An effective method for the direct crystallization of xylonic acid from fermentation broth of agricultural residue hydrolysate

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Abstract:

Xylose is the second most abundant sugar in nature and conversion of xylose to xylonic acid (XA) has become a research hotspot in recent years. Xylonic acid can be applied in the equivalent market niche of gluconic acid, due to their similar physical properties. XA bioproduction and application presents certainly a promising approach for xylose valorization in lignocellulose biorefinery, while the XA crystallization is so far still an unattainable goal. In this paper, an original method was proposed and experimentally investigated for the first time to get XA crystals from fermentation broth. In the XA crystallization, methanol was introduced as buffer-cum-solvent. The H_2SO_4 was dropwise added into the methanol solution dissolving potassium xylonate that is beforehand produced by fermentation. During H_2SO_4 blending with methanol, the strong and polar H_2SO_4 leads to XA diffusion and aggregation. Overall, 2.21 g of xylonic acid crystals was obtained per gram of 98% H_2SO_4 acidification. NMR detection showed that over 99% purity of XA crystals was finally obtained at the yields of 67.2% from xylonate. The integrated process could provide a practicable and effective technology for the high-qualify XA preparation from xylose derivations and lignocellulose biomass biorefinery.

Keyword: Xylonic acid crystals; xylose; Crystallizing separation; Acidification; Methanol; Microbial fermentation.

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1. Introduction

Xylose is the second most abundant sugar in nature after glucose[1]. The main applications of xylose from biomass are conversion to furfural through chemical routes and use as a hard-to-metabolize carbon source for production of chemicals by microorganisms[2-4]. However, in recent years, biomass processing for the production of value-added chemicals has come to the forefront of biological engineering research[2, 5, 6]. The importance of XA has been recognized and conversion of xylose to xylonic acid has become a research hotspot. The best known aldonic acid used industrially is gluconic acid, with a yearly consumption of 60,000 tons. The production of various aldonic acids like gluconic acid, lactobionic acid and xylonic acid have been extensively studied and are currently applied industrially with great success[7-9]. XA has a molecular structure similar to versatile gluconic acid and can replace it in several applications. Owing to its excellent chelating capacity in alkaline conditions, XA is used to increase the resistance of cement to cope with abnormal climacteric condition and improves concrete dispersion. Moreover, the production of XA from xylose by biotechnological processes has advantages over that of gluconic acid because it uses xylose as carbon source instead of glucose. From the economic standpoint, the use of xylose derived from lignocellulosic biomass as carbon platform is also promising. Only a few microorganisms, including *Pseudomonas aeruginosa, Pseudomonas fragi, and Gluconobacter oxydans*, have the ability to convert xylose to high titer xylonic acid. In addition to construction industry, xylonate bioproduction is

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currently a promising approach for the valorization of xylose constitute and lignocellulose biorefinery industry because of the abroad applications as pharmaceutical raw materials and paper manufacture. For example, a precursor of 1,2,4-butanetriol, an efficient biocatalyst for organic transformations, xylonic acid and viscose fibers can be blended to obtain a fiber fabric with cooling effect[1, 10, 11]. Moreover, owing to its excellent chelating capacity in alkaline conditions, it is used to increase the capacity to cope with abnormal climacteric conditions[11]. This versatile compound has been identified by the US Department of Energy (DOE) as one of the 30 most promising high-value chemicals to be produced from renewable biomass[12-14]. The more important issue is the fantastic and extremal high values in the end-product titer and yield of xylonate by whole-cell catalysis of lignocellulosic xylose broth[1, 6, 11, 14]. Zhou et al. reported a significant yield of xylonic acid (XA, 586.3 g/L) from xylose broth using COS-SSTR technology[6]. The bioconversion of xylose to XA has a promising future for industry because of its high efficiency and output. But so far, no commercial production of xylonic acid has been established. The reason is mainly because bacteria strains produce many other fermentation broth impurities resulting in difficult to produce high-grade XA. The subsequent purification of XA from broth is challenging.

Several studies have reported bioproduction of XA with high yield by *Gluconobacter oxydans*, an engineered strain with unique incomplete oxidation ability. As a gram-negative obligate aerobic bacterium, is known for rapidly and incompletely oxidizing sugars and sugar-alcohols. The bacterial strain of *G. oxydans*

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had proved to be a suitable microorganism for the bioconversion of xylose to XA. At the same time, as a substrate, xylose has high stability, high solubility, low cost, and almost no biotoxicity than furan chemicals for biochemical reactions. The biological production rate of XA remains at a high level, and the goal of continuous high XA production can be achieved through cellular recycling. Studies done with *Gluconobacter oxydans*, showed that the conversion to xylonic acid occurs in the periplasmic space, When the conversion to xylonic acid occurs in the periplasmic space the sugar never passes to the cytosol and is not used as substrate for growth purposes. In this study, we used *G. oxydans* for the incomplete bio-oxidation of xylose to XA to obtain a high titer of xylonate broth. The high titer of xylonic acid in the fermentation broth reduces the proportion of impurities, reduces difficulties in the purification stage and facilitates the formation of XA crystals.

The high-purity preparation is the first step for the industrial production and commercial application of XA and relative derivate[15-17]. And when it comes to purification of products, the XA crystallization in aqueous phase system is still an unattainable goal, mainly because hydrocolloids unique stability. Additionally, *Gluconobacter oxydans* possesses promiscuous oxidizing enzymes, which can convert xylose and many sugars and even sugar alcohols present in their specific acids, various chemical components in the fermentation broth making difficult the downstream processes for purification[18-21]. Xylonates purified by activated carbon adsorption or membrane separation are still contaminated by the culture medium and cell metabolites in the fermentation broth, making it difficult to achieve high purity

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goal. In order to realize the applications of high-value biomass transformation, we explored the possibility of solvents as crystallization medium, to judge from the XA unusual high solubility, and investigated XA crystallization process. It has long been known that solvents have important effects within various crystallization system[22-25]. In industrial manufacturing, thiamine hydrochloride is produced by thiamine nitrate using reactive crystallization in methanol[25]. In pharmaceutical purification industry, ethanol was often used as solvent in industrial crystallization of avermectin B1a. Regarding the unique stability of hydrocolloids, it is of great significance to find a suitable solvent for XA crystallization.

So far, only several literatures had revealed the crystallization of XA. As the major employed downstream processes for XA crystallization, activated charcoal treatment and solvent extraction from fermentation broth with a purity of 89.4% was described by Liu[26, 27]. After significant efforts and a total failure in experimental trails according to the above reported methods, we finally found the XA crystallization in organic solvents seems to provide a promising direction to solve purity predicament. Therefore, the objective of this study was first time to develop an efficient and green technical approach for the high-purity of xylonic acid crystals preparation from microbial fermentation broth.

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2. Materials and methods

2.1. Microorganism and chemicals

The strain of *G. oxydans* NL71 derived from ATCC621 was stored on agar medium containing 50g/L sorbitol, 5g/L yeast extract and 15g/L sorbitol/L[6]. The bacteria were grown in a 250-mL Euclidean flask, which contained 50 mL of nutrient medium (100 g/L sorbitol and 10 g/L yeast extract), and incubated at 30°C and 220 rpm for 24-36 hours. A refrigerated centrifuge (AvantiJ-26XP at 8000 rpm 5683g for 5min, Beckman Coulter) was used to harvest for 5 minutes. Since xylose exhibits no matrix inhibition to *G. oxydans*, resting cell catalysis was carried out by batch feeding operation with high titer xylose. The starting concentration of xylose is 100 g/L and each batch feed is 100 g/L. All chemicals were purchased from Sigma-Aldrich Co. LTD.

2.2. Resting cell catalysis and multi-stage crystallization precipitation step of XA

The resting cell catalysis process was carried out in the air compression bioreactor. Based on the air open bioreactor, an aired stirred tank reactor (ASTR) was developed to improve the biocatalytic performance of cell[28-30]. The stirred fermentation tank was connected with the compressed air under the inlet pressure of 0.01-0.02MPa. The biomass concentration was evaluated by determination of optical density (OD600) with spectrometer. The cell density was equivalent to OD_{600} = 4. A refrigerated centrifuge (AvantiJ-26XP, Beckman Coulter, USA) was used to separate fermentation broth and harvest cells at 5683 g for 5 min[18]. The fermentation pH value was adjusted to around 5.5 by the neutralizer addition with 50 % KOH and

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50%NaOH. The temperature was set to 30 ℃. The dissolved oxygen set point was 20 % saturation and the maximum agitation speed was 500 rpm. The resting cell catalysis was carried out by a fed-batch fermentation with 1 L fresh fermentation medium, containing $MgSO_4$ (0.5 g/L), KH_2PO_4 (1 g/L), K_2HPO_4 (2 g/L), $(NH_4)_2SO_4$ (5 g/L), yeast extract (15 g/L) and xylose at 30 ℃[18].

After fermentation, 100 mL of potassium xylonate fermentation broth was evaporated at 70℃ for 2 h and then freeze-dried for 12 h. The freeze-dried potassium xylate was added to a 50 mL conical flask with methanol and stirred thoroughly for 30min until the solid salt was completely dissolved. 98% sulfuric acid was added slowly dropwise to the xylonate-methanol solution and the solution was layered. Solid-liquid separation was conducted after 4 h to obtain XA crystals. Samples were periodically taken, centrifuged and filtrated through filters before analysis.

2.3. Determination of K_2SO_4 solubility by static method

A magnetic stirrer with a constant temperature function was used to measure the solubility of K_2SO_4 in methanol by static method. For this, 0.5 g K_2SO_4 was added in 20 mL methanol at varied temperatures within 30~70 ℃, followed by subsequent precipitation in a dynamic equilibrium state. The solubility of K_2SO_4 in methanol was obtained by testing the mass difference in specific temperature[28, 31-33].

The solubility of K_2SO_4 under acidification conditions was measured at room temperature, for which 0.5 g K_2SO_4 was added in 20 mL methanol and 0.38 g 98% H_2SO_4 was added dropwise, and the remaining solid mass was weighed after 30 min of thorough mixing. The solubility of K_2SO_4 under ultrahigh hydrogen ion

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concentration conditions was measured at room temperature, for which 0.5 g K_2SO_4 was added to 0.38 g 98% H_2SO_4.

2.4. Analytical methods

XA was determined on Agilent 1260 HPLC equipped with RI detector and Aminex HPX-87H column (Bio-Rad). Through the XA standard sample configuration of different concentrations after HPLC analysis to make a working curve, and then take the sample to be tested, according to the working curve can be found out the real content of XA in the sample, so as to calculate the purity. The purity of XA was determined by HPLC with the standard errors within 1.0 % for the duplicate samples. We use 20 min of 0.6 mL/min of 5.0 mM H_2SO_4 as the mobile phase. A Bruker Avance III HD 600 MHz spectrometer equipped with a 5 mm BBO probe was used to measure the NMR (600 MHz) spectra of crystals using a combined pulse proton decoupling sequence. XA (100 mg) was dissolved in 0.55 mL D_2O-d6 and the solution was transferred to a Shigemi microtube and characterized at 25℃. The acquisition parameters were pulse width: 30° ; relaxation delay: 2 s; acquisition time: 0.9044 s. A total of 200 scans were collected[21, 34].

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3. Results and discussion

3.1 Xylonate potassium fermentation and preliminary purification

G. oxydans, one of representative obligate aerobic bacterium, usually use oxygen as the final electron acceptor for driving a serial of oxidation reactions[18, 35-37]. During the biocatalysis process, D-Xylose is first oxidized to release and form xylono- -lactone, an equilibrium form of xylonate, by the catalysis of xylose dehydrogenase which is bound to cytoplasmic membrane[1, 6, 38]. XA is easily soluble in water because the -COOH and -OH of the molecule are hydrophilic groups, and these groups interact with polar water molecules to form a hydration layer that surrounds the molecule to form a hydrocolloid, which weakens the force between XA molecules and causes crystallization difficulty. The affinity hydrocolloids between XA molecules and solvent molecules are stabilized in water by two factors: charge and water film. We were able to combine bioprocesses with in-situ cell recycling, crystallization and electrodialysis for the semi-continuous whole-cell catalysis production of high purity glycolic acid in our previous works. However, we did not get any XK/sodium xylonate/calcium xylonate/XA crystals by crystallization in aqueous solution. The purity of the obtained salt solid was approximately 94% by HPLC. The best purity HPLC (Supplementary Fig. 1a shows the HPLC spectrum of dried XK) results were obtained from coupled purification of filtration and activated carbon adsorption, but results were poor in NMR spectrum (Supplementary Fig. 1b, 1c). Xylonates purified by activated carbon adsorption or membrane separation are still contaminated by the culture medium and cell metabolites in the fermentation broth, making it difficult to

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achieve high purity goal. In order to attain high purity goal and overcome crystallization barrier, we attempt to crystallize XA in the organic solvents and found that the purified XK under strong alkaline condition (pH=12) was soluble in methanol solution. Methanol is the alcohol that requires the least amount of dissociation energy, so it has the largest variety of inorganic salts dissolved. In order to crystallize xylonic acid, we expect to obtain crystals of xylonic acid by acidifying xylonate in methanol. The direct acidification of the xylonate by adding a high concentration of inorganic acid will directly carbonize all the solid, therefore methanol plays the role of buffer-cum-solvent in the XA production.

Considering the catalytic efficiency and the solubility properties of xylonates, we compared the initial reaction rates of xylose/XA in two main neutralizers-NaOH, KOH to understand the quantitative effects of various neutralizers on resting cell catalysis and bioconversion performances. The results (Fig. 1) show nearly identical high productivity and conversion was achieved for NaOH (57.8 g/L), KOH (58.1 g/L) in 10 h. Although the catalytic efficiencies of the two neutralizers were similar, the solubility of the formed salts differed, with potassium xylonate almost completely dissolved in methanol and sodium xylonate slightly dissolved in methanol. In general, the solubility of sodium salts is somewhat greater than that of potassium salts, but the opposite conclusion is reached for the solubility of xylonate in methanol solvent. It was speculated that polarity of methanol may be the main reason.

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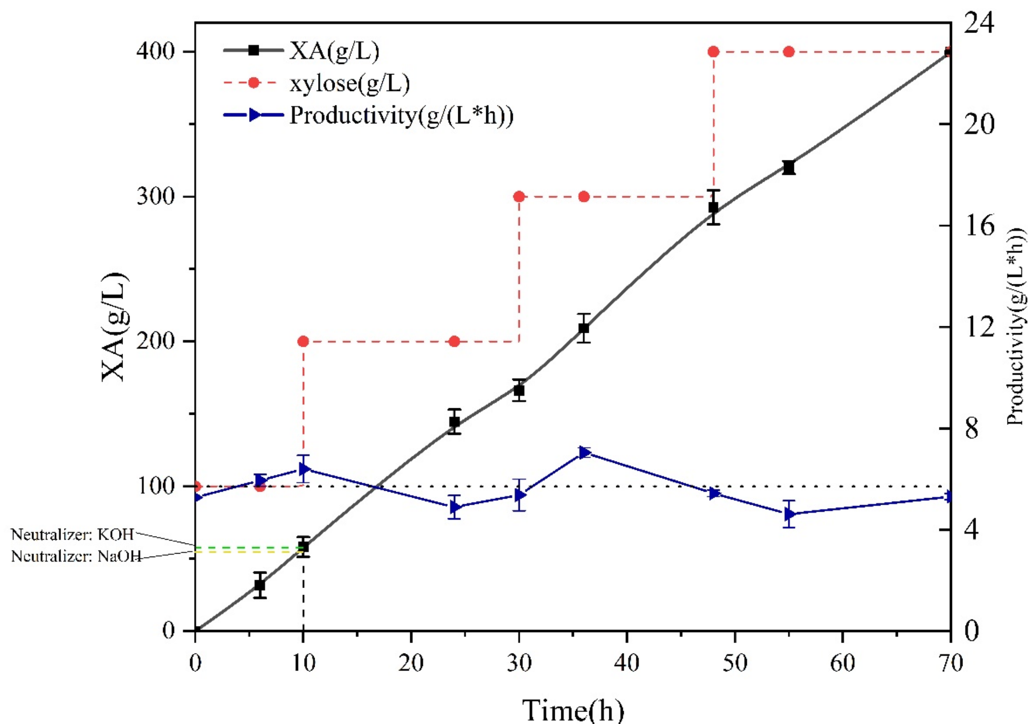

Figure 1. Diagram of bioprocess for the XK production

As an obligate aerobic bacterium, G. oxydans heavily depends on adequate oxygen supply for bio-oxidation. Therefore, we conducted the fermentation process in an aerated and stirred bioreactor. The pH value of broth was maintained at 5.5 by adding 50% KOH as the neutralizer. It is evident from Fig. 1 that the XK production was remain about 6 g/L/h during the entire fermentation process. The rate of production of xylonic acid is independent of xylose titer. Almost no xylose residue after catalytic completion and no addition of other sugar alcohols during the fermentation process further alleviates the difficulty of subsequent purification. Finally, 397.1 g/L XK accumulated after 70 h. The high titer of xylonic acid in the fermentation broth reduces the proportion of impurities, reduces difficulties in the

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237 purification stage and facilitates the formation of crystals.

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3.2 Multistage crystallization and separation of XA

An appropriate mass of XK and methanol were mixed in a reactor after a period of time, H_2SO_4 was added slowly into the suspension, the reactor was kept in a certain temperature. Cooling down the temperature when the reaction finished, and XA started to crystallize. In the process of the crystallization of XA, crystals of XA have been obtained via S2 growth by the acidification. It was interesting to observe that the crystallization process was connect with the time. For intuitive analysis, we divided the 24 h into three segments, 0–4 h, 4-16 h and 16-24 h, respectively, corresponding to 1, 2, and 3 stage (Fig. 2). In first 4 h, a total production of 0.84 g XA was achieved with the yield of 67.2%.

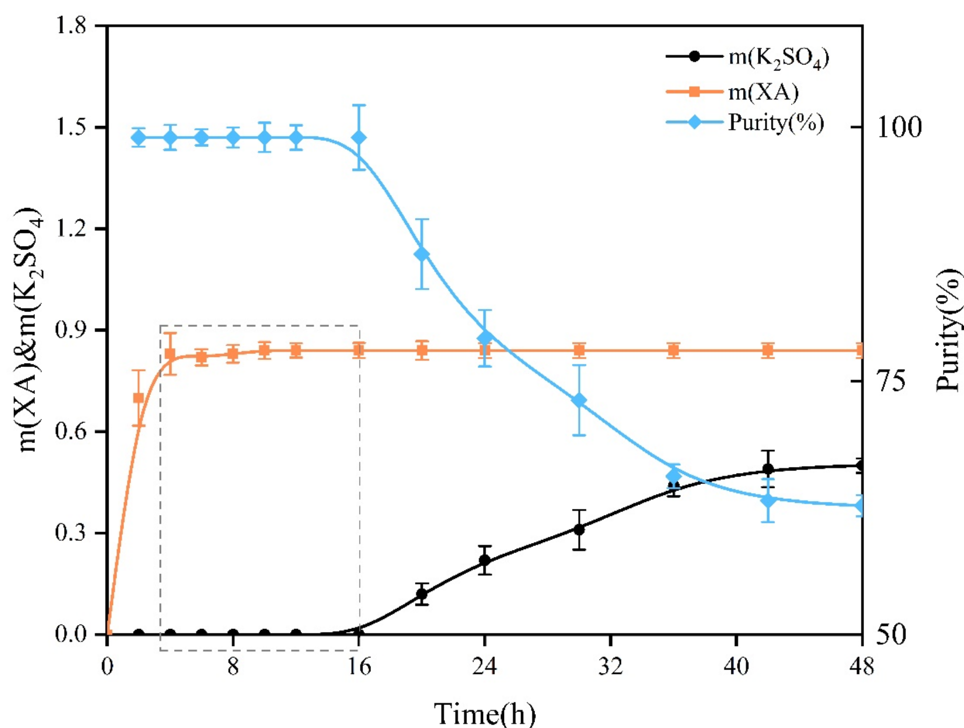

Figure 2. The crystallization performances of XA

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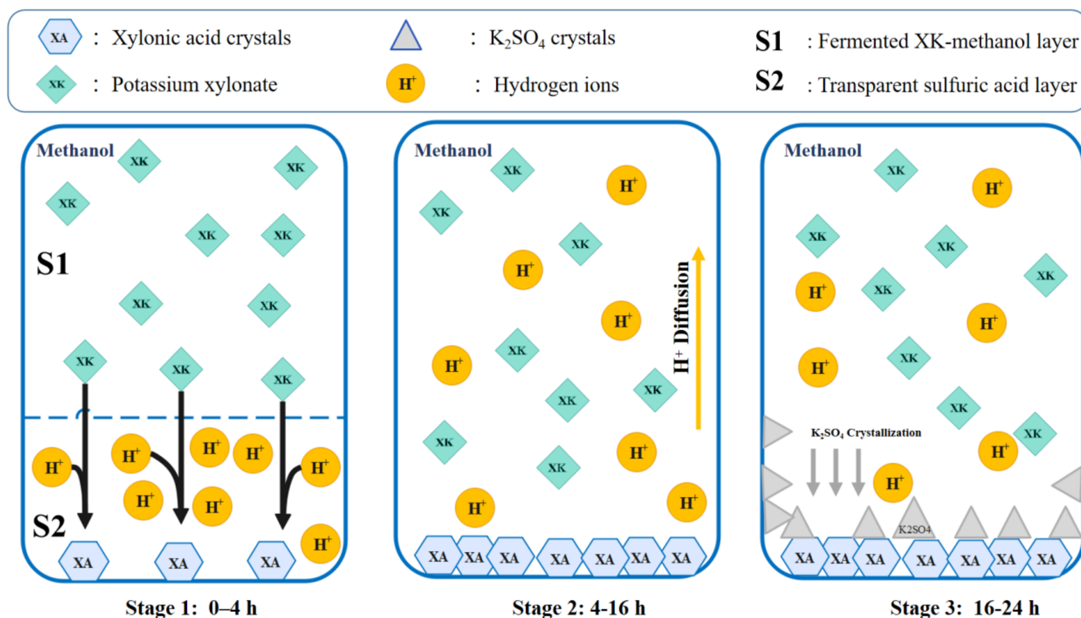

Figure 3. Process analysis of multistage crystallization model

As shown in Fig. 3, after solid mass analysis, the production of XA crystals in methanol approached a limit at 4 h. At this time point, the solid-liquid separation device can be used to synchronize the crystallization with in-situ separation. After four hours, the XA crystallization process was stopped, and solid mass in the crystallization reactor was found to be no increasing in second phase (4-16 h). In the second stage, mainly the diffusion of the ions, the solution will gradually mix and enter a constant state. After 16 h, the overall purity of solid XA decreases continuously due to the increase in mass and drops to 62.7% at 48 h. During this third stage, a total of 0.5 g of solid mass increase is formed.

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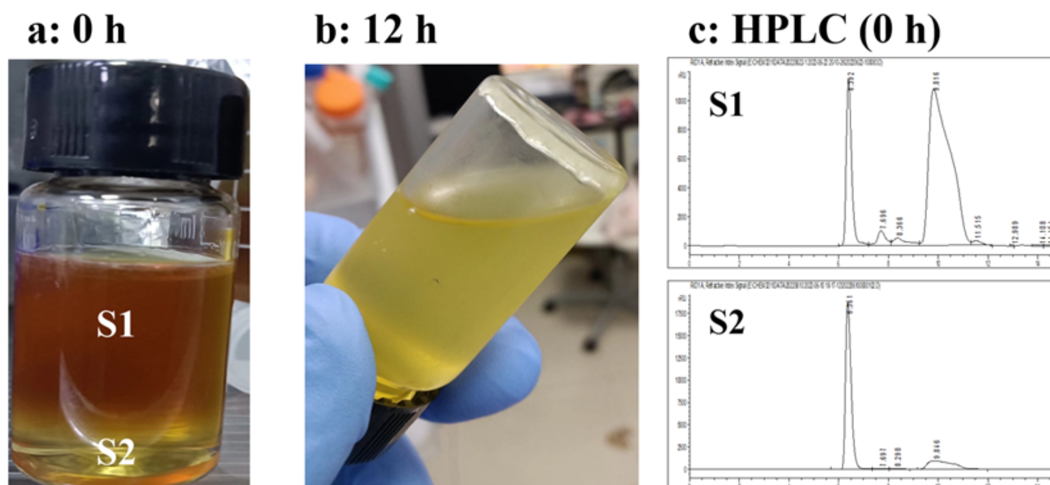

Figure 4. The image of XA crystallization (a) Crystallization in 0 h (b) Image of XA crystal in 12 h (c) HPLC detection of XK in methanol

The XA crystals produced by reactive crystallization in methanol is platelike (Fig. 4b). In addition, no increase in the mass of XA crystals in the second and third stage and the secondary formation granular crystals was considered as K_2SO_4. In the experiments on the solubility of K_2SO_4 in methanol, we found that the solubility of K_2SO_4 is extremely low, while K_2SO_4 can be dissolved in high titer of acid. It is speculated that due to the high concentration of acid gathered in the crystalline layer K_2SO_4 cannot precipitate while XA gradually crystallization, and with the formation of XA crystals, the acid concentration decreases and K_2SO_4 gradually precipitates. The acid inhibits the precipitation of K_2SO_4 and facilitates the crystallization of XA, but with the crystallization of XA, the decrease of hydrogen ion concentration will lead to the unavoidable precipitation of K_2SO_4 and will definitely contaminate the XA crystals. Therefore, we need to stage the whole process of crystallization to avoid contamination of XA crystals.

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In the whole crystallization process, 0.38 g of 98% sulfuric acid was used to acidify a total of 0.84 g of xylonic acid crystals, the acidification yield was 67.2%, and 0.5 g of K_2SO_4 crystals were produced in third stage. In the whole crystallization process, 2.21 g of xylonic acid crystals was obtained per gram of 98% sulfuric acid acidification.

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3.3 Process analysis of multistage crystallization model

To understand the possible crystallization process of XA, we examined the transparent sulfuric acid layer S2 and the yellow fermented XK-methanol layer S1 by HPLC (Fig. 4a, 4c). It was interesting to observe that the XK in S2 was significantly lower than the S1. The XK in S2 is only 4.76% of the S1. In the first stage, mainly the crystallization of xylonic acid which is crystallized in the S1 due to the higher density of 98% sulfuric acid. By measuring the solubility of K_2SO_4 in methanol (Fig. 5), we found that K_2SO_4 was almost insoluble in methanol, and the solubility of K_2SO_4 in methanol was only 0.175 g/L at 50 °C, while no crystals of K_2SO_4 were produced during the first stage of crystallization.

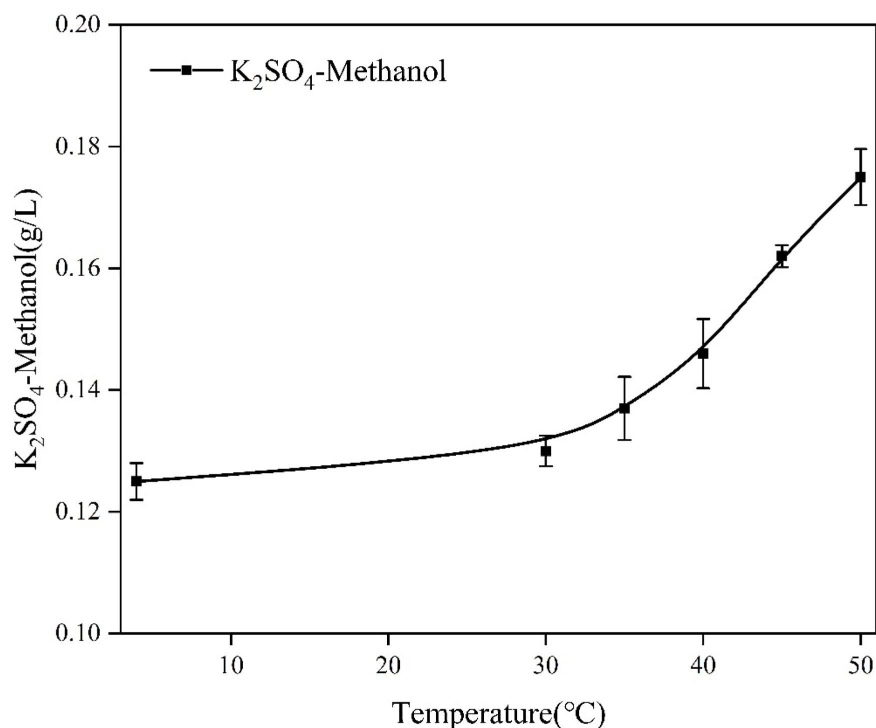

Figure 5. Solubility curves of K_2SO_4 with different temperature in methanol

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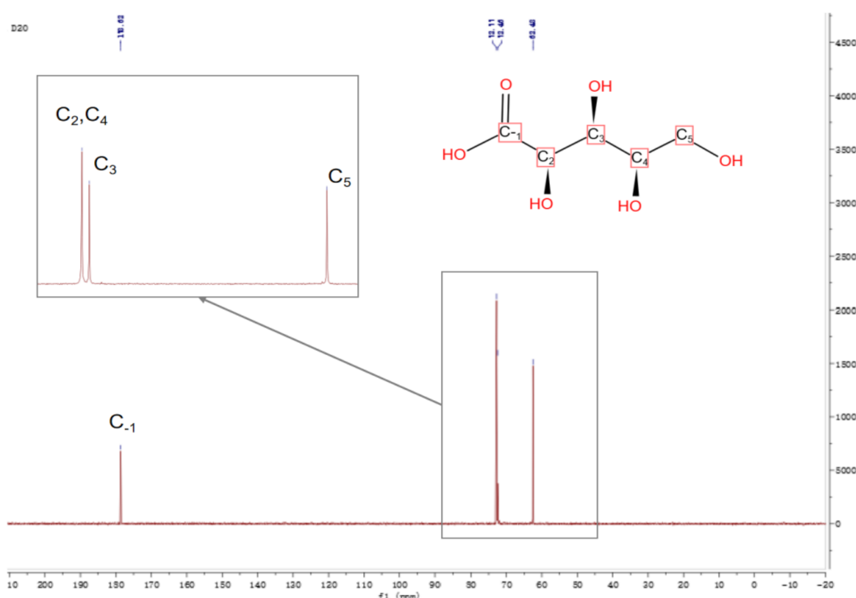
The solubility experiments proved that K_2SO_4 is completely soluble under ultrahigh hydrogen ion concentration conditions (98% H_2SO_4), partly dissolved in methanol solution with high hydrogen ion concentration. We believe that the ultrahigh concentration of hydrogen ions inhibits the crystallization process of K_2SO_4. The xylonate ions combined with hydrogen ions rapidly to crystallization. In the second stage, mainly the diffusion of the ions, the solution will gradually mix and enter a constant state. Next, we performed NMR to characterize the XA crystal purity and evaluated the crystallization result (Fig. 6). Thus, we obtained highly pure XA crystals by NMR analysis. To the best of our knowledge, XA crystals from fermentation broth are first reported. Totally, a green biotechnology was developed for the industrial production of the high-purified CG solid from resting cell catalysis and bio-oxidation of xylose.



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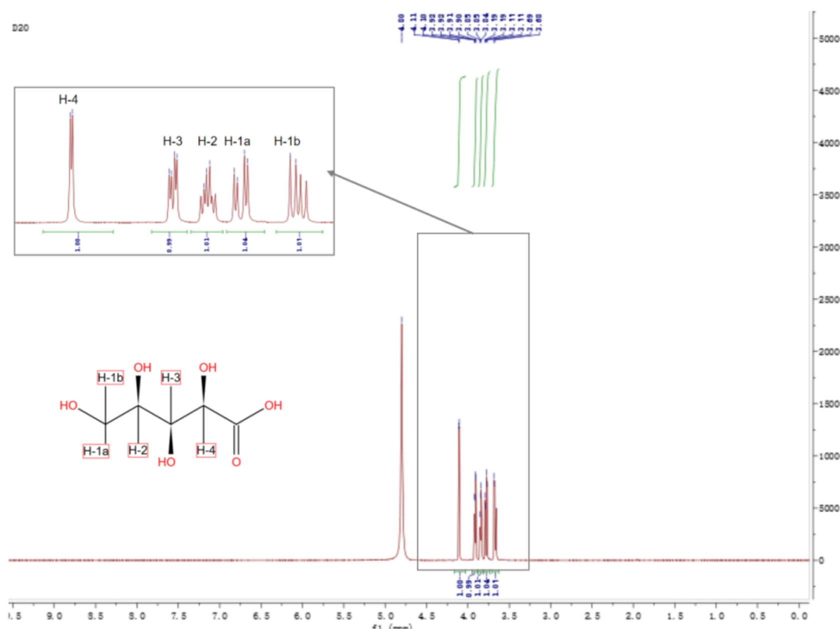

Figure 6. The ^{13}C-NMR and ^{1}H-NMR diagram of XA were measured on a Bruker Avance III HD 600 MHz spectrometer

^{13}C-NMR (151 MHz, D2O) δ 178.62 (C-1), 72.77 (C2, C4), 72.46 (C3), 62.48 (C5).

^{1}H-NMR (600 MHz, D2O), 4.11 (1H-4), 3.91 (1H-3), 3.88 – 3.80 (1H-2), 3.78 (1H-1a), 3.67 (1H-1b).

Due to the slow nucleation rate of K_2SO_4, no crystals are precipitated at second stage while XA crystals are not dissolved and the overall mass remains unchanged as the solution still maintains a high concentration of hydrogen ions[6, 13, 23, 26]. And in the subsequent third stage process, K_2SO_4 gradually crystallized due to the decreasing hydrogen ion concentration. Due to the chemical environment of the high concentration of hydrogen ions, some of the K_2SO_4 is still dissolved in methanol. The solubility of K_2SO_4 (5.1 g/L) under this simulated acidification condition is 38.5 times higher than that under neutral condition (0.13 g/L). In the third stage at 48 h precipitate 0.5 g K_2SO_4, at this time the concentration of K_2SO_4 in the solution is 4

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g/L. Next, we performed NMR evaluated the crystallization result and validated the multi-stage crystallization model (Supplementary Fig. 2). Heterogeneous peaks in the NMR spectrum under the influence of K_2SO_4 crystals.

During the staged crystallization, the third stage crystallization becomes the biggest drawback of this process. Although we can distinguish the difference between XA crystals and K_2SO_4 crystals from the crystal morphology, XA and K_2SO_4 cannot be separated after entering the third stage, so the solid-liquid separation is completed quickly after the completion of the second stage crystallization to obtain XA crystals and prevent the precipitation of K_2SO_4 in the third stage. The precipitation of K_2SO_4 crystals throughout the crystallization process is unavoidable, but we can still select the corresponding crystallization stage according to the precipitation time of K_2SO_4 in methanol to obtain XA crystals without K_2SO_4 contamination. This choice solves the problem of small-scale production of XA crystals, but for industrial production of xylonic acid more consideration is needed for recrystallization of the solution.

3.4 Integration technology of XA crystal production from xylose

The performances of XA crystallization in methanol system were compared with those of our previous work and other XA research studies. The total yields and purity for XA/xylonates productions in this and previous studies and results in ref. are summarized in Table 1.

Substrate	Strain	Process	XA production (g L^{-1})	Purity (%)	Ref.
Xylose	*G. oxydans* NL71	Fed-batch	586	-	6

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Xylose	*G. oxydans* NL71	Fed-batch	100	>70%	[1]
Sugarcane bagasse hydrolysate	*C. glycerinogenes* CgTT-Y4.2A. SYXB	Batch; shake fask	44.5	-	[10]
Corncob hydrolysate	*E. coli* W3110 (XGL4)	Fed-batch; bioreactor with 4-L operating volume	91.2	-	[39]
Xylose	*P. sacchari*	Fed-batch	390	-	[14]
Xylose	*E. coli*	Fed-batch	39.2	89.4	[27]
Xylose	*K. pneumoniae*	Fed-batch	103	-	[16]
Xylose	*G. oxydans NL71*	Fed-batch	400	99	This work

Table 1. Native strains and purification reported for xylonic acid production

Gluconobacter appears as the most promising microorganism for the production of xylonic acid when considering production titers in a defined medium. Additionally, *G. oxydans* possesses promiscuous oxidizing enzymes, which can convert xylose and many sugars and even sugar alcohols present in hydrolysates in their specific acids, making difficult the downstream processes for purification of the compounds. No addition of other sugar alcohols during the fermentation process further alleviates the difficulty of subsequent purification. In order to attain high purity goal and overcome crystallization barrier, we attempt to crystallize XA in methanol. The direct acidification of the xylonate by adding a high titer of inorganic acid will directly carbonize all the solid, therefore methanol plays the role of buffer-cum-solvent in the XA production. Due to the high concentration of acid gathered in the crystalline layer K_2SO_4 cannot precipitate while XA gradually crystallization, and with the formation

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of XA crystals, the acid concentration decreases and K_2SO_4 gradually precipitates. The precipitation of K_2SO_4 crystals throughout the crystallization process is unavoidable, but we can still select the corresponding crystallization stage according to the precipitation time of K_2SO_4 in methanol to obtain XA crystals without K_2SO_4 contamination. Therefore, in terms of XA production, the method had the following advantages: (a) This method avoids the hindrance of the ultrahigh solubility of XA on purity. (b) While get rid of the harsh reaction conditions of chemical methods, the low boiling point of methanol facilitates solvent recycling. (c) few technologies have been reported that can product high purity XA of over 90% especially from microbial fermentation broth. Herein, in this work, we demonstrate crystals of XA have been prepared in the acidification process with methanol as the system media.

For commercial applications, XA crystallization in reused xylonate-methanol should be considered, while finding better green acidification reagents. In our future work, biomass conversion coupled with crystallization technology, efficient utilization of low-cost feedstocks such as biomass hydrolysates instead of xylose as substrate will be further investigated, since the mixed five contents of C6 and C5 degraded sugars usually coexist in the medium that is oxidable for corresponding aldonic acids by whole-cell catalysis.

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4. Conclusions

The major bio-resource for xylonic acid production through either chemical or biotechnological paths is pentose-rich hemicellulosic hydrolysates. As an important practical approach to lignocellulose biorefinery, the bioconversion of xylose to XA has a promising future, especially for the valorization of xylose bioconversion to versatile platform biochemicals of xylonic acid. To render fermentation broth capable of producing high-purity XA, methanol was introduced as buffer-cum-solvent and sulfuric acid converted XK to XA crystals. Due to the high titer of acid collected in the crystalline layer K_2SO_4 cannot precipitate while XA gradually crystallization, and with the formation of XA crystals, the acid concentration decreases and K_2SO_4 gradually precipitates. The precipitation of K_2SO_4 crystals throughout the crystallization process is unavoidable, but we can still select the corresponding crystallization stage according to the precipitation time of K_2SO_4 in methanol to obtain XA crystals without K_2SO_4 contamination. In the whole crystallization process, 0.38 g of 98% sulfuric acid was used to acidify a total of 0.84 g of xylonic acid crystals, the acidification yield was 67.2%. Multi-stage crystallization system developed by the integration of bioreactor, acidification and crystallization facilitated in effectively achieve the direct bio-conversion of xylose into high-purity xylonic acid crystals though combined bioprocess.

Acknowledgements

The research was supported by the National Natural Science Foundation of China

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397 (32171730, 32211530071).

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