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Improving Xanthan Fermentation in a Mechanically Stirred Aerated Fermenter via External Loop

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Abstract: Xanthan fermentation processes were investigated in a 5 L Mechanically Stirred Aerated Fermenter (MSAF) with and without external loop. Experimental data showed that the process with external loop had higher final xanthan gum concentration, shorter fermentation time and lower energy consumption.

Key words: Xanthan fermentation, MSAF, external loop, productivity, energy consumption

Introduction

Xanthan gum is a high molecular weight natural expolysaccharide produced by *Xanthomonas campestris* using carbohydrates. Because of its excellent rheological, physical and chemical properties, this biopolymer has been widely used as a thickening or stabilizing agent in oil, food, textile, cosmetic and pharmaceutical industries and its output is now the largest of natural expolysaccharides (Garcia-Ochoa *et al.*, 2000). Unfortunately, xanthan fermentation is probably the most complex fermentation process in terms of rheological property and associated mixing, power consumption, mass and heat transfer problems (Zhao *et al.*, 1994). This makes xanthan fermentation become the bottleneck in xanthan gum production. In order to improve productivity and shorten fermentation time, extensive research work has been carried out in optimizing xanthan fermentation conditions and developing new bioreactors (Lo *et al.*, 2001; Francisco *et al.*, 2001). Although a lot of bioreactors, such as airlift fermenter, centrifugal packed bed fermenter and static mixing fermenter, have been used for xanthan fermentation in laboratory or pilot scale, the Mechanically Stirred Aerated Fermenter (MSAF) is still mostly used in industrial scale (Garcia-Ochoa *et al.*, 2000). However, the conventional MSAF can't meet the demand of xanthan fermentation in two aspects. On the one hand, it is unable to adjust with xanthan fermentation process and has a high energy consumption because the fermentation broth starts out as a low-viscosity Newtonian fluid and ends up a highly viscous non-Newtonian fluid. On the other hand, large dead spaces will occur in the MSAF because of high viscosity and strong pseudo-plasticity of the fermentation broth during the later stage of xanthan fermentation, which leads to low final xanthan gum concentration, long fermentation time, low volumetric productivity and high

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energy consumption (Zhu, 1992). How to eliminate the dead spaces and make it adjustable become the key point of improving xanthan fermentation in the MSAF. Some useful researches have been conducted to eliminate the dead spaces by modifying the internal structure in the MSAF, such as combining different impellers together and adopting more efficient spargers. These measures have achieved certain effect in improving xanthan gum productivity and decrease the energy consumption. However, these modifications make the structure of the MSAF more complicated and its maintenance more difficult, moreover, the modified fermenter is still unable to adjust with the fermentation process. The objective of this study is to eliminate the dead spaces in the MSAF and, at the same time, make it adjustable conveniently to fit the whole xanthan fermentation process. In this work the effect of external loop on the final xanthan gum concentration, the dissolved oxygen and the energy consumption of xanthan fermentation was investigated and some comparisons were made between the xanthan fermentation process with and without external loop.

Materials and Methods

Thirty batches of xanthan gum fermentation were carried out in the MSAF with and without external loop respectively and the given numbers are the mean values.

The schematic diagram of experimental apparatus for xanthan fermentation is shown in Fig. 1. The experimental apparatus mainly consisted of two parts: a 5 L KMJ-5 type MSAF and a SHG-100 type peristaltic pump. The MSAF, equipped with pH, dissolved oxygen, temperature and foam probes, was produced by Mituwa Co., Japan and its detailed structure was described by Zhu (1992). The peristaltic pump was from Shanghai Research Institute of Chemical Industry and its maximal flow rate was kept during the xanthan fermentation with external loop. The relationship between its maximal flow rate and xanthan gum concentration is shown in Fig. 2.

Micro-organism, Medium and Culture Conditions

Xanthomonas campestris SUB-11 was used throughout this study. The stock cultures were maintained on YM agar plates at 4°C and transferred to fresh plates every four weeks to avoid the micro-organism degradation. The inoculum preparation was by means of micro-organism transfer from stock cultures to a fresh agar plate and grew for 48 h at 28°C. Following this period, single colonies

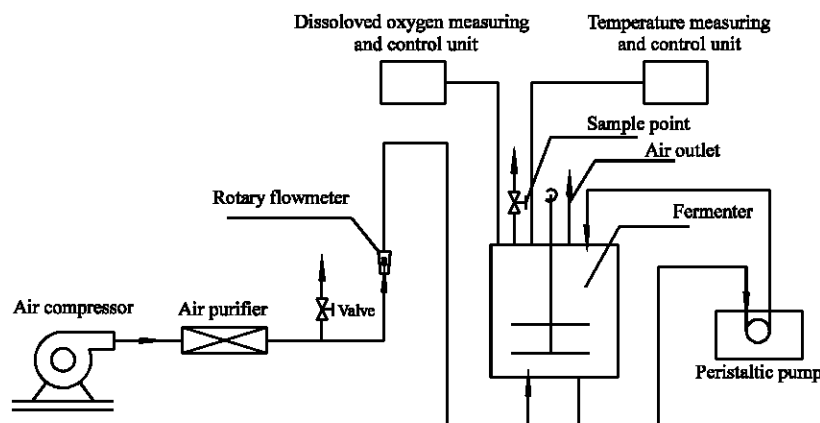


Fig. 1: The schematic diagram of experimental apparatus for xanthan fermentation

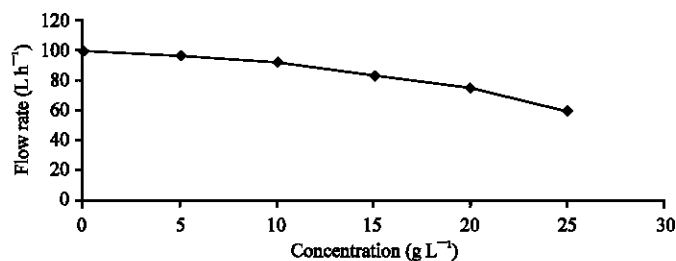


Fig. 2: The relationship between the maximal flow rate of the peristaltic pump and xanthan gum concentration

were transferred to a 500 mL shaking flask with 200 mL inoculum medium. The flask was placed on a orbital shaker with a shaking diameter 5 cm and a shaking frequency 200 rpm and incubated at 28°C for 48 h. This was used as inoculum for fermenter fermentation. The 5 L MSAF with 3.5 L production medium was inoculated and incubated at 28°C for 48 h. During the fermenter fermentation, the air flow rate and stir speed were kept at 3.5 L min⁻¹ and 900 rpm, respectively. Small samples were taken from the MSAF every 4 h for later analytical usage. As for the xanthan fermentation in the MSAF with external loop, the peristaltic pump started and its maximal flow rate was kept after the fermenter fermentation was carried out for 24 h. To the fermenter fermentation without external loop, the peristaltic pump was kept closed throughout the fermentation.

The compositions of culture medium were as follows (g L⁻¹):

- The YM agar plate medium: D-glucose 10, bacteriological peptone 3, yeast extract 3, malt extract 3, agar 24.
- The inoculum medium: sucrose 15, bacteriological peptone 5, yeast extract 3, sodium glutamate 2.4, KH₂PO₄ 1, NaCl 1, FeSO₄ 0.1.
- The production medium: corn starch 30, corn steep liquor 2.8, sodium glutamate 2.4, CaCO₃ 4, NaCl 1.
- Each medium was autoclaved at 121°C for 20 min after pH was adjusted to 7 by addition of 1 M NaOH or 1 M HCl.

Analytical Methods

Determination of Cell Concentration

This was done by dry-cell weigh estimation. The sample taken from the fermenter was diluted to xanthan gum concentration less than 10 g L⁻¹. The cell was collected after centrifugation at 5000×g for 15 min. The supernatant remained for later usage and the biomass was washed twice with distilled water to remove the traces of xanthan gum before following another centrifugation. Finally, the cell was dried in an oven at 70°C for 24 h and weighted.

Determination of Xanthan Gum Concentration

The supernatant after determining cell concentration was added two volumes of ethanol to precipitate xanthan gum. The supernatant after xanthan gum precipitation was kept for total sugar analysis. The precipitated gum was washed twice with ethanol, dried in an oven at 70°C for 24 h and weighted.

Determination of Total Sugar Concentration

The supernatant after xanthan gum precipitation was added some 6 M HCl and boiled for 30 min to hydrolysis the dissolved starch to reducing sugar. The solution was adjusted to neutral by addition of 2 M NaOH and used to determine the concentration of total sugar based on the method using 3,5-dinitrosalicylic acid reagent (DNS method) (Miller, 1959).

Measurement of Dissolved Oxygen and Energy Consumption

The dissolved oxygen was measured by the dissolved oxygen measuring and control unit in the MSAF. The total energy consumption during the xanthan fermentation process was measured by a kilo-watt-hour meter.

Results and Discussion

Effect of External Loop on Xanthan Fermentation Process

In order to explore the effect of external loop on xanthan fermentation in the MSA, the xanthan fermentation processes with and without external loop were studied and compared. Because no dead spaces existed during the initial 24 h of xanthan fermentation, the peristaltic pump started and the external loop began after the fermenter fermentation was carried out for 24 h. Figure 3 shows that the cell concentration with external loop was higher than that without external loop from 28 to 44 h. The cell concentration began to decrease at 36 h for xanthan fermentation with external loop, but at 28 h without external loop. The reason is that the external loop could partly eliminate the dead spaces where the bacteria were limited by dissolved oxygen and unable to maintain their normal metabolism. Figure 4 shows that the total sugar concentration with external loop was lower than that without external loop. This is because external loop partly eliminated the dead spaces and made more bacteria consume the carbon source to maintain their normal metabolism. Figure 5 shows the xanthan gum concentration with external loop was higher than that without external loop. The final xanthan gum concentration with or without external loop was $24.3 \pm 0.42 \text{ g L}^{-1}$ and $22.4 \pm 0.39 \text{ g L}^{-1}$, respectively. For the xanthan fermentation process with external loop, the xanthan gum concentration almost kept constant since 42 h, so the fermentation time could be shortened to 42 h. The higher final xanthan gum concentration and shorter fermentation time for the process with external loop were attributed to its partly-eliminated dead spaces where the bacteria could normally synthesize xanthan gum. Figure 6 shows the dissolved oxygen with external loop was lower than that without external loop. The external loop partly eliminated the dead spaces and made the bacteria in these spaces able to consume the

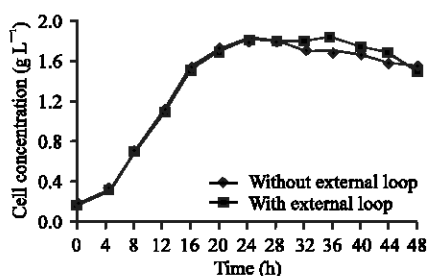


Fig. 3: The time courses of cell concentration for xanthan fermentation with and without external loop

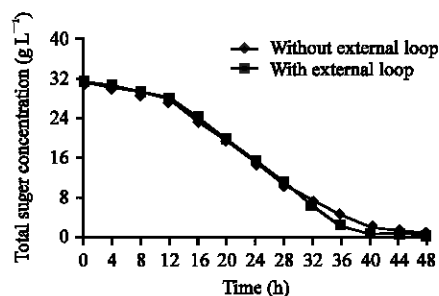


Fig. 4: The time courses of total sugar concentration for xanthan fermentation with and without external loop

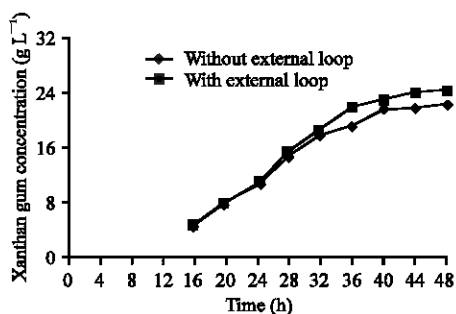


Fig. 5: The time courses of xanthan gum concentration for xanthan fermentation with and without external loop

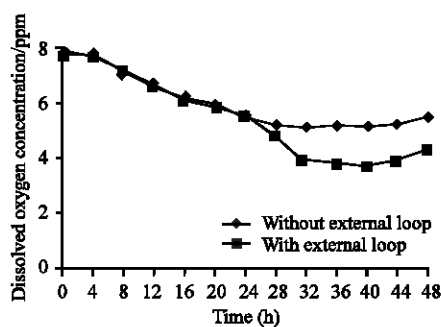


Fig. 6: The time courses of dissolved oxygen concentration for xanthan fermentation with and without external loop

oxygen normally. The result was that the bacteria in the fermenter would consume more oxygen. However, the external loop had almost no effect on oxygen transfer coefficient in the fermenter, this made the dissolved oxygen in the fermenter lower than that without external loop.

Effect of External Loop on Total Energy Consumption

The total energy consumption for xanthan fermentation process with external loop includes the energy consumption of aeration, agitation and circulation. However, the total energy consumption without external loop only includes the energy consumption of aeration and agitation. The complicated

rheological property of fermentation broth makes it difficult to study the energy consumption during the xanthan fermentation process. In order to simplify the complexity, the total energy consumption for the whole fermentation process was measured by a kilo-watt-hour meter. The total energy consumption with or without external loop was 2.50 ± 0.21 and 2.81 ± 0.23 kwh, respectively. Considering that the external loop made the final xanthan gum concentration increase from 22.4 to 24.3 g L^{-1} , the total energy consumption via external loop was saved by about 18% based on unit xanthan gum production.

Conclusions

The results of this study suggested that the external loop could improve the xanthan gum fermentation in the MSAF. It made the final xanthan gum concentration increase from 22.4 to 24.3 g L^{-1} , the fermentation time shorten from 48 h to 42 h and the total energy consumption saved by about 18% based on unit xanthan gum production.

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