Bioresource Technology 176 (2015) 121–128

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Establishment and assessment of an integrated citric acid–methane production process

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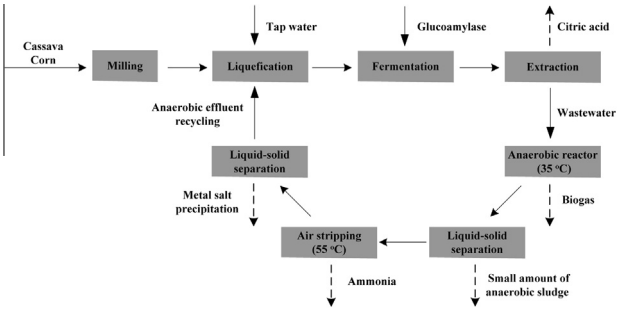
HIGHLIGHTS

- An improved integrated citric acid–methane production process was proposed.
- Anaerobic digestion performed efficiently and stably in recycling process.
- Citric acid production was slightly lower than fermentation with tap water.
- Excessive Na^+ contained in digestate was the major inhibitor for proposed process.
- The process could eliminate wastewater discharge and reduce water consumption.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 September 2014
Received in revised form 6 November 2014
Accepted 8 November 2014
Available online 15 November 2014

Keywords:
Citric acid
Anaerobic digestion
Air stripping
Glucoamylase
Sodium

ABSTRACT

To solve the problem of extraction wastewater in citric acid industrial production, an improved integrated citric acid–methane production process was established in this study. Extraction wastewater was treated by anaerobic digestion and then the anaerobic digestion effluent (ADE) was stripped by air to remove ammonia. Followed by solid–liquid separation to remove metal ion precipitation, the supernatant was recycled for the next batch of citric acid fermentation, thus eliminating wastewater discharge and reducing water consumption. 130 U/g glucoamylase was added to medium after inoculation and the recycling process performed for 10 batches. Fermentation time decreased by 20% in recycling and the average citric acid production (2nd–10th) was 145.9 ± 3.4 g/L, only 2.5% lower than that with tap water (149.6 g/L). The average methane production was 292.3 ± 25.1 mL/g $COD_{removed}$ and stable in operation. Excessive Na^+ concentration in ADE was confirmed to be the major challenge for the proposed process.

1. Introduction

Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid), one of the most important organic acids produced by fermentation today, is widely used in food, beverage, chemical and metallurgical industries (Ates et al., 2002; Chang and Holtzapple, 2000; Karthikeyan and Sivakumar, 2010; Mattey, 1992). In 2010, approximately one million tons of citric acid was produced in China and approximately 500–600 million tons of extraction wastewater with high concentration of chemical oxygen demand (15,000–20,000 mg/L) and low pH (4.5–4.8), was generated which has seriously restricted the development of citric acid industry (Zhi et al., 2010). Biochemical and physical chemistry methods have

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http://dx.doi.org/10.1016/j.biortech.2014.11.024
0960-8524/

been widely studied for citric acid extraction wastewater treatment (Li et al., 2013b; Yang et al., 2006). In the conventional process of biochemical methods, extraction wastewater was usually treated by anaerobic digestion followed by aerobic digestion (Colleran et al., 1998). However, aerobic digestion needs high capital investment and operation costs. Many scholars recently have used *Photosynthetic bacteria* and *Chlorella vulgaris* for extraction wastewater treatment (Kayombo et al., 2003; Li et al., 2013a; Valderrama et al., 2002). Although these methods operate simply and capital investments are low, the effluent could not meet the national discharge standard and still needs further treatment. Fenton's reagent, emulsion liquid membrane, microwave radiation and other physio-chemical methods were also used to treat citric acid extraction wastewater (Li et al., 2013a). The efficiency of above methods is high, however complexity of operation and large amount of inorganic reagents consumption make it not suitable on a large industrial scale.

As above end treatment methods are not satisfactory, direct recycling of extraction wastewater has been developed by many researchers. However, metal ions and pigment substances contained in wastewater were found to inhibit the growth of *Aspergillus niger* and reduce citric acid production. Pretreatment of wastewater with acidic cation exchange resin and activated carbon adsorption respectively was found to improve citric acid production (Qi et al., 2001). Although these methods could completely solve the problem of extraction wastewater, high costs and complicated regeneration made them unable to be used in industrial-scale production. Therefore, the disposal of wastewater is still a hard task for the citric acid industry.

Learning from experiences of cleaner production in ethanol industry (Wang et al., 2013, 2014), an integrated citric acid–methane production process was proposed in this laboratory to solve above problems (Xu et al., 2014a). Cassava and corn starch in this process are used in citric acid production, while unused materials (fiber and pectin) and metabolites of *A. niger* in fermentation are converted to biogas through mesophilic anaerobic digestion. The biogas can be used to produce electricity and heat while the ADE was recycled for the next citric acid fermentation, thereby avoiding the wastewater discharge and decreasing the water resource consumption.

It has been confirmed that ammonia and metal ions contained in ADE could significant inhibit citric acid fermentation and cation exchange resin was used to remove them in our previous study (Xu et al., 2014a). However, it was not quite suitable for industrial-scale production because of its high costs and complicated regeneration. Air stripping at 55 °C was an efficient method to remove ammonia and part of metal ions (Zn^{2+}, Mn^{2+}, Fe^{3+} and Ca^{2+}) to relieve the inhibition in ADE (Xu et al., 2014b). Therefore, an improved process has been established in this paper. ADE was treated with air stripping to remove ammonia and followed by liquid–solid separation to remove metal ion precipitation before making mash for the next citric acid fermentation. Glucoamylase was added to medium after inoculation to completely counteract the rest of inhibition in ADE. This improved process replaced complicated cation exchange resin operation and reduced the operation costs, making it more suitable for industrial-scale application. Moreover, it proved the technical feasibility of cleaner production in citric acid industrial and provided reference for other submerged fermentation industries in management of the wastewater.

2. Methods

2.1. Strain and seed culture conditions for citric acid fermentation

A. niger used for citric acid fermentation in this study was obtained from our laboratory and potato dextrose agar (PDA) slants maintained at 4 °C in a refrigerator was used for its preservation.

Cassava powder (starch content is 65–70% (w/w); size is approximate 0.45 mm) provided by the Henan Tianguan Co. Ltd., China, was used to prepare seed culture liquefied mash. 100 g cassava powder was mixed with 400 mL tap water and the slurry pH was adjusted to 6.0 with 30% (w/w) sulfuric acid or 10% (w/v) sodium hydroxide. 10 U/g high-temperature amylase (20,000 U/mL, Genencor China Co. Ltd.) was then supplemented, followed by heating up slurry to 100 °C for 2 h. Water loss during liquefaction was made up with sterile water and 0.1% (w/v) ammonium sulfate was supplemented as nitrogen source for spore germination. The slurry was adjusted pH to 5.5 and autoclaved at 115 °C for 20 min. Conidia from a 7-day-old potato dextrose agar slant were used for inoculation. 10 mL of a spore suspension in sterile water, containing approximate 6×10^6/mL of conidia, was added to the 80 mL sterile inoculation medium in a 1000 mL shake flask. The shake flask was incubated on a rotating shaker (200 rpm) at 36 ± 1 °C for 20 h before the seed culture was incubated for citric acid fermentation.

2.2. Citric acid fermentation

80 g cassava powder and 20 g corn powder (starch content is 75–80% (w/w); size is approximate 0.45 mm) provided by the Henan Tianguan Co. Ltd., China, were mixed with 450 mL ADE or tap water to prepare the fermentation media. The liquefaction operation was the same to that of seed medium and the initial total sugar of fermentation media was adjusted to approximate 155 g/L. The liquefied mash was autoclaved at 115 °C for 20 min and then 15% (v/v) seed broth (6/40 mL) was inoculated in a 500 mL shake flask to start the fermentation. The fermentation was conducted at 260 rpm, 37.5 ± 1 °C for 92 h. All the shake flask experiments were conducted in triplicate. Recycling experiments were employed in an agitator bioreactor (Korea bioreactor Co. Ltd.) with 5-L total capacity. Tap water was used to make mash for the first batch of citric acid fermentation and performed as control. Seed broth at 15% of fermentation volume (450 mL/3 L) was inoculated to fermentor. At the end of the fermentation, its extraction wastewater was treated through anaerobic digestion and the ADE was further treated and recycled in the later batches (2nd–10th) which was termed as recycling batches. In recycling batches, 130 U/g glucoamylase (130,000 IU/mL, Genencor China Co. Ltd.; mixed enzymes contained pullulanase) was added after inoculation. Temperature was maintained at 37.5 ± 1 °C and fermentation time was 72 h. Dissolved oxygen during fermentation was controlled by using 3-bladed impellers operating at 600 rpm with an aeration rate of 2 vvm. Samples were collected in an interval of 12 h.

In air stripping treatment, ADE was stripped with 12 L/min air at 55 °C in thermostatic water bath for 6 h. The lost water in striping was made up with tap water. Then effluent was centrifuged (4000*g*, 20 min) and the supernatant was used for citric acid fermentation.

2.3. Methane production condition

Upflow anaerobic sludge blanket (UASB) reactor (Shanghai Daming, China) with a working volume of 5 L was used in methane production in this study. Temperature was maintained at 35 ± 1 °C using circulator bath and 30% of the mesophilic anaerobic granular sludge (ratio of volatile suspended solid (VSS) to total suspended solid (TSS) is approximate 0.6 and specific methanogenic activity is more than 100 mL/g VSS/d, provide by Yixing Xielian Biological Chemical Co. Ltd., China) was inoculated in reactor. Extraction wastewater obtained from the extraction operation of medium at the end of fermentation in recycling batches was used as influent.