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Biohydrogen production by the psychrophilic G088 strain using single carbohydrates as substrate

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ABSTRACT

The interest in hydrogen as an energy carrier has intensified the search of novel approaches for new production processes, among which biohydrogen stands out. In this study, the production of biohydrogen by psychrophilic G088 strain ([EU636029]) closely related to *Polaromonas rhizosphaerae* ([EF127651]) was evaluated using xylose, glucose, fructose, galactose, lactose or sucrose as carbon source. Biohydrogen production was performed in 120 ml serological bottles with a production medium containing 2.75 g/l tryptone, 0.25 g/l yeast extract, and 20 g/l of each carbohydrate. Results showed that G088 strain produced biohydrogen using all the evaluated substrates, ranging from 91.7 to 439.8 ml for lactose and glucose, respectively. However, glucose was the substrate with the highest consumption rate, accompanied with the maximum values of biohydrogen production rate and biohydrogen yield of 19.3 ml/l/h and 1.7 mol H_2/mol glucose, respectively. Analysis of the secreted metabolites from psychrophilic strain cultivations showed that ethanol and organic acids were the main by-products. Our results demonstrate that G088 strain has potential to be used for developing new biotechnological processes for biohydrogen production.

Keywords: Biohydrogen, psychrophilic bacteria, potential hydrogen producers, carbohydrate metabolism.

1. INTRODUCTION

Environmentally friendly energy carrier and sources are the most highlighted topic in the energy and environmental sector. The current global energy demand is mostly dependent on reserves of fossil fuel uses [1]. In recent years, various studies have been conducted to obtain a sustainable source of energy that can replace fossil fuels and its negative impact on the environment. In this regard, hydrogen has found as a promising clean and environmental friendly energy carrier [2], also its energy value is 122 kJ/g, which is 2.75 times higher than hydrocarbon fuels [3] and upon oxidation hydrogen produces water [4].

Hydrogen is a valuable energy carrier, an important feedstock to the chemical industry, and useful in detoxifying a wide range of water pollutants. As an energy carrier, it is especially attractive due to its potential to be used to power chemical fuels [5]. In industry, hydrogen is used for hydrogenation of many products, including heavy oils in gasoline production, foods, and ammonia for fertilizer [6]. Nowadays, hydrogen is mainly produced by reforming fossil fuels. Therefore, hydrogen currently is neither renewable nor carbon-neutral. Instead, hydrogen manufacturing has a large greenhouse-gas footprint. Society needs to gain the enormous benefits hydrogen can offer without incurring the greenhouse-gas costs [5].

Among various hydrogen production processes, biological method is known to be less energy intensive; it can be carried out at room temperature and pressure [7]. Dark fermentation is one of the main biological processes in which microorganisms utilize carbohydrates to produce biohydrogen in anaerobic fermentation conditions

[8]. However, low yields and production rates have been the mail barriers for practical applications [9]. Most of the studies addressing fermentative hydrogen production operate on anaerobic digesters at mesophilic (24-40°C), thermophilic (40-65°C) or hyperthermophilic (>80°C) [10] temperatures. Whereas, in our knowledge, only two studies have been reported on the biohydrogen production using psychrophilic bacteria [11-12].

Psychrophiles have slower metabolism rates and higher catalytic efficiencies than mesophiles [13], the high activity of psychrophilic enzymes at low and moderates temperatures offers potential economic benefits due to the substantial energy savings in large-scale processes that would not require the expensive heating of reactors [14]. In addition, the temperature range prevents the risk of microbial contamination [13]. These advantages make the psychrophilic bacteria a good candidate for biohydrogen production. The current interest of biotechnology on these bacteria may not have been realized sufficiently. Nevertheless the current applications of these bacteria are focused to the food, bioremediation and environmental technologies [15].

In this study, the effectiveness of biohydrogen production from single carbohydrates using a psychrophilic G088 strain closely related to *Polaromonas rhizosphaerae* was assessed. This microorganism was isolated from samples of glacier sediment from Antarctica (incluir cita. Folia Microbiol (2011) 56:209–214). Carbohydrates assessed were glucose, xylose,…., which are currently obtained from industrial waste such as cheese whey, cellulosic and hemicellulosic hydrolysate. Currently there is only one study reporting the biohydrogen production

from psychrophilic bacteria isolated from Antarctica, which was reported by our own research group [12].

2. MATERIAL AND METHODS

2.1 *Strain and Culture media*

In this study, the psychrophilic G088 strain obtained of samples of glacier sediment from Antarctica was used. The accession number EU636050 and closest relativity of this strain according to NCBI is *Polaromonas rhizosphaerae* [EF127651] [16]. The strain was grown routinely in YPG agar plates [0.25 g/l Bacto-tryptone (Difco), 0.25 g/l yeast extract (Difco), 0.25 g/l glucose (Sigma) and 15 g/l Bacto-agar (Sigma)] and maintained at 4°C. Six carbohydrates were used as substrate (xylose, glucose, fructose, galactose, sucrose or lactose). Biohydrogen production experiments were done in a rich production medium containing 2.75 g/l Bacto-tryptone (Difco), 0.25 g/l yeast extract (Difco) and 20 g/l of the corresponding carbohydrate mentioned above (Sigma) [17].

2.2 *Biohydrogen production experiments*

To evaluate the hydrogen production by the G088 strain, preinocula were grown in rich production medium under anaerobic conditions at XºC. Cells were harvested, centrifuged, washed and inoculated into 120 mL anaerobic serological bottles (Prisma, DF, Mex) containing 110 ml of production medium with 20 g/l of the respective carbohydrate supplemented with 1 ml/l of trace elements solution (0.015

g/l $FeCl_3.4H_2O$, 0.00036 g/l $Na_2MoO_4.2H_2O$, 0.00024 g/l $NiCl_2.6H_2O$, 0.0007 g/l $CoCl_2.6H_2O$, 0.0002 g/l $CuCl_2.2H_2O$, 0.0002 g/l Na_2SeO_3, 0.01 g/l $MgSO_4$). The cultures were started at an optical density at 600 nm (OD_{600nm}) of 1, pH adjusted at 6.8 and were incubated at 20°C and 150 rpm [18]. All experiments were carried out in triplicate.

2.3 *Analytical methods*

The hydrogen produced was measured by water displacement with NaOH 1N in an inverted burette connected to serological bottles with rubber tubing and a needle and validated by Gas chromatography using a thermal conductivity detector (cita del primer paper de luis manuel). All the experiments were carried out in triplicate. Samples of 1 ml were taken at different times during fermentation, then were diluted and filtered through a 0.22 mm membrane (Millipore, Bedford, Massachusetts, USA) [12]. Remaining substrate, xylose, glucose, fructose and galactose and several metabolites such as succinic acid, lactic acid, acetic acid and butanol were analyzed by High Performance Liquid Chromatography (HPLC, Infinity LC 1220, Agilent Technologies, Santa Clara CA USA) using a Refraction Index Detector, and a column Phenomenex Rezex ROA (Phenomenex, Torrance, CA, USA) at 60–°C, and using 0.0025 M H_2SO_4 as mobile phase at 0.41 ml/min. Sucrose was analyzed by the colorimetric method for determination of sugars and related substances [19] and lactose was analyzed by the 3, 5-dinitrosalicylic acid (DNS) method [20]. Ethanol, butyric acid, propionic acid, and acetone were analyzed in a Gas Chromatograph (GC, 6890N Network GC System, Agilent Technologies Wilmington, DE, USA) using a flame ionization detector (Agilent

Technologies Wilmington) . The column used was a capillary column HP-Innowax with the following dimensions: 30 m x 0.25 mm i.d. x 0.25 m film thickness (Agilent, Wilmington, DE, USA). Temperatures of the injector and flame ionization detector (FID) were 220 and 250–°C respectively. Helium was used as carrier gas at a flow rate of 25 ml/min. The analyses were performed with a split ratio of 5:1 and a temperature program of 25°C for 10 min to 280°C, and was maintained at this temperature for a final time of 10 min [12].

3. RESULTS

3.1 *Fermentation of pentoses*

We conducted an experiment with xylose as substrate to evaluate the hydrogen production because of the importance of this sugar as the main pentose obtained from the hydrolysis of hemicellulosic materials. Fig. 1 shows a typical batch culture of G088 strain using xylose as single substrate. As observed, xylose started to be consumed 16 h after initiating the culture, causing a lag-phase of 16 h, meanwhile the hydrogen production started at 34 h of incubation with a volume of 8.7 ml. The hydrogen production attained its maximum volume at 333 h with 349.9 ml; however at that point there was still 5 g/l of substrate. The maximum hydrogen production rate reached using xylose was 13.4 ml/l/h at 202 h, which is 31% lower than using glucose. As well as the highest yield achieved in this fermentation was 1.4 mol H_2/ mol xylose, which is approximately 18% lower than the yield attained by the culture using glucose as substrate (Table 1).

3.2 *Fermentation of hexoses*

Lignocellulosic biomass contains 70-80% carbohydrates and could serve as the ideal feedstock for fermentative hydrogen production [22]. In this regard we evaluated the capability of G088 strain to metabolize glucose, which is currently obtained by hydrolysis of starch, cellulose and hemicellulosic materials [21], as well as fructose that is mainly extracted from a fructan called inulin [23]. In addition galactose was tested too, which the same as glucose, is obtained from hemicellulosic material (lignocellulose). All of them constitute the major component of biomass.

3.2.1 *Glucose*

In the case of the cultures using glucose as substrate, its consumption started at 20 h as seen in figure 2. The beginning of hydrogen production was 43 h after the culture started, with a volume of 12 ml. Afterward, the maximum hydrogen production was attained after the exponential phase, generating a final volume of 439.8 ml in 306 h. In the middle point of the fermentation, the glucose concentration was 6.9 g/l, and as expected G088 strain utilized completely the available substrate since no glucose was detected in the medium at the end of the fermentation. Moreover, productivity and yield were 19.3 ml/l/h and 1.7 mol H_2/ mol glucose respectively (table 1).

3.2.2 *Fructose*

The fermentation of fructose by G088 strain had duration of 386 h, of which 50 h corresponded to the lag-phase (Fig. 3). Unlike glucose, where its consumption started at 20 h, the consumption of fructose began at 40 h, and the hydrogen production started 21 h later, with a volume of 51.33 ml of hydrogen after having consumed 2.8 g/l of available substrate. Moreover at 302 h the higher productivity was reached with a value of 19.7 ml/l/h. The maximum hydrogen volume was 388.1 ml, achieved after 352 h of the fermentation; also at this point fructose was not detected. Maximum yield reached was 1.37 mol H_2/ mol fructose. The maximum hydrogen production rate in this culture (19.7 ml/l/h) was similar to the rate reached using glucose as substrate (19.3 ml/l/h). Whereas, the hydrogen yield attained using fructose was only 23.5 % lower than the obtained from cultures using glucose.

3.2.3 *Galactose*

The galactose fermentation presented a lag-phase of 44 h, nonetheless the hydrogen production started at 69 h with volume of 5.66 ml. Moreover, after 279 h of cultivation, approximately the 50% of available galactose was consumed by G088 strain. The maximum production rate was obtained at 361 h with 5.28 ml/l/h, which is approximately 3.6-times lower than using glucose. On the other hand, the higher yield was 1.32 mol H_2/ mol galactose which is similar to the yield obtained using fructose as substrate (Table 1). Furthermore, almost at the end of the

196 fermentation at 568 h, the maximum hydrogen production was measured,
197 registering a volume of 293.3 ml. In addition, the galactose started to be consumed
198 from the beginning of the fermentation, and it was depleted until 592 h.

199

200 **3.3 *Fermentation of disaccharides***

201 Sucrose and lactose are typical disaccharides, the first one is obtained from
202 molasses, a by-product of sugar industry that is obtained as a thick syrup sugar
203 extraction [24], and the latter is obtained from cheese whey, a by-product
204 generated during cheese production, which represents an 85-90% of the total
205 volume of processed milk [18]. Therefore we evaluated the potential use of these
206 carbohydrates as raw material for hydrogen production.

207 **3.3.1 *Sucrose***

208 The fermentation of sucrose lasted for about 350 h. Due to the lag phase of 20 h
209 (data not shown), hydrogen production began 34 h after the cultivation started. The
210 hydrogen production attained 201.7 ml in 351 h. This production represents
211 approximately the 50% the hydrogen produced from glucose. The maximum
212 hydrogen production rate 5.6 ml/l/h was considerably lower than using glucose
213 (19.3 ml/l/h), however the yield obtained in this culture was 1.6 mol H_2/mol
214 sucrose, which is close to the yield achieved using glucose (Table 1).

215 **3.3.2 *Lactose***

216 In these cultures, the lag-phase lasted 39 h. The biohydrogen production started
217 after 44 h of culture. Unlike culture using sucrose, the fermentation using lactose

218 took about 10 h to produce the first 9.3 ml. However the exponential phase lasted
219 about 138 h and the maximum hydrogen production achieved by G088 strain was
220 91.7 ml after 302 h of fermentation. On the other hand, the highest production rate
221 and yield were 5.5 ml/l/h and 1.5 mol H_2/ mol lactose, respectively (Table 1). This
222 production rate is similar to the one attained using sucrose, however it is
223 significantly lower than the maximum hydrogen production rate registered with
224 glucose (19.3 ml/l/h).

225

226 **3.4 *Comparison of hydrogen production***

227 Table 1 shows that using lactose as substrate for G088 strain resulted in a poor
228 hydrogen production (91.7 ml) and a low production rate (5.5 ml/l/h), however with
229 this substrate the yield of 1.5 mol H_2/mol lactose could be considerable. Highest
230 yield reached was 1.7 mol H_2/mol glucose with glucose, followed by the culture
231 using sucrose with similar yield of 1.6 mol H_2/ mol sucrose. Moreover the maximum
232 hydrogen production rate achieved was 19 ml/l/h using either glucose or fructose.
233 On the other hand, the highest hydrogen volume obtained, was using glucose
234 (439.8 ml), followed by the fermentation with fructose (388.1 ml) and the culture
235 with xylose (349.9 ml). The analysis of variance (ANOVA) showed that there was
236 no significant difference ($p < 0.05$) between glucose, which is the substrate with the
237 highest hydrogen production, in comparison with xylose and fructose. Galactose
238 and sucrose fermentations produced hydrogen volumes in a range of 201.7 ml and
239 293.3 ml respectively. However, the analysis of variance indicated that these two

240 substrates are statistically different. Moreover their production rates were
241 approximately 3.5 times lower than using glucose.

242

243 **3.5 *Fermentative metabolites***

244 Hydrogen formation is accompanied with volatile fatty acids or solvent production
245 during an anaerobic digestion process. Table 2 shows the excreted metabolites
246 found in the culture at the end of the culture. In each fermentation with different
247 single substrate, the presence and concentration of metabolites varied. For
248 instance, higher concentration of ethanol was detected in the culture with xylose,
249 followed by the culture with glucose, while in cultures using lactose was not
250 detected. In the case of the production of butyric acid, the fermentation with
251 glucose achieved the highest concentration (2.515 g/l), and for the rest of the
252 cultures a concentration lower than 0.9 g/l was registered. On the other hand, the
253 acetic acid presence on the fermentation with fructose was remarkably high, with
254 2.314 g/l, while on the other cultures; the concentration of this metabolite was
255 shown to be below of 0.8 g/l. A similar observation was found same for the
256 propionic acid, whose highest concentration was detected on the fermentation with
257 xylose as substrate (2.028 g/l), followed by the fermentation using glucose, as for
258 the remaining fermentations, the concentrations of this metabolite were in small
259 quantities. Succinic acid was found on the fermentations using glucose, galactose,
260 lactose and sucrose, being the fermentation with galactose the one that attained
261 the highest concentration (4.921 g/l). In addition, the presence of other solvents
262 was detected, such as butanol and acetone. In the case of butanol, the highest

concentration 0f 1.482 g/l was detected in the cultures with glucose, followed by the cultures with fructose (1.338 g/l). On the other hand, acetone was detected only in the cultures with glucose and fructose with 1.509 g/l and 0.547 g/l, respectively.

4. DISCUSSION

The Polar Regions such as Antarctica represent a vast source of novel psychrophilic microorganisms. Psychrophilic bacteria and their enzymes are of commercial interest because their possibility of use at low temperatures [12]. However their application in biohydrogen production has just begun. The organic materials and residues currently constitute a large source of biomass, which includes agricultural crops and their waste by-products, wood and wood waste, food processing waste, aquatic plants, algae and effluents produced in the human habits [21]. Consequently production of biohydrogen from renewable resources would become major and attractive future source of energy. In accordance glucose, xylose, fructose, galactose, sucrose, and lactose were explored as substrates because they are available in large amounts on the compounds mentioned above.

Only within the past few years it has been recognized that psychrophilic microorganisms and their products or enzymes provide a large reservoir of potentially novel biotechnological exploitation [15]. However, hydrogen production

285 by these microorganisms has not been extensively explored, representing a new
286 alternative in the biohydrogen production field. To our knowledge there is only one
287 report on the use of psychrophilic bacteria isolated from Antarctica for biohydrogen
288 production, which was reported by our own group [12]. Other study by Debowsky
289 et al. [11] assessed the biohydrogen production using psychrophilic strains isolated
290 from underground water and demersal lake water samples. These studies were
291 carried out at temperatures of 25°C and 20°C respectively, showing that hydrogen
292 production via dark fermentation is possible by psychrophilic bacteria at ambient
293 temperatures.

294 Current fermentative biohydrogen production processes are carried out mainly at
295 mesophilic (24-40°C), thermophilic (40-65 °C) or hyperthermophilic (>80 °C)
296 temperatures [10]. In addition, glucose, sucrose and starch mixtures are the most
297 commonly used substrates [25]. Although these processes generate high yields,
298 they have the disadvantage of requiring large amounts of energy to maintain the
299 optimum process temperature. Table 3 shows the hydrogen production yields at
300 different temperatures using different substrates and microorganisms. For example
301 Chin et al. [26] assessed the hydrogen production using *Clostridium actobutylicum*
302 at 37 °C obtaining a yield of 2.0 mol H_2/ mol glucose, while Mizuno et al. [27]
303 employed Clostridium sp. at 35°C reaching a hydrogen yield of 0.85 mol H_2 /mol
304 glucose. In other study by Ishikawa et al. [28] the hydrogen production by a
305 *Escherichia coli* MCI3-4 strain that overproduce hydrogen because of its deficiency
306 in lactate production, reached a yield of 1.2 mol H_2/mol glucose at 37°C.
307 Comparing these results it is clear that the yield of 1.7 mol H_2/ mol glucose

achieved by psychrophilic G088 strain lies between the yields obtained under conditions of mesophilic temperature. However, our process has the advantage of requiring approximately 15°C lower than the aforementioned processes. Moreover Lo et al. [29] examined the hydrogen production with seven hydrogen-producing pure strains at 37°C with xylose as substrate, those results showed that *Clostridium butyricum* CGS5 was the best hydrogen producer on xylose, with a yield of 0.73 mol H_2/ mol xylose. On the other hand the yield reached by G088 strain was 1.73 times higher than the reported above. In this regard other substrates as fructose have been evaluated under mesophilic conditions as shown by Wu et al. [30] where the maximum yield attained by anaerobic sludge at 37°C was 0.56 mol H_2/ mol fructose, this result is considerably lower compared to the yield of 1.3 mol H_2/mol fructose obtained by G088.

As mentioned before, the biohydrogen production can also be carried out at thermophilic and hyperthermophilic temperatures. The high temperatures provide some advantages, such as low viscosity, better mixing, less risk of contamination, and higher reaction rates [25]. Nevertheless, these attributes are overshadowed by the high energy consumption required in these processes. Nowadays the most desired processes are those in which the hydrogen can be produced with the minimum amount of energy input.

The yields by cultures of thermophiles and hyperthermophiles are reported in a range between 0.87 and 4.0 mol H_2/mol hexose [31] (Table 3). The minimum yield corresponds to a published report by Shaw et al. [32], in which a culture of *Thermoanaerobacterium saccharolyticum* YS485 at thermophilic conditions (55

°C), reached a yield of 0.87 mol H_2/ mol hexose. In addition to this, recent studies reported regarding the hydrogen production with hyperthermophiles. For example Van Niel et al. [33] reported a hydrogen yield of 3.3 mol H_2/mol substrate using either *Caldicellulosiruptor saccharolyticus* on sucrose (70–°C) or *Thermotoga elfii* on glucose (65–°C). The highest yield was obtained by *Thermotoga maritima* at 80 °C using glucose as substrate with 4 mol H_2/mol glucose reported by Schröder et al. [34]. Contrary to these studies our operating temperature was 20–°C, which allow us to obtain a considerable yield at temperatures close to room.

Currently, the cost of hydrogen generated from biological processes is very high, and one of the key aspects in the biohydrogen production is the use of a feasible substrate. In this regard, potential substrates intended for a sustainable biohydrogen production, must be not only abundant and readily available but, also, cheap and highly biodegradable, such agro industrial and food waste, which meet all these requirements [35]. In this respect, the psychrophilic G088 strain showed a high hydrogen production using glucose (439.83 ml), fructose (388.16 ml) and xylose (349.9 ml) as substrate. These monosaccharides form part of a wide variety of agricultural residues, and hence, these results suggest that the biohydrogen production with G088 strain can be coupled to the use of lignocellulosic feedstock. Therefore, the utilization of wastes to generate hydrogen energy could reduce the costs of production, making the hydrogen gas more available and cheaper.

5. CONCLUSIONS

Biohydrogen has gained attention due to its potential as a sustainable alternative to the conventional methods for hydrogen production. However the current processes demand external energy input to maintain the optimal fermentation temperature, which represents a disadvantage by the main reason that the most desired processes are those in which the hydrogen can be produced with the minimum amount of energy demand. This study has shown the feasibility of the psychrophilic G088 strain isolated from Antarctica and which closely related to *Polaromonas rhizosphaerae*, to produce biohydrogen at low temperature. The yields obtained in this study are comparable to those reported for mesophilic and thermophilic microorganisms. On the other hand, glucose, xylose and fructose are the best substrates for biohydrogen production by the G088 strain. In consequence, this strain could be used to the use of agroindustrial wastes, which contain these three monosaccharides in large amounts. Additional investigation is necessary to find the optimal conditions to operate the biohydrogen production process using complex substrates with psychrophilic microorganisms.

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500

501 **Table 1**. Comparative hydrogen production by G088 strain using different

502 substrates.

Substrate	Production (ml)	Hydrogen production rate (ml/l/h)	Yield (mol H_2/ mol substrate)
Glucose	439.8 ± 64.25	19.3 ± 0.30	1.7 ± 0.28
Fructose	388.1 ± 17.82	19.7 ± 2.61	1.3 ± 0.06
Xylose	349.9 ± 34.97	13.3 ± 3.29	1.4 ± 0.12
Galactose	293.3 ± 8.03	5.2 ± 0.40	1.3 ± 0.03
Sucrose	201.7 ± 9.16	5.6 ± 0.15	1.6 ± 0.11
Lactose	91.7 ± 8.14	5.5 ± 0.58	1.5 ± 0.09

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507 **Table 2.** Fermentative metabolites produced by the psychrophilic strain G088 at the end of

508 each fermentation with different substrates in g/l.

	Glucose	Xylose	Fructose	Galactose	Lactose	Sucrose
Succinic acid	3.883	0	0	4.921	0.2143	0.181
Lactic acid	0	0	0	3.85	0	0
Acetic acid	0.675	0.1003	2.314	0.422	0.1871	0.8002
Propionic acid	0.916	2.0288	0.0811	0.0977	0.11347	0.0872
Butyric acid	2.515	0.854	0.6535	0.0976	0.8541	1
Butanol	1.482	0	1.338	0	0.2767	0.3075
Ethanol	0.506	1.22	0.1045	0.1879	0	0.1504
Acetone	1.509	0	0.5471	0	0	0

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513 **Table 3.** Comparative hydrogen production yields at different temperatures using

514 different substrates and microorganisms.

Microorganism	T (°C)	Working volume (ml)	Culture type	Substrate	Maximum hydrogen yield (mol H_2/ mol substrate)	Reference
Thermotoga maritima	80	100	Batch	Glucose	4	[34]
Caldicellulosiruptor saccharolyticus	70	1000	Batch	Sucrose	3.3	[33]
Thermotoga elfii	65	1000	Batch	Glucose	3.3	[33]
Thermoanabacterium saccharolyticum YS485	55	8	Batch	Cellobiose	0.87	[32]
Clostridium acetobutylicum	37	850	Batch	Glucose	2.0	[26]
Escherichia coli MC13-14	37	20	Batch	Glucose	1.2	[28]
Clostridium butyricum CGS5	37	150	Batch	Xylose	0.73	[29]
Anaerobic sludge	37	3860	Continuous	Fructose	0.56	[30]
Clostridium sp.	35	2300	Continuous	Glucose	0.85	[27]
G088	20	110	Batch	Glucose	1.7	This study
G088	20	110	Batch	Fructose	1.3	This study
G088	20	110	Batch	Xylose	1.4	This study

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Figure Captions

Fig. 1. Batch culture of psychrophilic G088 strain closely related to *Polaromonas rhizosphaerae* using xylose as substrate. Hydrogen production (●) and xylose consumption (▲).

Fig. 2. Batch culture of psychrophilic G088 strain closely related to *Polaromonas rhizosphaerae* using glucose as substrate. Hydrogen production (●) and glucose consumption (▲).

Fig. 3. Batch culture of psychrophilic G088 strain closely related to *Polaromonas rhizosphaerae* using fructose as substrate. Hydrogen production (●) and fructose consumption (▲).

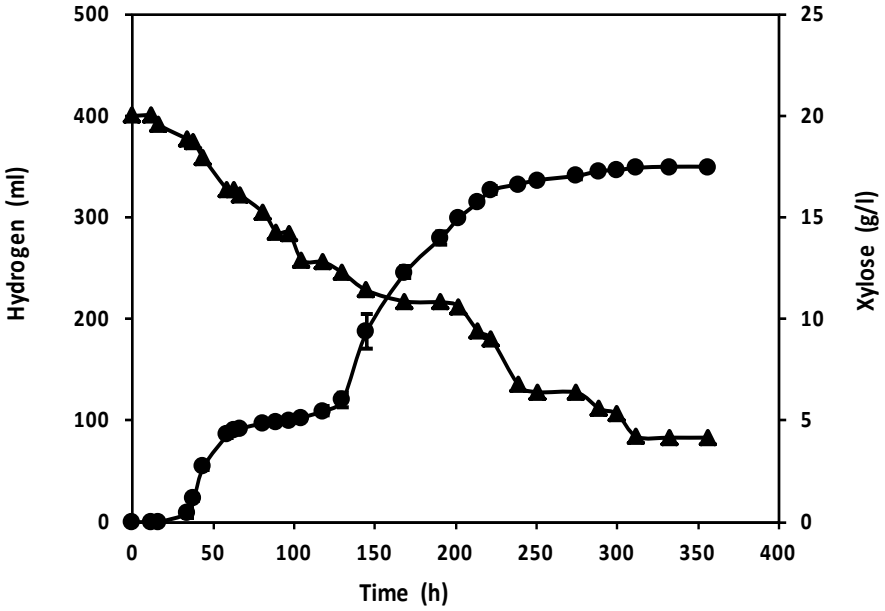
Hydrogen (ml)
Xylose (g/l)
Time (h)
0
100
200
300
400
500
0
5
10
15
20
25
0
50
100
150
200
250
300
350
400

Fig. 1

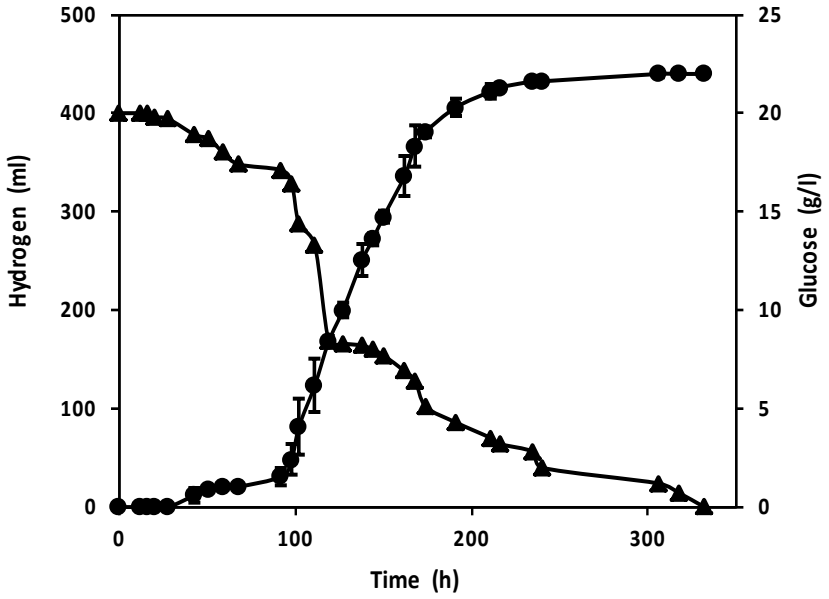
500
400
300
200
100
0
Hydrogen (ml)
25
20
15
10
5
0
Glucose (g/l)
0
100
200
300
Time (h)

Fig. 2

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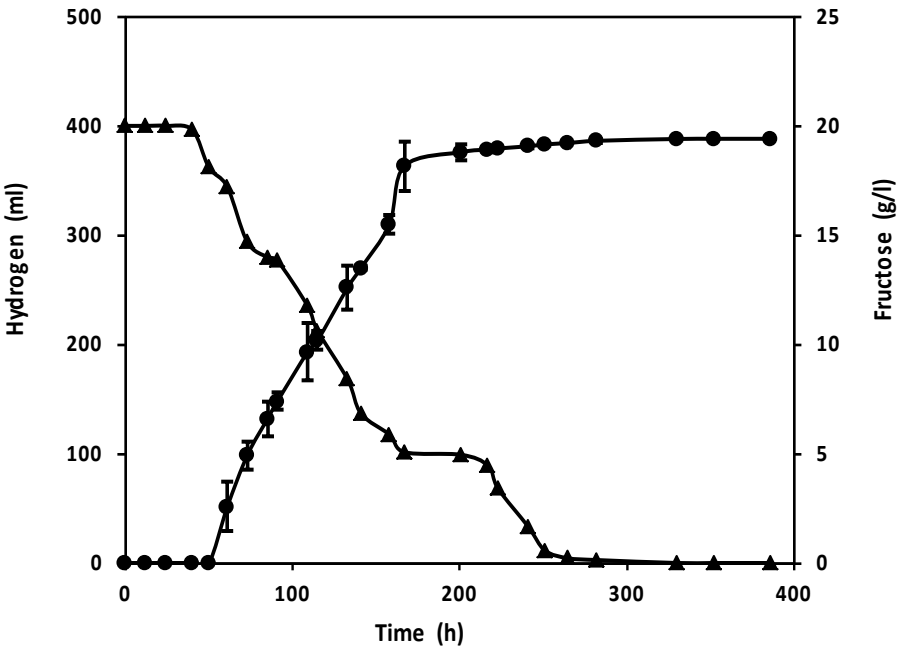

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548 Fig. 3.