

Cell free xanthan gum production using continuous recycled packed fibrous-bed bioreactor-membrane

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ABSTRACT

Although the xanthan gum has been produced as a commercial commodity, the biomass isolation and its recovery are still challenging. This study revealed the xanthan gum production by fermentation of *Xanthomonas campestris* DSMZ using glucose as a carbon source in an immobilised batch and a continuous recycled packed fibrous-bed bioreactor-membrane (CRPBFBM). The pure cotton fibre was used to immobilise the microbial cell biomass and to isolate from the liquid phase containing medium and xanthan gum. The cellulose acetate membrane with 0.45 µm was used to recover the xanthan gum. The batch fermentation showed that the immobilisation technique gave higher xanthan gum concentration at 20g/L than the free moving cell without immobilisation at 18g/L. The CRPBFBM produced the highest xanthan gum concentration at 18.7 g/L at the dilution rate of 1.44 d⁻¹. The highest production rate of CRPBFBM was 0.475 g/L-h. Further research needs to be conducted to ascertain the stability of the *Xanthomonas Campestris* DSMZ during a long period of continuous fermentation as well as up scaling the CRPBFBM.

Keywords: *Xanthan gum, packed fibrous-bed bioreactor, membrane process, and immobilisation*

INTRODUCTION

It is a common knowledge that the commercial production of exopolysaccharides is produced by the batch fermentation. Basically, the chemical and physical separations called precipitation and centrifugation is used to recover the exopolysaccharides from the fermentation broth. The same process is also applied to the xanthan gum production. The findings of studies examining the various parameters and variables of xanthan gum production such as nutrients composition and feeding technique, temperature, pH, agitation, and foaming reduction, showed significant improvements especially for the substrate conversion.

Studies by Yong et al [1996; 1998] indicated superior results with the immobilisation of *Xanthomonas campestris* onto suitable supports with the immobilization ensured the natural isolation of the *X. campestris* from the liquid containing nutrients and products to produce a cell-free liquid broth. Despite that, the drawback of immobilisation was some significant amount of the xanthan gum trapped in the bed and was not easily separated from the cells. Furthermore, the immobilisation technique still encounters a classic problem of mixing where the viscosity of liquid increases throughout the fermentation cycle. This causes the immobilised cell to get less oxygen as it grows up.

Although much work has been done, more studies need to be conducted to isolate the biomass and to recover the product. As part of a continuing studies of the centrifugal fibrous-bed bioreactor for cell-free xanthan gum process as reported by Yong [1996; 1998], in this paper we reveal the combination process of the continuous recycled packed fibrous-bed bioreactor and membrane separation for enhancing the oxygen transfer as well as for producing the cell-free xanthan gum.

MATERIALS AND METHODS

Bacterial Strain

X. campestris strain supplied by DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) was used in this study. The dried pellet in a sterile glass ampoule was dehydrated in the skim milk before being streaked on the YDC agar slant. The YDC agar slants contained 10 g/L yeast extract, 20 g/L glucose, 20 g/L calcium carbonate, and 20g/L agar. The agar slant was initially adjusted to pH 7 and then grown at 28 °C for 24 hours. The agar slant was then stored at 4 °C. Similar technique was also employed by other researchers. The *X. campestris* strain was maintained in an active and stable condition by transferring them every fortnight a month into a new agar slant.

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Inocula Preparation

The seed culture of *X. campestris* in the form of liquid was prepared before fermentation process. One litre of the liquid seed culture medium contained 10g/L of yeast extract, 20g/L of glucose, 20g/L of calcium carbonate, and water. The ingredient was heated and stirred on the magnetic stirrer-heater to form a homogenous liquid. 50 mL of the medium was then transferred into 150 mL of the conical flask. The flask containing the medium was covered with the aluminium foil and then was autoclaved. The sterile medium was adjusted to pH 7. After cooling to the ambient temperature, a sterile loopful of inoculum was streaked on the agar slant and was submerged into the liquid medium to initiate the strain growth. The liquid seed culture medium was clamped onto the shaker, and then incubated at 200 rpm and 28 °C for 24 hours. After incubation the liquid seed culture was stored at 4 °C.

The packed bed and free cell batch fermentations

The determination to investigate the possibility of using the packed bed is a basic factor in the success of the continuous recycled packed fibrous-bed bioreactor membrane (CRPFBMB) development. Once the packed bed fermentation performed in the batch process has successfully adsorbed the biomass, the CRPFBMB are performed.

A total of 125 and 200 mL conical flasks were used as the batch bioreactor. Different packing densities of packed beds of 0.026 and 0.039 g-cotton per mL of medium, and a control experiment without beds were studied. The packed bed was prepared by loosely packing the defined weight of the cotton fibre into silicone tube with an internal diameter of 5 mm as a support. Fine holes were made on the tube surface to allow the medium and oxygen to penetrate into the cotton. The packed cotton fibre with the tube was then cut into 5 cm lengths.

After preparation of the bed, the batch fermentation process was started by filling-in 0.026 and 0.039 g-cotton/g-medium of the packed bed cotton into the 125mL conical flask along with 25 mL of medium containing 30g/L of glucose, 3 g/L of yeast extract, 2 g/L K_2HPO_4 and 0.1 g/L $MgSO_4 \cdot 7H_2O$. The bioreactor was autoclaved twice at 121 °C and they were left to cool. The bioreactor was then clamped into the incubator shaker, agitated at 200 rpm, and the temperature and pH were maintained at 30 °C and at pH 7 respectively. After 15 minutes, 2.5 mL of seed culture of *X. campestris* was inoculated into the bioreactor and then the fermentation process was allowed to proceed. Each of the bioreactor was sampled every day and the longest fermentation duration was seven days.

Packed fibrous-bed bioreactor-membrane

Figure 1 shows a schematic diagram of the used packed fibrous-bed bioreactor membrane. A reflux glass tube measuring 470 mm in length and 22 mm in internal diameter was improvised as the packed bed bioreactor. Inside the reflux glass tube, the packed pure cotton fibre was inserted with a total cotton weight of 4.16 g. Water at 30 °C was circulated outside the reflux glass for constant temperature in the CPBBM. The vacuum filter system was used as a dead end filtration system.

One litre sterile medium containing 10g/L of glucose, 3g/L of yeast extract, 2 g/L K_2HPO_4 , and 0.1 g/L $MgSO_4 \cdot 7H_2O$ was prepared in a 2 litre mixing tank. To start up, the CPBBM was sterilised at 121 °C for 30 min by using sterilized air. The sterilised medium was fed from the bottom of the bioreactor continuously using a peristaltic pump (Masterflex 07523-40 drive and 77200-60 pump head) at three flow rates; 1, 2 and 3 mL/min that equal to dilution rates of 1.44d⁻¹, 2.88d⁻¹, and 4.32d⁻¹ respectively. The effluent was filtered by the vacuum filter system using a 0.45 µm cellulose acetate membrane to recover the xanthan gum. The membrane filtrate was recycled into the mixing tank as shown in Figure 1. The filtered air was also supplied at a constant flow rate of 30 mL/min into the packed bed bioreactor from the bottom and through the same line the medium was also fed. After 15, the packed bed bioreactor was charged 50 mL seed culture. The glucose concentration in the medium was increased to 30g/L after one day, and then the harvesting was started thereafter. The membrane filter was replaced every day and 10 mL filtrate sampled for xanthan gum and glucose concentrations measurement.

Determination of biomass and xanthan gum concentration

The fermentation broth produced by the batch processes were diluted using distilled water to lower the viscosity, and then 20 mL aliquots were transferred into micro-centrifuge tubes. The micro-centrifuge tubes containing aliquots were centrifuged at 11,000 rpm for 30 minutes at 4 °C. After centrifugation, two fractions were formed, supernatant containing xanthan gum, and biomass deposited as a pellet. The biomass pellet was re-suspended with water for washing and then re-centrifuged to re-precipitate the biomass. The biomass deposited at the bottom of tubes was dried in the oven at 60 °C for two hours and weighted to get the dry mass to show the relative performance of the cotton fibre in retaining the cells. Supernatants were mixed with 2/3 (v/v) isopropanol, re-centrifuged at 11,000 rpm for 45 minutes at 4 °C to completely precipitate xanthan gum before removing the solvent and water from the top portion. The precipitated xanthan gum collected from all samples was

dried overnight in the oven at 50 to 60 °C in the pre-weighed micro-centrifuge tube. The concentration of xanthan gum was determined as the dry weight of xanthan gum per litre culture medium.

For xanthan gum estimation produced by the CRPFBBM system, the pre-weighed cellulose acetate membrane used in the CRFBBM which was replaced every day was used. The cellulose acetate membrane was dried in the oven for two hours at 60 °C and then was weighted to estimate the dry mass of the xanthan gum. The purity of the xanthan gum was examined by re-suspending it into distilled water, and then re-centrifuged to see if the biomass was constant.

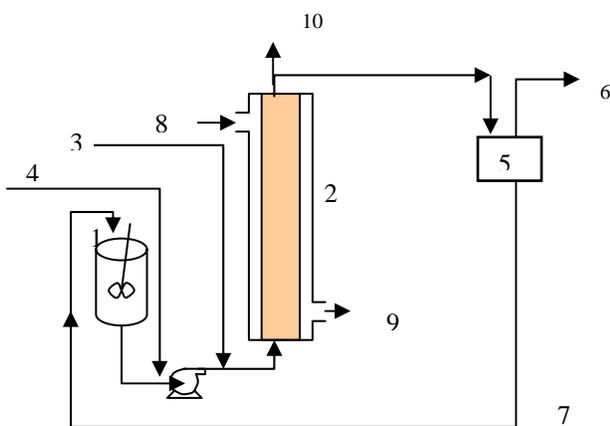


Figure 1: Schematic representation of experimental set up: 1. Mixing tank with magnetic stirrer, 2. Packed-bed bioreactor, 3. Sterilized air, 4. Inocula, 5. Membrane filter, 6. Concentrated cell-free xanthan gum, 7. Permeate substrate and medium recycled stream, 8, 9. Water 10. Air out.

RESULTS AND DISCUSSION

The packed bed and free cell batch fermentations

Figure 2 and 3 show the effects of packing density on the concentration of biomass in the liquid phase and the xanthan gum production throughout seven days batch fermentation. As can be seen in Figure 2, after 4 days of fermentation, the biomass concentration in the liquid phase was almost adsorbed. The 0.039g/litre packing density showed almost complete biomass adsorption. In Figure 3, results also indicated that 0.039 g/litre packing density produced significantly higher xanthan gum concentration at 20.63 g/litre. Krishnaiah et al. (2006)

also obtained similar xanthan gum concentration using glucose as a substrate. Low xanthan gum production in the free cell fermentation (control experiment) could be due to the stress caused by movement of the cell thus reducing the activity as compared to the immobilized cells. The immobilized cell may also actively produce the xanthan gum by staying still on the packed bed support. The same finding was reported by Yang et al. (1998). This is also supported by the fact that the natural behaviour of *X. campestris* is to the gum while staying attached to plant surfaces.

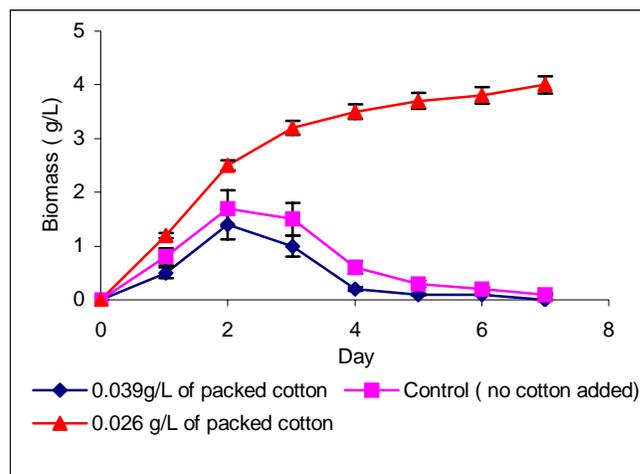


Figure 2: Biomass adsorption (immobilization) on different packing densities at 30 °C

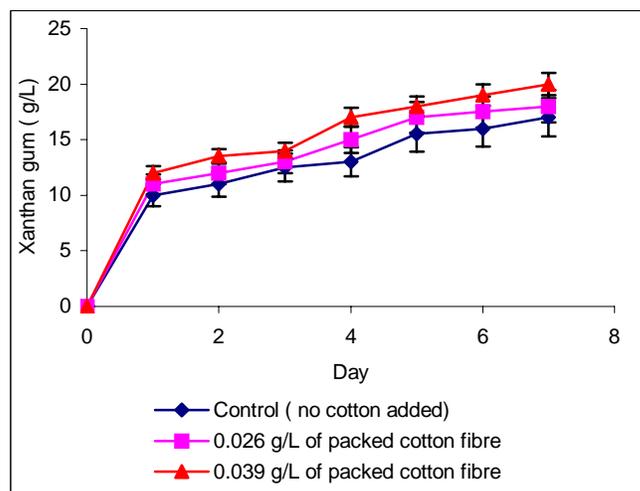


Figure 3: Xanthan gum production by different packing densities

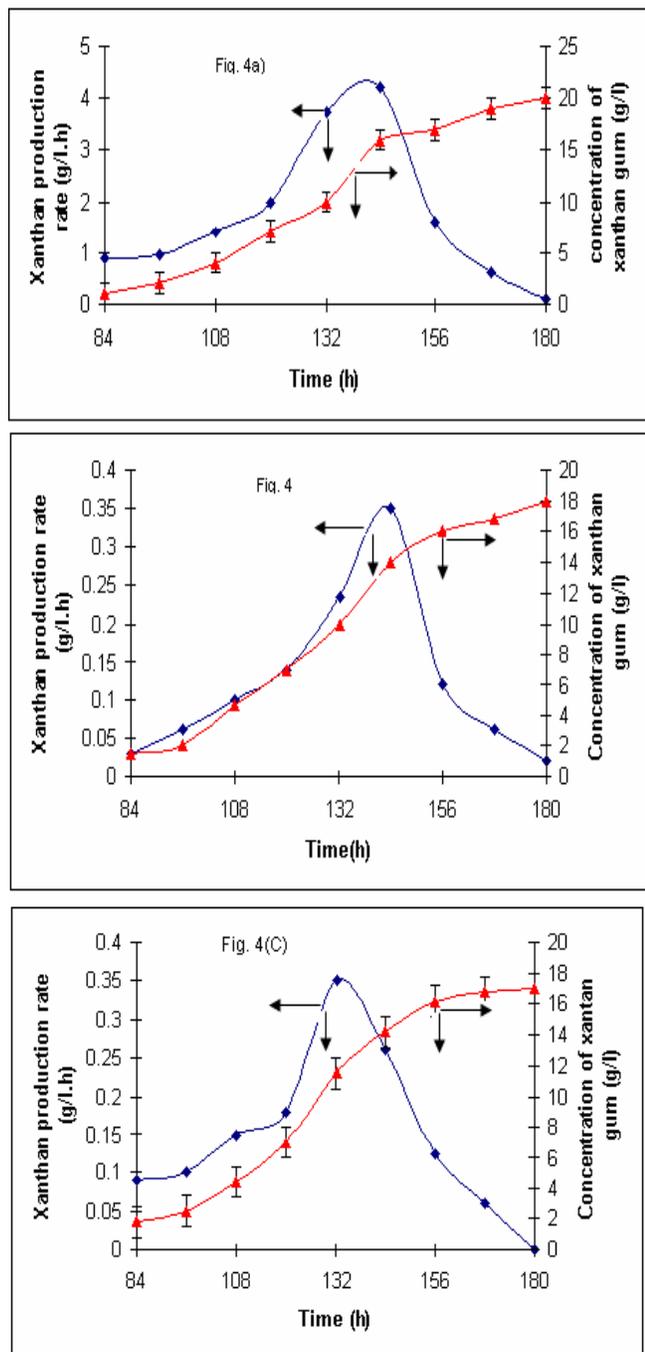


Figure 4: Xanthan gum production by immobilized cells in packed bed reactor at dilution rates of (a) $1.44d^{-1}$, (b) $2.88d^{-1}$, and (c) $4.32d^{-1}$

Continuous recycled packed fibrous-bed membrane bioreactor (CRPFMBB)

Xanthan gum purity

The xanthan gum purity from day four to day seven recovered by the membrane filtration system for the CRPFMBB was found to be greater than 98%. Lo et al. (1996) also reported the same finding that the cell free xanthan gum broth by immobilizing in fibrous matrix supports did not significantly foul the membrane during the entire period.

The effect of dilution rate

Figure 4 (a), (b), (c) show sets of fed-batch continuous fermentation with a dilution rate of $1.44 d^{-1}$, $2.88 d^{-1}$, and $4.32 d^{-1}$ while other parameters remained the same. The concentration of xanthan gum was highest at 144 hour. The continuous production of xanthan gum produced by *X. campestris* in a packed-bed bioreactor, equipped with $0.2 \mu m$ pore size cellulose acetate membrane module, was successfully performed over a period of 180 hour each set. In fed-batch continuous fermentation, the kinetics of fermentation and production of xanthan gum were investigated at three dilution rates, $1.44d^{-1}$, $2.88d^{-1}$ and $4.32d^{-1}$. The best fermentation performance was obtained at a dilution rate of $1.44 d^{-1}$ where the concentration of the xanthan gum produced was $18.7 g/L$. The production was low when the dilution rate was high. The highest production rate of the fed-batch continuous fermentation in this research work was obtained at $0.475 g/L-h$.

CONCLUSIONS

The optimal conditions for cell growth and xanthan gum biosynthesis are known to be quite different. Cells present in the xanthan fermentation broth can be separated by using cell adsorption to fibres provided that the xanthan gum has been produced. This adsorption process would allow high purity cell free xanthan gum for industrial application. The highest fermentation rate was obtained at a dilution rate of $1.44d^{-1}$. About $18 g/L$ to $20.6 g/L$ of xanthan was produced from the immobilised batch fermentation while the fed-batch production gave 17.2 to $18.7 g/L$ of xanthan gum.

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