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1 D-lactic acid production by *Sporolactobacillus inulinus* YBS1-5 with simultaneous
2 utilization of cottonseed meal and corncob residue

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12 Abstract

13 D-lactic acid, is an important organic acid produced from agro-industrial wastes by *S.*

14 *inulinus* YBS1-5 was investigated to reduce the raw material cost of fermentation.

15 The YBS1-5 strain could produce D-lactic acid by using cottonseed meal as the sole

16 nitrogen source. For efficient utilization, the cottonseed meal was enzymatically

17 hydrolyzed and simultaneously utilized during D-lactic acid fermentation. Corn cob

18 residues are rich in cellulose and can be enzymatically hydrolyzed without

19 pretreatment. The hydrolysate of this lignocellulosic waste could be utilized by strain

20 YBS1-5 as a carbon source for D-lactic acid production. Under optimal conditions, a

21 high D-lactic acid concentration (107.2 g/L) was obtained in 7-L fed-batch fermenter,

22 with an average productivity of 1.19 g/L/h and a yield of 0.85 g/g glucose. The optical

23 purity of D-lactic acid in the broth was 99.2%. This study presented a new approach

24 for low-cost production of D-lactic acid for an industrial application.

25 **Keywords** D-lactic acid; *Sporolactobacillus inulinus*; Cottonseed meal; Corn cob

26 residue

27 1. Introduction

28 D-lactic acid (D-LA) is an important and multifunctional organic acid that has
29 traditional applications in pharmaceutical, chemical and cosmetic industries. Presently,
30 the demand for D-LA is significantly increasing due to its wide application in
31 manufacturing poly (lactic acid) as a feedstock monomer (Fukushima et al., 2007). To
32 date, almost all D-LA produced globally is manufactured by fermentation using
33 refined sugars and yeast extract (YE) (Abdel-Rahamn et al., 2011). According to
34 Coelho et al. (2011)⁵, the cost of raw materials represents 68% of the total cost of
35 lactic acid production. Thus, development of an efficient and cost-effective process
36 for D-LA fermentation from cheap and non-food materials is highly desired.

37 Lignocellulosic waste represents a potentially inexpensive and renewable carbon
38 source for the large-scale fermentation of D-LA due to its abundance, low price, high
39 polysaccharide content, and renewability (Abdel-Rahamn et al., 2011). Until now,
40 some lignocellulosic wastes including corn stove (Zhang et al., 2013), curcuma longa
41 residue (Nguyen et al., 2013) and hardwood pulp (Hama et al., 2015) have been used
42 for D-LA production by *Lactobacillus* species. *Sporolactobacillus inulinus* is a
43 homo-fermentation organism and mainly converts glucose to D-LA. Besides, it
44 appears to have lower nutritional requirements compared to other lactic acid
45 producing bacteria (Wang et al., 2011). However, there are few studies on utilization
46 of lignocellulosic waste as carbon source by *S. inulinus* for D-LA production. In
47 China, nearly 20 million tons of corncobs are produced annually and the
48 hemicellulose fraction of corncob is generally extracted by dilute-acid treatment for

49 xylitol and furfural production, thereby approximately half million tons of corncob
50 residue (CCR) are generated as solid waste annually. On the other hand, after dilute
51 acid hydrolysis, CCR is rich in cellulose and its lignocellulose structure is readily
52 disrupted (Gu et al., 2014). Therefore, the cost intensive pretreatment is not necessary
53 for enzymatic hydrolysis. For these merits, CCR has been used for L-LA production.
54 Xie reported CCR hydrolysate was efficiently used by *B. coagulans* ZX25 for L-LA
55 production. The final L-LA concentration reached to 52 g/L within 54 h (Xie et al.,
56 2015). Shen used the immobilized cells of *L. delbrueckii* to produce 107.5 g/L L-LA
57 from CCR hydrolysate (Shen and Xia, 2006). To our best knowledge, no research has
58 been reported on D-LA fermentation via CCR as substrate.

59 Most of lactic acid-producing bacteria are known to be fastidious so the lactic
60 acid fermentation step requires not only sugars but also other nutrients including
61 amino acids, peptides and vitamins (Wang et al., 2011). To supply all these ingredients,
62 YE has been used, resulting in increased production costs (Nancib et al., 2005).
63 Alternatives to YE, based on agro-industrial waste, have been studied intensively,
64 such as soybean meal (Nguyen et al., 2013), cottonseed meal (Li et al., 2013) and
65 peanut meal (Wang et al., 2011). However, compared with the studies on L-lactic acid
66 production, information on D-LA fermentation from agro-industrial wastes is limited
67 (Liang et al., 2015). Therefore, an efficient method to increase D-LA yield and titer by
68 *S. inulinus* with simultaneous utilization of lignocellulosic and agro-industrial wastes
69 still needs to be developed.

70 In our previous study, the efficient mutant strain *S. inulinus* YBS1-5 produced

71 125.3 g/L D-LA from glucose and YE (Xu et al., 2010; Sun et al., 2015). This strain
72 was also found to be a robust microorganism to inhibitors in lignocellulose
73 hydrolysates (Bai et al., 2015). Therefore, the main objectives of this study were as
74 follows: 1) to screen out an inexpensive nitrogen source from agro-industrial wastes
75 and optimize its treatment mode for efficient D-LA production, 2) to evaluate the
76 feasibility of D-LA production by strain YBS1-5 using CCR as carbon source.

77 **2. Materials and Methods**

78 2.1. Chemicals

79 Cottonseed meal, soybean meal, peanut meal and corn wine dregs were purchased
80 from Tianyuan Food Processing CO., Ltd. (Xuzhou, China). Yeast extract was
81 purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). CCR was
82 kindly provided by Prof. Ouyang (Nanjing Forestry University, China). Commercially
83 available neutral protease (No RY03002) with an activity of 1×10^4 U/g according to
84 the manufacturer's data, was obtained from Jiangsu RuiYang BioTech co. Ltd
85 (Jiangsu, China). The cellulase was purchased from Imperial Jade Bio-technology Co.,
86 Ltd. (Ningxia, China). The filter paper activity and β -glucosidase activity of this
87 cellulase were 283 FPU/g and 525 CBU/g, respectively. All other chemicals used
88 were of analytical grade and commercially available.

89 2.2. Microorganism and the seed cultivation conditions

90 *Sporolactobacillus inulinus* YBS1-5 (CCTCC. M2012516) was obtained by
91 combining two mutation methods, low-energy ion implantation treatment and
92 atmospheric and room temperature plasma treatment (Xu et al., 2010; Sun et al.,

93 2015). The seed medium contained: yeast extract (2 g/L), peptone (2 g/L), glucose (20
94 g/L), corn steep liquor (5 ml/L), KH_2PO_4 (1 g/L), MgSO_4 (0.2 g/L), wheat bran (2
95 g/L) and CaCO_3 (14 g/L). The medium was anaerobically cultured for 16 h at 37 °C
96 at 150 rpm and then inoculated with 10% (v/v) into Erlenmeyer flasks or fermenter
97 for D-LA production.

98 2.3. Enzymatic saccharification for CCR hydrolysate preparation

99 The CCR was subjected to enzymatic saccharification by the commercial
100 cellulase with dosages of 15 FPU/g dry CCR. Enzymatic hydrolysis was carried out at
101 50 °C and pH 4.8 for 48 h with 10% (w/v) of CCR loading. The resultant hydrolysates
102 were isolated by centrifugation at 12000 g, and then the concentrations of total
103 reducing sugar, glucose, xylose, acetic acid furfural, 5-hydroxymethylfurfural (HMF),
104 and total phenolic content in the supernatant were determined. Finally, this
105 supernatant was concentrated to the desired sugar concentration by rotary evaporation
106 and was utilized as carbon source for D-LA fermentation.

107 2.4. Effect of different nitrogen sources on D-LA production

108 The effects of different nitrogen sources on D-LA fermentation were determined
109 in a medium containing 150 g/L glucose. The pH was maintained by addition of 90
110 g/L CaCO_3 . According to analysis of total nitrogen, the total nitrogen concentration
111 (w/w) in yeast extract, soybean meal, peanut meal, cottonseed meal and corn wine
112 dregs were 5.8%, 4.5%, 7.7%, 7.9% and 4.9%, respectively. Therefore, quantities of
113 above nitrogen sources were 5, 6.4, 3.8, 3.7 and 5.9 g/L, which correspond to a
114 nitrogen concentration of 0.3 g/L. Fermentations were carried out in the 250 ml shake

115 flask containing 120 ml fermentative medium at 37 °C and 150 rpm.

116 2.5. Effect of the concentrations of protease and cottonseed meal on D-LA production

117 In order to improve the utilization of cottonseed meal, the simultaneous
118 enzymatic hydrolysis and fermentation strategy was performed in D-LA fermentation.

119 To study the optimal protease concentration, different concentration of neutral
120 protease were added while the initial concentration of cottonseed meal was 3.7 g/L in
121 the medium in the shake flask.

122 The effect of cottonseed meal dosage on D-LA production was determined at
123 concentrations of 3-6 g/L in the medium supplemented with appropriate concentration
124 of neutral protease.

125 2.6. Effect of CCR hydrolysate concentration on D-LA production

126 The CCR hydrolysate supernatant was concentrated to a total reducing sugar
127 concentration of about 100 g/L and 150 g/L, respectively. The concentrations of
128 glucose, xylose and inhibitors in two concentrated supernatants were also determined.
129 Then, initial CCR hydrolysate supernatant and two concentrated supernatants were
130 utilized as carbon source for D-LA fermentation in the shake flask. In control medium,
131 the corresponding concentrations of glucose and xylose were added.

132 2.7. Fed-batch fermentation by *S. inulinus* YBS1-5 using CCR hydrolysate as
133 feedstock

134 Fed-batch fermentation was carried out in a 7-L bioreactor (Winpact, Major
135 Science, USA) containing 2 L fresh medium. The initial total reducing sugar
136 concentration in CCR hydrolysate was about 100 g/L. During the fermentation, when

137 the concentration of glucose decreased below 50 g/L, two liters of the concentrated
138 CCR hydrolysate with about 200 g/L of total reducing sugar was fed. The culture pH
139 was automatically maintained at about 5.5 by adding CaCO₃. The temperature was
140 controlled at 37 °C. Samples were collected periodically to determine the
141 concentrations of D-LA, glucose and xylose, and optical purity of D-LA.

142 2.8. Analytical methods

143 The components of CCR were determined according to the National Renewable
144 Energy laboratory (NREL, Golden, CO) analytical methods for biomass (Kristensen
145 et al., 2007). The total reducing sugar concentration was determined by the
146 dinitrosalicylic acid (DNS) method (Miller 1959). The total nitrogen concentration
147 was measured by Kjeldahl method (Kjeldahl 1883). According to our previous
148 methods, the concentrations of glucose, xylose, acetic acid, vanillin and
149 syringaldehyde in the hydrolysate and fermentation broth were analyzed by HPLC
150 with a refractive index detector using an Aminex HPX-87H column (300 mm × 7.8
151 mm) (Sun et al., 2015; Bai et al., 2015). The concentrations of furfural and HMF were
152 detected with a C-18 column (250 mm × 4.6 mm) and a UV detector set at 210 nm
153 (Bai et al., 2015). The concentration and optical purity of D-LA were measured with a
154 chiral column (150 mm × 4.6 mm, SUMICHIRAL OA-5000) at 254 nm (Sun et al.,
155 2015). The concentration of the total phenolic compounds (TPC) was estimated
156 colorimetrically by the Folin-Ciocalteu method (Bai et al., 2015).

157 3. Results and discussion

158 3.1. Effects of nitrogen source on D-lactic acid production

159 Because lactic acid production depends strictly on cell growth, the type of
160 nitrogen source has a significant impact on D-LA production (Sikder et al., 2012). In
161 order to replace YE with another inexpensive nutrient source, four agro-industrial
162 wastes were used to evaluate the effects of different nitrogen sources on D-LA
163 production by *S. inulinus* YBS1-5 (Fig. 1). The results indicated that the production of
164 D-LA was markedly influenced by the type of nitrogen source used and YE achieved
165 the highest D-LA production (125.3 g/L). The four agro-industrial wastes also were
166 utilized by strain YBS1-5. When cottonseed meal was used as a nitrogen source, the
167 D-LA concentration reached 77.4 g/L, which was comparatively higher than
168 concentrations obtained with soybean meal, peanut meal and corn wine dregs.
169 Cottonseed meal, which is regenerated as a waste after cotton treatment, is rich in
170 proteins and vitamins (Li et al., 2013). However, only few lactic acid bacteria possess
171 a complex proteolytic system to make full use of proteins in the agro-industrial waste.
172 Li (2013) reported that *S. laevolacticus* DSM442 was unable to efficiently metabolize
173 cottonseed meal directly, and only 20 g/L D-LA accumulated during the fermentation
174 process. Lu et al. (2009) also found that soybean meal could not be significantly used
175 by *L. delbrueckii* HG106 for D-LA fermentation. In a few studies, crude wastes were
176 hydrolyzed by the protease to improve the utilization efficiency (Wang et al., 2011; Li
177 et al., 2013). Therefore, in the further research, we hydrolyzed the cottonseed meal
178 with a neutral protease for D-LA fermentation.

179 3.2. Effect of the concentrations of protease and cottonseed meal on D-LA production

180 Conventionally, protein hydrolysis and fermentation are separated, which not

181 only increases the additional treatment steps, but also might destroy or remove the
182 nutritional components (Wang et al., 2011). In order to overcome these deficiencies,
183 simultaneous enzymatic hydrolysis and fermentation strategy was carried out. To
184 determine the optimum amount of protease required, different concentrations of
185 neutral protease were added. As shown in Fig. 2a, the concentration of D-LA
186 dramatically increased in proportion with the dose of neutral protease. Maximum
187 D-LA production (120.3 g/L) was obtained with the neutral protease concentration of
188 810 U/g cottonseed meal, which was 57.3% higher than that without protease
189 supplementation. Further protease addition resulted in a decrease in D-LA production,
190 which was consistent with many previous reports (Wang et al., 2011; Li et al., 2013).
191 The yield also presented a similar trend with the maximum value of 0.88 g/g glucose
192 when protease was added at a concentration of 810 U/g cottonseed meal.

193 The optimum concentration of cottonseed meal required for D-LA production
194 was determined (Fig. 2b). Initially, the D-LA concentration increased with the amount
195 of cottonseed meal added, and then decreased when cottonseed meal concentration
196 was higher than 3.7 g/L. Maximum D-LA production reached to 121.3 g/L, which was
197 close to that obtained with YE. The results indicated that *S. inulinus* YBS1-5 could
198 utilize inexpensive and abundant agricultural wastes to produce D-LA. Furthermore,
199 the simultaneous hydrolysis and fermentation strategy used in this study would
200 simplify the operation process and reduced damage of the nutrients, thereby proves to
201 be cost-effective.

202 In comparison to L-LA production, only a small number of studies have been

203 performed to produce D-LA from agro-industrial wastes. Li et al. (2013) also used
204 cottonseed meal as a nitrogen source for D-LA production with the simultaneous
205 hydrolysis and fermentation strategy. The optimum concentrations of cottonseed meal
206 and protease were 40 g/L and 10000 U/L. In a study by Wang et al. (2011), a D-LA
207 concentration of 149 g/L was obtained using *Sporolactobacillus* sp. strain CASD in a
208 medium that contained 40 g/L of peanut meal and 15000 U/ml of protease. In this
209 study, the optimum concentrations of cottonseed meal and protease for D-LA
210 production were 3.7 g/L and 3000 U/L, respectively. To the author's knowledge,
211 compared with previous reports, *S. inulinus* YBS1-5 exhibited the lowest nutrient
212 requirement to produce D-LA.

213 3.3. Effect of CCR hydrolysate concentration on D-LA production

214 The major composition of CCR was cellulose (61.1%), lignin (30.3%), and
215 hemicellulose (3.5%). After enzymatic hydrolysis at 10% substrate loading, the total
216 reducing sugar concentration in the CCR hydrolysate was 67.7 g/L which included
217 62.5 g/L glucose and 3.3 g/L xylose. The concentrations of total reducing sugar,
218 glucose and xylose in two concentrated hydrolysates were also determined and shown
219 in Table 1. The effects of these three CCR hydrolysates on D-LA production were
220 analyzed. As shown in Fig 3a, with unconcentrated CCR hydrolysate as carbon source,
221 the D-LA concentration and productivity reached to 51.9 g/L and 1.41 g/L/h, which
222 were similar to those of control medium. As increasing the total sugar concentration
223 of CCR hydrolysate to 98.3 g/L, the concentration of D-LA reached to 74.3 g/L, but
224 the productivity decreased slightly to 1.31 g/L/h. When CCR hydrolysate was

225 concentrated to total sugar of 146 g/L, the concentration of D-LA increased to 102 g/L.
226 However, strain YBS1-5 had a long lag phase and productivity was only 0.92 g/L/h of
227 D-LA, which was only 64.7% of the productivity from the corresponding control
228 medium.

229 Many reports had indicated that sugar osmotic pressure could influence cell
230 growth and metabolites production (Hama et al., 2015; Ouyang et al., 2013). To
231 investigate the reason of the long fermentation time, refined sugars, glucose and
232 xylose with the corresponding concentrations to CCR hydrolysates, were used for
233 D-LA production (Fig. 3b). The results indicated there was no significant inhibition
234 under the tested total sugar concentrations. Therefore, it is suggested that high total
235 sugar concentration in CCR hydrolysate was not the reason for the decrease of D-LA
236 productivity. Further, the effect of lignocellulosic inhibitors on D-LA fermentation
237 was investigated. In previous work, *S. inulinus* YBS1-5 had high tolerance to acetate
238 and furan derivatives (Bai et al., 2015). However, phenolic compounds acted as major
239 inhibitors for D-LA production (Bai et al., 2015). In this study, the typical inhibitors in
240 the CCR hydrolysate were measured. The concentrations of furfural and HMF were
241 below 0.05 g/L (Table 1). The highest concentration of acetic acid was at around 0.1
242 g/L. These inhibitor levels were not high enough to give obvious inhibition on D-LA
243 fermentation (Bai et al., 2015). On the other hand, phenolic compounds usually have
244 low solubility in water and could possibly precipitate on CCR even after multiple
245 washing steps. Gu et al. (2014) reported that a very high total phenolic content (TPC)
246 of 5.59 g/L in the whole slurry of CCR hydrolysate and strain *S. cerevisiae* DQ1

247 exhibited the poor fermentability during the SSF. The results showed that the TPC
248 content increased from 0.43 g/L to 0.95 g/L when the liquid CCR hydrolysate was
249 concentrated. The D-LA productivity decreased with TPC levels, which indicated that
250 phenolic compounds were responsible for the low fermentation rate of *S. inulinus*.
251 However, in concentrated CCR hydrolysate with the highest TPC concentration, strain
252 YBS1-5 produced 105.3 g/L of D-LA, which was equivalent to 90.5% of that in the
253 corresponding control experiment. These results suggested that although phenolic
254 compounds in the hydrolysate reduced fermentation productivity, they did not
255 obviously induce the decrease in D-LA production.

256 Effect of four agro-industrial wastes on productivity and concentration of D-LA
257 using unconcentrated CCR hydrolysate as carbon source was also performed. As
258 shown in Fig. S1 (see supplementary material), cottonseed meal still resulted in more
259 D-lactic acid yield and productivity in comparison with the other nitrogen sources.

260 3.4. Fed-batch fermentation by *S. inulinus* YBS1-5 from CCR hydrolysate

261 Pulse fed-batch strategy was used to improve the D-LA productivity of *S. inulinus*
262 YBS1-5 from CCR hydrolysate. As shown in Fig 4, the fermentation process was
263 completed within 90 h, and the final D-LA concentration reached 107 g/L, at this
264 point, the glucose was completely exhausted. The average productivity, yield, and
265 optical purity were 1.19 g/L/h, 0.85 g/g glucose and 99.2%, respectively. During
266 fermentation, xylose accumulated and remained unused. According to the calculation,
267 a total of 586 g total sugars including 553 g of glucose were added in whole
268 fermentation process. As the final fermentation volume was 4 L, the average

269 concentrations of total sugar and glucose were 146 g/L and 138 g/L. Compared to the
270 results of batch fermentation with an initial total sugar concentration of 146 g/L in
271 concentrated CCR hydrolysate, the fermentation time of the fed-batch fermentation
272 was shortened by 22 h and the productivity was increased by 29.3%.

273 The economics of D-LA production depends on many factors. Of which, the cost
274 of raw materials is very significant (Abdel-Rahman et al., 2013). The most used
275 microorganisms for D-LA production from cheap and renewable wastes were
276 *Lactobacillus* strains, and the D-LA concentration obtained was usually below 100
277 g/L (Table 2). Although *Sporolactobacillus* species mainly produce D-LA, there are
278 only few studies on their potential ability to produce D-LA from inexpensive and
279 renewable materials. Recently, Wang (2011) reported that peanut meal was a better
280 nutrient compared to YE for D-LA production by *Sporolactobacillus* sp. CASD. The
281 final D-LA concentration reached 207 g/L within 54 h. The *S. laevolacticus* DSM442
282 could produce D-LA at a concentration of 144 g/L by using cottonseed meal as the
283 sole nitrogen source (Li et al., 2013). Although a high concentration of D-LA was
284 obtained in these two studies, the carbon source used was glucose. The present study
285 demonstrated that *S. inulinus* YBS1-5 could efficiently utilize CCR hydrolysates and
286 cottonseed meal as the substrates to produce D-LA. Since glucose is always costly
287 substrate compared to hydrolyzed CCR and cellulase, a cost estimation of nitrogen
288 source in this study was carried out. The prices of the materials and enzymes used
289 were obtained from suppliers, and the amount of materials and enzymes consumed
290 were calculated from the fermentation parameters. As Table 3 showed, the total costs

291 of cottonseed meal and neutral protease for D-LA production were \$ 0.02 per kg
292 D-LA. Compared with the conventional process using YE, the cost is reduced by 95%.
293 Also, our studies were favorable to the reuse of waste resources.

294 **4. Conclusion**

295 Efficient production of D-LA from cheap and renewable wastes by *S. inulinus*
296 YBS1-5 was performed in this study. The CCR hydrolysate and cottonseed were used
297 as carbon source and nitrogen source, which potential reduced the cost of the medium.
298 After optimization of the fermentation conditions, an economical and environmentally
299 friendly strategy for polymer-grade D-LA production was established.

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404 **Figure Legends**

405 Fig. 1. Comparison of the various nitrogen sources used for D-LA production by *S.*

406 *inulinus* YBS1-5 in a shake flask. The concentration of glucose was 150 g/L. Error

407 bars represent standard deviation of three replicated experiments.

408 Fig. 2. Effects of neutral protease concentration (A) and cottonseed meal

409 concentration (B) on D-LA production by strain YBS1-5 in a shake flask. The

410 concentration of glucose was 150 g/L. Symbols: D-LA concentration (column); yield

411 (■). Error bars represent standard deviation of three replicated experiments.

412 Fig. 3. Effects of CCR hydrolysate concentration (A) and total reducing sugar

413 concentration in control (B) on D-LA production by strain YBS1-5 in a shake flask.

414 The concentrations of cottonseed meal and neutral protease were 3.7 g/L and 800 U/g

415 cottonseed meal. Symbols: D-LA concentration (column); yield (■). Data shown are

416 means of three replicated experiments \pm standard error.

417 Fig. 4. The fed-batch fermentation for D-LA production by *S. inulinus* YBS1-5 from

418 concentrated CCR hydrolysate in a 7-L bioreactor. Symbols: glucose concentration

419 (■), D-LA production (▲), xylose concentration (●). Data shown are means of three

420 replicated experiments \pm standard error.

Table 1 Concentrations of sugars and inhibitors in unconcentrated CCR hydrolysate and two concentrated CCR hydrolysates

Concentrations	Unconcentrated CCR hydrolysate	Concentrated CCR hydrolysate 1	Concentrated hydrolysate 2
Total reducing sugar (g/L)	67.7	98.3	145.9
Glucose (g/L)	62.5	92.2	138.4
Xylose (g/L)	3.3	5.1	7.5
Furans (g/L)	ND	ND	furfural 0.03 g/L HMF 0.05 g/L
Acetic acid (g/L)	ND	0.05	0.1
TPC (g/L)	0.43	0.64	0.95

ND=not detected

Table 2 Comparison of D-LA production from agricultural residues by different organisms

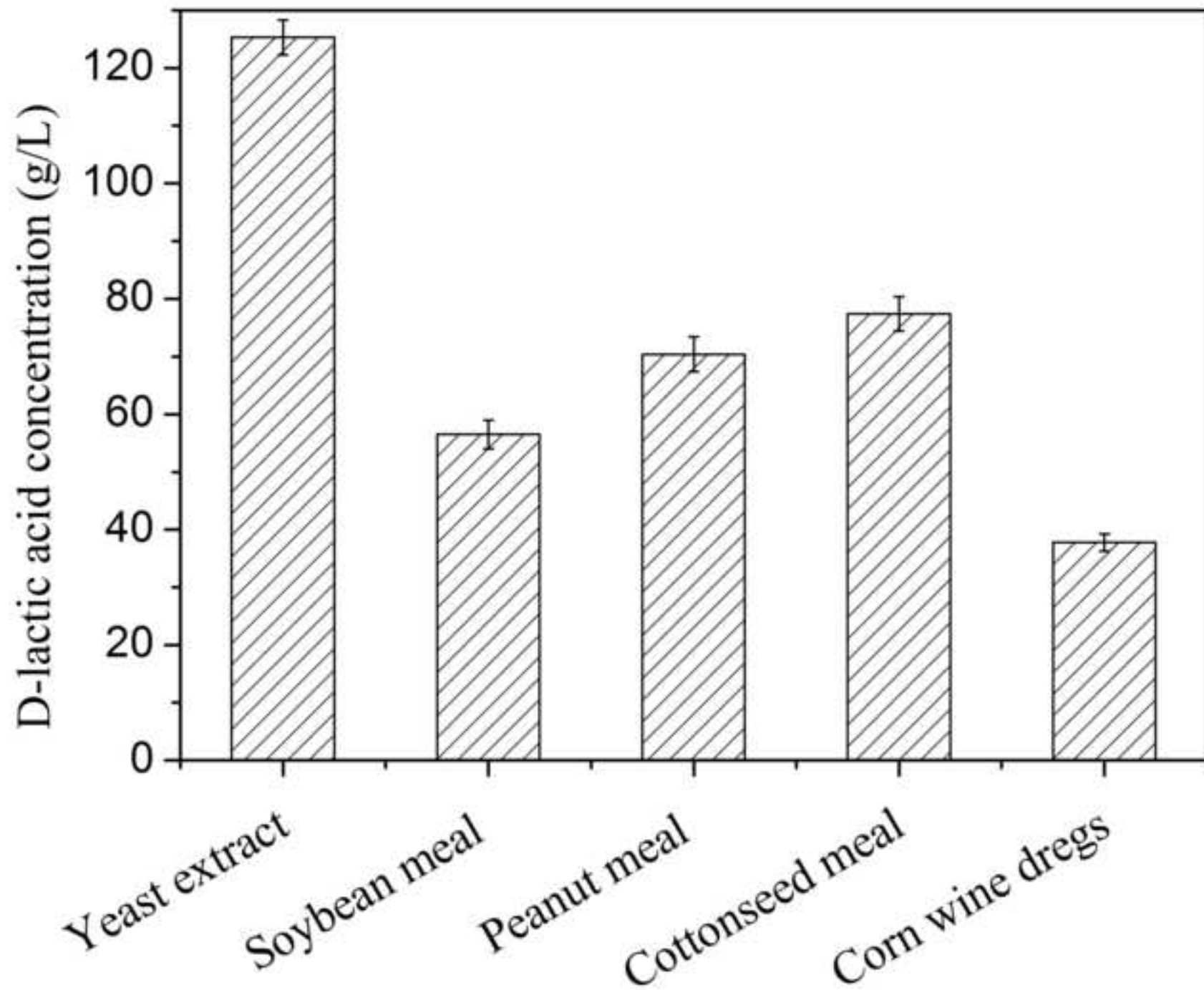
Microorganisms	Substrates	D-LA concentration (g/L)	Yield (g/g glucose)	Time (h)	Reference
<i>L. coryniformis</i> ATCC25600	Pretreated cardboard; YE	23.4	0.56	48	Yanez et al., 2005
<i>L. delbrueckii</i> IFO 3202	Rice bran; YE	28	0.8	36	Tanaka et al., 2006
<i>L. delbrueckii</i> LD2008	Rice saccharificate; YE	62.6	0.70	96	Lee, 2007
<i>L. delbrueckii</i> HG106	Rice saccharificate; Wheat bran + YE	90.8	0.91	60	Lu et al., 2009
<i>L. lactis</i> RM2-2*4	Cellulose; YE	73	0.73	48	Singhvi et al., 2010
<i>L. mesenteroides</i> B512	Sugarcane juice; Yeast autolysate	60.2	0.51	48	Coelho et al., 2011
<i>Sporolactobacillus</i> sp. CASD	Glucose; Peanut meal	207	0.93	60	Wang et al., 2011
<i>L. coryniformis</i> ATCC 25600	Hydrodictyon reticulatum; YE	36.7	0.46	48	Nguyen et al., 2012
<i>L. coryniformis</i> ATCC 25600	Waste curcuma longa; Soybean meal	91.6	0.65	60	Nguyen et

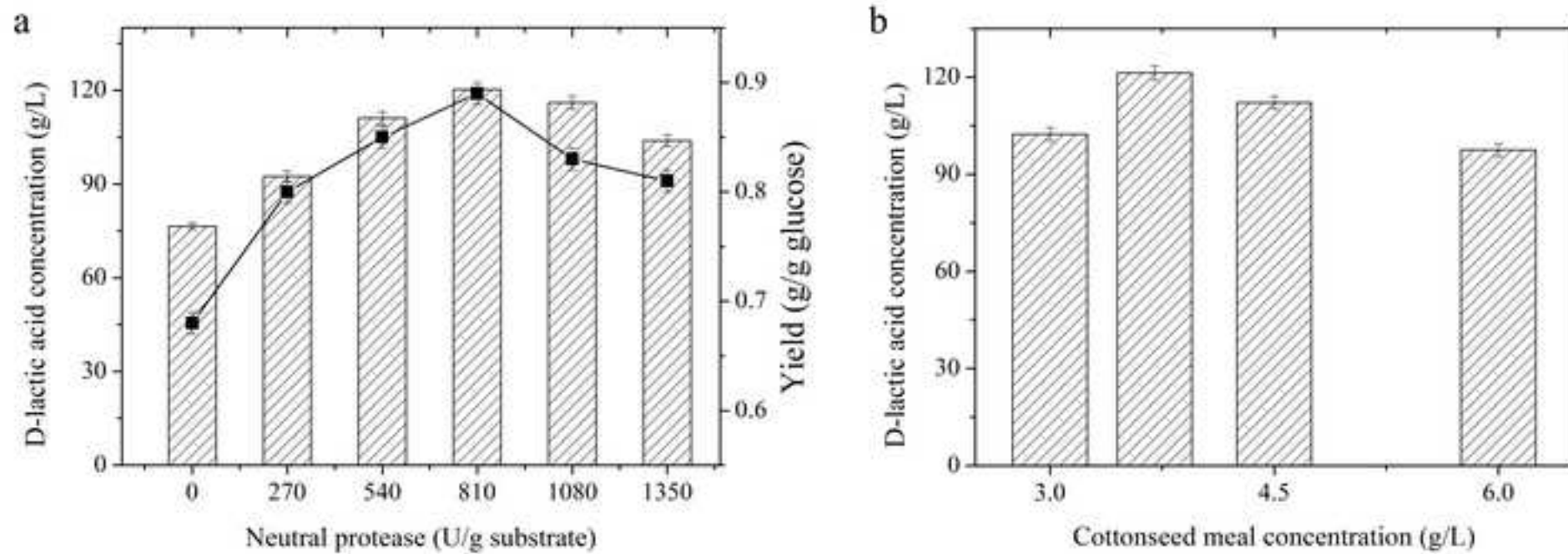
					al., 2013
<i>L. lactis</i> RM2-2*4	Hydrolyzed cane sugar; YE+Peptone	84.7	0.93	30	Singhvi et al., 2013
<i>S. laevolacticus</i> DSM 442	Glucose; Cottonseed meal	144.4	0.96	35	Li et al., 2013
<i>L. delbrueckii</i> ATCC 9649	Corn stove; YE	20.1	0.58	72	Zhang et al., 2013
<i>L. lactis</i>	Casein whey permeate; Casein hydrolysate	24.3	0.49	47	Prasad et al., 2014
<i>Lactobacillus plantarum</i>	Hardwood pulp; Barely extract	102.3	0.88	144	Hama et al., 2015
<i>S. inulinus</i> YBS1-5	Corn cob hydrolysates; Cottonseed meal	107.2	0.85	90	This study

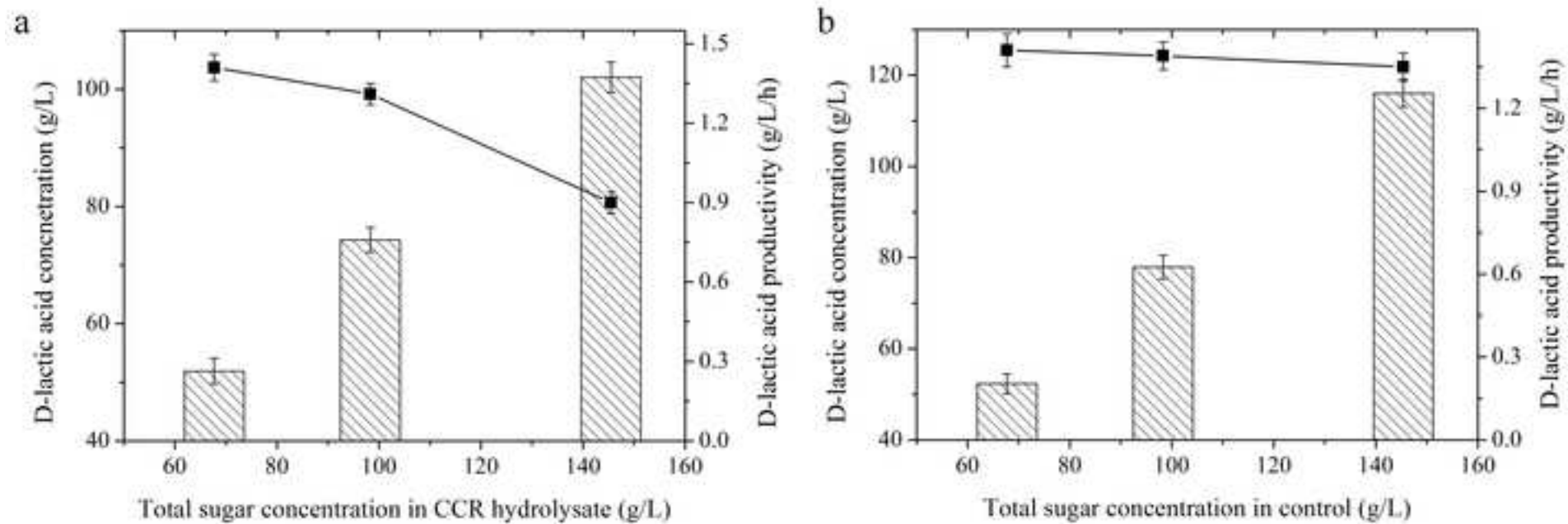
Table 3 Estimated nitrogen source cost for production of D-lactic acid by *S. inulinus* YBS1-5

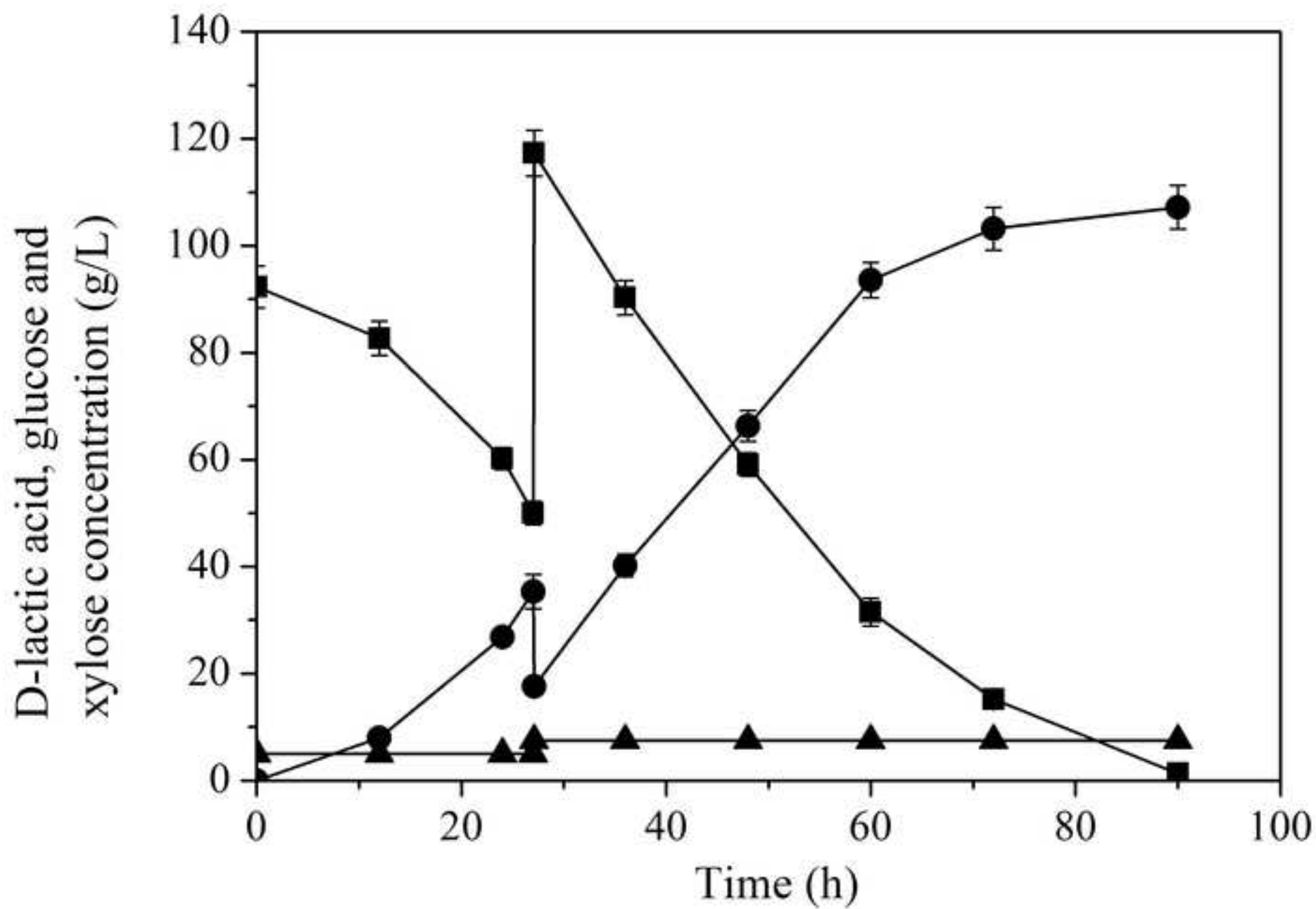
Group	Material	Price (US \$/kg)	Used in the fermentation (kg/kg D-LA)	Unite price (US \$/kg D-LA)
1	YE	11.25	0.04	0.45
2	Cottonseed meal	0.38	0.03	0.01
	Neutral protease	48.9	2.8×10^{-4}	0.01
Total price				0.02

The exchange rate of US dollar to Chinese Yuan (RMB) is set to approximately 6.4 according to the average exchange rate in 2015.









Highlights

S. inulinus YBS1-5 had low nutrient requirement for D-lactic acid production

Efficient D-lactic acid production from cheap cottonseed meal was demonstrated

Corn cob residue was suitable for D-lactic acid fermentation by strain YBS1-5

A combined use produced 107.2 g/L D-lactic acid with a yield of 0.85 g/g

