C for 24 h, and finally packed into resealable bags for future use.

2.2 Experimental setup and procedure

2.2.1 Freeze-thaw pretreatment

2 g of the CSP was accurately weighed and transferred into a 50 mL centrifuge tube. Distilled water was then added to the samples at a solid-to-liquid ratio (w/w) of 1:10.

The tubes were subsequently subjected to freezing at -20°C for 24 hours. Following the freezing process, the samples were retrieved and thawed at 25°C for 12 h. A control group was established using samples that were not subjected to the freeze-thaw cycle (CK). Each treatment was replicated three times to ensure the reliability of the results.

2.2.2 Anaerobic Fermentation of Corn Straw

The experiment employed 100 mL anaerobic bottles as fermentation reactors with a working volume of 60 mL. After freeze-thaw pretreatment, distilled water was added to bring the total volume to 59 mL. Nitrogen gas was purged through the headspace for 1 minute to remove oxygen. The bottle was then sealed tightly with a rubber stopper and an aluminum cap, and sterilized at 121 °C for 20 minutes. After cooling to room temperature, 1 mL of the hydrogen-producing bacterium *Clostridium guangxiense* G1^T was inoculated, and the culture was incubated in a constant-temperature water bath shaker at 37 °C and 120 rpm for 48 hours. For the control group (CK), 2 g of untreated corn straw and 59 mL of distilled water were added to the anaerobic bottle. After nitrogen purging and sterilization, the same strain (*Clostridium guangxiense* G1^T) was inoculated, and the culture was then incubated under the same conditions. Each treatment was replicated three times.

2.2.3 Anaerobic Fermentation with Coal Gangue

Anaerobic bottles containing 2 g of freeze-thaw pretreated corn stover and 59 mL of water were supplemented with coal gangue at different particle sizes (10, 20, 40, 80, and 100 mesh) and concentrations (0, 1, 5, 10, 30, 50, 70 g/L). 1 mL of hydrogen-producing bacteria *Clostridium guangxiense* G1^T was inoculated for anaerobic fermentation under the same conditions as described in Section 2.2.2.

2.3 Energy Conservation and Emission-Reduction Assessment Method

Calculate the standard coal equivalent and coal-fired carbon dioxide emissions corresponding to hydrogen production using Formulas (1) and (2) [17]:

$$Q_C = Q_B \times E \quad (1)$$

$$C_C = 3.67 \times C \times q_C \times E_C \times 94\% \quad (2)$$

where Q_C denotes standard coal (kg), Q_B represents hydrogen production (m³), E indicates the conversion factor between hydrogen and standard coal (with a value of 0.3329 kg/m³), C_C denotes carbon dioxide emissions from coal combustion (t), 3.67 represents the ratio of the molecular weight of carbon dioxide to the atomic weight of carbon, C represents standard coal consumption (kg), q_C

indicates the calorific value of standard coal(0.0209 TJ/t [18]), E_c signifies the carbon content per unit calorific value of standard coal (26.37 t-c/TJ), and 94% represents the carbon oxidation rate of standard coal.

2.4 Measurement Methods

The samples were centrifuged at 8000 rpm for 5 min, and the supernatant was taken and filtered to determine reducing sugars and SCOD. Reducing sugars were determined using the 3,5-Dinitrosalicylic acid method at 540 nm[19], and SCOD was determined using a fast digestion method at a wavelength of 600 nm[20]. The pH and oxidation-reduction potential (ORP) values were measured using a pH meter (PHS-3E). The gas yield was determined by the drainage method[21]. Volatile fatty acids (VFAs) were determined using gas chromatography(Varian CP-3800) [22]. The surface structure of untreated and pretreated corn straw was observed using scanning electron microscope (JSM-IT1800 scanning electron microscope of Japan Electronics Corporation). The microscope was operated at an acceleration voltage of 3 kV, providing a resolution of 3 and 10 μm . The samples were ground with KBr, pressed into pellets, and analyzed using a Fourier Transform Infrared Spectrometer (IRTracer-100 spectrometer from Shimadzu Corporation, Japan). The scanning wavelength range was set to 400 to 4000 cm^{-1} , with a resolution of 4 cm^{-1} [23].

3 Results and discussions

3.1 Effect of Freeze-Thaw Pretreatment on Hydrogen Production from Corn Straw Anaerobic Fermentation

Pre-treatment disrupts the crystalline structure of cellulose, releasing more soluble substances, which leads to increased reducing sugar yield and SCOD. Consequently, these two parameters serve as paramount indicators for assessing pre-treatment efficiency[24]. As shown in the Fig. 1, pretreatment of corn straw resulted in significant increases in both reducing sugar release and SCOD. The reducing sugar yield in the hydrolysate reached 177.43 mg/g, which was 66.96% higher than that of the control. Similarly, the SCOD value increased to 185.83 mg/g, marking a 68.94% enhancement compared to the untreated group.

To verify the effect of freeze-thaw pretreatment on the microstructure of corn straw, surface morphology was observed using a scanning electron microscope (SEM) for both untreated and freeze-thaw-pretreated corn straw. The control group (Fig. 1a and Fig. 1b) exhibited intact structure with tightly ordered surface arrangement and no apparent damage. Following freeze-thaw pretreatment, corn straw exhibited noticeable cavities in its external structure. The overall block-like structure was disrupted, resulting in numerous irregular and fragmented structures. This indicates that freeze-thaw pretreatment disrupted the dense external barrier of corn straw, enhancing the accessibility of cellulose and hemicellulose and accelerating the hydrolysis rate of corn straw.

Fourier transform infrared (FTIR) spectroscopy was used to scan corn straw samples before and after freeze-thaw pretreatment over the wavenumber range of 4000–400 cm^{-1} . By analyzing changes in surface functional groups and chemical bonds of lignin, cellulose, and hemicellulose before and after pretreatment, the effects of freeze-thaw pretreatment on the chemical composition of corn straw were inferred. The FTIR results are shown in the figure. The shapes and positions of the absorption peaks of corn straw remained largely consistent before and after pretreatment. However, certain characteristic peaks showed varying degrees of enhancement or attenuation. The absorption peak at 3382 cm^{-1} is attributed to the stretching vibration of –OH groups in cellulose[7]. The peak intensity of pretreated corn straw is significantly reduced here, indicating that the hydrogen bonds connecting cellulose, hemicellulose, and lignin molecules have been broken. The peak at 1728 cm^{-1} arises from the C = O stretching vibration of the carbonyl group in hemicellulose, while the peak at 900 cm^{-1} corresponds to the β -glycosidic linkages between glucose units in cellulose[25]. Both peaks are characteristic of cellulose. After freeze-thaw pretreatment of corn straw, the vibrations at these characteristic peaks intensified, indicating a significant increase in the relative cellulose content. The peak observed at 1632 cm^{-1} is associated with the C–O stretching vibration of the phenolic ring in lignin[26]. Following freeze-thaw pretreatment, the intensity of this peak diminished, indicating partial removal of lignin during the process. These results demonstrate that freeze-thaw pretreatment effectively disrupts the dense lignin structure in corn straw by breaking intermolecular hydrogen bonds among cellulose, hemicellulose, and lignin, thereby improving microbial accessibility and hydrolysis efficiency.

Following pretreatment, the anaerobic fermentation of corn straw yielded significantly higher hydrogen production (10.2 mL/g(hydrogen production/corn straw weight), a 27.5% increase) and VFA concentration (0.6 g/L, a 63.8% increase) compared to the control. This enhancement was primarily attributed to the production of acetic acid and butyric acid. Acetic acid, from 0.001 g/L, reached 0.15 g/L, while butyric acid increased from 0.66 g/L to 1.05 g/L. Furthermore, the pretreatment increased the proportion of (butyric acid + acetic acid) among the fermentation end products, with their combined share stabilizing at approximately 70%.

The pH of the final fermentation product from anaerobic fermentation of corn straw before and after pretreatment were 4.84 and 4.95, respectively. This is because the hydrogen production process during corn straw anaerobic fermentation generates large amounts of volatile fatty acids, leading to a drop in pH. When the pH falls below the hydrogen production pH range, the activity of hydrogen-producing bacteria decreases, and these bacteria cease utilizing the straw for hydrogen production.

The redox potential at the end of fermentation in the control group was – 230 mV, while the redox potential of the final product from pretreated corn straw remained around – 280 mV. This indicates that pretreatment reduces the redox potential of the reaction system. A lower redox potential promotes the growth and metabolism of specific microbial dehydrogenase systems and ferredoxin, enhancing the activity of anaerobic fermentation microorganisms and thereby increasing hydrogen production during anaerobic fermentation [27].

3.2 Effect of Coal Gangue Addition on Hydrogen Production

3.2.1 Effect of Coal Gangue Particle Size

The effects of adding coal gangue with different particle sizes on hydrogen production and VFAs concentration during corn straw anaerobic fermentation are shown in the Fig. 2a and Fig. 2b. The results indicated that supplementing with coal gangue enhanced both hydrogen yield and VFAs concentration, suggesting a promotive effect on the growth and metabolism of hydrogen-producing microorganisms. As the particle size was reduced from 10 to 100 mesh, both hydrogen production and VFAs concentration first increased and then decreased, peaking at 11.9 mL/g and 1.65 g/L, respectively, with an 80-mesh addition. Among these, butyric acid and acetic acid constitute the primary components of the fermentation broth, with the combined percentage of (butyric acid + acetic acid) consistently around 95%. Different particle sizes of the added material exhibited varying promotional effects. This may be attributed to the fact that as the particle size of coal gangue decreases, its surface active sites and specific surface area increase accordingly. This enhances the concentration and release rate of Fe(III) within the anaerobic fermentation system, thereby accelerating its conversion to Fe(II). Consequently, the bioavailability of coal gangue is improved[28]. However, when the particle size of coal gangue is reduced to 100 mesh, its huge specific surface area and high surface energy cause it to agglomerate readily, preventing it from remaining stably in the reaction system over extended periods.

The pH of the fermentation system is a critical indicator in anaerobic hydrogen production, as it directly influences the activity of hydrogen-producing microorganisms and hydrogenase enzymes. To evaluate the effect of coal gangue particle size on system pH, the end-fermentation pH was measured (as shown in the Fig. 2c). Under an initial pH of 6.5, the final pH remained stable above 4.7. Furthermore, as coal gangue particle size decreased, the final fermentation product pH exhibited a slight increase, rising from 4.75 to 5.00. This may be attributed to the iron content in the coal gangue reacting in an acidic environment, simultaneously generating hydroxide ions, which elevates the pH of the final fermentation product[13]. The redox potential of the final product decreases as the particle size of coal gangue diminishes. When the particle size of coal gangue is reduced from 10 mesh to 100 mesh, the redox potential of the final product decreases from -223 to -325 mV, which can sustain the growth and metabolism of anaerobic microorganisms[29]. The change in redox potential is inversely proportional to the change in pH of the final fermentation product.

3.2.2 Effect of Coal Gangue Dosage

To investigate the effect of different concentrations of coal gangue on hydrogen production during anaerobic fermentation of corn straw, coal gangue was added to the anaerobic fermentation system at concentrations ranging from 0 to 70 g/L. The results are shown in the Fig. 3a. When the coal gangue dosage ranged from 0 to 30 g/L, hydrogen production gradually increased. At a gangue dosage of 30 g/L, hydrogen yield peaked at 14.2 mL, representing a 31.5% increase compared to the control group.

Further increases in coal gangue dosage did not enhance hydrogen production, which instead slightly decreased. The Fe(III) released from coal gangue can be reduced to Fe(II) required for microbial growth and metabolism. Additionally, coal gangue contains trace elements that support microbial growth and metabolism. Both factors enhance the activity of hydrogen-producing bacteria, thereby increasing hydrogen production. In this study, hydrogen production exhibited a trend of first increasing and then decreasing with rising coal gangue dosage. This occurred because, at excessively low coal gangue dosages, the Fe(III) concentration in the reaction system was also too low, resulting in suboptimal promotion of anaerobic hydrogen production from corn straw by coal gangue. As the coal gangue dosage increased, more Fe(III) was released, satisfying the growth and metabolic needs of microorganisms and enhancing the metabolic activity of anaerobic microorganisms. On the other hand, the Fe(III) released from coal gangue can participate in electron transfer between microbial cells. Higher concentrations of Fe(III) enhance the electron transfer rate, thereby boosting the hydrogen production efficiency of anaerobic microorganisms[30]. However, excessively high concentrations of coal gangue release excessive iron(III), hindering microbial contact with the substrate. Furthermore, excessive iron(III) may damage microbial cell walls and even exert toxic effects, thereby inhibiting the growth and metabolism of anaerobic microorganisms[31].

As shown in the Fig. 3b, with increasing coal gangue dosage, the concentration of total volatile fatty acids in the fermentation broth first increased and then decreased, reaching a maximum of 1.63 g/L at 30 g/L. In the control group, butyric acid constituted 78% of total VFAs, while this proportion increased to 85% after adding 30 g/L of coal gangue. These results indicate that coal gangue promotes hydrogen production by enriching butyric acid in the fermentation broth, thereby providing more effective nutrition for hydrogen-producing bacteria and enhancing hydrogen yield.

The pH of the final fermentation product increased from 4.63 to 5.19 as the coal gangue dosage was raised from 0 to 70 g/L (Fig. 3c). This indicates that coal gangue addition effectively elevated the system pH, providing a buffering capacity against acidification and creating a more suitable environment for anaerobic microbes[32]. Similarly, the redox potential change inversely correlates with the pH change of the final product, decreasing as the coal gangue dosage increases and remaining within the range of -210 to -343 mV.

3.3 Energy Conservation and Emission-Reduction Effects of Adding Coal Gangue to Anaerobic Digestion Systems

The environmental benefits of anaerobic fermentation for hydrogen production primarily lie in the fact that the hydrogen produced during fermentation is a zero-carbon energy source. During combustion, it produces only water and does not emit carbon dioxide or other pollutants. Therefore, it can replace fossil energy and reduce carbon dioxide emissions. Using the experimental data, the amount of hydrogen gas produced per ton of pre-treated corn straw and the corresponding standard coal quantity were calculated, thereby determining the corresponding reduction in carbon dioxide emissions. The calculation results are shown in Table 1. Under identical anaerobic fermentation conditions, the addition

of 30 g/L of 80 mesh coal gangue yielded 14.2 m³ of hydrogen, displacing approximately 4.7 kg of standard coal and reducing carbon dioxide emissions by 8.98 kg. This represents a 32.4% greater reduction in carbon emissions compared to the control group. These research results indicate that adding coal gangue can significantly reduce carbon dioxide emissions, enhance energy conservation and emission-reduction capabilities, and achieve collaborative emission reduction through the "waste treatment - reuse of waste" approach. Compared to other processes (biogas, bioethanol), anaerobic fermentation for hydrogen production not only effectively reduces carbon emissions but also offers the most favourable economic returns and energy output [33]. As hydrogen equipment manufacturing technology continues to advance and research and development in hydrogen transportation and storage technologies progress, the overall benefits of anaerobic fermentation for hydrogen production will continue to increase.

Table 1 Energy conservation and emission-reduction capabilities			
Control group	Hydrogen Production (m ³)	Standard coal (kg)	Coal CO ₂ Emissions (kg)
	10.8	3.6	6.8
Add coal gangue	14.2	4.7	9.0

4 Conclusion

Freeze-thaw pretreatment effectively altered the microstructure of corn straw by disrupting the hydrogen bonds between cellulose, hemicellulose, and lignin. Anaerobic fermentation of the pretreated straw resulted in a 27.5% increase in hydrogen production. The addition of coal gangue to the anaerobic fermentation system further stimulated the growth and metabolism of hydrogen-producing microorganisms, elevating the hydrogen yield to a maximum of 14.2 mL/g representing a 31.5% increase over the control. Under the combined effect of freeze-thaw pretreatment and coal gangue addition, hydrogen production increased by 77.5% compared with that of untreated corn straw. Concurrently, this approach reduced carbon dioxide emissions by 32.4%, establishing a synergistic waste-to-waste treatment model for effective emissions reduction.

Declarations

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Author Contribution

N.and Y. made substantial contributions to the conception or design of the work and the acquisition, analysis and interpretation of data; N.and Y. drafted the work. N. revised it critically for important intellectual content;P. and L. approved the version to be published; N.and Y. agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data availability

The datasets used during the current study are available from the corresponding author on reasonable request.

References

1. Lang LX, Chen YB, Liu Y, Wu JX, Yu YK, Wang Y, Chen XP, Zhang ZL (2023) Changes in spatial patterns of biomass energy potential from biowaste in China from 2000 to 2020. *Front Energy Res* 11:1109530. <http://dx.doi.org/10.3389/fenrg.2023.1109530>
2. Li X, Du YY, Chang HM, Ai J, Zhao Y (2024) Life cycle environmental impacts of five technological routes for straw utilisation in China. *Biomass Bioenergy* 183:107157. <http://dx.doi.org/10.1016/j.biombioe.2024.107157>
3. Zhong YM, Ragauskas AJ, Zheng Y, Meng XZ, Zhou Y, Lin YQ (2025) A review on the pretreatment of straw biomass by using biogas slurry. *Process Saf Environ* 195:106843. <http://dx.doi.org/10.1016/j.psep.2025.106843>
4. Gao YA, Guo MK, Wang DD, Zhao DQ, Wang MX (2023) Advances in extraction, purification, structural characteristics and biological activities of hemicelluloses: A review. *Int J Biol Macromol* 225:467–483. <http://dx.doi.org/10.1016/j.ijbiomac.2022.11.099>
5. Jia GS, Innocent MT, Yu Y, Hu ZX, Wang XF, Xiang HX, Zhu MF (2023) Lignin-based carbon fibers: Insight into structural evolution from lignin pretreatment, fiber forming, to pre-oxidation and carbonization. *Int J Biol Macromol* 226:646–659. <http://dx.doi.org/10.1016/j.ijbiomac.2022.12.053>
6. Sahil S, Nanda S (2025) Process Intensification of Anaerobic Digestion of Biowastes for Improved Biomethane Production: A Review. *Sustainability* 17(14):6553.

<http://dx.doi.org/10.3390/su17146553>

7. Sun JH, Deng YF, Li SH, Xu WY, Liu GQ (2022) Enhanced efficiency of enzymatic hydrolysis of wheat straw via freeze-thaw pretreatment. *Environ Sci Pollut R* 29(37):56696–56704.
<http://dx.doi.org/10.1007/s11356-022-18893-w>
8. Yang LY, Li XJ, Yuan HR, Yan BB, Yang GX, Lu Y, Li J, Zuo XY (2023) Enhancement of biomethane production and decomposition of physicochemical structure of corn straw by combined freezing-thawing and potassium hydroxide pretreatment. *Energy* 268:126633.
<http://dx.doi.org/10.1016/j.energy.2023.126633>
9. Qi N, Wang Y, Zhao X, Han X, Dong L, Hu X (2022) Characteristic and calculation on the co-contribution in the bio-H₂ energy recovery enhancement with low temperature pretreated peanut shell as co-substrate. *Environ Res* 212:113169.
<http://dx.doi.org/10.1016/j.envres.2022.113169>
10. Li J, Wachemo AC, Yuan HR, Zuo XY, Li XJ (2019) Natural freezing-thawing pretreatment of corn stalk for enhancing anaerobic digestion performance. *Bioresour Technol* 288:121518.
<http://dx.doi.org/10.1016/j.biortech.2019.121518>
11. Yan XY, Peng P, Li X, Zhou XD, Chen LX, Zhao F (2025) Unlocking anaerobic digestion potential via extracellular electron transfer by exogenous materials: Current status and perspectives. *Bioresour Technol* 416:131734. <http://dx.doi.org/10.1016/j.biortech.2024.131734>
12. Baek G, Kim J, Lee C (2019) A review of the effects of iron compounds on methanogenesis in anaerobic environments. *Renew Sustainable Energy Rev* 113:109282.
<http://dx.doi.org/10.1016/j.rser.2019.109282>
13. Hwang Y, Sivagurunathan P, Lee MK, Yun YM, Song YC, Kim DH (2019) Enhanced hydrogen fermentation by zero valent iron addition. *Int J Hydrogen Energy* 44(6):3387–3394.
<http://dx.doi.org/10.1016/j.ijhydene.2018.06.015>
14. Liu R, Wang S (2025) Research progress on the comprehensive utilization of coal gangue. *Int J Coal Prep Util* 591–605. <http://dx.doi.org/10.1080/19392699.2025.2467736>
15. Qi N, Zhao YN, Zhang CX, Xu Y, Wei ZX, Bao HX, Wang J (2025) Research on Volatile Fatty Acids Production During the Anaerobic Digestion of Corn Stover Waste in Northeast China: Freeze-Thaw Pretreatment and Effect of Initial pH. *Bioenergy Res* 18(1):66. <http://dx.doi.org/10.1007/s12155-025-10866-4>
16. Zhao X, Li DY, Xu SH, Guo ZH, Zhang Y, Man L, Jiang BH, Hu XM (2017) *Clostridium guangxiense* sp nov and *Clostridium neuense* sp nov., two phylogenetically closely related hydrogen-producing species isolated from lake sediment. *Int J Syst Evol Microbiol* 67(3):710–715.
<http://dx.doi.org/10.1099/ijsem.0.001702>
17. Zhao K, Wei Q, Bai M, Shen M (2024) Study on the Environmental Impact and Benefits of Incorporating Humus Composites in Anaerobic Co-Digestion Treatment, *Toxics*, p. 360
18. Gao MX, Wang DM, Wang H, Wang XJ, Feng YZ (2019) Biogas potential, utilization and countermeasures in agricultural provinces: A case study of biogas development in Henan Province,

- China. *Renew Sustainable Energy Rev* 99:191–200. <http://dx.doi.org/10.1016/j.rser.2018.10.005>
19. Hu JJ, Jing YY, Zhang QG, Guo J, Lee DJ (2017) Enzyme hydrolysis kinetics of micro-grinded maize straws. *Bioresour Technol* 240:177–180. <http://dx.doi.org/10.1016/j.biortech.2017.02.078>
 20. Jiang SS, Yu D, Xiong F, Lian XY, Jiang XY (2023) Enhanced methane production from the anaerobic co-digestion of food waste plus fruit and vegetable waste. *Environ Sci Pollut R* 30(27):70592–70603. <http://dx.doi.org/10.1007/s11356-023-27328-z>
 21. Liu YW, Li X, Tan Z, Yang CP (2021) Inhibition of tetracycline on anaerobic digestion of swine wastewater. *Bioresour Technol* 334:125253. <http://dx.doi.org/10.1016/j.biortech.2021.125253>
 22. Xie J, Chen YZ, Duan X, Feng LY, Yan YY, Wang F, Zhang XZ, Zhang ZG, Zhou Q (2019) Activated carbon promotes short-chain fatty acids production from algae during anaerobic fermentation. *Sci Total Environ* 658:1131–1138. <http://dx.doi.org/10.1016/j.scitotenv.2018.12.280>
 23. Ji XX, Ma H, Tian ZJ, Lyu GJ, Fang GG, Chen JC, Saeed HAM (2017) Production of Xylose from Diluted Sulfuric Acid Hydrolysis of Wheat Straw. *BioResources* 12(4):7084–7095. <http://dx.doi.org/10.15376/biores.12.4.7084-7095>
 24. Lü JL, Wang Z, Tao M, Zheng H, Lou CG, Yang SS, Liu XL (2024) Fermentation of Rice Straw and Its Hydrolysate with Sludge for Ethanol Production. *Bioenergy Res* 17(4):2259–2267. <http://dx.doi.org/10.1007/s12155-024-10771-2>
 25. Lopes AMD, Joao KG, Rubik DF, Bogel-Lukasik E, Duarte LC, Andreus J, Bogel-Lukasik R (2013) Pre-treatment of lignocellulosic biomass using ionic liquids: Wheat straw fractionation. *Bioresour Technol* 142:198–208. <http://dx.doi.org/10.1016/j.biortech.2013.05.032>
 26. Chen DY, Gao DX, Huang SC, Capareda SC, Liu XY, Wang Y, Zhang T, Liu YY, Niu WS (2021) Influence of acid-washed pretreatment on the pyrolysis of corn straw: A study on characteristics, kinetics and bio-oil composition. *J Anal Appl Pyrol* 155:105027. <http://dx.doi.org/10.1016/j.jaap.2021.105027>
 27. Liu C-G, Xue C, Lin Y-H, Bai F-W (2013) Redox potential control and applications in microaerobic and anaerobic fermentations. *Biotechnol Adv* 31(2):257–265. <http://dx.doi.org/10.1016/j.biotechadv.2012.11.005>
 28. Li XM, Liu TX, Li FB, Zhang W, Zhou SG, Li YT (2012) Reduction of structural Fe(III) in oxyhydroxides by *Shewanella decolorationis* S12 and characterization of the surface properties of iron minerals. *J Soils Sediments* 12(2):217–227. <http://dx.doi.org/10.1007/s11368-011-0433-5>
 29. Li D, Jiao CX, He W, Yan ZY, Yuan YX, Li ZD, Guo YN, Liu XF (2016) Comparison of micro-aerobic and anaerobic fermentative hydrogen production from corn straw. *Int J Hydrogen Energy* 41(12):5456–5464. <http://dx.doi.org/10.1016/j.ijhydene.2016.01.141>
 30. Andriamanohiarisoamanana FJ, Ihara I, Yoshida G, Umetsu K (2022) Comparative effects of ferric hydroxide and (semi) conductive iron oxides on the anaerobic digestion of oxytetracycline-contaminated dairy manure. *J Environ Manage* 310:114731. <http://dx.doi.org/10.1016/j.jenvman.2022.114731>
 31. Rambabu K, Bharath G, Banat F, Hai A, Show PL, Nguyen THP (2021) Ferric oxide/date seed activated carbon nanocomposites mediated dark fermentation of date fruit wastes for enriched

- biohydrogen production. *Int J Hydrogen Energy* 46(31):16631–16643.
<http://dx.doi.org/10.1016/j.ijhydene.2020.06.108>
32. Zhang YC, Xiao LL, Wang SN, Liu FH (2019) Stimulation of ferrihydrite nanorods on fermentative hydrogen production by *Clostridium pasteurianum*. *Bioresour Technol* 283:308–315.
<http://dx.doi.org/10.1016/j.biortech.2019.03.088>
33. Lin HT, Zhang MH, Chauhan BS, Ayed H, Khadimallah MA, Tang XD, Mahariq I (2024) Development, kinetic analysis, and economic feasibility of different Corn Stover-driven biorefineries to produce biohydrogen, bioethanol, and biomethane: A comparative analysis. *Renew Energy* 237:121625.
<http://dx.doi.org/10.1016/j.renene.2024.121625>

Figures

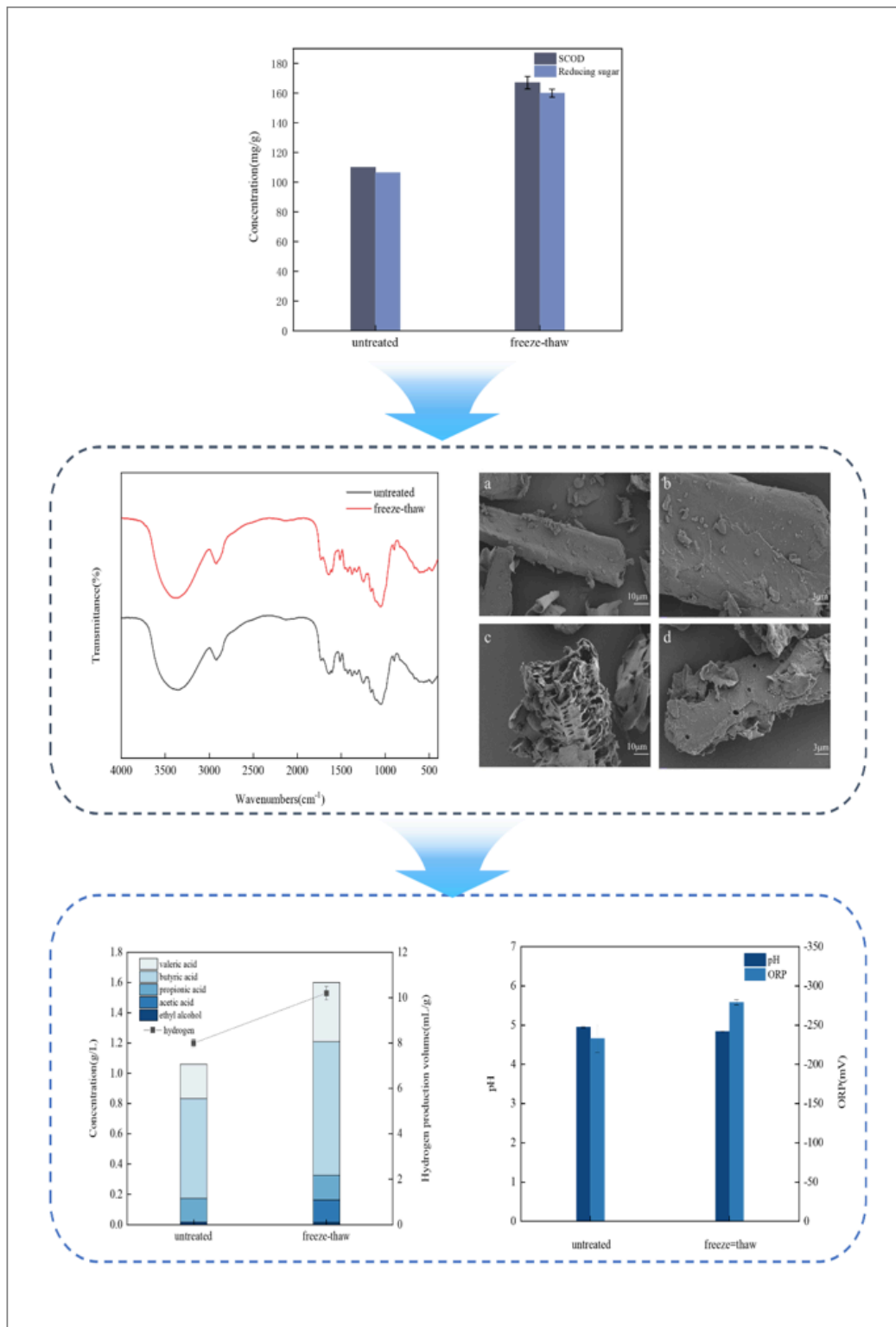


Figure 1

Changes in corn straw structure and anaerobic digestion system stability before and after freeze-thaw pretreatment((a) and (b) untreated corn straw; (c) and (d) corn straw after experimental optimal conditions)

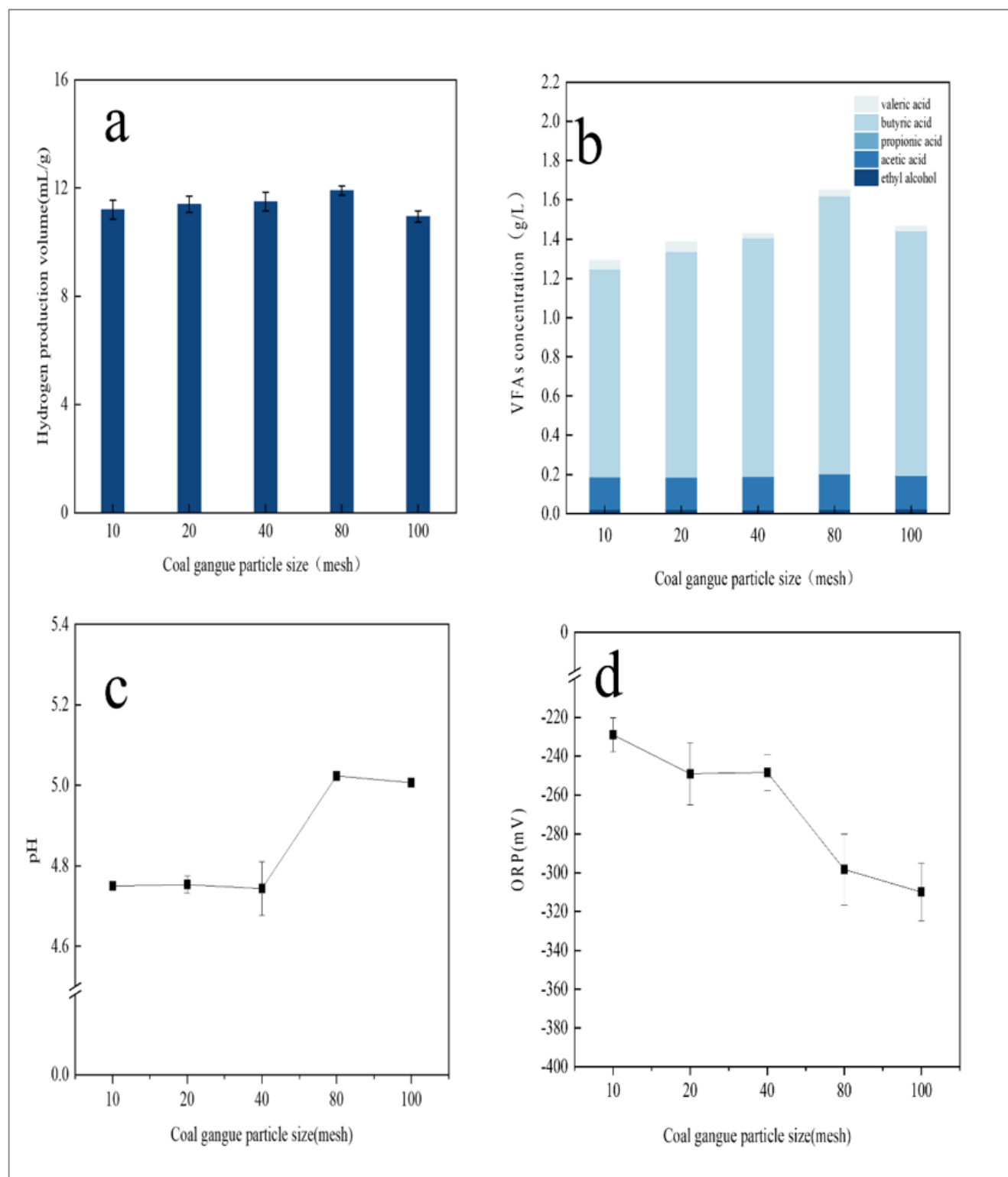


Figure 2

Effect of Coal Gangue Particle Size on Hydrogen Yield and System Stability in Anaerobic Fermentation of Corn Straw

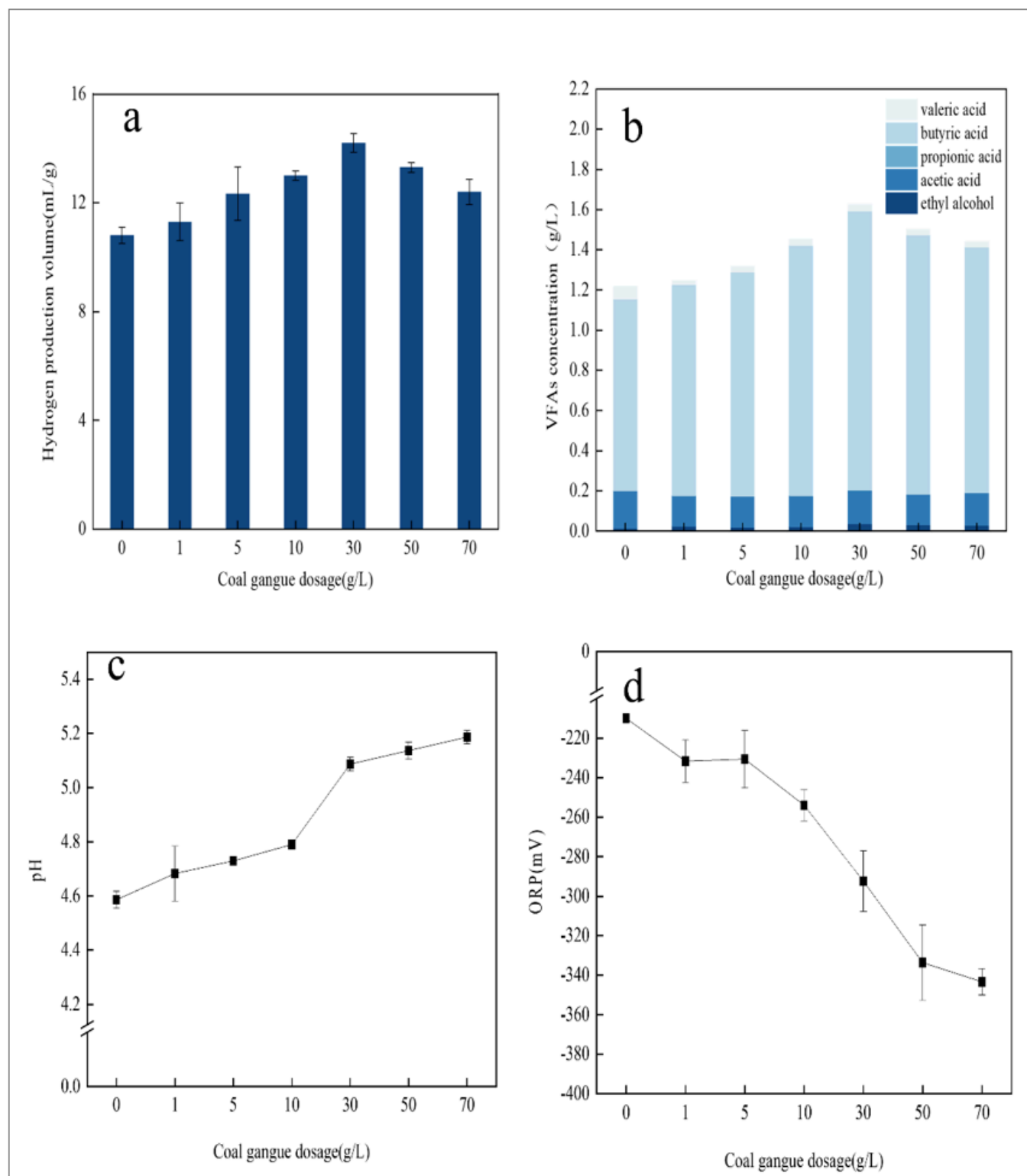


Figure 3

Effect of Coal Gangue Dosage on Hydrogen Yield and System Stability in Anaerobic Fermentation of Corn Straw