Potato peel waste for fermentative biohydrogen production using different pretreated culture

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Abstract

How to manage potato peel waste sustainably has been an issue faced by the potato industry. This work explored the feasibility of potato peel waste for biohydrogen production via dark fermentation, and investigated the effects of various inoculum enrichment methods (acid, aeration, heat-shock and base) on the process efficiency. It was observed that the hydrogen production showed a great variation when using various inoculum enrichment methods, and the aeration enriched inoculum obtained the maximum hydrogen yield of 71.0 mL/g-VS_{added} and VS removal of 28.9%. Different enriched cultures also exhibited huge variations in the bacterial community structure and metabolic pathway. The highest abundance of *Clostridium sensu stricto* fundamentally contributed to the highest process efficiency for the fermenter inoculated with aeration treated culture. This work puts forward a promising strategy for recycling potato peel waste, and fills a gap in the optimal inoculum preparation method for biohydrogen fermentation of potato peel waste.

Keywords: Biohydrogen; potato peel waste; dark fermentation; metabolic pathway; microbial community

1. Introduction

Potato is vital in human diet around the world, which is the fourth agricultural crop. Potatoes need to be peeled before eating, which commonly generates 15-40% byproduct, i.e. potato peel waste (Arapoglou et al., 2010). According to the potato yield, the annual amount of global potato peel waste would exceed 50 million tons. Therefore, considerable effort has been taken into the management of potato peel waste in the food industry.

Dark fermentation has been confirmed to be a bright approach for handling organic wastes, including food waste (Han et al., 2015), agroforestry waste (Yang and Wang, 2018a), residual glycerin (Faber and Ferreira-Leitao, 2016), waste activated sludge (Yang and Wang, 2017), livestock manure (Xing et al., 2010), algal biomass (Srivastava et al., 2021), antibiotic fermentation residue (Yang et al., 2019b), and wastewater (Ren et al., 2018). This technology is receiving increasing attention, because it can realize the dual benefits of waste treatment and clean hydrogen production. Besides the aforementioned wastes, potato peel waste also exhibits enormous potential to be used as feedstock for fermentative biohydrogen production, since such type of waste contains high quantities of fermentable sugars, including starch, cellulose, and hemicellulose (dos Santos et al., 2016). Previous investigations mostly concentrated on using potato peel waste for biomethane production through anaerobic digestion (Soltaninejad et al., 2022; Liang and McDonald, 2015). Actually, the anaerobic digestion process has some disadvantages, such as long retention time, the generation of H_2S, and high carbon emission (Mishra et al., 2021). Comparing

with anaerobic digestion, dark fermentation may be a better technology for treating potato peel waste, because of its much lower retention time, significant higher heating value of the produced biogas, and lower H_2S and carbon emission (Yang and Wang, 2018a; Han et al, 2012). Nevertheless, there has been limited studies performing on utilizing potato peel waste for fermentative hydrogen production, and associated understanding of potato peel waste-based biohydrogen production is rare.

For dark fermentation, inoculum is another crucial factor of process efficiency, in addition to the factor of feedstock types. The inoculum of biohydrogen fermentation can be classified into pure culture and mixed culture. Comparing with pure culture, mixed culture is more efficient, applicable and economical for biohydrogen fermentation, because of the collaborative functions of different types of microbes (Wang and Yin, 2017), especially when real wastes are used as the feedstock. Unfortunately, the sources of mixed culture (e.g. anaerobically digested sludge and livestock manure compost) contain both biohydrogen-producing bacteria and biohydrogen-consuming bacteria. Accordingly, it is necessary to pretreat the source of mixed culture to eliminate biohydrogen consumers, while enriching the hydrogen-producing bacteria. So far, mixed culture has been successfully prepared by many kinds of pretreatment approaches (Wang and Yin, 2017), including base, aeration, heat-shock, acid, UV radiation, electric field, chemical inhibitors, ultrasonication, ionizing radiation, microwave, and their combinations. However, the effectiveness of inoculum pretreatment methods is highly dependent on the types of feedstocks. For example, when the same anaerobically digested sludge was used as

45 the source of mixed culture for biohydrogen fermentation, base pretreatment was most
46 effective for antibiotic fermentation residues (Yang et al., 2019b), while the most
47 effective pretreatment method for grass residue was acid (Yang and Wang, 2018a).
48 Additionally, even when the same feedstock was used for fermentative hydrogen
49 production, inconsistent outcomes have been reported. Ren et al. (2008) found that
50 aeration pretreated sludge reached the highest hydrogen production from glucose,
51 while Wang and Wan (2008) reported that heat-shock pretreated sludge obtained the
52 maximum biohydrogen production from glucose. This suggests more thorough
53 research is needed to understand the influence of inoculum pretreatment methods
54 towards the dark biohydrogen fermentation of potato peel waste.

55 Furthermore, most investigations concentrated on the process performances,
56 including hydrogen production, the utilization ratio of substrate, and the distribution
57 of liquid metabolites, when comparing different inoculum pretreatment methods for
58 biohydrogen fermentation, while paid little attention to microbial community structure
59 in the fermenter. Actually, the process performances of biohydrogen fermentation
60 essentially depend on microbes enriched by inoculum pretreatment (Hallenbeck,
61 2009). Therefore, figuring out the microbe community for the reactors inoculating
62 various pretreated cultures is of great significance for understanding the underlying
63 mechanism for the different biohydrogen fermentation performances.

64 This work aimed to assess the feasibility of utilizing potato peel waste for
65 fermentative hydrogen production, and experimentally investigated the impact of
66 various inoculum pretreatment methods on the process performance. According to

67 literature review (Wang and Yin, 2017), base, acid, heat-shock and aeration were
68 selected as pretreatment methods for preparing the inoculum in this work, due to their
69 ease of operation and high efficiency. The hydrogen productivity, substrate utilization
70 and liquid metabolites formation were comprehensively examined for these four
71 inoculum pretreatment methods. In addition, microbial community dynamics with
72 various inoculum pretreatment methods, including diversity, richness and composition,
73 were analyzed to elucidate the mechanisms behind the process performance observed.
74 This work contributes to a better understanding of the technical feasibility of potato
75 peel waste-based biohydrogen production for future up-scaling research.
76

77 **2. Materials and methods**

78 **2.1. Feedstock**

79 Raw potato peel waste was collected from a university canteen (Beijing, China).
80 The main characteristics of the potato peel waste were: moisture content, 80.1%;
81 volatile solids (VS) content, 859.3 mg/g-dry weight; and carbohydrate content, 665.7
82 mg/g-VS. The potato peel waste was dried in an oven, and then milled to about
83 20-mesh prior to be used as the fermentation feedstock.

84 **2.2. Inoculum preparation**

85 The sludge from an anaerobic digester was used as the inoculum source in this
86 work. The conditions of the four inoculum pretreatment approaches (heat-shock,
87 aeration, base and acid) were selected according to previous investigations (Yang et
88 al., 2019b; Yang and Wang, 2018a). In detail, the heat-shock treatment was performed

by boiling the sludge at 100℃ for 15 minutes. The aeration treatment was carried out by continuously aerating the seed sludge for 24 hours. Acid and base treatments were carried out in the conditions of pH of 3.0 and pH of 10.0 for 24 h, respectively. After various pretreatment, the sludge samples were centrifuged and washed with deionized water for several times. Afterwards, the four treated sludge samples were adopted as the inoculum in the following biohydrogen fermentation.

2.3. Batch fermentation

A series of 150 mL bottles were used to conduct the batch hydrogen fermentation experiment in triplicate. For each fermenter, the working volume was set at 100 mL, containing 2 g of potato peel waste, 30 mL of various pretreated inoculums, 60 ml of deionized water, and 10 mL nutrient solution. After the mixing, the pH of all fermenters was adjusted to 7.0 (Yang et al., 2019c). Then, N_2 gas was pumped through each fermentation reactor to create an anaerobic environment, and finally transferring into a shaker operated at 120 rpm and 37 ℃ for biohydrogen production. Because the seed sludge used in the present experiment was anaerobically digested sludge, dark fermentation inoculating the untreated sludge was actually a biomethane-producing process that could consume the produced hydrogen during the methanogenesis, and thus was not set as the control (Yang et al., 2019b).

2.4. Analytical methods

The APHA Standard Methods were used to test the VS concentration and moisture content. Total carbohydrate of the fermentation sample was tested with the phenol-sulfuric acid method. Acetate, butyrate and propionate in the fermentation

liquor were filtered and measured by a high-performance liquor chromatograph as previously reported (Yang et al., 2019a). The total amount of biogas produced from each reactor was determined every two hours by displacement of NaOH solution. Hydrogen fraction in the biogas was analyzed by a gas chromatograph with the carrier gas of argon (Yang et al., 2019a).

2.5. Microbial community analysis

When the biohydrogen fermentation inoculating various treated cultures terminated, DNA was extracted (soil DNA extraction kit) from the fermentation samples to determine the bacterial community (Li et al., 2022). After the PCR amplification (515F-806R, V4 region of the bacterial 16S rRNA gene) and purification, an Illumina MiSeq PE250 platform was used to sequence the PCR amplicons (Yang and Wang, 2018b). The clean sequences were grouped into the OTUs with a 97 percent similarity level to examine the taxonomic classification. The "Shannon" and "Simpson" indices were calculated for evaluating the diversity of the bacterial OTUs (Sun et al., 2022). The "Chao" and "Ace" indices were calculated for evaluating the richness of the bacterial OTUs.

2.6. Kinetic analysis

The Cone equation (Eq. (1)) was employed to fit the cumulative hydrogen production data for different pretreated inoculums (Yang and Wang, 2018a).

$$\mathrm{H} = \frac{P}{1 + (k_{hyd}t)^{-n}} \quad (1)$$

Where, H – cumulative hydrogen production at t (mL); P – cumulative hydrogen production potential (mL); k_{hyd} – hydrolysis rate constant (h^{-1}); t – fermentation time (h); n – shape factor.

3. Results and discussion

3.1. Hydrogen production

The potato peel waste has a high sugar content of 665.7 mg/g-VS, which suggests a great potential for fermentative biohydrogen production. In the present work, methane was not detected in the biogas, which indicated that all four pretreatments effectively contained the methanogenic activity of the seed sludge. Fig. 1 shows biohydrogen generation over time for the fermenters inoculating various treated cultures. As is apparent from Fig. 1, the fermentation process finished within 46 h in this work, and the hydrogen production process for the four inoculums followed three distinct phases: lag, rapid and slow. However, there was a big difference in the lag phase for different pretreated inoculums, which were 4, 10, 6 and 6 h for the aeration, heat-shock, acid and base treated sludge, respectively. This variation may be due to different reactions of the seed sludge microbes to the different treatment conditions. The aeration pretreated inoculum showed the shortest lag phase, while the inoculum obtained by heat-shock treatment exhibited the longest lag phase. Similarly, other studies also observed that the inoculum with heat-shock pretreatment exhibited the longest lag phase for producing biohydrogen among various pretreated inoculums (Yang et al., 2019b; Luo et al., 2022). The longest lag time for the

154 inoculum with heat-shock pretreatment may be because the retained microorganisms
155 after the high-temperature treatment required more time to recover the activity
156 compared to the other pretreatment conditions. In addition, it has been shown that
157 slow-growing spore-formers were commonly enriched by heat-shock pretreatment
158 (Wang and Yin, 2017), which could be another reason for the long lag time.

159 **Fig. 1**

160 To further understand the hydrogen production processes for various pretreated
161 inoculums, the Cone model was employed to analyze the cumulative hydrogen
162 production data (Table 1). For cumulative hydrogen production potential (P), the
163 aeration pretreated inoculum achieved the highest value, followed by the base
164 pretreated inoculum, the acid pretreated inoculum, and the heat-shock pretreated
165 inoculum. The highest P value for the system with aeration pretreated inoculum may
166 be due to that the aeration treatment enriched the most effective bacterial community
167 for hydrogen production. For the hydrolysis rate constant (k_{hyd}), the aeration
168 pretreated inoculum also achieved the highest value among the four pretreated
169 inoculums. Hydrolysis is the limiting step for fermentative hydrogen production from
170 solid wastes (Yang and Wang, 2018b). Accordingly, the highest rate of hydrolysis may
171 be a factor facilitating the hydrogen production for the aeration pretreated inoculum.

172 **Table 1**

173 After the fermentation, hydrogen yields were 70.1, 64.9, 50.8 and 40.2 mL/g-VS
174 for the groups with aeration, base, acid and heat-shock treated inoculums, respectively.
175 The difference in hydrogen yield for these four groups may be attributed to the

differences in bacterial community structure and fermentation pathway. Clearly, the inoculum with aeration pretreatment achieved the highest yield of biohydrogen ($p<0.05$), followed by the base, acid and heat-shock pretreated inoculums. This was similar to the study by Ren et al. (2008), which also observed that aeration pretreated inoculum achieved the highest hydrogen production from glucose among four pretreated inoculums (aeration, acid, heat-shock and alkaline). However, some other studies showed different results. For example, Yang et al. (2018a) utilized ryegrass as the feedstock for fermentative biohydrogen generation, and found that acid pretreated inoculum achieved the highest hydrogen yield among five pretreated inoculums (acid, aeration, gamma radiation, base and heat-shock). Luo et al. (2022) compared various pretreatment approaches (aeration, base, electric-shock, acid, 2-bromoethanesulfonate, heat and free nitrous acid) for enriching hydrogen-producing inoculum, and found that base pretreated inoculum reached the highest hydrogen yield for dark fermentation of food waste. This inconsistency may be associated with the different characteristics of the feedstocks used in these studies.

The hydrogen yield of dark fermentation of potato peel waste (71.0 mL/g-VS) was higher compared to some other wastes, including municipal solid waste (17.2 mL/g-VS) (Paillet et al., 2021), waste activated sludge (13.0 mL/g-VS) (Wang et al., 2018), fallen leaves (30.5 mL/g-VS) (Yang et al., 2019a), antibiotic fermentation residue (17.8 mL/g-VS) (Yang et al., 2019b), and tofu residue (42.5 mL/g-VS) (Ali et al., 2022). This clearly confirms that potato peel waste shows great potential in producing biohydrogen.

198 **3.2. Substrate utilization**

199 In this work, biohydrogen was generated from the utilization of available
200 substrates in the potato peel waste. Fig. 2 depicts the total organics removal for the
201 four groups with different pretreated inoculums. The ratios of total organics removal
202 were 15.8%, 19.4%, 24.3% and 28.9% for the fermenters inoculated with heat-shock,
203 acid, base and aeration treated sludge, respectively (Fig. 2). Obviously, the inoculum
204 with aeration pretreatment obtained the maximum organics removal, indicating that,
205 in addition to the maximum hydrogen yield, the aeration pretreated inoculum
206 achieved the most effective waste reduction as well. As a result, it can be concluded
207 that aeration pretreatment was the most effective approach for preparing biohydrogen
208 fermentation inoculum for potato peel waste. The maximum VS removal for potato
209 peel waste (28.9%) was higher than those of biohydrogen fermentation fed with food
210 waste (25.5%) (Pu et al., 2019), antibiotic fermentation residue (17.8%) (Yang et al.,
211 2019b), and rice straw (13%) (Kim et al., 2013).

212 **Fig. 2**

213 Carbohydrate is the dominant component of potato peel waste, and it was also
214 the main substrate that could be used for producing biohydrogen. Consequently, the
215 carbohydrate utilization efficiency for different pretreated groups was also evaluated
216 (Fig. 2). The efficiencies of carbohydrate utilization were 42.7%, 51.0%, 62.5% and
217 69.2% for the fermenters inoculating the heat-shock, acid, base and aeration treated
218 sludge, respectively. The aeration treated inoculum also achieved the maximum
219 carbohydrate utilization, and the rank of the carbohydrate utilization efficiency was in

agreement with both the biohydrogen production and organics removal among the four groups, indicating that enhanced utilization of carbohydrates contributed to a higher biohydrogen yield and waste reduction (Hallenbeck, 2009).

3.3. Liquid metabolites formation

Metabolites produced in liquid phase of dark fermentation are the vital indicator for distinguishing the fermentation pathways. Fig. 3 illustrates the contents of dominant liquid metabolites for the groups inoculating various treated cultures. The total concentrations of liquid metabolites (butyrate, propionate and acetate) were 3357.3, 5779.8, 5329.3 and 5371.5 mg/L for the fermenters inoculating the heat-shock, acid, base and aeration treated cultures, respectively, with the yields being 1953.5, 3363.1, 3125.5 and 3101.0 mg/g-VS, respectively. The lowest yield of metabolites in the heat-shock pretreated system may result from the low efficiency of substrate utilization. These organic acids produced can be further utilized for generating electricity through microbial fuel cells, biological denitrification, and synthesis of complex polymers (Bhatia and Yang, 2017).

Fig. 3

For the distribution of liquid metabolites, acetate, propionate and butyrate accounted for 52.9%, 1.8% and 45.4%, 47.7%, 30.6% and 21.7%, 47.8%, 12.8% and 39.5%, and 59.4%, 4.7% and 35.9% of total metabolic products for the fermenters inoculating the acid, heat-shock, base and aeration treated cultures, respectively. Clearly, the distribution of liquid metabolites shows a big difference among the four groups, implying that there was a great variation in the metabolic pathway when

242 inoculating different treated cultures. Other studies also found that different pretreated
243 inoculums exhibited a great variation in the distribution of liquid metabolites (Dessì et
244 al., 2018; Yin et al., 2014; Yang et al., 2019b), which may be attributed to the different
245 bacterial communities enriched by different pretreatments. According to the
246 metabolites distribution, it can be deduced that the fermenter inoculating the
247 heat-shock treated culture was predominated by the mixed-acid type fermentation,
248 and the other fermenters were predominated by the butyrate-type fermentation. In
249 addition, it should be mentioned that, compared to other fermenters, the proportion of
250 propionate was significantly higher in the fermenter inoculated with the heat-shock
251 treated culture. Generally, there is no hydrogen produced in dark fermentation with
252 generating propionate as the liquid metabolite, and propionate is even
253 disadvantageous to hydrogen fermentation process (Hallenbeck, 2009). Accordingly,
254 the low hydrogen production for the heat-shock pretreated group was also probably
255 link to the higher propionate proportion.

256 **3.4. Microbial community analysis**

257 To elucidate how inoculum pretreatment methods influence the biohydrogen
258 production from dark fermentation of potato peel waste, microbial communities in the
259 fermenters were analyzed and compared. Fig. 4 illustrates the microbial diversity and
260 richness for the groups with the inoculums enriched by the different pretreatment
261 methods. In Fig. 4a, the Shannon index follows the order of aeration > base > acid >
262 heat-shock, while the Simpson index follows the order of heat-shock > acid > base >
263 aeration. This indicates that the aeration pretreated fermenter had the highest level of

264 bacterial diversity, followed by the base, acid and heat-shock pretreated fermenters.
265 This phenomenon may be due to that the harsh high-temperature condition of the
266 heat-shock pretreatment eliminated more types of microbes in the seed sludge
267 compared to the other pretreatment methods. Moreover, the microbial diversity is
268 positively correlated with the biohydrogen yield, indicating that high diversity was
269 beneficial for maintaining the stability and promoting the efficiency of microbial
270 ecosystem, probably due to the collaborative functions between the different types of
271 bacteria (Tilman et al., 2006). According to the indices of Ace and Chao (Fig. 4b), the
272 group with the aeration pretreated culture had the highest microbial richness as well,
273 while the group inoculating the heat-shock treated culture presented the lowest value,
274 which was in accordance with the microbial diversity result.

275 **Fig. 4**

276 To further illustrate the influence of different inoculum pretreatment methods on
277 the community structure, the bacterial composition was compared at the genus level
278 (Fig. 5). It can be clearly seen that different inoculum pretreatment approaches
279 resulted in a significant variation in the bacterial community, which would have a
280 direct impact on the fermentation performances.

281 **Fig. 5**

282 In the present work, *Clostridium sensu stricto* was the predominant
283 biohydrogen-producing genus (Fig. 5), with the abundances of 21.9%, 26.9%, 32.2%
284 and 39.5% for the fermenters inoculated with heat-shock, acid, base and aeration
285 treated cultures, respectively. Interestingly, the ranking of *Clostridium sensu stricto*

286 abundance is consistent with the ranking of biohydrogen yield among the four groups.
287 Existing studies have shown that *Clostridium* sp. can use many kinds of organics for
288 efficient biohydrogen production, such as starch (the main component of potato peel
289 waste), cellulose, glucose, hemicellulose, and sucrose (Wang and Yin, 2021; Chen et
290 al., 2021a). As a result, the genus *Clostridium sensu stricto* may be mainly responsible
291 for biohydrogen evolution in the present study, and the highest content of *Clostridium*
292 *sensu stricto* corresponds to the highest hydrogen production efficiency for the
293 fermenter inoculating the aeration pretreated culture. It was also supported by Pearson
294 correlation analysis, which further showed that both organics utilization and
295 biohydrogen yield exhibited a significant positive correlation with *Clostridium sensu*
296 *stricto* (Fig. 6). Similar correlation has also been found by existing studies, i.e. the
297 performances of biohydrogen fermentation were positively correlated with the
298 abundance of *Clostridium sensu stricto* (Yin and Wang, 2018; Zhang et al., 2015).
299 *Acetomicrobium*, *Candidatus Caldatribacterium* and *Anaerosporobacter* were the
300 other three hydrogen-producing genera in this work (Jeong et al., 2007; Soutschek et
301 al., 1984; Wang et al., 2022), with the main liquid metabolic product being acetate.
302 The abundances of these three genera were 1.3%, 0.3%, 2.4% and 6.9% for the groups
303 inoculated with the heat-shock, acid, base and aeration treated cultures (Fig. 5),
304 respectively. Clearly, the aeration pretreatment also enriched the highest abundance of
305 the three biohydrogen producers mentioned above, which was also beneficial for
306 biohydrogen production.

307 **Fig. 6**

Besides the hydrogen-producing genera, *Enterococcus* and *Lactococcus* were detected in this work, with the abundances of 5.1% and 0.1%, 1.1% and 3.6%, 4.3% and 0.0%, and 3.8% and 0.0% in the fermenters inoculating the heat, acid, base, and aeration treated cultures, respectively. *Enterococcus* and *Lactococcus* belong to lactic acid bacteria (Chen et al., 2021b), which can consume available substrates and secrete bacteriocins during the fermentation process. Accordingly, the presence of lactic acid bacteria is disadvantageous to hydrogen production, and a higher level of these two genera might lead to the lower biohydrogen productivities for the fermenters inoculating the heat-shock and acid treated cultures.

Low abundances of *Proteiniphilum*, *Syntrophomonas*, *Keratinibaculum* and *Dialister* were also detected in the present study. The genus *Proteiniphilum* is obligately anaerobic and proteolytic, which can use peptone as energy source, while cannot use sugars (Chen and Dong, 2005). The fermentation products of the genus *Proteiniphilum* are mainly composed of acetate and propionate. The genus *Syntrophomonas* is syntrophic and strictly anaerobic, which can metabolize fatty acids into acetate when co-culturing with methanogens (Zhang et al., 2004). The genus *Keratinibaculum* is proteolytic, which is capable of fermenting proteins into acetate, propionate and butyrate (Huang et al., 2013), while growing weakly when fed with some sugars, such as xylose and glucose. The genus *Dialister* is obligately anaerobic, gram-negative, non-sporing and non-fermentative (Downes et al., 2003). The genera mentioned above have been frequently detected in anaerobic fermentation reactors, while their roles in biohydrogen fermentation of potato peel waste is still unknown.

3.5. Implications for potato peel waste disposal

How to appropriately handle potato peel waste has become a challenge faced by the potato industry (Ncobela et al., 2017). Using potato peel waste for biohydrogen production can realize the production of clean energy and the treatment of such waste, which could bring enormous economic and environmental benefits. This work demonstrates that it is potential to use potato peel waste for biohydrogen production, showing a relatively high hydrogen yield. In addition to biohydrogen, dark fermentation of potato peel waste can generate a high content of volatile fatty acids as well (Fig. 3), which brings a bonus for this process, because these volatile fatty acids are valuable chemicals with a wide range of application possibilities (Feng et al., 2022).

However, this study just figured out the technical feasibility of potato peel waste for dark fermentative biohydrogen production and found the optimal inoculum enrichment approach. As a microbial process, a variety of factors can influence the efficiency of biohydrogen fermentation, such as temperature, pH and mixing rate. These factors need to be systematically studied for process optimization to increase the fermentative efficiency and economics in future works. Additionally, towards scale-up applications, continuous operation of potato peel waste fermentation should be tested, and some process enhancement methods (e.g. additives and bioaugmentation) are suggested to be explored to further improve the biohydrogen productivity. Finally, the economic feasibility and environmental impacts of potato peel waste fermentation should also be evaluated for the deployment of continuous

352 systems for practical potato peel waste treatment.

353

354 **4. Conclusions**

355 This work confirms that it is feasible to use potato peel waste for producing
356 renewable biohydrogen via dark fermentation. The hydrogen yield showed a big
357 variation when inoculating various pretreated cultures. The group with aeration
358 treated inoculum achieved the highest substrate utilization (VS removal of 28.9%) and
359 hydrogen yield (71.0 mL/g-VS). Various treated inoculums presented a huge
360 difference in the metabolic pathway and bacterial composition. More enrichment of
361 *Clostridium sensu stricto* was the fundamental factor contributing to the best process
362 performances for the aeration pretreated fermenter. This study provides an effective
363 and eco-friendly way for recycling potato peel waste.

364

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Figure Captions

Fig. 1 Biohydrogen production over time for the groups with different pretreated inoculums

Fig. 2 The efficiencies of total organics removal and carbohydrate utilization for the groups with different pretreated inoculums

Fig. 3 The concentrations of dominant liquid metabolites after the fermentation with different pretreated inoculums

Fig. 4 The diversity (a) and richness (b) of bacterial OTUs for the fermenters with different pretreated inoculums

Fig. 5 Bacterial community at genus level for the groups with different pretreated inoculums

Fig. 6 The correlation between the identified genera and the hydrogen fermentation performances

Tables

Table 1 Kinetic analysis of cumulative hydrogen production for different pretreated inoculums

Parameters	Inoculum pretreatment methods			
	Aeration	Base	Acid	Heat-shock
P (mL)	126.8	114.9	86.4	69.3
k_{hyd} (h^{-1})	0.054	0.040	0.052	0.043
n	3.86	6.53	7.39	5.91
R^2	0.990	0.982	0.996	0.997

Figures

Fig. 1

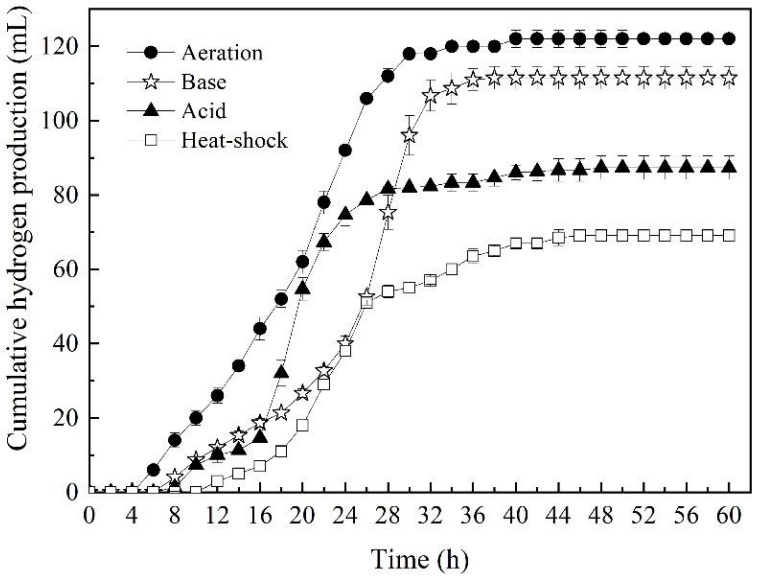


Fig. 2

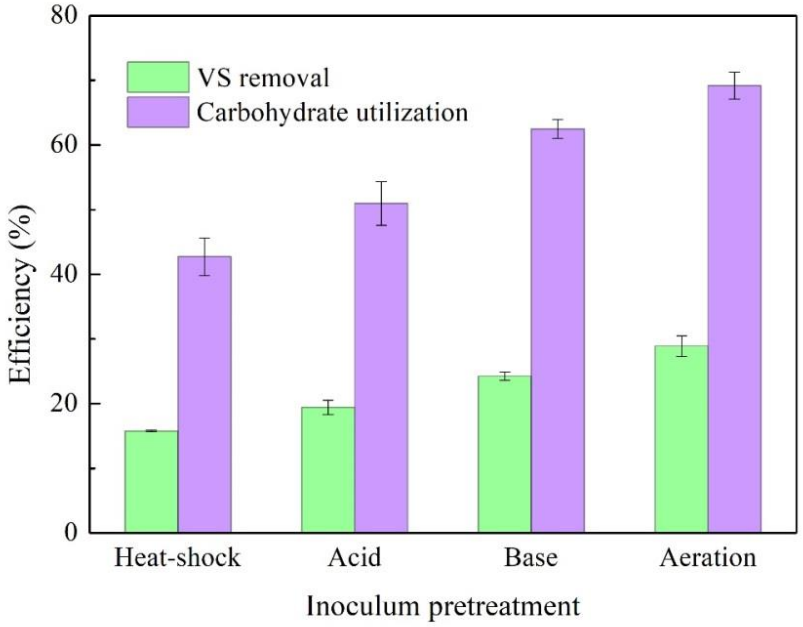

80
60
40
20
0
Efficiency (%)
VS removal
Carbohydrate utilization
Heat-shock
Acid
Base
Aeration
Inoculum pretreatment

Fig. 3

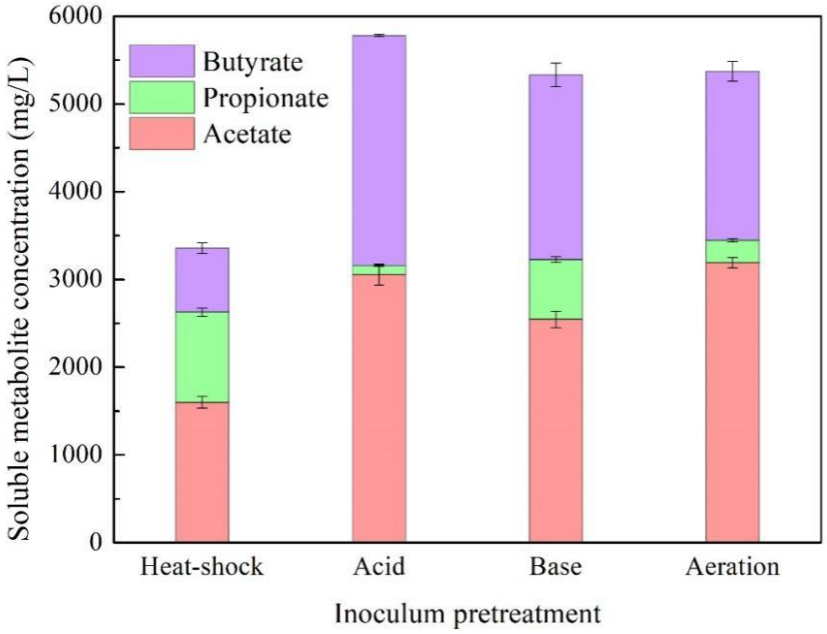

Soluble metabolite concentration (mg/L)
6000
5000
4000
3000
2000
1000
0
Butyrate
Propionate
Acetate
Heat-shock
Acid
Base
Aeration
Inoculum pretreatment

Fig. 4

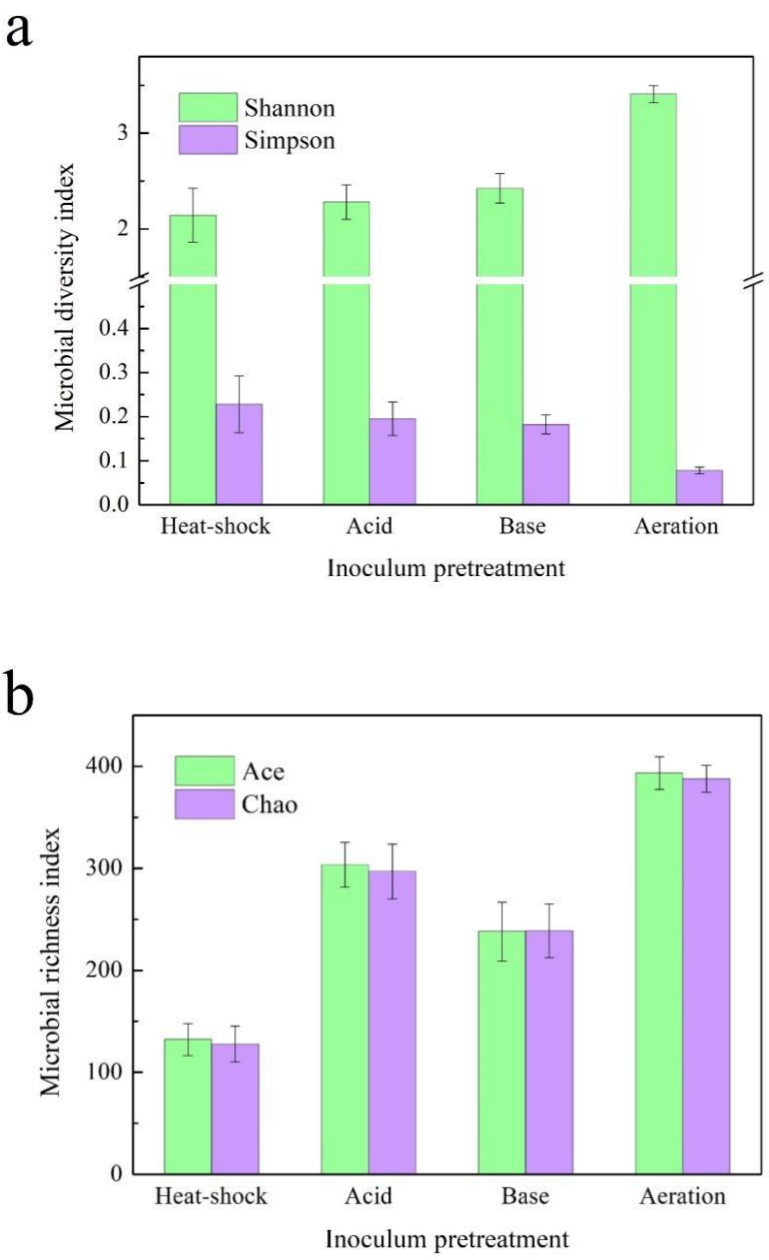

a
Shannon
Simpson
Microbial diversity index
3
2
0.4
0.3
0.2
0.1
0.0
Heat-shock
Acid
Base
Aeration
Inoculum pretreatment
b
Ace
Chao
Microbial richness index
400
300
200
100
0
Heat-shock
Acid
Base
Aeration
Inoculum pretreatment

Fig. 5

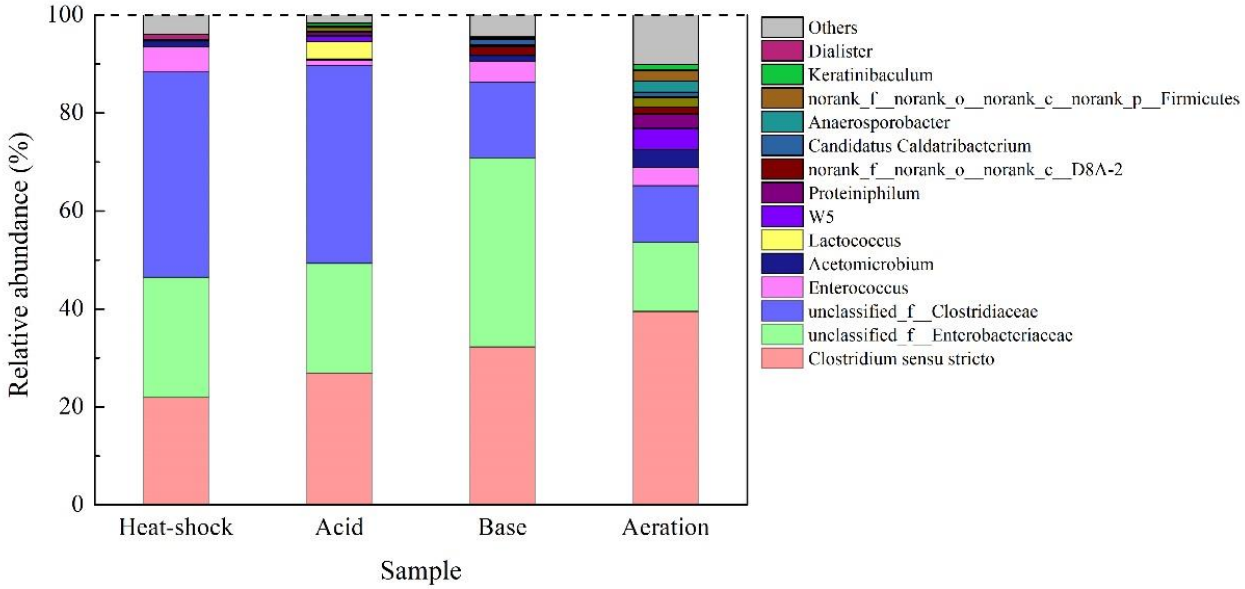
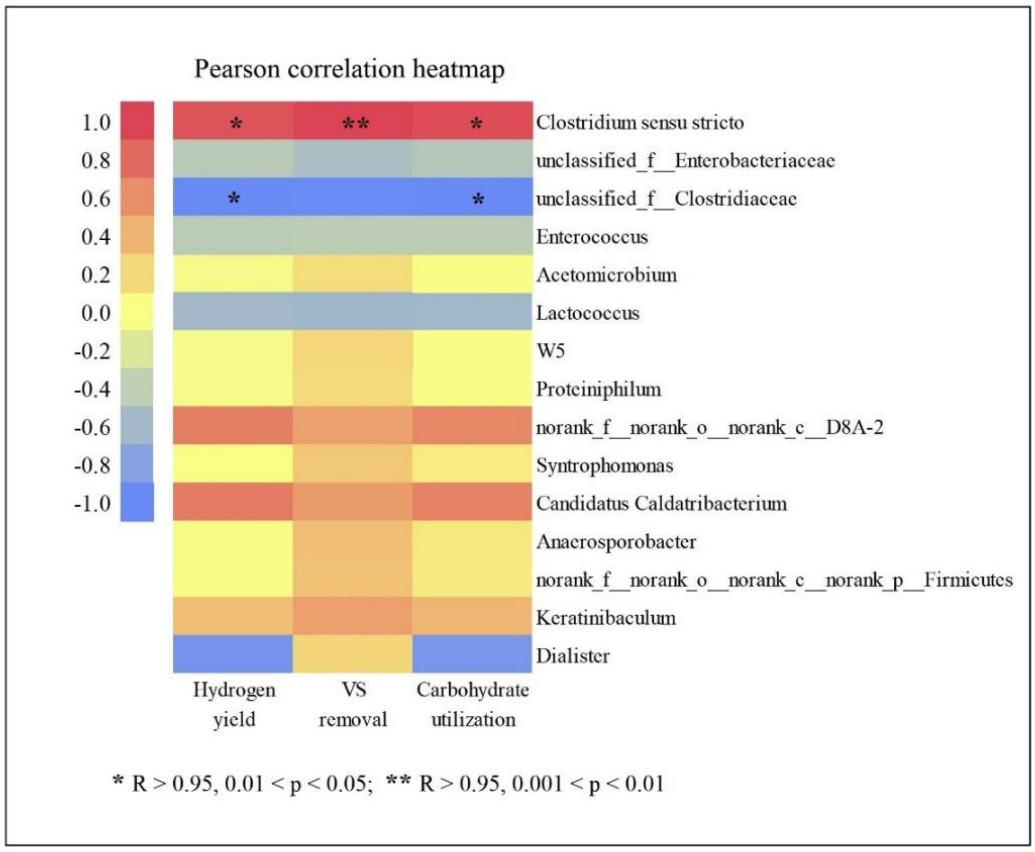

100
80
60
40
20
0
Relative abundance (%)
Heat-shock
Acid
Base
Aeration
Sample
Others
Dialister
Keratinibaculum
norank_f__norank_o__norank_c__norank_p__Firmicutes
Anaerosporobacter
Candidatus Caldatribacterium
norank_f__norank_o__norank_c__D8A-2
Proteiniphilum
W5
Lactococcus
Acetomicrobium
Enterococcus
unclassified_f__Clostridiaceae
unclassified_f__Enterobacteriaceae
Clostridium sensu stricto

Fig. 6


Pearson correlation heatmap
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
Clostridium sensu stricto
unclassified_f__Enterobacteriaceae
unclassified_f__Clostridiaceae
Enterococcus
Acetomicrobium
Lactococcus
W5
Proteiniphilum
norank_f__norank_o__norank_c__D8A-2
Syntrophomonas
Candidatus Caldatribacterium
Anaerosporobacter
norank_f__norank_o__norank_c__norank_p__Firmicutes
Keratinibaculum
Dialister
Hydrogen yield
VS removal
Carbohydrate utilization
* R > 0.95, 0.01 < p < 0.05; ** R > 0.95, 0.001 < p < 0.01