Journal of Renewable Materials
DOI:10.32604/jrm.2020.08761
Research Article

Tech Science Press

Mannonic Acid and Bio-Ethanol Production from Konjac Using a Two-Step Bioprocess with *Candida Shehatae* and *Gluconobacter Oxydans*

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Received: 5 October 2019; Accepted: 25 October 2019

Abstract: *Amorphophallus konjac* is rich in glucomannan, which can be hydrolyzed into glucose and mannose, thereby acting as an economic raw material for the acquisition of glucose and mannose. The total sugar yield was 91.2% when konjac powder was treated with 0.75% hydrochloric acid at 121°C for 1 h. Thus, dilute acid hydrolysates of konjac powder were used as a carbon source for obtaining value-added products. Here we showed that the microbial production of ethanol and mannonic acid was obtained by employing *Candida shehatae* (*C. shehatae*) and *Gluconobacter oxydans* (*G. oxydans*). Through a step-by-step bioprocess, glucose is the first selectively converted to ethanol by *C. shehatae*, which enables *G. oxydans*-mediated biocatalysis of mannose to mannonic acid. Finally, approximately 100 g ethanol and 340 g mannonic acid were produced starting from 1 kg refined konjac powder. The results demonstrated the feasibility of this bioconversion method for producing mannonic acid starting from crude hydrolysates of konjac powder.

Keywords: Konjac; mannonic acid; ethanol; *Gluconobacter oxydans*; *Candida shehatae*

1 Introduction

Meeting the growing demand for energy required by various industrial processes and providing sustainable sources of raw materials for chemical industries are major challenges of the twenty-first century [1, 2]. Currently, sugar acids emerged as an ideal option to solve these requirements in a sustainable manner and they attracted considerable attention because of their potential applications in food, chemicals, cosmetics, and cement additive manufacture [3, 4]. In addition, they play a crucial part in the production of high-valued chemicals, including biofuels and biopolymers, through physical chemistry or metabolic engineering technologies [5]. The most well-known sugar acid is gluconic acid, with an annual output of approximately 87,000 metric tons worldwide [6]. Mannonic acid, another sugar acid, is like gluconic acid, and can be used in a wide variety of applications, including as a chemical, chelator, dispersant, and retardant for oil wells [7].

The lignocellulosic biorefinery, equipped with a thriving selection of techniques for converting lignocellulosic materials into valuable fuels, bio-chemicals, and advanced materials, is gradually industrialized [8-10]. Currently, sugar substrates are subjected to the recent advancements in biomass

hydrolysis to obtain glucose, xylose, arabinose, galactose, or mannose [11, 12]. So far, acid hydrolysis has been used to release various sugars into the hydrolysates. *Amorphophallus konjac* is common and abundant in Asia, and it usually contains 49-60% glucomannan fiber, 10-30% starch, 2-5% insoluble fiber, 5-14% crude protein, and 3-5% ash [13, 14]. The konjac glucomannan represents the main component and is composed of β-1,4-linked D-glucose and D-mannose units at a ratio of approximately 1:1.6 [15]. Thus, it can act as an economic raw material to obtain mannose.

The commercial success of producing gluconic acid/gluconate using emerging technologies in microbial catalysis can be applied to the production of other sugar acids, such as galactonic acid and xylonic acid [16-18]. In addition, the methods used for high-level production of gluconic acid and xylonic acid may provide a guideline for mannonic acid production [2, 19, 20]. Reports on microbial production of mannonic acid are few. Therefore, the objective of this study is to explore a cost-effective strategy to bio-produce mannonic acid using low-cost konjac as a starting feedstock. *Gluconobacter oxydans* (*G. oxydans*), a Gram-negative and obligate aerobic bacterium, is well-known for its rapid and incomplete oxidation of sugars and sugar-alcohols [21, 22]. *G. oxydans* is widely used in industrial processes, such as in the production of 1,3-dihydroxyacetone and sorbose [23]. Hence, *G. oxydans* is used for incomplete bioconversion of mannose to mannonic acid. *G. oxydans* usually catalyzes the simultaneous conversion of glucose to the corresponding carboxylic and ketonic acids. Separation and purification of these mixed products is difficult because of their highly similar characteristics. Thus, to acquire mannonic acid from konjac, glucose was selectively removed via yeast fermentation. Although both mannose and glucose are metabolized, glucose is used as first, thus repressing mannose utilization, a phenomenon called carbon catabolite repression (also known as glucose repression) [24]. The ethanol produced by glucose fermentation can be easily removed. Hence, the objective of this study was to develop a two-step bioprocess to produce mannonic acid from konjac as raw material. The basic features of this process may provide a guideline for an efficient and comprehensive utilization of konjac.

2 Experimental

2.1 Microorganisms

G. oxydans NL71, originating from the strain ATCC 621, was routinely maintained on sorbitol-agar plates (sorbitol 50 g/L, yeast extract 5 g/L, agar 15 g/L) at 4°C [25].

Candida shehatae NL33 (*C. shehatae*), provided by the Biochemical Engineering Research Institute of Nanjing Forestry University, was routinely maintained on glucose-agar plates (glucose, 10 g/L; peptone, 5 g/L; yeast extract, 3 g/L and agar 10 g/L) at 4°C.

2.2 Fermentation by G. oxydans

The inoculum of *G. oxydans* NL71 was prepared in a 250 mL Erlenmeyer shaker flask containing 50 mL medium (sorbitol 50 g/L, yeast extract 5 g/L), and cultured for 24-36 h at 200 rpm and 30°C. The inoculum culture, with an approximate cell density of 4.0 g/L, was then subjected to centrifugation (6000 rpm, 6 min) to collect *G. oxydans* cell pellet. The culture medium for mannose fermentation consisted of 2.0 g/L *G. oxydans* cells, 2.5 g/L yeast extract, 0.1 g/L $MgSO_4$, 2.0 g/L K_2HPO_4, and 1.0 g/L $(NH_4)_2SO_4$, and the pH was controlled by the addition of $CaCO_3$ powder [26]. Fermentation was performed in 250 mL Erlenmeyer shaker flasks containing 50 mL simulation medium or hydrolysate and cultured at 200 rpm and 30°C. The carbon source content in the simulation medium was represented by 30 g/L glucose and 50 g/L mannose. The fermentation medium used for verifying the effect of ethanol contained 50 g/L mannose.

2.3 Hexose Fermentation by C. Shehatae

The inoculum of *C. shehatae* NL33 was prepared in a 250 mL Erlenmeyer shaker flask containing 50 mL medium (20 g/L glucose; 20 g/L xylose; 20 g/L peptone; 10 g/L yeast extract) and cultured for

24 h at 170 rpm and 30°C. The inoculum culture, with an approximate cell density of 10 g/L, was then subjected to centrifugation (3000 rpm, 10 min) to collect *C. shehatae* cell pellet. Fermentation using simulation medium or hydrolysates was performed at 30°C in 250 mL Erlenmeyer flasks with 50 mL medium containing 1-8 g/L *C. shehatae* cells, 0.1 g/L $MgSO_4$, 0.1 g/L $ZnCl_2$, 0.2 g/L $CaCl_2$, 2.5 g/L yeast extract, and 1.0 g/L $(NH_4)_2SO_4$. The solution was incubated on a rotary shaker at 170 rpm. The carbon source content in the simulation medium was represented by 30 g/L glucose and 50 g/L mannose.

2.4 Sequential Fermentation

The sequential fermentation medium contained 0.1 g/L $MgSO_4$, 0.1 g/L $ZnCl_2$, 0.2 g/L $CaCl_2$, 2.5 g/L yeast extract, 2.0 g/L K_2HPO_4 and 1.0 g/L $(NH_4)_2SO_4$, and the pH was adjusted by adding $CaCO_3$ powder. The density of the inoculum was 2 g/L *C. shehatae* NL33 and 2 g/L *G. oxydans* NL71, and they were sequentially inoculated in a 250 mL Erlenmeyer shaker flask containing 50 mL medium. Ethanol fermentation was performed as first at 170 rpm and 30°C; when all glucose was consumed, the *C. shehatae* cells were removed by centrifugation prior to mannose fermentation and the ethanol was removed by distillation in a BÜCHI rotary evaporator (R-200, BÜCHI Shanghai Trading LLC, Shanghai, China) at 70°C and 1.60×10^4 Pa. Next, mannose fermentation was performed at 200 rpm and 30°C.

2.5 Preparation of Hydrolysates

Konjac powder was supplied by Weixin Biotechnology Corporation (China, Henan). The mass concentration of hydrochloric acid in liquid solution was 0.75% (w/v) and the konjac powder/solution mass ratio was 1/10. The reaction was performed in a 500 mL high-pressure agitated reactor (TGYF-C, Qiuzuo Corporation, Shanghai, China) at 121°C and 60 rpm for 60 min. After acid hydrolysis, the solid and liquid fractions were separated after the reaction. The liquid fraction was named acid hydrolysates. The pH was adjusted to 6.5 with $Ca(OH)_2$ powder and the suspension was filtered through a glass fiber filter.

2.6 Analytical Methods

Ethanol concentration was estimated using high performance liquid chromatography (HPLC) (Agilent 1260) equipped with an Aminex Bio-Rad HPX-87H column with 5 mmol/L H_2SO_4 at 0.6 mL/min as the mobile phase. Mannose, glucose, gluconic acid, mannonic acid, and 2-ketogluconic acid (2-KGA) were analyzed on high performance anion-exchange chromatography (Thermo ICS-5000) linked to a CarboPacTM PA 200 column with 25 mmol/L sodium hydroxide as eluents and a flow rate of 0.25 mL/min [27]. The yield of mannonic acid was calculated from the end production of mannonic acid divided by the initial mannose concentration, and multiplied by the constant of 0.918, which is the conversion factor for mannose to equivalent mannonic acid based on the stoichiometric balance. Three parallel assays were performed for each sample.

3 Results and Discussion

3.1 Hydrolysis of Konjac Powder and Composition Analysis

The chemical composition of the original and untreated konjac powder was determined using the method of the National Renewable Energy Laboratory [28]. The total polysaccharide content (glucomannan) was confirmed as high (94.1%). Furthermore, the ratio of mannose to glucose was approximately 1.7:1. This lignocellulosic resource is an attractive material for acquiring mannose because of its high carbohydrate content. The remaining components (approximately 6%) of the original untreated konjac powder, which include proteins, fats, and lignins, were not quantified. The treatment of konjac powder with 0.75% (w/v) hydrochloric acid (using a konjac powder/acid solution mass ratio of 1/10) was performed at 121°C for 60 min. After acid hydroysis, the total sugar (mannose and glucose) yield was 91.2%. The results indicated that H^+ was able to depolymerize glucomannan. Anyway, glucose and mannose concentrations in the acid hydrolysates were 34.2 g/L and 51.6 g/L, respectively. In conclusion,

342 g glucose and 516 g mannose could be obtained from 1 kg konjac powder after acid pretreatment. This indicated that the acid hydrolysates of konjac powder could provide an appropriate feedstock for acquiring mannose, which could be subsequently used for mannonic acid production.

3.2 Simultaneous Metabolism of Mannose and Glucose by G. oxydans

G. oxydans incompletely oxidizes various sugars to the corresponding sugar acids. Thus, simultaneous metabolism of glucose and mannose using *G. oxydans* was investigated in simulation medium (their concentration was like the one in the hydrolysates, which contained 50 g/L mannose and 30 g/L glucose). As shown in Fig. 1, *G. oxydans* was able to oxidize glucose and mannose to the corresponding sugar acids or keto-sugar acids. The curves of the fermentation process indicated that the presence of glucose repressed mannose conversion because glucose was utilized as first, followed by mannose. Glucose was first transformed to gluconic acid with a yield of 91.1% in 12 h; thereafter, the levels of mannose and gluconic acid started to considerably decrease, whereas mannonic acid and 2-KGA were simultaneously generated. In other words, mannose and gluconic acid were simultaneously oxidized to produce reducing hydrogen when glucose was consumed. After 48 h of fermentation, 18.1 g/L 2-KGA and 39.2 g/L mannonic acid were produced from the oxidation of gluconic acid and mannose, respectively. Therefore, co-production of gluconic acid and mannonic acid was difficult. Furthermore, gluconic acid was converted to other byproducts. Thus, in a mixed medium, the desired fermentation result was difficult to achieve, and separation/purification of the products was challenging because of the complicated nature of the byproducts. To reduce the byproduct formation and improve the purity of mannonic acid from konjac hydrolysates, the introduction of *C. shehatae* into the system was considered for selectively fermenting glucose to ethanol, which is easy to remove.

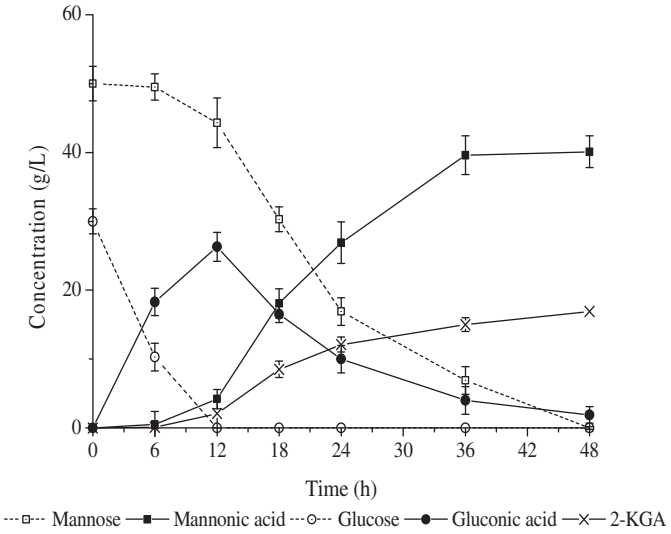

Figure 1: Reaction profile of mannose and glucose simultaneous fermentation by *G. oxydans*

3.3 Hexose Fermentation by C. shehatae

The profile of simultaneous mannose and glucose fermentation by different cell counts of *C. shehatae* is shown in Fig. 2 (1 g/L, 2 g/L, 4 g/L, and 8 g/L *C. shehatae* were separately loaded into the simulation medium containing 50 g/L mannose and 30 g/L glucose). The results showed that *C. shehatae* efficiently fermented glucose and mannose. The fermentation curves revealed that glucose and mannose metabolism

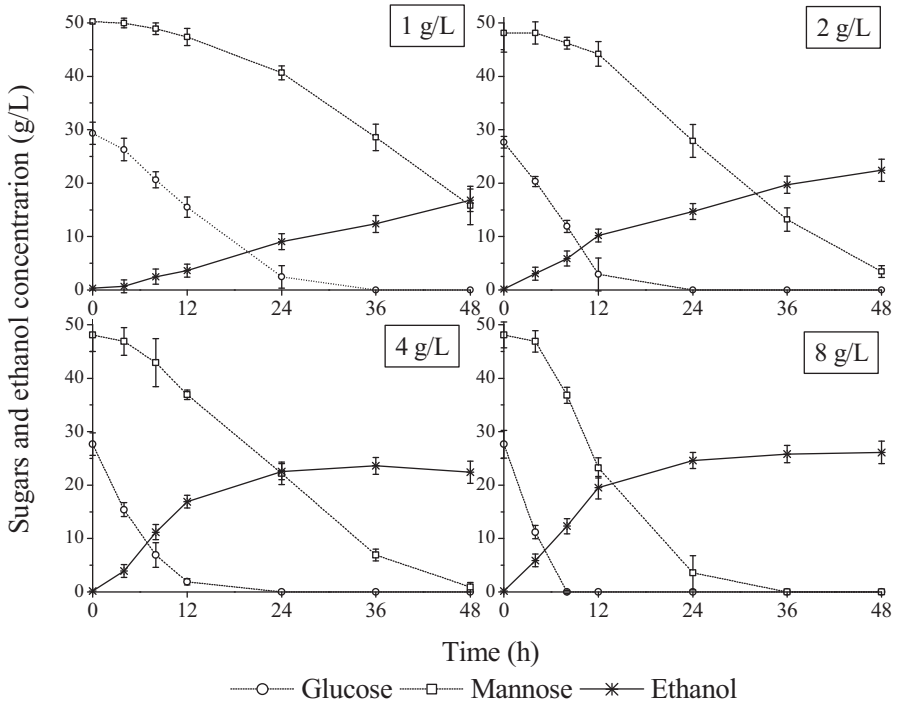

Figure 2: Mannose and glucose fermentation by *C. shehatae* at different cell concentration

followed a diauxic pattern, in which glucose was metabolized as first, followed by mannose. This phenomenon is related to glucose repression [29].

In other words, when *C. shehatae* is grown in a medium containing a mixture of glucose and other fermentable sugars, the presence of glucose represses the metabolism of other carbon sources [24]. Although all sugars were used up at the end of the fermentation process, mannose utilization started when most of the glucose was consumed. Glucose was completely and rapidly consumed with higher *C. shehatae* cell concentration, but the loss rate of mannose also increased. We inferred that the low rate of mannose utilization was inevitable even if glucose was not completely consumed. In the case of 2 g/L *C. shehatae* cell concentration, glucose concentration at 12 h was below 3 g/L and 44.5 g/L mannose remained unutilized in the medium. Furthermore, the amount of ethanol accumulated was 11.1 g/L. In this case, fermentation was manually stopped, and glucose was rapidly fermented to ethanol, which was easily removed. Meanwhile, mannose was effectively preserved in the broth. In conclusion, our results demonstrate that ethanol fermentation by *C. shehatae* is a feasible and practical method of removing glucose from hydrolysates. Concurrently, mannose was preserved in the medium for downstream biotransformation to mannonic acid.

3.4 Evaluation of the Effect of Ethanol on G. oxydans-mediated Fermentation

Zhou et al., reported that the performance of *G. oxydans* on xylonic acid production is restricted in the presence of ethanol [30]. Ethanol production during glucose fermentation is inevitable when *C. shehatae* is used for removing glucose, and approximately 11 g/L ethanol accumulated in the broth in this study. Thus, to evaluate the effect of ethanol on the productivity of *G. oxydans*-mediated fermentation, 0 g/L (as control) and 11 g/L ethanol were separately added to the medium containing 50 g/L mannose as the sole carbon source.

As shown in Fig. 3a, mannose was rapidly and completely metabolized by a 2 g/L inoculum of *G. oxydans* in ~24 h. Therein, the highest accumulation of mannonic acid was 41.9 g/L, with a yield of

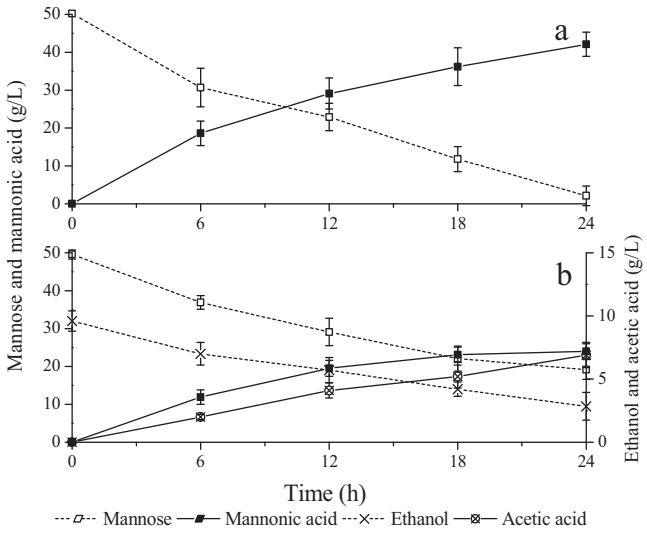

Figure 3: Effect of ethanol on mannose fermentation using *G. oxydans*. a): Mannose fermentation without ethanol; b): Mannose fermentation in the presence of 10 g/L ethanol in the medium

76.9% in 18 h. Ethanol can be oxidized to acetic acid at a low rate [31], which restricts the performance of mannose metabolism (Fig. 3b). The efficiency of *G. oxydans* fermentation evidently dropped when 10 g/L ethanol was loaded into the medium, and only 21.2 g/L mannonic acid was generated after 24 h fermentation. Our hypothesis was that ethanol or acetic acid exerted a negative effect on cell membrane permeability or dehydrogenase activity, reducing the bioconversion performance of *G. oxydans*. Thus, ethanol should be separated from the system before mannose fermentation.

3.5 Two-Step Bioprocess using C. shehatae, G. oxydans, and Konjac Hydrolysates

Our objective was to convert the mannose derived from lignocellulose to mannonic acid. In our initial experiments in this work, glucose was efficiently transformed to ethanol by *C. shehatae* and mannose was oxidized to mannonic acid by *G. oxydans*. Konjac powder hydrolysates typically contain significant amounts of mannose and glucose after acid treatment. Thus, to obtain mannonic acid from konjac, the two-step fermentation approach using *C. shehatae* and *G. oxydans* was performed using the acid-hydrolysates of konjac powder as the starting solution. Furthermore, to avoid further mannose metabolism, *C. shehatae* cells and ethanol were removed by centrifugation and distillation, respectively, after the first step of ethanol fermentation when glucose concentration was below 5 g/L. Ethanol was collected as the distillate and xylose and arabinose were retained in the broth. Then, the initial volume of the broth was reconstituted by adding distilled water. The detailed results of the experiment are shown in Fig. 4. In the presence of 2 g/L *C. shehatae*, glucose was consumed within 16 h and 10.2 g/L ethanol was produced from 29.1 g/L glucose and 8.2 g/L mannose.

Noticeably, glucose utilization rate was slightly decreased in paralleled with the decrease in the loss rate of mannose and in contrast to the results observed in the simulation medium. We hypothesized that the complicated chemical composition of hydrolysates might be responsible for the downward trend. Treatment with dilute hydrochloric acid resulted in the release of mannose and glucose. However, small amounts of inhibitors (such as 5-hydroxymethyl furfural and levulinic acid) were detected in the prehydrolysates [32, 33]. In addition, pH adjustment to neutrality prior to ethanol fermentation increased the salinity, which was an adverse factor for yeast cell growth. Overall, these factors might be the reason of downregulating the performance of *C. shehatae* [32]. Nonetheless, 42.1 g/L mannose could be

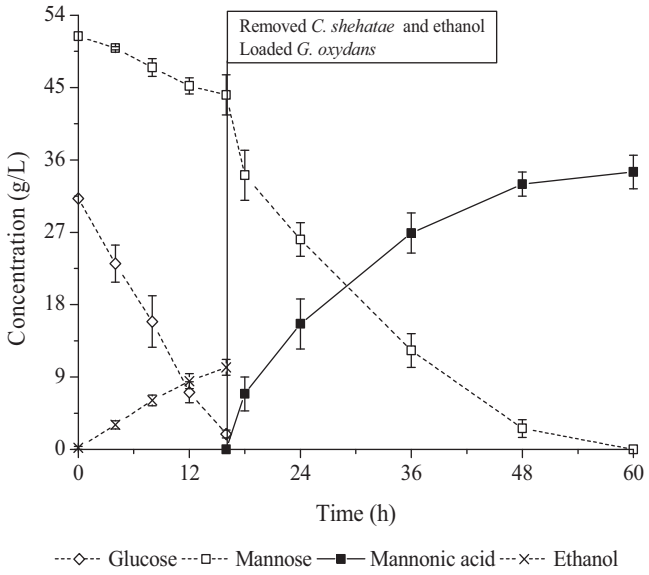

Figure 4: Profile of the two-step bioprocess using the real konjac powder hydrolysate as feedstock

preserved for subsequent bio-catalysis by *G. oxydans*. After the first fermentation step, *C. shehatae* cells were removed by centrifugation (3000 rpm, 10 min). Then, the solution underwent vacuum rotary evaporation for ethanol distillation. The volume and ethanol concentration of the distillate and residual fractions were measured after 1 h distillation. Approximately 93% ethanol was distilled and collected in a round flask. Finally, ethanol was recovered as the distillate and mannose was retained in the broth and the broth initial volume (50 mL) was reconstituted by adding distilled water. Then, *G. oxydans* was loaded into the broth for mannonic acid production. The detailed results of the experiment are shown in Fig. 4. Finally, 34.2 g/L mannonic acid were produced, indicating that the fermentative capability of *G. oxydans* was restricted.

The production of mannonic acid was lower than that obtained after the fermentation of pure mannose. Although the two-step bioprocess could not achieve the desired yield of mannonic production, it still provided a technology for bio-production of mannonic acid from konjac powder hydrolysates. As shown in Fig. 5, approximately 340 g mannonic acid and 100 g ethanol were obtained from 1 kg konjac powder. Overall, this integrated process for bio-production of mannonic acid and ethanol from a biomass resource is inexpensive and feasible and will simultaneously benefit subsequent studies on the application of mannonic acid.

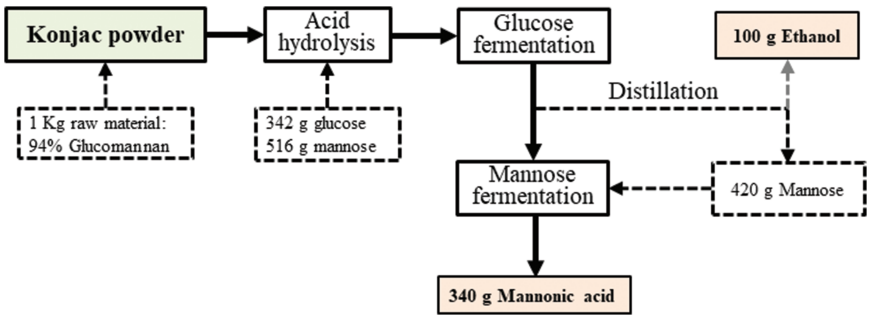

Figure 5: A mass balance for mannonic acid and ethanol production process from konjac powder

4 Conclusions

Konjac, a glucose and mannose rich resource, is increasingly recognized as a source of value-added products. A two-step fermentation involving ethanol fermentation by *C. shehatae*, followed by mannonic acid fermentation by *G. oxydans*, was successfully performed. At the end of the entire process, approximately 100 g ethanol and 340 g mannonic acid were produced starting from 1 kg refined konjac powder. This strategy offers a guideline for further studies to achieve the desired yield of mannonic and ethanol production. Overall, microbial fermentation is an interesting alternative for the bioremediation of konjac and key features of this bioprocess may be useful for efficient and comprehensive utilization of konjac.

Acknowledgement: The authors acknowledge the financial support from the National Natural Science Foundation of China (31901270), and the Scientific Research Start-up Funds of Nanjing Forestry University, China (163030127).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Dowdells, C., Jones, R. L., Mattey, M., Benčina, M., Legiša, M., Mousdale, D. M. (2010). Gluconic acid production by *Aspergillus terreus*. *Letters in Applied Microbiology, 51(3),* 252–257. DOI 10.1111/j.1472-765X.2010.02890.x.
2. Toivari, M. H., Nygard, Y., Penttila, M., Ruohonen, L., Wiebe, M. G. (2012). Microbial D-xylonate production. *Applied Microbiology and Biotechnology, 96(1),* 1–8. DOI 10.1007/s00253-012-4288-5.
3. Chun, B. W., Dair, B., Macuch, P. J., Wiebe, D., Porteneuve, C. et al. (2006). The development of cement and concrete additive: based on xylonic acid derived via bioconversion of xylose. *Applied Biochemistry and Biotechnology, 131(1–3),* 645–658. DOI 10.1385/ABAB:131:1:645.
4. Werpy, T., Petersen, G., Added, T. V. (2004). *Top Value-Added Chemicals from Biomass Volume I—Results of Screening for Potential Candidates from Sugars and Synthesis Gas*. Oak Ridge: US Department of Energy.
5. Hildebrand, A., Schlacta, T., Warmack, R., Kasuga, T., Fan, Z. (2013). Engineering *Escherichia coli* for improved ethanol production from gluconate. *Journal of Biotechnology, 168(1),* 101–106. DOI 10.1016/j.jbiotec.2013.07.033.
6. Sauer, M., Porro, D., Mattanovich, D., Branduardi, P. (2008). Microbial production of organic acids: expanding the markets. *Trends in Biotechnology, 26(2),* 100–108. DOI 10.1016/j.tibtech.2007.11.006.
7. Ramachandran, S., Fontanille, P., Pandey, A., Larroche, C. (2006). Gluconic acid: properties, applications and microbial production. *Food Technology and Biotechnology, 44(2),* 185–195.
8. Lin, W., Chen, D., Yong, Q., Huang, C., Huang, S. (2019). Improving enzymatic hydrolysis of acid-pretreated bamboo residues using amphiphilic surfactant derived from dehydroabietic acid. *Bioresource Technology, 293,* 122055. DOI 10.1016/j.biortech.2019.122055.
9. Huang, C., Wang, X., Liang, C., Jiang, X., Yang, G. et al. (2019). A sustainable process for procuring biologically active fractions of high-purity xylooligosaccharides and water-soluble lignin from Moso bamboo prehydrolyzate. *Biotechnology for Biofuels, 12(1),* 189. DOI 10.1186/s13068-019-1527-3.
10. Chen, W., Luo, M., Liu, C., Hong, S., Wang, X. et al. (2019). Fast microwave self-activation from chitosan hydrogel bead to hierarchical and O, N co-doped porous carbon at an air-free atmosphere for high-rate electrodes material. *Carbohydrate Polymers, 219(1),* 229–239. DOI 10.1016/j.carbpol.2019.05.033.
11. Yoon, M. H., Lee, Y. W., Lee, C. H., Seo, Y. B. (2012). Simultaneous production of bio-ethanol and bleached pulp from red algae. *Bioresource Technology, 126,* 198–201. DOI 10.1016/j.biortech.2012.08.102.
12. Zhang, Z., Donaldson, A. A., Ma, X. (2012). Advancements and future directions in enzyme technology for biomass conversion. *Biotechnology Advances, 30(4),* 913–919. DOI 10.1016/j.biotechadv.2012.01.020.

13. Kato, K., Matsuda, K. (1969). Studies on chemical structure of Konjac Mannan. *Agricultural and Biological Chemistry, 373(10),* 567–571.
14. Cui, T., Wu, T., Liu, R., Sui, W., Wang, S. et al. (2019). Effect of degree of konjac glucomannan enzymatic hydrolysis on the physicochemical characteristic of gluten and dough. *ACS Omega, 4(6),* 9654–9663. DOI 10.1021/acsomega.9b00061.
15. Tester, R., Al-Ghazzewi, F. (2017). Glucomannans and nutrition. *Food Hydrocolloids, 68,* 246–254. DOI 10.1016/j.foodhyd.2016.05.017.
16. Taraboulsi, F. A. Jr, Tomotani, E. J., Vitolo, M. (2011). Multienzymatic sucrose conversion into fructose and gluconic acid through fed-batch and embrane continuous processes. *Applied Biochemistry and Biotechnology, 165(7–8),* 1708–1724. DOI 10.1007/s12010-011-9389-6.
17. Kuivanen, J., Dantas, H., Mojzita, D., Mallmann, E., Biz, A. et al. (2014). Conversion of orange peel to L-galactonic acid in a consolidated process using engineered strains of *Aspergillus niger*. *AMB Express, 4(1),* 33. DOI 10.1186/s13568-014-0033-z.
18. Zhou, X., Zhou, X., Tang, X., Xu, Y. (2018). Process for calcium xylonate production as a concrete admixture derived from in-situ fermentation of wheat straw pre-hydrolysate. *Bioresource Technology, 261,* 288–293. DOI 10.1016/j.biortech.2018.04.040.
19. Kuivanen, J., Mojzita, D., Wang, Y., Hilditch, S., Penttila, M. et al. (2012). Engineering filamentous fungi for conversion of D-galacturonic acid to L-galactonic acid. *Applied and Environmental Microbiology, 78(24),* 8676–8683. DOI 10.1128/AEM.02171-12.
20. Zhang, H., Han, X., Wei, C., Bao, J. (2017). Oxidative production of xylonic acid using xylose in distillation stillage of cellulosic ethanol fermentation broth by *Gluconobacter oxydans*. *Bioresource Technology, 224,* 573–580. DOI 10.1016/j.biortech.2016.11.039.
21. Zhou, X., Zhou, L., Xu, Y. (2017). Improvement of fermentation performance of *Gluconobacter oxydans* by combination of enhanced oxygen mass transfer in compressed-oxygen-supplied sealed system and cell-recycle technique. *Bioresource Technology, 244,* 1137–1141. DOI 10.1016/j.biortech.2017.08.107.
22. Prust, C., Hoffmeister, M., Liesegang, H., Wiezer, A., Fricke, W. F. et al. (2005). Complete genome sequence of the acetic acid bacterium *Gluconobacter oxydans*. *Nature Biotechnology, 23(2),* 195–200. DOI 10.1038/nbt1062.
23. Deppenmeier, U., Hoffmeister, M., Prust, C. (2002). Biochemistry and biotechnological applications of *Gluconobacter* strains. *Applied Microbiology and Biotechnology, 60(3),* 233–242. DOI 10.1007/s00253-002-1114-5.
24. Sánchez, S., Bravo, V., Castro, E., Moya, A. J., Camacho, F. (2002). The fermentation of mixtures of d-glucose and d-xylose by *Candida shehatae*, *Pichia stipitis* or *Pachysolen tannophilus* to produce ethanol. *Journal of Chemical Technology and Biotechnology, 77(6),* 641–648. DOI 10.1002/jctb.622.
25. Miao, Y., Zhou, X., Xu, Y., Yu, S. (2015). Draft genome sequence of *Gluconobacter oxydans* NL71, a strain that efficiently biocatalyzes xylose to xylonic acid at a high concentration. *Genome Announcements, 3(3),* e00615-15. DOI 10.1128/genomeA.00615-15.
26. Buchert, J., Puls, J., Poutanen, K. (1988). Comparison of *Pseudomonas fraoi* and *Gluconobacter oxydans* for production of xylonic acid from hemicellulose hydrolyzates. *Applied Microbiology and Biotechnology, 28(4–5),* 367–372. DOI 10.1007/BF00268197.
27. Wang, X., Xu, Y., Lian, Z., Yong, Q., Yu, S. (2014). A one-step method for the simultaneous determination of five wood monosaccharides and the corresponding aldonic acids in fermentation broth using high-performance anion-exchange chromatography coupled with a pulsed amperometric detector. *Journal of Wood Chemistry and Technology, 34(1),* 67–76. DOI 10.1080/02773813.2013.838268.
28. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J. et al. (2012). *Determination of Structural Carbohydrates and Lignin in Biomass: Laboratory Analytical Procedure*. Golden, Colorado: National Renewable Energy Laboratory.
29. Gancedo, J. M. (1998). Yeast carbon catabolite repression. *Microbiology and Molecular Biology Reviews, 62(2),* 334–361.

30. Zhou, X., Huang, L., Xu, Y., Yu, S. (2017). A two-step bioprocessing strategy in pentonic acids production from lignocellulosic pre-hydrolysate. *Bioprocess and Biosystems Engineering, 40(11),* 1581–1587. DOI 10.1007/s00449-017-1814-y.
31. Gupta, A., Singh, V. K., Qazi, G. N., Kumar, A. (2001). *Gluconobacter oxydans*: Its biotechnological applications. *Journal of Molecular Microbiology and Biotechnology, 3(3),* 445–456.
32. Palmqvist, E., Hähn-Hägerda, B. (2000). Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. *Bioresource Technology, 74(1),* 25–33. DOI 10.1016/S0960-8524(99)00161-3.
33. Huang, C., Sun, R., Chang, H. M., Yong, Q., Jameel, H. et al. (2019). Production of dissolving grade pulp from tobacco stalk through SO_2-ethanol-water fractionation, alkaline extraction, and bleaching processes. *BioResources, 14(3),* 5544–5558.