Study on production of organic acid rust remover from passion fruit fermented by *Aspergillus Niger*

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Abstract. Organic acid and rust remover based on citric acid were gradually praised by the industry because of its environmental protection and good derusting effect. In this paper, the production cycle of citric acid by *Aspergillus Niger* shaking flask fermentation was studied with Passion Fruit/Shell, and the rust removal effect of the product was preliminarily verified. The cultivation factors included Passion Fruit/Shell 20%+Sucrose 5%, 35 °C, pH 5.0-5.5, shaking table 150 r/min, the fermentation cycle could be completed in 4-5 days, and the pH in fruit fermentation broth decreased to 2.7. At room temperature, the derusting effect of 1 time diluted fruit fermentation broth equal ed that of 3% commercial citric acid, the rust removal time could prolonged properly at low temperature. The resultshad a guiding significance for producing organic rust remover from Passion Fruit by *Aspergillus Niger*.

1. Introduction

Citric acid (CA) is a kind of tricarboxylic acid compound, which is one of the main metabolic products of organisms. It mainly exists in lemon, citrus, pineapple and other fruits, widely used in medicine, chemical industry, cosmetics, etc [1]. It is also an environmental desulfurization adsorbent. In recent years, organic acid environmental rust remover represented by citric acid has become a researc hotspot because it does not contain toxic and harmful substances, environment-friendly, good rust removal andcorrosion inhibition effect [2-4]. For example, the application of scale removal rust in steel wire hot-dip galvanizing production[5] and thermal production operation, the new rust removal solution for rapid cleaning of stainless steel oxide scale at room temperature [6], and the study of removing heavy metals from soil by leaching with citric acid fermentation broth, etc [7]. Citric acid is mainly extracted from natural plants. There are two main methods of industrial production, synthetic method and biological fermentation method. Now days, liquid submerged fermentation by *Aspergillus niger* is the main method [8-9]. Citric acid is mainly produced by starch fermentation at home and abroad [10-11], but the cost of raw materials and energy are high. It is very important to find cheap substitutes for raw materials to reduce production costs [12]. In recent years, scholars have explored the production of citric acid from biomass cellulose such as *Aspergillus niger* fermentation degradation straw [13-15], apple pomace[16], in order to expand raw materials and reduce industrial costs.

In this paper, citric acid was produced by *Aspergillus Niger* fermentation with abandoned tropical fruit peels (shells) such as Passiflora as raw materials. Appropriate technological conditions and rust removal effects were explored to provide reference for the development of new raw materials and promote the green cycle development of local tropical fruit industry.
2. Experimental materials and methods

2.1. Experimental materials

2.1.1 Fermentation materials and equipment
Main reagents: *Aspergillus Niger* strains were purchased from Guangdong Microbial Collection Center. Passion fruit was bought in the agricultural market.
Main equipment: Electronic balance, Vertical pressure steam sterilizer, Ultra-clean worktable, HYG-II rotary constant temperature speed control shaker.

2.1.2 Activation of *Aspergillus Niger* and preparation of spore suspension
*Aspergillus Niger* inclined medium, plate medium and liquid seed medium were formulations of PDA medium. The activation of *Aspergillus Niger* and the preparation of spore suspension referenced Sun Jianqiu [17], \(10^7\) spore suspensions were prepared for reserve.

2.1.3 Basic medium for shaking flask fermentation
The whole Passiflora fruit or shell raw materials were crushed by a crusher to form a basic shaking flask fermentation medium, which contained 20% fruit /shell+5% sucrose+75% water. The initial pH was adjusted to 5.5. Every conical bottle (250 ml) was filled with 100 ml liquid, sterilized 121°C for 30 minutes with 0.1 MPa and cooled for reserve.

2.1.4 Rust Removal materials
Fermentation broth: Passion fruit fermentation products were filtered through 8 layers of gauze packed in conical bottle sterilization at high temperature, kept in refrigerator. Commodity standard citric acid solution: 3% (mass) citric acid solution was prepared, pH 2.5-3.0, which was used as standard control. Fermentation Rust Removal Solution: Dilute the original fermentation liquor into 1, 2 and 3 times of the original liquor for reserve. Rust iron sheet: 0.5*2*2cm square, provided by the fitter room of the school training center.

2.2. Experimental methods

2.2.1 Determination of fermentation period of passion fruit
After sterile operation on super-clean worktable, 2 ml of activated *Aspergillus Niger* spore suspension was absorbed by sterile pipette and transferred to the prepared fermentation medium. Shaking-bed fermentation was carried out at 30°C for 5 days at a rotating speed of 150 r/min. The pH value of fermentation broth and the acidity of citric acid in fermentation broth were determined every 12 hours from the 2nd day. Citric acid acidity determination reference Zhou Jing [11]. Fermentation filtrate 1 mL was diluted properly. Phenolphthalein was used as indicator and titrated with standard 0.1429 mol/L NaOH[18]. The acidification degree was evaluated by titrated NaOH volume, and the optimum period of citric acid fermentation by *Aspergillus Niger* was determined.

2.2.2 Effect of nitrogen source on citric acid fermentation
The effects of different nitrogen sources on fermentation culture were investigated. Adding 1.0% ammonium sulfate, urea, peptone and soybean powder to the shake flask fermentation medium of Passion Fruit as exogenous nitrogen source, the other culture conditions were the same as 2.2.1, to determine the best nitrogen source. Subsequent experiments were conducted to prepare fermentation broth under these conditions.

2.2.3 Rust removal test
The rusty iron sheets were weighed and put into 100 mL rust removal liquid at room temperature, 2 sheets in each concentration, and soaked for 0.5-3 h. The quality of iron sheet was measured every 0.5
h, and the effect of rust removal was evaluated according to the weight reduction and appearance. Refrigerate at 4 degrees was used to simulate the rust removal effect at lower temperature.

3. Experimental results and analysis

3.1. Determine of the fermentation period of CA

CA fermentation period was determined by determining the change of pH value and acidity of fermentation broth during shaking flask fermentation of Passion Fruit. It was found that the pH value of Passiflora hull decreased gradually from 5.5 to 2.7 during the culture of 96 h, and then increased slowly to about 3.0.

![Figure 1 Growth cycle pH curve](image)

The determination of acid production (by NaOH titration) showed that the formation of citric acid was basically consistent with the change of pH value. After 36-96 hours, the organic acids in the culture medium increased gradually and reached the maximum value. After that, the growth rate was slow, indicating that the fermentation had been completed. According to the change of pH value and acid production during fermentation, it showed that the fermentation period of Aspergillus Niger was 96-108 h, and the suitable pH value for citric acid transformation from Passion shell is 2.7-3.0. The fermentation law of Passiflora fruit was similar to that of fruit shell. Because of the sugar content in fruit juice, the transformation of organic acid in the early stage was higher slightly than shell. However, the content of organic acid in the later stage of fermentation decreases rapidly and the corresponding pH value increases gradually, which may be related to the rapid growth of bacteria in the earlier stage and the acceleration of senescence and explanation in the later stage. Considering that the residual Passion Fruit and shell are mostly abandoned and not used, the residual Passion Fruit (shell) could be developed to produce citric acid by fermentation, and 5-10% sugar source supplemented appropriately.
3.2. Comparison of rust removal efficiency of fermentation broth and commercial citric acid

The effect of rust removal was investigated between fermentation broth and standard citric acid solution on iron lumps of different quality (110g, 108g, 112g, 115g). The ratio of rust mass to original mass changes with time was shown in figure 3-4.
It showed (Figure 3) that the quality of iron sheet diluted by 1 times of fermentation broth decreased significantly from 0 h to 1.5 h at room temperature, and tended to be stable after 1.5 h, and its rust removal effect was equivalent to 3% citric acid. The quality of iron flakes in other fermentation broth also decreased, but the decreasing range and speed were lower than that of the former, which may be due to the lower concentration and shorter soaking time. It could conclude that the best time for rust removal was 1-1.5 h. Figure 4 showed that the time of rust removal should prolonged to improve the efficiency of rust removal in winter or at low temperature.

4. Conclusions
In this paper, the production of organic rust remover from Passion Fruit by Aspergillus Niger fermentation was studied, and the fermentation process was preliminarily determined. Citric acid fermentation conditions were passion fruit (shell) 20%+sucrose 5%, 35 °C, 150r/min. During the fermentation, the pH gradually decreased from 5.5 to 2.7 after cultured 84-96h, and then slowly increased. It indicated the fermentation was completed, and the organic acid accumulated was also the highest. Therefore, the fermentation cycle was generally 4-5d, and the final acid yield was 2.8%. Preliminary evaluation of rust removal effect: Compared with the commercial citric acid standard sample, it was found that the fermentation broth could be used for rust removal without separation and purification, and the effect was obvious.

The effect of 1-fold diluent effect was equivalent to that of 3% commercial citric acid. The best time of rust removal is 1-1.5 h. The time of rust removal should prolong at low temperature to improve the effect of rust removal. By studying, the basic conditions of citric acid fermentation were obtained and the citric acid conversion rate was better. However, how to optimize the citric acid fermentation process and apply it to industrial production needs further exploration and research.

5. References
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