



Characteristic and calculation on the co-contribution in the bio-H₂ energy recovery enhancement with low temperature pretreated peanut shell as co-substrate

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ABSTRACT

Bio-H₂ production from organic wastewater together with lignocellulose wastes not only achieved the H₂ energy recovery, but also be beneficial to carbon emission reduction and carbon neutralization. In order to obtain higher energy recoveries, promotion attempts were performed in bio-H₂ fermentation with low temperature (−80–0 °C) pretreated peanut shell powder (PSP) as co-substrate. A maximum H₂ production of 109.2 mL was obtained as almost double of the sum from the same amount of untreated PSP and glucose as sole substrate. The enhancement was co-contributed by 44% from PSP supplementary, 35% from low-temperature pretreatment, and 2.8% from buffer effect and acidification, respectively, and realized through C/N balancing, PSP conversion influencing, fermentative pH buffering and time prolonging. The experimental results uncovered the co-contribution realization ways of supplementing low-temperature pretreated lignocellulose wastes in the bio-H₂ fermentation system, and provided mechanism support for application potential of low-temperature pretreatment on lignocellulose wastes in cold regions.

1. Introduction

In the context of “Carbon emission peak and Carbon neutralization”, the traditional fossil energy system will be gradually replaced by renewable and clean energies in the near future (Zhang et al., 2021). Among kinds of alternative energy sources, hydrogen energy is considered to be the most suitable one for the replacement because of its specific characteristics of the highest unit energy content (120 MJ/kg or 33.6 kWh/kg) and completely non-carbon emission oxidation product of H₂O (Zhang et al., 2021; Geng et al., 2021; Yamamoto et al., 2021).

Besides the chemical and physical methods, H₂ gas could also be produced through biological processes from organic wastes with functional microorganisms, which achieved pollutants treatment and removal, as well as the H₂ energy recovery and carbon emission reduction. Although the bio-H₂ production has advantages like mild

reaction conditions, organic wastes recycling and environmental benefits (Wang and Yin, 2019), there are still difficulties in the wide promotion and application since shortages in H₂ generation efficiencies and operation cost at present (Yamamoto et al., 2021). Therefore, investigations on efficient bio-H₂ producers enrichment, fermentative condition optimization and low-cost substrates selection have been carried out (Li et al., 2021).

Lignocellulose agricultural wastes is considered as one of promising fermentative substrates for bio-H₂ production because of its economic feasibility and environmental benefits (Contreras-Dávila et al., 2017). Cellulose and hemicellulose, the main components of lignocellulose wastes, were good substrates for H₂ fermentative functional microbes but wrapped by the unusable lignin (Hu et al., 2017). Thus, attempts, such as physical and chemical processes or their combination, were carried out in the lignocellulose de-structuring to release reducing

Abbreviations: PSP, Peanut shell powder; PSPG, Group of PYG medium with PSP; PSPN, Group of PYG medium without glucose but PSP; G, Group of PYG medium; RT, Room temperature; DNS, 3,5-dinitrosalicylic acid; TRS, Total reducing sugar; VFAs, Volatile fatty acids.

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sugars and promote the conversion efficiency (Huo et al., 2015; Janke et al., 2017; Schneider et al., 2017). Comparing with other pretreatment methods, low temperature treatment has advantages like non-chemicals addition and environment friendly (Dong et al., 2019; Hiden, 2017). A maximum hydrolysis efficiency of 19.4% was obtained from the $-18\text{ }^{\circ}\text{C}$ pretreated barley straw with an ethanol process efficiency up to 120.75% (Rooni et al., 2017). Our previous study also suggested that low temperature pretreatment ($-80\text{--}0\text{ }^{\circ}\text{C}$) significant promoted the reducing sugar releasing by delaminating and peeling the fiber of lignocellulose (Qi et al., 2020). Although low temperature pretreatment was observed effective in lignocellulose conversion, details on that, such as the realizing process, contribution analysis and pretreated temperature below $-20\text{ }^{\circ}\text{C}$ were still obscure.

In our previous study, a 70% H_2 yield enhancement was realized in the fermentation with peanut shell powder (PSP) as supplementary substrate comparing to the sum of that from PSP and glucose as sole substrate (Qi et al., 2018). On the basis of aforementioned studies, the low temperature ($-80\text{--}0\text{ }^{\circ}\text{C}$) pretreated PSP was used as the co-substrate for bio- H_2 recovery enhancement in this study, obtaining an almost doubled bio- H_2 yield. The possible promoting factors for bio- H_2 production enhancement were deduced, and contribution rates were comprehensively calculated, which would provide a unique analysis for material and energy metabolism in the bio- H_2 generation process.

2. Materials and methods

2.1. Inoculation and substrate

Clostridium guangxiense ZGM211^T, a butyrate-type fermentative H_2 producer, was used as the inoculum and pre-cultured with PYG medium (Qi et al., 2018; Zhao et al., 2017). Co-substrate of peanut shells, collected from the Sihe Grain Processing Mill, Linghai, China, was air-dried, grinded to $<1\text{ mm}$ powder and stored at room temperature (RT, $25\text{ }^{\circ}\text{C}$) for further tests as our previous report (Qi et al., 2018).

2.2. Low temperature pretreatment and batch experiment

The low temperature pretreatment was performed with 100-mL plastic bottles containing 5 g PSP and 50 mL liquid medium with an initial pH 7.0 in the low temperature freezer at 0, -20 , -50 and $-80\text{ }^{\circ}\text{C}$, respectively, for 12 h (Qi et al., 2020). After the pretreatment, mixtures were respectively transferred into 100-mL anaerobic bottles, stripped oxygen by blowing-in high-purity argon, sealed up with rubber stoppers and sterilized at $121\text{ }^{\circ}\text{C}$ for 15 min (Qi et al., 2018). Group of PYG medium with PSP was set as PSPG, and PYG medium without glucose but PSP was set as PSPN. All H_2 production tests were performed at an inoculum rate of 2% (v/v) in the air-bath shaker at $35\text{ }^{\circ}\text{C}$ and 120 rpm for 48 h. Gas production was measured with the drainage method (Qi et al., 2018).

2.3. H_2 production contribution calculation

The PSP supplementary, temperature pretreatment, and acid buffer and acidification effects were respectively calculated as follows.

$$\text{Contribution of supplementary PSP} = \frac{H_{\text{PSPG}} - H_{\text{G}}}{H_{\text{PSPG}}} \quad (1)$$

$$\text{Contribution of temperature pretreatment} = \frac{H_{\text{PSPG}} - H_{\text{RT}}}{H_{\text{PSPG}}} \quad (2)$$

$$\text{Contribution of acid buffer effect and acidification} = \frac{P_{\text{PSPG}} - P_{\text{G}}}{P_{\text{PSPG}}} \quad (3)$$

where H_{PSPG} , H_{G} and H_{RT} were the H_2 production from PSPG, glucose and untreated PSP, respectively; P_{PSPG} and P_{G} were the end-point pH from PSPG and glucose, respectively.

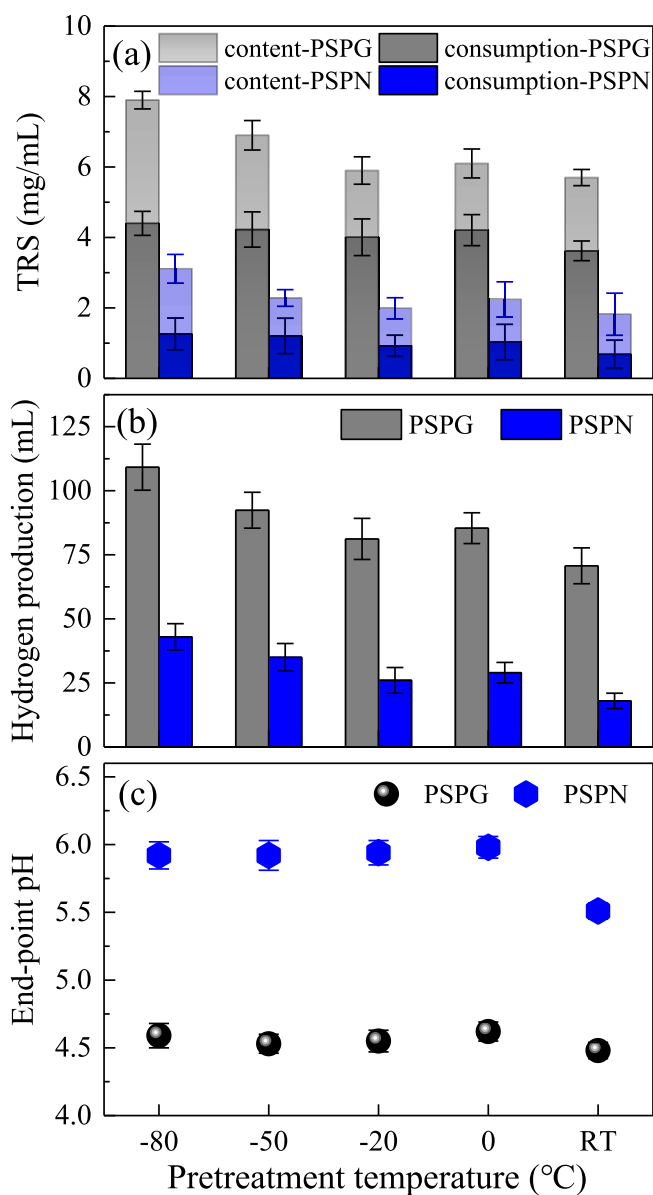


Fig. 1. Bio- H_2 production from low temperature pretreated PSP as sole- (PSPN) or co- (PSPG) carbon substrate. (a) TRS content and consumption; (b) H_2 production; (c) End-point pH.

2.4. Energy calculation

The energy output was calculated by the following formula (Soltan et al., 2017):

$$E_o = HP \times C_v \times \rho \quad (4)$$

where E_o and C_v were energy output and calorific value of hydrogen, respectively. In this study, C_v was the lowest heating value of hydrogen ($1.4 \times 10^5\text{ kJ/kg}$). HP was the hydrogen production (L). ρ was the density of hydrogen ($8.9 \times 10^{-5}\text{ kg/L}$).

The energy value of PSP (E_{PSP}) and glucose (E_{G}) was calculated based on the modified Dulong Formula as shown in Eq. (5) (Lin et al., 2019),

$$E_{\text{PSP}} / E_{\text{G}} = 337C + 1419 \times (H - 0.125O) + 23.26N \quad (5)$$

where C (45.79%), H (5.98%), O (46.77%), and N (0.5%) represented the respective weight percentage of each element in PSP (Torres-García et al., 2020).

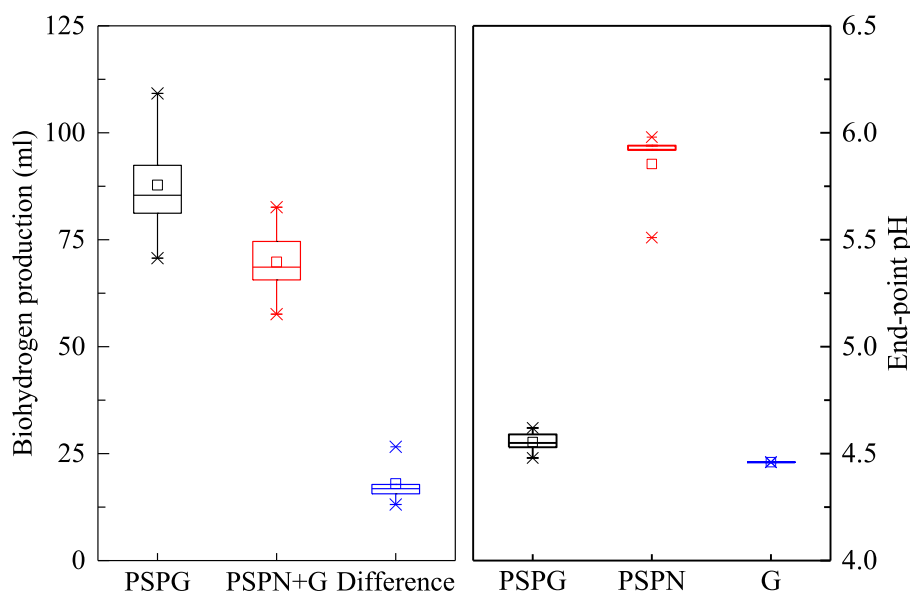


Fig. 2. The H₂ production from PSPN, PSPG and glucose (G), and the end-point pH. Left: Bio-H₂ production difference between PSP as sole or co-carbon substrate; Right: End-point pH.

The energy conversion efficiency (η) was defined as the ratio of the heating value of hydrogen to the total heating value of the added substrate,

$$\eta = \frac{E_o}{m_{PSP} \times E_{PSP} + m_g \times E_g} \times 100 \quad (6)$$

2.5. Analytical methods

The total reducing sugar (TRS) in the liquid was determined by the 3,5-dinitrosalicylic acid (DNS) method at 540 nm (Hu et al., 2017). The pH was measured using a pH meter (S220, Mettler-Toledo, Switzerland) (Qi et al., 2018). H₂ content was determined by a gas chromatograph (Agilent 7890 A, USA) with the capillary GS-CARBONPLOT (Zhao et al., 2011).

3. Results and discussions

3.1. Bio-H₂ recovery from sole or co-substrate

3.1.1. H₂ recovery from PSP as sole carbon substrate

As shown in Fig. 1a & b, the TRS content of 3.11 mg/L and TRS consumption of 1.26 mg/L were both peaked with -80 °C pretreatment. The H₂ yields from low temperature pretreated PSP were ranged from 43 to 26 mL with temperature increasing, and peaked at -80 °C, which was nearly 2.4 folds of that from unpretreated PSP. It was indicated that the low-temperature pretreatment was beneficial for stimulating H₂ yield. The end-point pH from pretreated PSP was at pH 5.98–5.92 (Fig. 1c). The low values of end-point pH were caused by the production of volatile fatty acids (VFAs) during the fermentation process, including acetic acid and butyric acid (Zhao et al., 2017). VFAs produced in the fermentative process were found having an acidification effect on the TRS releasing from lignocellulose, that about 3.1% TRS was obtained from treated PSP by fermentation liquid byproducts (Qi et al., 2019). The results inferred that the acidification was one of the reasons for TRS releasing and H₂ yield increasing from PSP.

3.1.2. H₂ recovery from PSP as co-substrate

With the pretreatment temperature shifting, the TRS content and consumption were respectively ranged at 5.9–7.9 mg/L and 4.0–4.4 mg/L, and peaked at -80 °C (Fig. 1a). The maximum H₂ yield of 109.2 mL

was obtained with pretreatment at -80 °C, which was 54.46% higher than that from PSP at RT (Fig. 2). It meant that low temperature pretreatment was effective in enhancing the H₂ yield from PSPG. The end-point pH 4.53–4.62 in pretreated PSP groups was a little higher than glucose sole carbon substrate groups of pH 4.46 and untreated PSP as co-substrate of pH 4.48 (Fig. 2). The results indicated that pH could be buffered by PSP supplementary as co-substrate, and the buffer effect was stimulated by the pretreatment temperature decreasing. As shown in the Fig. S1 in the Supplementary, with the pretreatment temperature decreasing, the delamination and peeling in the fibers of PSP were obtained more obvious, which could provide more reaction contact area for VFAs and PSP, and resulted in the buffer effect stimulation (Qi et al., 2020). pH was one of the key factors in the fermentation process, since it has significant relationship with the functional bacteria growth and reaction processes, even the fermentation termination (Wang and Wan, 2009). What's more, the fermentation duration could be prolonged by the acid buffer effect of PSP supplementary (Qi et al., 2019). Thus, the acid buffer effect of lignocellulose wastes as co-substrate was beneficial to the fermentation process and bio-H₂ yield enhancement.

3.1.3. Comparison between sole and co-substrate of PSP

As shown in Fig. 2, the H₂ yield from PSPG was nearly 1.9 folds higher than the sum of those from untreated peanut shell (PSPN) and glucose (G) as sole carbon substrate. The result indicated that PSP supplementary as co-substrate was significantly effective in H₂ yield enhancement. The end-point pH in the PSPG group was lower than that of PSPN, and changed more slightly with pretreatment temperature shifting. The relatively stable and low pH were conducive to the PSP acidification for TRS releasing and H₂ production. The slightly higher end-point pH values in PSPG groups than that of glucose (G) could be explained with the acid buffer effect (Huang et al., 2016; Qi et al., 2019). Together with the previous investigation results on the structure changes after low temperature pretreatment, contributions of the PSP as co-substrate to the H₂ yield enhancement could be summarized into four parts: PSP supplementary, low temperature pretreatment, acid buffer and acidification effects (Qi et al., 2018, 2019).

Table 1
Contributions analysis for bioenergy recovery from lignocellulose wastes as co-carbon substrate.

Biogas	Co-carbon substrate		Temperature pretreatment			Acid buffer & acidification		Reference
	Biomass	CR*	Biomass	Temp.**	CR	Biomass	CR	
H ₂	cassava residue	45.2%	–	–	–	cassava residue	0.7%	Chavadej et al. (2019)
CH ₄	rice straw	33.7%	–	–	–	–	–	Yong et al. (2015)
CH ₄	<i>Hydrilla verticillata</i>	40.0%	–	–	–	–	–	Kainthola et al. (2019)
CH ₄	fruit & vegetable waste	22.4%	–	–	–	–	–	Wang et al., 2018b
H ₂	–	–	maize leaves	130	50%	–	–	Ivanova et al. (2009)
CH ₄	–	–	washed vinegar residue	160	29.8%	–	–	Ran et al. (2018)
Biogas	–	–	rice straw	90/180	3%	–	–	Wang et al. (2018a)
H ₂	peanut shell	44%	peanut shell	–80	35%	peanut shell	2.8%	this study

–: not mentioned; *: contribution rate (CR); **: Unit of temperature is °C.

3.2. Contributions on H₂ recovery enhancement

3.2.1. Contribution from PSP supplementary

The contribution of PSP to the H₂ production was calculated at 44% as the difference of H₂ yield between the group of PSPG at RT and group G (glucose as sole carbon substrate) as in Eq. (1). It was suggested to be realized through adjusting C/N ratio, increasing bio-H₂ yield, and improving the utilization efficiency (Chen et al., 2015; Yong et al., 2015). In Chavadej’s study, the H₂ production was improved by 45.2% with cassava residue as co-substrate (Table 1) (Chavadej et al., 2019). The methane yield from the co-digestion of rice straw and *hydrilla verticillata* was nearly 40% higher than that of mono-digestion, which was caused by the metabolic pathway improvement with co-substrates (Kainthola et al., 2019). The slight difference in enhancement rates, such as in this study, was suggested due to the different co-substrate supplementary and experiment condition.

3.2.2. Contribution from temperature pretreatment

The contribution of low temperature pretreatment to the H₂ production was calculated about 35% as Eq. (2), which was the difference of H₂ production between groups of PSPG and RT. The bio-H₂ yield from 130 °C pretreated maize leaves was about 50% enhanced due to the cell destruction by the high temperature pretreatment (Ivanova et al., 2009). The enhancement in Ivanova’s study was a little higher than that in the present study, which was suggested due to the higher hemicellulose content of 34.32% in maize leaves than peanut shell (Intani et al., 2016; Qi et al., 2018). Comparing to the high temperature, pretreatment temperature below 0 °C would result in a considerable reduction in phenolic acid complement, significant contribution to arabinoxylan moieties solubilization and cell wall destruction, as well as the enhancement of bacterial accessibility (Merali et al., 2013; Qi et al., 2020).

3.2.3. Contribution from acid buffer effect and acidification

As calculated in Eq. (3), the contributions from acid buffer effect and acidification in RT and –80 °C were about 0.4% and 2.8%, respectively, which were represented by the difference of end-point pH between groups of PSPG and G. The contributions of acid buffer effect and acidification from –80 °C pretreated PSP was 7 folds of that from untreated PSP. The results indicated that acid buffer effect and acidification were effective in the bio-H₂ production enhancement, and significantly enhanced by low temperature pretreatment. As shown in Table 1, the end-point pH was about 0.7% raised under the optimal cassava residue concentration of 1200 mg/L in Chavadej’s investigation (Chavadej et al., 2019), which was higher than that of RT but lower than that of –80 °C pretreated PSP in this study. The end-point pH raising was caused by the partial adsorption of VFAs in the PSP fermentation. Meanwhile, the PSP was acidified by the adsorbed VFAs to further destroy structures of lignocellulose and release the TRS, and then be converted into additional bio-H₂ by stain ZGM 211. As the supplementary co-substrate, PSP not only helped to balance the improper C/N ratio and slow down the rapid decline of pH in fermentation processes, but also provided more substrate for TRS releasing (Kainthola et al., 2019). The acid buffer effect and acidification of PSP prolonged the fermentation time, provided a proper fermentative condition, and promoted co-substrates conversion efficiency and H₂ yield.

3.2.4. Co-contribution analysis at optimal pretreatment condition

In the fermentation process, the cellulose structure of PSP co-substrate was destroyed in the low-temperature pretreatment process and resulted in the TRS release increasing. The PSP co-substrate was acidified by the fermentative byproduct of VFAs, which promoted the further release of TRS, buffered the rapid decline of pH, and finally prolonged the fermentation time. The combination of above effects co-contributed to the significant promotion of H₂ yield enhancement.

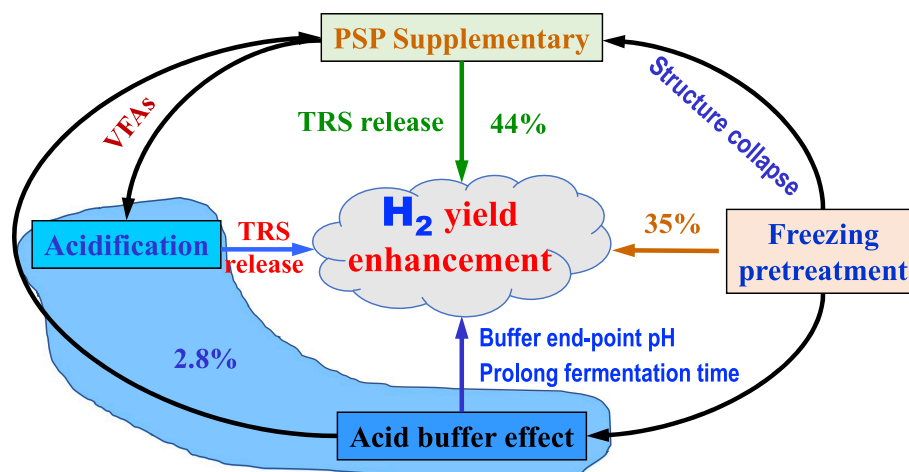


Fig. 3. Calculations on the co-contribution in the H₂ production enhancement.

Table 2
Energy conversion efficiencies from low temperature pretreated PSP to H₂.

Substrate	Energy output (kJ/kg)	Conversion efficiency (%)
Glucose	84.59	0.47
PSP	38.45	0.29
20°C-PSP	151.02	1.01
0°C-PSP	182.41	1.22
-20°C-PSP	173.44	1.16
-50°C-PSP	197.37	1.32
-80°C-PSP	233.25	1.56

The H₂ production from the optimal PSPG with -80 °C pretreatment was nearly 1.9 folds enhanced comparing to the sum of that from untreated PSP and glucose. The enhancement was co-contributed by PSP supplementary, low-temperature pretreatment, as well as the acid buffer and acidification effects of PSP, with the calculated contribution rates of 44%, 35% and 2.8%, respectively (Fig. 3). Two hypotheses were proposed on the co-contribution of 81.8% that less than 100%. One was that there were other contributors for H₂ yield enhancement. The other one was that the suggested contributors in this study overlapped, complemented and reinforced to each other. Based on the calculation, the latter hypothesis was inferred with higher possibility.

3.3. Co-contribution calculation on energy conversion

As shown in Table 2, the maximum energy output of 233.25 kJ/kg was obtained from -80 °C pretreated PSP, while from PSP and glucose as sole carbon substrate were 38.45 and 84.59 kJ/kg, respectively. According to the section 3.2.4, the energy output enhancement of 110.21 kJ/kg could be calculated by the difference between the output energy from -80 °C pretreated PSP and the sum of output energy from untreated PSP and glucose as sole carbon substrate. It was calculated being co-contributed by PSP supplementary, low temperature pretreatment, as well as the acid buffer effect and acidification at 48.49, 38.57 and 3.09 kJ/kg, respectively.

The energy conversion efficiency of untreated PSP was calculated as 1.01%, which was a little higher than Lin's report of 0.7% with seaweed *Saccharina latissima* (Lin et al., 2019). The results could be attributed to the substrate difference of seaweed and PSP. The PSP with low temperature pretreatment at -80 °C led to the highest energy conversion efficiency of 1.56%, which was nearly 1.5 or 2.1 folds enhanced than the untreated PSP as co-substrate or the sum of untreated PSP and glucose as sole carbon substrate. The 0.8% enhancement of energy conversion efficiency was contributed from PSP supplementary of 0.35%, low temperature pretreatment of 0.28%, as well as acid buffer and acidification effects of 0.02%.

Although the reducing sugar yield from low-temperature pretreated lignocellulose wastes was less than that with physical and chemical treatments, or high-temperature pretreatment, low-temperature means were not energy intensive and large demand for chemical reagents. Therefore, studies on low-temperature pretreatment of lignocellulose in areas with high latitude or low temperature in winter is not only helpful to reduce the cost of bio-H₂ production and enhance substrates conversion efficiency, but also of great significance to "Carbon emission peak and Carbon neutralization".

4. Conclusions

Supplementary with low-temperature (-80-0 °C) pretreated PSP as co-substrate was effective in enhancing the fermentative H₂ yield, and provided the potential of low-cost and natural application to bio-H₂ recovery from lignocellulose wastes in areas with high latitude or low temperature in winter. The H₂ yield and energy conversion efficiency from optimal temperature of -80 °C pretreated PSP as co-substrate were almost double of the sum from the same amount of untreated PSP and glucose as sole carbon substrate. The bio-H₂ recovery enhancement was

deduced by the co-contribution from PSP supplementary, low temperature pretreatment, acid buffer effect and acidification, respectively, and realized through C/N balancing, PSP conversion influencing, fermentative pH buffering and fermentation time prolonging. These contributors overlapped, complemented and reinforced each other.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2022.113169>.

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