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Xanthan Production on Polyurethane Foam and Its Enhancement by Air Pressure Pulsation

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Abstract In this study, we evaluated the feasibility of solid-state fermentation (SSF) on polyurethane foam (PUF) for xanthan production. The effects of air pressure pulsation (APP) on biomass accumulation and final xanthan concentration were also studied. Under suitable conditions (15% inoculum, 0.5-cm (side length) PUF cubes, 15 mL medium per gram cubes and 4.5 cm bed depth), the broth was dispersed on the PUF as a film. When the initial glucose concentration in the media was low (20 and 40 g L^{-1}), there was no significant difference between the final xanthan concentration in static SSF and submerged fermentation (SMF). When high initial glucose concentrations (60 and 80 g L^{-1}) were used, the final gum concentrations in SSF were much higher than those in SMF. When the APP technique was applied in xanthan production with a medium containing a high glucose concentration (80 g L^{-1}), the oxygen consumption rate of *Xanthomonas campestris* was significantly enhanced at the later stages of fermentation, and both the biomass and xanthan concentration were improved. The results indicated that SSF on PUF is suitable for xanthan preparation, especially when the initial glucose concentration ranged from 60 to 80 g L^{-1}. Those results also demonstrated that APP technology can be used to enhance xanthan yields.

Keywords Xanthan gum · Solid-state fermentation · Inert support · Air pressure pulsation · *Xanthomonas campestris*

Introduction

Xanthan gum is an extracellular heteropolysaccharide produced by *Xanthomonas campestris.* Because of its unique rheological properties, such as high viscosity and pseudoplasticity, xanthan has wide applications in industries such as food, pharmaceuticals, and oil and in other industries [1].

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Xanthan is usually produced industrially by submerged fermentation (SMF) [1–4]. Because of the high viscosity of xanthan gum-containing solutions, there is stagnant fluid in the fermentor when the gum concentration is high. The oxygen transfer resistance caused by these stagnant zones is a primary restriction in xanthan production (SMF) [1, 5–8].

Compared with SMF, solid-state fermentation (SSF), which is defined as the growth of microorganisms on solid substrates or supports in the absence of free-flowing water [9], often has several advantages: good aeration, low energy consumption, and high yields [10–12]. Currently, many products are produced by this technique, such as enzymes [13–15], organic acids [16], and antibiotics [17].

Stredansky et al. [18] demonstrated the feasibility of xanthan production using SSF. In their study, spent malt grains were used as the inert support. As it was difficult to separate the gum from spent malt grains, polyurethane foam (PUF), which has been used in the production of organic acids [19], enzymes [20], and antibiotics [21], was chosen as the inert carrier for the present study. Compared with other materials such as spent malt grains and ion-exchange resins, PUF has more homogeneous porous structure, and PUF can be reused. More importantly, the post-treatment is simpler as the broth in the PUF cubes can be collected easily without dilution.

Like SMF, some methods should to be used to enhance the transfer process in SSF. In the present study, air pressure pulsation (APP), a novel strategy for enhancing oxygen transfer using the force of normal air pressure [22–24], was introduced to xanthan production using SSF. The pressure change can enhance the oxygen transfer between air and liquid. When the pressure was high, more oxygen was dissolved in the liquid absorbed on the substrate or support than under normal atmospheric pressure, while when the pressure was suddenly reduced to the ambient atmospheric pressure, the gas flow could be significantly improved [25]. Recently, the SSF bioreactor based on APP has been successfully used in the production of enzymes [26, 27] and bio-pesticides [23] because of the good aeration achieved by this system. Zeng et al. [26] demonstrated that the respiration of *Aspergillus niger* was enhanced by this technique. Chen et al. [23] applied the technique in an industrial-scale SSF fermentor (70 m^3) for production of *Bacillus thuringiensis* and found that gas transfer was improved by APP because the porosity of the substrate was improved.

The aim of the present study was to evaluate the suitability of SSF on PUF for xanthan preparation and to determine the effects of APP on xanthan yields when using solid-state cultivation.

Materials and Methods

Microorganisms and Inoculum Preparation

X. campestris CGMCC 1.1781, obtained from the China General Microbiological Culture Collection Center, was used in the present study. The bacteria were cultured on potato dextrose agar slants (pH 6.5) at 30°C for 72 h and preserved at 4°C for short-term storage (less than 2 weeks).

In the present study, suspension of *X. campestris* was used as inoculum. To prepare the inoculum, 10 mL of sterile distilled water was added to agar culture in test tube, and the bacterial lawn was scraped with an inoculation needle under strict aseptic conditions. Then the liquid in test tube was transferred into a sterile Erlenmeyer flask containing glass beads (diameter 0.5 cm), and the suspension was prepared by shaking [28]. The cell concentration of the suspension was about 4.8×10^8 cells per millilitre (determined by a haemacytometer).

Media and Inert Supports

To determine the suitable SSF condition, the basal medium was used which had the following composition [29] of 30 g L^{-1} glucose, 1 g L^{-1} NH_4NO_3, 2 g L^{-1} yeast extract powder, 0.25 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.1 g L^{-1} $Na_2HPO_4{\cdot}12H_2O$, and 3 g L^{-1} $CaCO_3$ (Table 1).

The media for the comparison of SMF, static SSF, and APP-SSF contained different glucose concentrations as specified (including 20, 40, 60, and 80 g L^{-1}), and all media had the same ratio of carbon to nitrogen and $CaCO_3$ as the basal medium (Table 2).

The PUF, which was used as an inert support, was cut into cubes of desired side lengths (including 0.5, 1.0, and 1.5 cm), washed twice with tap water, and dried in an oven before use.

Media and PUF cubes in all experiments were sterilised in an autoclave at 121°C for 15 min before use.

Determination of Suitable SSF Culture Conditions

The experiments to determine the suitable SSF culture conditions were carried out in 150-mL beakers (6 cm in diameter) containing PUF cubes, and the beakers were cultured in an incubator. After the beakers containing PUF cubes were sterilised and cooled to room temperature, basal media and inoculum were added in. Then the PUF cubes in these beakers were stirred using a glass stick under strict aseptic conditions.

The process parameters were varied: inoculum size (5–25%), side length of PUF cubes (0.5–1.5 cm), volume of added moistening medium per gram PUF cubes (5–25 mL), and bed depth (1.5–6 cm) (Table 1).

The procedure, adopted for the determination of xanthan production, was to evaluate the effects of individual parameters while keeping all other parameters constant (Table 1). All the beakers were cultured at 30°C for 72 h.

The experiments were performed in triplicate. Average values and the standard deviation are reported.

Table 1 Parameters and nutrition concentrations used in the experiment to determine the suitable SSF condition.

		Test 1	Test 2	Test 3	Test 4
Parameters	Inoculum amount (*v*/*v*) (%)	5, 10, 15, 20, 25	15	10	15
	Side length of PUF cubes (cm)	0.5	0.5, 1.0, 1.5	0.5	0.5
	Added medium per gram PUF (mL)	10	10	5, 10, 15, 20, 25	10
	Bed depth (cm)	3	3	4.5	1.5, 3, 4.5, 6
Nutrition concentration (g L^{-1})	Glucose	30	30	30	30
	NH_4NO_3	1	1	1	1
	Yeast extract powder	2	2	2	2
	$MgSO_4{\cdot}7H_2O$	0.25	0.25	0.25	0.25
	$Na_2HPO_4{\cdot}12H_2O$	0.1	0.1	0.1	0.1
	$CaCO_3$	3	3	3	3

Table 2 Four conditions (media concentrations) used in the experiments to compare the difference among SMF, static SSF, and APP-SSF.

Compound	Concentration (g L^{-1})			
Glucose	20	40	60	80
NH_4NO_3	0.67	1.33	2	2.67
Yeast extract powder	1.33	2.66	4	5.33
$MgSO_4 \cdot 7H_2O$	0.25	0.25	0.25	0.25
$Na_2HPO_4 \cdot 12H_2O$	0.1	0.1	0.1	0.1
$CaCO_3$	2	4	6	8

Static SSF and APP-SSF Performed in APP-Bioreactor

After the suitable conditions of SSF were determined, other SSF experiments (including static SSF and APP-SSF) were carried out in beakers too (the same as the beaker used in the experiment to determine the suitable SSF culture conditions), and the beakers were cultured in an APP-SSF bioreactor [30].

The APP-SSF bioreactor used in the present study was a 32.5-cm long, 33-cm diameter sealed horizontal-type cylinder, in which there was a shelf for holding beakers. Figure 1 shows the photograph and schematic diagram of the bioreactor.

The pressure variation in APP-SSF bioreactor was controlled by an electromagnetic valve connected to time relays. The periodical pulsation of air was regulated with a computer control system. Each period had three stages. First, the air inlet valve was on, the air outlet valve was off and the compressed, sterilised air entered the fermentation vessel rapidly until the air pressure reached 0.2 MPa. Then the inlet valve was closed, and the air pressure was maintained for 10 s. Finally, the outlet valve was opened, and the air pressure in the bioreactor decreased to the level equal to the air pressure outside the bioreactor. Then the next period began. The time interval between two periods was 20 min. When the static

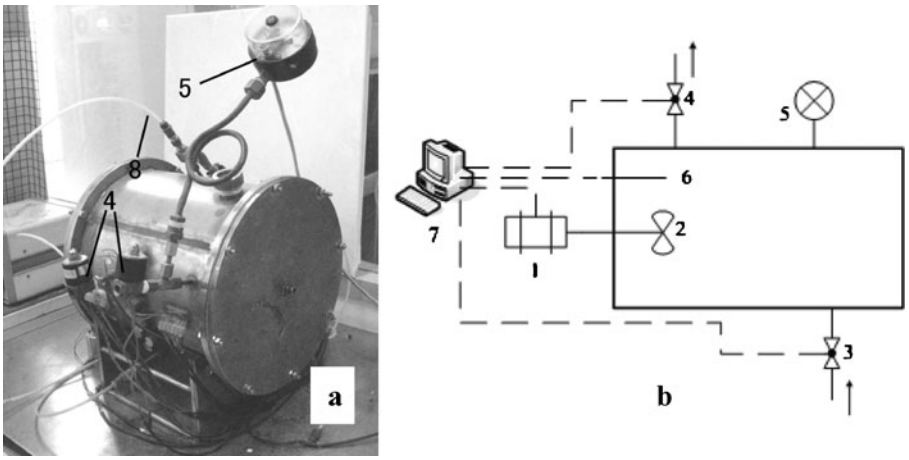

Fig. 1 The photograph and schematic diagram of APP-bioreactor used in present study. **a** Photograph of APP-bioreactor. **b** Schematic diagram of APP-bioreactor. Key parts to the process: (*1*) variable speed motor; (*2*) fan; (*3*) electric valve of air inlet; (*4*) electric valve of air outlet; (*5*) air pressure meter; (*6*) temperature probes; (*7*) computer; (*8*) circulation loop connect to online gas analyser

SSF was performed in the bioreactor, both the inlet valve and outlet valve were off, and the air in bioreactor was changed once every 4 h.

The temperature and relative humidity (RH) of the air entering the bioreactor were maintained at 30±1°C and 90–97% RH, respectively.

The values of process parameters such as inoculum size, side length of PUF cubes, volume of added moistening medium per gram PUF cubes, and bed depth were obtained from the results of "Determination of Suitable SSF Culture Conditions".

The experiments were performed in triplicate. Average values and the standard deviation are reported.

Submerged Fermentation

SMF in shake flasks was carried out in 250-mL Erlenmeyer flasks containing 30 mL of medium, inoculated with a 10% (*v*/*v*) inoculum. Incubation was done at 30°C on a rotatory shaker (220 rpm) for 72 h. The experiments were performed in triplicate. Average values and the standard deviation are reported.

SMF in stirred-tank bioreactors was performed in 3-L NBS BioFlo 110 bioreactors (New Brunswick Scientific, USA). The working volume was 2 L. The inoculum was 10% (*v*/*v*). The temperature, airflow rate, and stirrer rate were set to 30°C, 1 vvm and 600 rpm, respectively [2]. All SMF experiments performed in stirred-tank bioreactors were replicated twice, and average values are presented.

Biomass, Glucose, and Xanthan Concentration Determinations

In liquid cultures, the pH was adjusted to 3 at the end of fermentation with an HCl solution (5 mol L^{-1}) to remove the residual $CaCO_3$ in the broth. Then 2 mL of the broth was used to determine the biomass and sugar concentrations. The sample was diluted by a factor of 10 with deionised water. A 10-mL portion of the diluted sample was centrifuged at 14,000×*g* for 30 min at 5°C. The pellet was re-suspended in deionised water and analysed with a spectrophotometer to obtain the biomass concentration [31]. The rest of the diluted sample was used to determine the glucose concentration using the dinitrosalicylic acid reagent method [32]. After the biomass and sugar concentrations were determined, the rest of the broth was precipitated with three volumes of 95% ethanol to determine the xanthan concentration. The precipitation was filtered and dried at 60°C for 24 h. The dried sample was then weighed to calculate the raw polysaccharide concentration. The xanthan concentration was determined as the difference between the concentration of raw polysaccharide and the total biomass.

In solid-state cultivation, biomass, sugar, and xanthan concentrations were determined after the broth in PUF cubes was collected using a plastic 60-mL syringe, at the front end of which several holes of 1 mm diameter were punched. To obtain accurate values for the xanthan concentrations, three volumes of deionised water were added to dilute the broth in the PUF before it was collected. The biomass, glucose, and xanthan concentrations in the broth were calculated using the dilution proportion.

Determination of the Oxygen Consumption Rate

The oxygen consumption rate was defined as the oxygen concentration decrease per litre per hour in the bioreactor. The gas in the APP-bioreactors was vented through a venting air detection system and the O_2 concentration was measured with an S710 online gas analyser (SICK MAIHACK GmbH, Germany).

Data Analysis

Four one-way analyses of variance (ANOVA) were conducted to examine the statistical significance of the between-group differences for the four fermentation conditions (SMF in flasks, SMF in a stirred tank, static SSF, and APP-SSF). Following the ANOVAs, a post hoc test was performed to reveal the differences between each pair of operation conditions. The analyses were performed using the SPSS 10.0 statistical package (SPSS Inc., USA).

Observation by Microscope

A sample of the static SSF culture was observed using a microscope (XSZ-H3, CN) equipped with an optical imaging system (WV-CP230, JP) after the sample was cultured for 48 h.

Results and Discussion

Determination of Suitable SSF Culture Conditions

To determine the suitable culture conditions for static SSF, four factors, the amount of inoculum, the size of the PUF cubes, the ratio of media to cubes, and the bed depth on xanthan yields were investigated.

The effect of the inoculum amount on xanthan yields is shown in Fig. 2. Test 1 in Table 1 shows the tested inoculum amount and other constant process parameters. The xanthan concentration increased as the amount of inoculum increased up to 15% (*v*/*v*), and the maximum gum concentration was 16.1 g L^{-1}. When the amount of inoculum was over 15%, the xanthan concentration decreased with increases in the inoculum.

Figure 3 shows the effect of the size of PUF cubes on xanthan yield. In the present study, the PUF was cut into small cubes with side lengths of 0.5, 1.0, and 1.5 cm (test 2 in Table 1). The final concentration of xanthan gum decreased as the size of the cube

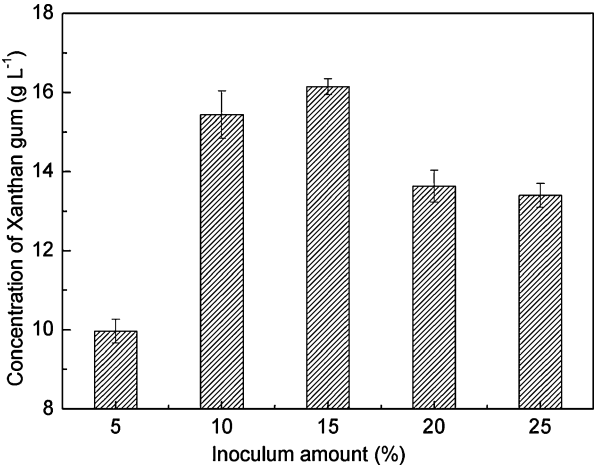

Fig. 2 Influence of inoculum amount on xanthan yield. Side length of PUF cubes, 0.5 cm, bed depth, 3 cm, moistening medium volume, 10 ml per gram PUF cubes, fermentation time, 72 h

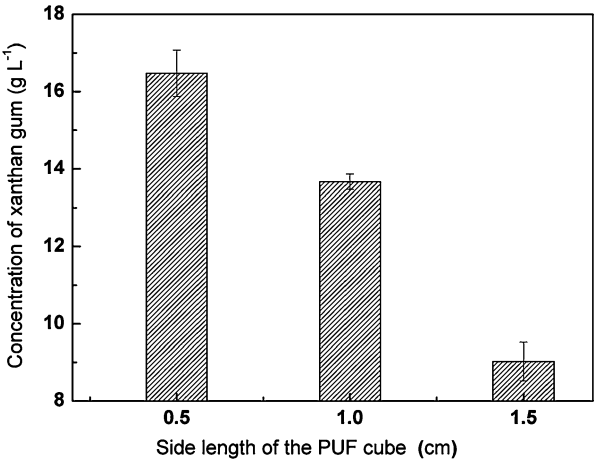

Fig. 3 Influence of the size of the inert support (PUF cubes) on the xanthan gum yield. Inoculom amount, 15% (*v*/*v*), bed depth, 3 cm, moistening medium volume, 10 ml per gram PUF cubes, fermentation time, 72 h

increased. When cubes with a side length of 0.5 cm were used, 16.5 g L^{-1} xanthan was obtained. When the side length was 1.5 cm, the xanthan concentration in the broth was only 9.0 g L^{-1}. The results suggest that under static conditions, smaller PUF cubes are better for xanthan production. Cubes with sizes smaller than 0.5×0.5×0.5 cm were not convenient to prepare, so we did not culture the *X. campestris* on smaller cubes.

Figure 4 shows the effect of the ratio of medium volume to PUF cubes on xanthan yield. Test 3 in Table 1 shows the tested medium amount per gram PUF and other constant process parameters. The xanthan yield appeared to decrease when the ratio of medium volume to PUF cubes increased, and the maximum xanthan amount obtained was 15.9 g L^{-1} at the ratio of 15:1 (mL:g). The observation that too much medium reduced xanthan gum yield may be due to the fact that the amount of medium exceeding the

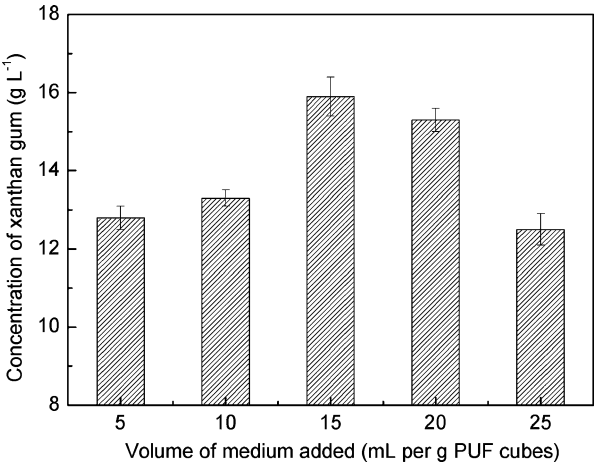

Fig. 4 Influence of moistening medium volume on the xanthan yield. Inoculom amount, 10% (*v*/*v*), side length of PUF cubes, 0.5 cm, bed depth, 4.5 cm, fermentation time, 72 h

dispersion capacity of the PUF restricted oxygen transfer from the environment to the interior of the PUF cubes.

The effect of bed depth on xanthan gum yield is shown in Fig. 5. Test 4 in Table 1 shows the tested depth and other constant process parameters. Xanthan yield increased as the bed depth increased up to 4.5 cm, and the maximum concentration obtained was 17.3 g L^{-1}. When the bed depth was over 4.5 cm, the gum yield decreased with increasing bed depth. The bed depth of the substrate was an important parameter. In the present study, oxygen was supplied by air infiltration. Bed depths that were too high negatively affected the respiration of the bacteria.

From the above results, we identified suitable conditions for xanthan fermentation under static SSF. These conditions are a 15% inoculum, 0.5-cm (side length) PUF cubes, 15 mL medium per gram PUF cube and a 4.5-cm bed depth.

Observation of Broth Distribution in PUF Cubes

In the present study, the distribution of broth in the PUF was observed by microscopy after 48 h of culture (static SSF). Figure 6a shows a micrograph of a PUF cube impregnated with broth that was distributed as film. Figure 6b shows a micrograph of a clear PUF cube.

The two micrographs revealed that there were many small pores (approximately 700 μm in diameter) supported by the polyurethane skeleton in the PUF. If the pores were modelled as spheres, the specific surface area of the PUF was about 4.5×10^3 m^2/m^3. Accordingly, the thickness of the broth distributed on the inner surface of the PUF was about 0.1 mm, given that the liquid-to-solid ratio was 10 mL per gram PUF cube. In SMF production of xanthan, the primary restriction was oxygen transfer resistance caused by stagnant regions, while when SSF was performed, the broth was dispersed over many and stable surface of the PUF cubes. The large inner surface and the thin broth film were helpful for oxygen transfer from the air to the liquid. Moreover, the gas phase above the inner surface of the PUF was continuous, which contributed to oxygen transfer from the outside to the inside. In addition, the recovery of broth distributed in PUF is not difficult. In the present study, broth was collected using a plastic 60-mL syringe, at the front end of which several holes of 1 mm diameter were punched. If an industry process is implemented, some equipment, such as

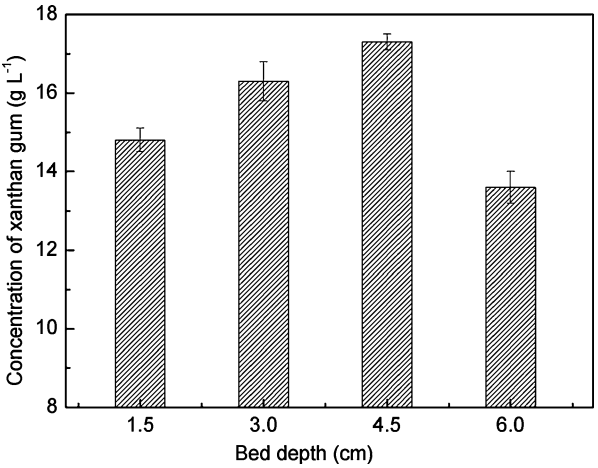

Fig. 5 Influence of bed depth on xanthan yield. Inoculom amount, 15% (*v*/*v*), side length of PUF cubes, 0.5 cm, moistening medium volume, 10 ml per gram PUF cubes, fermentation time, 72 h

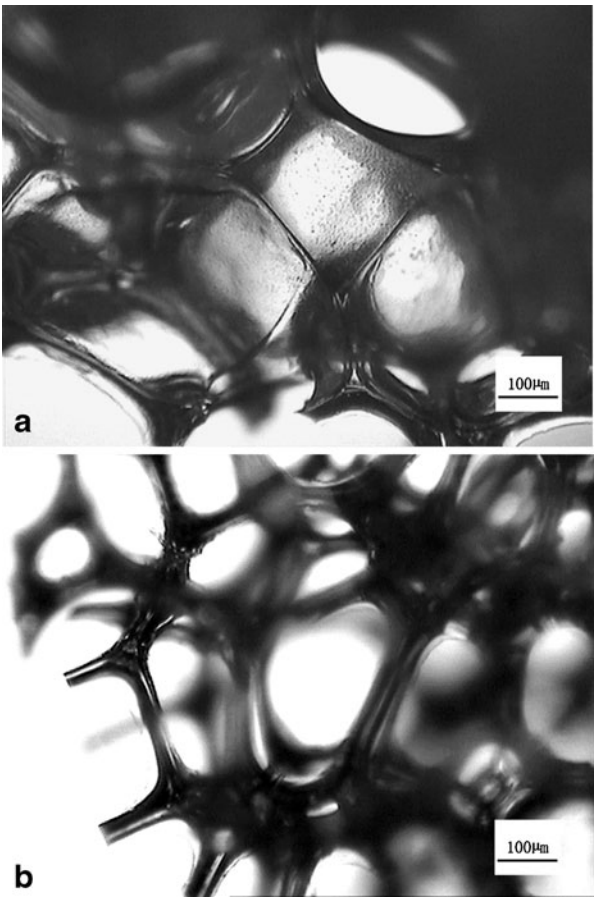

Fig. 6 Micrographs of the PUF used in SSF. **a** A micrograph of a PUF cube impregnated with broth cultured for 48 h. **b** A micrograph of a clear PUF cube

sugarcane crusher, can be used to separate the broth from PUF. Hence, PUF has the potential to be a good support for xanthan production.

Until recently, most of the microorganisms grown using SSF on inert supports were filamentous fungi [11]. The growth state of these fungi on these supports was similar to that shown in the micrograph of *A. niger* growing in PUF reported by Viniegra-González et al. [33], in which the pores were filled with mycelia. In the present study, the producing strain was the bacillus *X. campestris*. In contrast to filamentous fungi, which respire via aerial hypha, the bacteria were suspended in liquid broth and absorbed dissolved O_2. Hence, the methods for oxygen transfer enhancement used for SSF production of filamentous fungi, for example forced aeration and APP, might be more effective in xanthan production on PUF because these methods could enhance oxygen transfer from the air to the liquid film.

Comparison of the Xanthan Yields of Static SSF on PUF and SMF

To investigate the suitability of SSF on PUF for xanthan production, we compared the xanthan yields of static SSF and SMF. Because a carbon source concentration in batch

mode (SMF) of 40 g L^{-1} is commonly used [1], two high concentrations (60 and 80 g L^{-1}) and two low concentrations (20 and 40 g L^{-1}) were used in the present study. The results of SMF in shake flasks and in a stirred-tank bioreactor are summarised in Table 3.

Four one-way ANOVAs were conducted to examine the statistical significance of the between-group differences of the four fermentation conditions (SMF in flasks, SMF in a stirred-tank bioreactor, static SSF, and APP-SSF). Following ANOVA, a post hoc test was performed to reveal the differences between each pair of operation conditions. The results of the ANOVA are shown in Table 4.

Table 3 shows that for SMF, the difference between the xanthan final concentrations obtained from shake flasks and from the stirred-tank bioreactor was statistically insignificant. On the other hand, using the two types of fermentors, the biomass concentration increased as the glucose concentration increased from 40 to 80 gL^{-1}, but xanthan production did not increase. This is consistent with the results of a previous report [1, 6]. The reason of this phenomenon was that at the later stage, the region near the impeller was well mixed while the surrounding broth was stagnant. The stagnant region led to oxygen transfer resistance [5, 6].

The difference between xanthan final concentrations in static SSF and SMF (shake flask and stirred tank) were statistically insignificant when the initial glucose concentration was low (such as 20 and 40 g L^{-1}). When the glucose concentration was increased to 60 and 80 g L^{-1}, the post hoc test showed that the final xanthan concentrations obtained by static SSF were significantly higher than those obtained by SMF. Additionally, there was a positive correlation between the final gum concentration and the initial glucose concentration in static SSF. These results indicate that SSF on PUF is suitable for xanthan gum production, especially when media with high glucose concentrations (above 40 g L^{-1}) are used.

Table 3 Comparison of xanthan production among SMF, static SSF, and APP-SSF (when the glucose concentrations were 20 and 40 g L^{-1}, the fermentation time was 48 h; when glucose concentrations were 60 and 80 g L^{-1}, the fermentation time was 96 h).

		Initial glucose concentration (g L^{-1})	Xanthan concentration (g L^{-1})	Biomass concentration (g L^{-1})	Residual glucose (g L^{-1})	Xanthan yield from glucose (g g^{-1})
SMF	In shake flasks	20	11.21±0.71	1.01±0.21	1.45±0.20	0.56
		40	21.85±0.36	1.49±0.29	5.39±0.11	0.55
		60	22.81±0.89	2.59±0.08	21.30±0.05	0.38
		80	21.39±0.74	3.94±0.19	38.10±0.23	0.27
	In stirred-tank[a]	40	22.40	1.18	4.23	0.56
		80	23.24	3.66	35.35	0.29
SSF	Static SSF	20	11.79±0.53	0.98±0.24	0	0.59
		40	22.19±0.44	1.81±0.23	0.37±0.12	0.55
		60	30.73±0.98	2.88±0.33	0.46±0.11	0.51
		80	38.65±1.01	3.84±0.37	1.65±0.30	0.48
	APP-SSF	20	11.46±0.29	0.85±0.14	0	0.57
		40	22.90±0.47	2.23±0.44	0.38±0.08	0.57
		60	33.54±0.91	3.37±0.09	0.52±0.07	0.56
		80	42.62±0.77	4.67±0.23	1.78±0.13	0.53

[a] The SMF experiments performed in stirred-tank bioreactors were replicated twice, and averaged values are presented

Table 4 Results of one-way analysis of variance for the concentrations of xanthan, biomass, and residual sugar in four fermentation condition (SMF in flask, SMF in stirred-tank, static SSF, and APP-SSF).

	Xanthan concentration			Biomass concentration			Residual sugar concentration		
	df	MS	*F*	df	MS	*F*	df	MS	*F*
20	2	0.08	0.50	2	0.02	0.537	–	–	–
40	3	0.628	1.415	3	0.51	4.70*	3	19.78	365.10**
60	2	60.03	76.44**	2	0.471	11.182**	2	433.89	56810.82**
80	3	320.875	420.478**	3	0.64	4.24[a]	3	1135.11	370.24**

df degree of freedom, *MS* mean square

*$p<0.05$; **$p<0.01$

[a] Marginal significance ($p=0.053$)

When a high initial glucose concentration (60 and 80 g L^{-1}) was used, the difference between the final biomass concentrations obtained by static SSF and SMF (in shake flasks and in a stirred-tank bioreactor) were statistically insignificant, although the xanthan yields in static SSF were higher than those obtained for SMF (Table 3). On the other hand, the residual sugar concentrations obtained with static SSF were significantly lower than those obtained with SMF. This result indicates that the improvement of the final gum concentration was the result of the more efficient conversion of glucose to xanthan with static SSF.

As reported previously, oxygen transfer resistance in stagnant zones was the key limiting factor in xanthan fermentation (SMF) [1, 6], while the large surface area of the porous PUF cubes enhanced oxygen transfer. Hence, in this study, the reason for the high conversion rate of glucose to gum in SSF using media with a high glucose concentration might be the better aeration conditions.

Amanullah et al. investigated the influence of dissolved oxygen [5], agitation [34], and the glucose-feeding strategy [35] on xanthan yield. They concluded that improved yields cannot be achieved in batch SMF by increasing the initial glucose concentration above 50 g L^{-1} because it was found that when initial glucose concentration was 50 g L^{-1}, xanthan production was 33.1 g L^{-1} while when that increased to 54 g L^{-1}, bacteria growth was completely inhibited [35]. Some authors owned this to glucose inhibition [36]. However, in the present study, no significant glucose inhibition of bacteria growth was observed when SSF was performed and as the initial glucose concentration increased from 20 to 80 g L^{-1}, the final gum concentration increased from 11.79 to 38.65 g L^{-1}. Hence, making *X. campestris* acclimate to the elevated glucose concentration might be an advantage of SSF on PUF.

We also showed that when the media contained 60 or 80 g L^{-1} glucose, the xanthan yields from glucose in static SSF (0.51 and 0.48 g g^{-1}) were significantly lower than the results obtained when using the media containing 20 and 40 g L^{-1} glucose (0.59 and 0.55 g g^{-1}), although the values were much higher than those obtained with SMF (Table 3). This result is consistent with the report of Stredansky et al. [18] who studied xanthan fermentation under SSF on spent malt grains in a packed-bed bioreactor. The xanthan yield from sucrose in media containing 100 g L^{-1} sucrose (0.41 g g^{-1}) was significantly lower than the yield of cultures grown in media containing 50 g L^{-1} sucrose (0.63 g g^{-1}). Hence, when high initial glucose concentrations are used in SSF, the xanthan yield might be further improved.

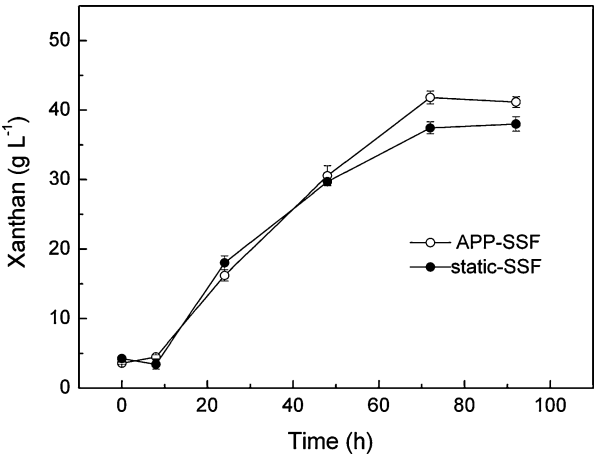

Fig. 7 Comparison of the fermentation curves for xanthan production under APP-SSF and static SSF using the medium containing 80 g L^{-1} glucose

Effects of APP on Xanthan SSF

The APP technique was used with xanthan fermentation (SSF) to improve the final gum concentration. To evaluate the effect of this technique, several aspects of static SSF and APP-SSF were compared. The fermentation curve and the oxygen consumption rate (OCR) obtained when using a high initial glucose concentration are shown in Figs. 7 and 8. The final gum concentrations, biomass concentrations, and residual sugar concentrations are summarised in Table 3. A one-way ANOVA and a post hoc test were also performed (Table 4).

The results of the ANOVA (Table 4) and post hoc test showed that when a low initial glucose concentration (20 and 40 g L^{-1}) was used, the difference between the final xanthan

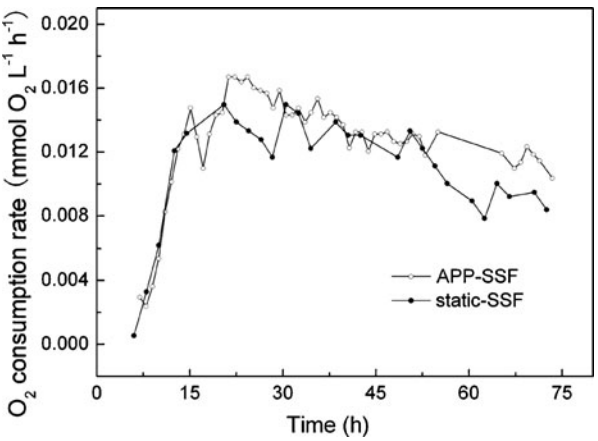

Fig. 8 Comparison of the oxygen consumption rate for xanthan production under APP-SSF and static SSF using the medium containing 80 g L^{-1} glucose

concentrations obtained with APP-SSF and static SSF was statistically insignificant; when the glucose was increased to 60 and 80 g L^{-1}, the final gum concentrations obtained with APP-SSF were higher than those obtained with static SSF. These results suggest that xanthan production is significantly enhanced by APP when the initial glucose concentration is high.

In the present study, final biomass concentration increased with the concentration of medium (carbon–nitrogen ratio was constant in all medium used in present study). When the glucose concentration was 60 and 80 g L^{-1}, the biomass concentration was much higher than that derived from low-concentration medium whether the APP was performed or not (Table 3). High biomass concentration certainly requires high oxygen uptake. Hence, as an effective strategy to enhance oxygen transfer, APP showed more significant effect when the medium concentration was higher.

Figures 7 and 8 show the comparison of the fermentation curves and the OCRs for APP-SSF and static SSF when using the medium containing 80 g L^{-1} glucose. The gum concentrations and the OCR for APP-SSF were higher than those for static SSF at the later stages of fermentation (after about 50 h). This observation is consistent with previous research [37], which showed that oxygen transfer resistance generally appears at the later stages of cultivation because of the increasing viscosity of the broth. The two figures (Figs. 7 and 8) suggest that there was oxygen resistance in the static SSF cultures when high glucose concentrations were used, although higher yields were obtained compared with SMF; the improvement in xanthan yield with APP-SSF occurred because APP increased the OCR of *X. campestris*.

As also shown in Table 3, the difference between the concentrations of residual sugar obtained with static SSF and APP-SSF were statistically insignificant when the glucose concentration in the media was 60 or 80 g L^{-1}. Thus, the enhancement of the final xanthan concentration was not due to greater utilisation of the carbon source. The reason for the higher final xanthan concentration might be that APP improved the product yield.

The post hoc test showed that when the initial glucose concentration in the media was 60 or 80 g L^{-1}, the biomass concentrations obtained with APP-SSF were higher than those obtained with static SSF (see Table 3) (when the glucose concentration was 40 g L^{-1}, the mean biomass concentration obtained with static SSF was higher than that obtained with APP−SSF; however, the difference was not statistically significant). These results indicate that APP improved the biomass accumulation. This is consistent with a previous report by Li et al. [38], who studied cellulase production using an APP-SSF bioreactor and found that not only the cellulase yield was enhanced, but also the biomass yield was 1.04 times higher than that of the control. There are two possible reasons for the improvement of biomass: (a) APP enhances oxygen transfer; (b) the cells are stimulated by the air pressure. Further work is required to prove those hypotheses.

Stredansky et al. [18] studied two methods, rotation and forced aeration, to enhance the xanthan yield under SSF and concluded that forced aeration was a good choice. However, even if the operation was performed, the yield of xanthan from sucrose at a high sucrose concentration (100 g L^{-1}) was 44.2%, which is lower than the yield obtained with cultures grown in media containing 50 g L^{-1} sucrose. In the present study, APP was performed to enhance the xanthan yield. When the glucose concentration was 80 g L^{-1}, the xanthan yield from glucose (0.53 g g^{-1}) was slightly lower than that obtained with the media containing 20 g L^{-1} glucose (0.57 g g^{-1}). Moreover, the final xanthan concentration in APP-SSF derived from media containing 80 g L^{-1} glucose was 42.62 g L^{-1}. This final gum concentration was not only higher than the corresponding concentration for static SSF, but it was also higher than the final xanthan concentrations obtained using the batch operation

(SMF) reported in previous research [34]. Hence, the APP technique is a more efficient method for increasing xanthan yields when using SSF.

Conclusions

SSF on PUF is suitable for xanthan preparation, especially when the initial glucose concentration is between 60 and 80 g L^{-1}. APP technology can be used to enhance xanthan production. Hence, SSF on PUF has the potential to be used in xanthan production.

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