Bioprocess Biosyst Eng (2011) 34:95–102
DOI 10.1007/s00449-010-0450-6

ORIGINAL PAPER

Enhanced welan gum production using a two-stage agitation speed control strategy in *Alcaligenes* sp. CGMCC2428

Hui Li · Hong Xu · Hao Xu · Sha Li · Han-Jie Ying · Ping-Kai Ouyang

Received: 15 April 2010 / Accepted: 2 July 2010 / Published online: 17 July 2010

Abstract Batch fermentative production of welan gum by *Alcaligenes* sp. CGMCC2428 was investigated under various oxygen supply conditions using regulating agitation speed. Based on a three kinetic parameters analysis that includes specific cell growth rate (μ), specific glucose consumption rate (q_s), and specific welan formation rate (q_p), a two-stage agitation speed control strategy was proposed to achieve high concentration, high yield, and high viscosity of welan. During the first 22 h, the agitation speed in 7.5 L fermenter was controlled at 800 rpm to maintain high μ for cell growth. The agitation was then reduced step-wise to 600 rpm to maintain a changing profile with stable dissolved oxygen levels and obtain high qp for high welan accumulation. Finally, the maximum concentration of welan was reached at 26.3 ± 0.89 g L^{-1} with a yield of 0.53 ± 0.003 g g^{-1} and the welan gum viscosity of 3.05 ± 0.10 Pa s, which increased by an average of 15.4, 15.2, and 20.1% over the best results controlled by constant agitation speeds.

Keywords Welan gum · *Alcaligenes* sp. CGMCC2428 · Agitation speed · Two-stage control strategy · Batch fermentation

H. Li · Hong Xu (✉) · Hao Xu · S. Li · P.-K. Ouyang
State Key Laboratory of Materials-Oriented Chemical Engineering, College of Food Science and Light Industry, Nanjing University of Technology, No. 5 Xinmofan Road, Gulou District, Nanjing 210009, China
e-mail: xuh@njut.edu.cn

H.-J. Ying
State Key Laboratory of Materials-Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, National Engineering Technique Research Center for Biotechnology, Nanjing University of Technology, No. 5 Xinmofan Road, Gulou District, Nanjing 210009, China

Introduction

Welan gum is an exopolysaccharide produced by the *Alcaligenes* species [1]. It is composed of tetrasaccharide repeating units of D-glucose, D-glucuronic acid, D-glucose, and L-rhamnose, with L-rhamnopyranosyl or L-mannopyranosyl side chains [2]. At least 85% of the repeat units also have an acetyl substituent at O(2) of the 3-linked glucose. Welan gum biosynthesis represents a multi-step process and essentially follows essentially the mechanisms established for other acidic heteropolysaccharides of Gram-negative bacteria [3]. The pathway can be divided into three different components: (1) the intracellular synthesis of sugar-activated precursors, (2) the assembly of the tetrasaccharide attached to a membrane-anchored C55-isoprenyl pyrophosphate carrier, and (3) the polymerization of the repeat units and export of the polysaccharide. Because of the interesting rheological properties of welan gum, it is used in a wide variety of applications, including as a suspending, stabilizing, emulsifying, and thickening agent in several areas such as food, coating materials, medicine, concrete additives, and enhanced oil recovery [4]. The *Alcaligenes* species is an aerobic microorganism, and oxygen supply is an essential factor in aerobic fermentation processes, particularly in polysaccharide production because the broth becomes highly viscous and limits mass and oxygen transfer that influence cellular activities and metabolite production [5].

A previous study found that oxygen supply was a critical factor for high-level production of microbial polysaccharides, such as xanthan gum, gellan gum, and hyaluronic acid. Garcia-Ochoa found that an increase in oxygen supply enhanced the xanthan gum production [6]. Interestingly, Giavasis offers another opinion on the effect of oxygen supply, stating that high aeration rates and vigorous

agitation enhanced the growth of *Sphingomonas paucimobilis*, a gellan gum producer. However, the increase in cells did not always lead to high gellan production. At very high agitation rates (1,000 rpm), growth was stimulated at the expense of biopolymer synthesis [7]. A similar phenomenon was observed in the study of hyaluronic acid fermentation [8, 9]. In addition, an increase in agitation means that more energy is consumed for the process, thereby increasing production cost. Moreover, very high agitation has also been shown to cause mechanical damage to polysaccharides [10]. On the other hand, growth under low oxygen supply condition is unable to satisfy the oxygen demand of the cells, and often leads to lower gellan production [5]. In addition, as with other microbial polysaccharides, the viscosity of welan gum is likely to be influenced by oxygen supply [11]. Therefore, it is necessary to set up a proper oxygen supply strategy to ensure efficient polysaccharide production with high concentration, high yield, and high viscosity.

In this article, a simple oxygen supply method based on agitation speed control was established and successfully used for the enhanced production of welan gum. The processes of welan gum fermentation using *Alcaligenes* sp. CGMCC2428 were compared at different oxygen supply conditions by changing agitation speed. Subsequently, based on the kinetic analysis of batch processes controlled by single-agitation speed, a two-stage agitation speed control strategy was designed and confirmed experimentally.

Materials and methods

Microorganism and media

Alcaligenes sp. CGMCC2428 used in this study was deposited at the General Microbiological Culture Collection Center in China. The seed medium contained 20 g L^{-1} glucose, 1 g L^{-1} yeast extract, 3 g L^{-1} peptone, 2 g L^{-1} $K_2HPO_4{\cdot}3H_2O$, 0.1 g L^{-1} $MgSO_4$ at pH 7.2–7.4. The fermentation medium comprised 50 g L^{-1} glucose, 8 g L^{-1} $(NH_4)_2SO_4$, 3 g L^{-1} $K_2HPO_4{\cdot}3H_2O$, and 0.4 g L^{-1} $MgSO_4$. The initial pH was adjusted to 7.2–7.4.

Culture methods

Alcaligenes sp. CGMCC2428 was first inoculated into 135 mL of fresh seed medium in 1 L flasks and aerobically incubated for 16 h with shaking at 200 rpm. Seed culture (3%, v/v) was then inoculated into the fermentation medium. Batch fermentation was carried out in a 7.5-L stirred fermenter (Rushton-style impeller, d. 6 cm; bioreactor i.d. 17.8 cm, height 32.1 cm, 4.5 L working volume, BioFlo 110, New Brunswick Scientific, USA). All cultivations were carried out at 30 °C, and pH was controlled at 7.0 automatically by adding 3 M NaOH.

The aeration rate was controlled at 1.0 vvm for all the experiments. The agitation speed was controlled at 400, 600, 800, and 1,000 rpm in the batch fermentation. The dissolved oxygen (DO) concentrations under different operation conditions were expressed in DO concentration (%), whereas 100% DO saturation level corresponded to an actual DO concentration of approximately 6.8 mg L^{-1} at 30 °C, 1.0 atm. These operation conditions were adopted in different batch fermentation experiments to investigate the effects of agitation speed on cell growth, welan gum production, and glucose consumption. The oxygen uptake rate (OUR) was determined using the dynamic method [6].

Analytical methods

Dry cell weight (DCW) was determined from at least three 10 mL cell suspensions that were harvested by centrifugation, washed with distilled water, and dried at 80 °C for 24 h to a constant weight. The concentration of glucose was analyzed using a biosensor equipped with glucose oxidase electrode (SBA-40C, Shandong Academy of Sciences, China). The DO concentration and pH were measured using indicators of the bioreactor. The concentration of welan gum was measured following a previously reported method [12].

The weight-average welan gum molecular weight produced by fermentation at the end of the process was measured using the Laurent method [13], in which single-point measurements were performed on diluted samples containing 20–50 mg mL^{-1} in 0.15 M NaCl at pH 7.0. The Mark-Houwink constants were $k = 3.6 \times 10^{-4}$ and $a = 0.78$. The viscosity of the polymer (1% in water, 25 °C) was measured by rotational viscometer (NDJ-1, Shanghai Hengping Scientific Instrument Company, China) using rotor No. 4 at 60 rpm. Each experiment was repeated three times, and the experimental errors were less than 4%.

Assay of welan gum chemical composition

Welan gum was hydrolyzed in H_2SO_4 (2 M) at 100 °C for 8 h, and then neutralized using $BaCO_3$. One part of the supernatant was carried on the derivative process as indicated in literature [14], while the reaction product was analyzed using the gas chromatography (Agilent 6890N, USA) with a flame ionization detector and fitted with a quartz capillary column (25 m × 0.32 mm i.d., 0.25 μm). Nitrogen was used as carrier gas. The injector was maintained at 260 °C, with an injection volume of 0.8 μL. The column was raised from 80 to 210 °C at 20 °C min^{-1}, and then increased to 280 at 10 °C min^{-1}. The chemical

composition of the hydrolyzed welan gum (D-glucose, L-rhamnose, and L-mannose) was identified through comparisons with internal standards (Sigma, USA).

Another part of the supernatant was analyzed using the ion chromatography (Dionex 2010i, USA) with a carbohydrate analysis column (150 mm × 3 mm i.d., CarboPac PA20, Dionex) using a pulsed amperometric detector. A total of 250 mM NaOH and NaAc were used as eluent with a flow rate of 0.9 mL min^{-1} at 30 °C. The chemical composition of the hydrolyzed welan gum (D-glucuronic acid) was determined based on this method.

Calculation of kinetic parameters

The specific cell growth rate (μ, h^{-1}), specific glucose consumption rate (q_s, h^{-1}), and specific welan gum formation rate (q_p, h^{-1}) were estimated from the experimental or fitted data of cell growth (χ, g L^{-1}), residual glucose concentration (s, g L^{-1}), and welan gum production (p, g L^{-1}) using Eqs. (1)–(3), respectively [15]. The fitted data were obtained by interposing the experimental data of cell growth, residual glucose concentration, or welan gum production at definite time ($dt = 0.1$ h) with the approximation method of cubic spline interpolation in Origin software (Version 7.5, OriginLab Corp., Northampton, MA, USA).

$$\mu = \frac{1}{\chi}\frac{d\chi}{dt} = \frac{1}{\chi}\lim_{\Delta t\to 0}\frac{\Delta\chi}{\Delta t} \quad (1)$$

$$q_s = -\frac{1}{\chi}\frac{ds}{dt} = -\frac{1}{\chi}\lim_{\Delta t\to 0}\frac{\Delta s}{\Delta t} \quad (2)$$

$$q_p = \frac{1}{\chi}\frac{dp}{dt} = \frac{1}{\chi}\lim_{\Delta t\to 0}\frac{\Delta p}{\Delta t} \quad (3)$$

Results and discussion

Effects of agitation speed on microbial welan gum production

The effects of agitation speed (400, 600, 800, and 1,000 rpm) on welan gum fermentation were investigated in the 7.5 L stirred fermenter (Fig. 1a–c). Figure 1c shows that the relatively higher maximum welan gum concentrations of 20.6 ± 0.65 and 22.8 ± 0.61 g L^{-1} were obtained at the agitation speeds of 600 and 800 rpm, respectively. The production of welan gum using *Alcaligenes* sp. CGMCC2438 was affected by agitation speeds. This suggests that either low (400 rpm) or high (1,000 rpm) agitation speed was not beneficial for welan gum production. Higher DCW was achieved at a higher agitation speed and the maximal DCWs obtained were 5.98 ± 0.21, 6.54 ± 0.22, 7.02 ± 0.22, and 7.50 ± 0.25 g L^{-1} at 400, 600, 800, and 1,000 rpm, respectively (Fig. 1a). Consistent with decreasing cell growth, the consumption of glucose declined from 1,000 to 400 rpm (Fig. 1b). The results demonstrate that the increase

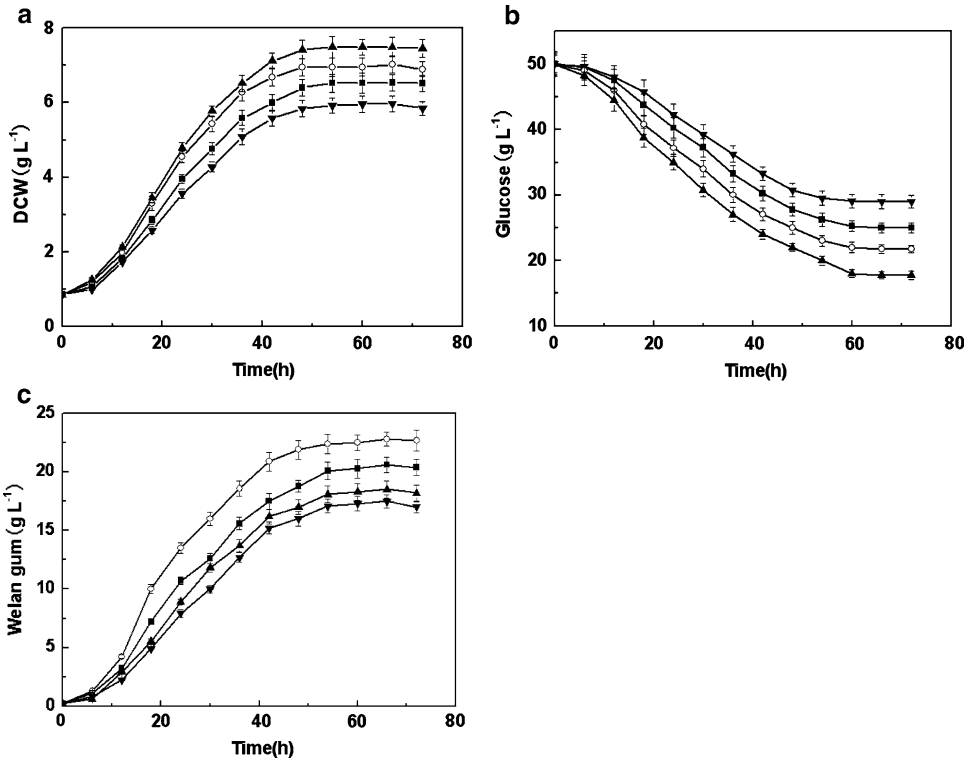

Fig. 1 Time profiles of cell growth (**a**), glucose concentration (**b**), welan gum concentration (**c**) in the cultivation of *Alcaligenes* sp. CGMCC2428 at different agitation speeds. 400 rpm (*filled inverted triangle*), 600 rpm (*filled square*), 800 rpm (*open circle*), 1,000 rpm (*filled triangle*)

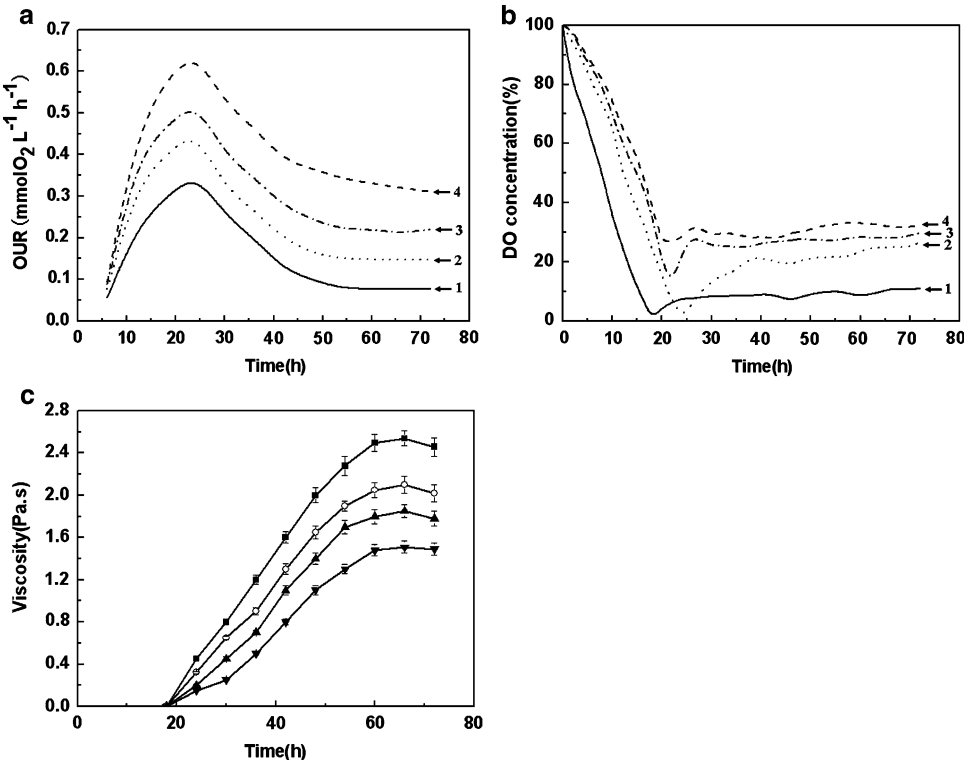

Fig. 2 Time courses of OUR (**a**), DO concentration (**b**), welan gum viscosity (**c**) in the cultivation of *Alcaligenes* sp. CGMCC2428 at different agitation speeds. *1*, 400 rpm (*filled inverted triangle*); *2*, 600 rpm (*filled square*); *3*, 800 rpm (*open circle*); *4*, 1,000 rpm (*filled triangle*)

in cells did not lead to high welan gum production at the highest agitation speed.

The measurement of welan gum viscosity suggests that the high or low agitation speed was not helpful for a high-viscosity welan gum production (Fig. 2c). Although the final welan gum concentration at the agitation speed of 800 rpm was higher compared with that in the agitation speed of 600 rpm, the previous welan gum viscosity was lower than the latter (2.10 ± 0.08 Pa s at 800 rpm and 2.54 ± 0.07 Pa s at 600 rpm). The reason may be because very high agitation causes mechanical damage to welan gum. To verify this speculation, we investigated the effect of different agitation speeds on welan gum molecular weight (Table 1). The molecular weight was only $(8.65 \pm 0.09) \times 10^5$ Da at a speed of 400 rpm. The welan molecular weight reached its maximum value of $(9.01 \pm 0.12) \times 10^5$ Da at a speed of 600 rpm, and then decreased as the agitation speed increased. The chemical composition of welan gum produced by fermentation with various agitation speeds was analyzed (Figs. 3, 4). The D-glucose, L-rhamnose, and L-mannose of welan gum chemical composition at speeds of 400 and 1,000 rpm were detected by gas chromatography (Fig. 3b, c). In addition, another composition of welan gum, D-glucuronic acid was determined using ion chromatography (Fig. 4). The results demonstrated that the welan gum chemical composition had no significant variation at different agitation speeds.

Table 1 The analysis of parameters at different agitation speed control strategies

Parameters	Agitation speed (rpm)				
	400	600	800	1,000	800 (0–22 h), 600 (after 22 h)
DCW (g L^{-1})	5.98 ± 0.21	6.54 ± 0.22	7.02 ± 0.22	7.50 ± 0.25	6.81 ± 0.26
Welan gum (g L^{-1})	17.5 ± 0.56	20.6 ± 0.65	22.8 ± 0.61	18.5 ± 0.74	26.3 ± 0.89
Welan gum productivity (g L^{-1} h^{-1})	0.27 ± 0.005	0.31 ± 0.006	0.35 ± 0.004	0.28 ± 0.003	0.40 ± 0.003
Welan gum yield (g g^{-1})	0.35 ± 0.004	0.41 ± 0.003	0.46 ± 0.005	0.37 ± 0.002	0.53 ± 0.003
Viscosity (Pa s)	1.51 ± 0.06	2.54 ± 0.07	2.10 ± 0.08	1.85 ± 0.06	3.05 ± 0.10
Welan gum molecular weight ($\times 10^5$ Da)	8.65 ± 0.09	9.01 ± 0.12	8.81 ± 0.10	8.73 ± 0.07	9.32 ± 0.13

Welan gum yield was expressed as g welan gum/g glucose utilized

Values are mean ± standard deviation ($n = 3$)

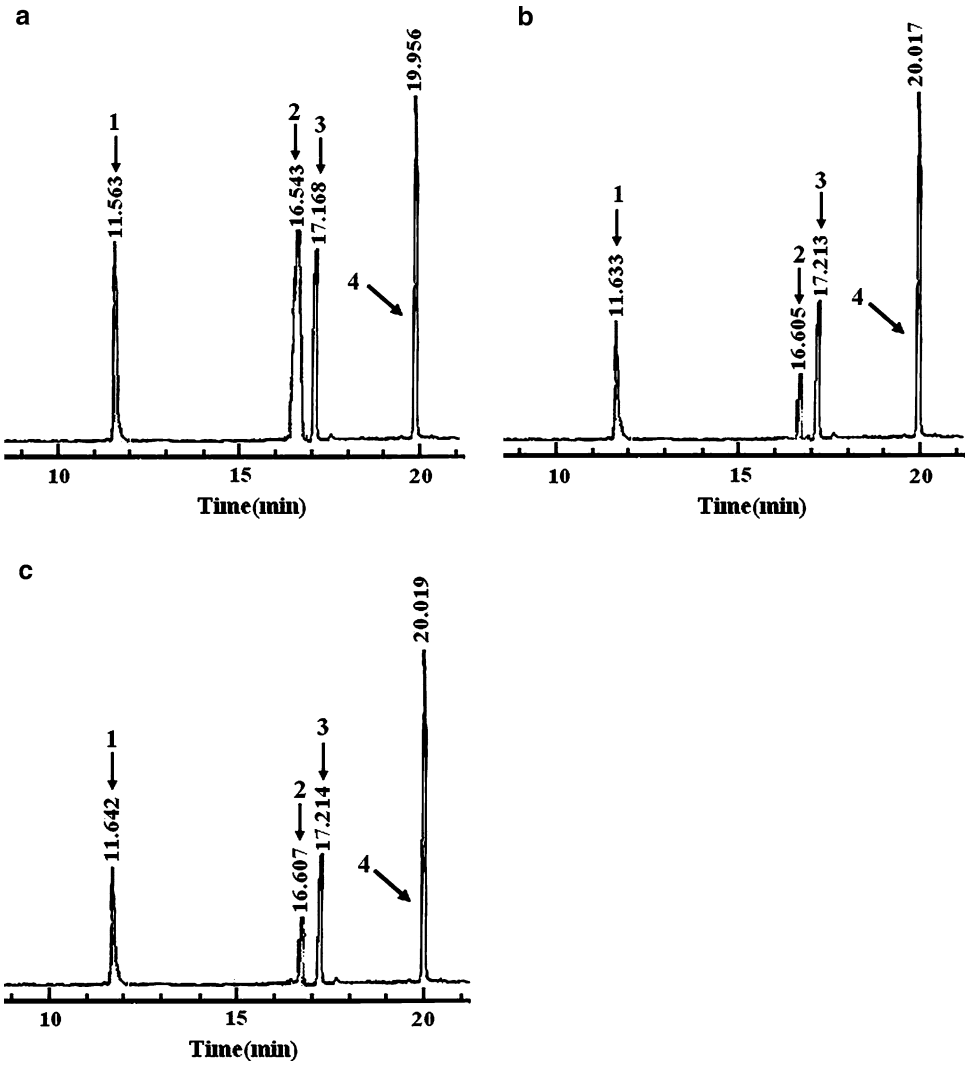

Fig. 3 Detection of D-glucose, L-rhamnose, and L-mannose of welan gum chemical composition produced by fermentation at different agitation speeds (400, 1,000 rpm) using gas chromatography. **a** Mixture reference substances and internal standard, **b** sample and internal standard (400 rpm), **c** sample and internal standard (1,000 rpm). *1*, L-rhamnose; *2*, L-mannose; *3*, D-glucose; *4*, inositol, internal standard

The kinetic courses of OUR and DO concentration during cultivations at various agitation speeds are displayed in Fig. 2a and b. The change trends of OUR during welan gum fermentation were similar to those of DO concentration. They increased with the increase in agitation speeds. The DO concentration reached its lowest values of 0.4, 1.1, 12.2, and 26% air saturation at the agitation speeds of 400, 600, 800, and 1,000 rpm, respectively. The corresponding maximal OUR values were 0.356, 0.461, 0.532, and 0.650 mmolO_2 L^{-1} h^{-1}. The change in DO concentrations at different agitation speeds was similar to that described by Li [16]. There was a rapid decrease in the first 20 h and stabilization at a constant level at the mid-growing period of cultivation. The results suggest that the OUR of cells was higher than the oxygen transfer rate in the first 20 h under various agitation speeds; subsequently, these two rates reached an equilibrium. Therefore, the mechanisms of controlling agitation speeds should be investigated further for agitation speeds to be beneficial not only for oxygen transfer but also for the production of high viscosity polysaccharide at the late phase of fermentation.

Kinetic analysis of welan gum fermentation at different agitation speeds

To analyze the kinetic characteristics of the two processes at the agitation speeds of 600 and 800 rpm, three kinetic parameters, μ, q_s, and q_p, were calculated based on the data in Fig. 1. Figure 5 shows that μ, q_s, and q_p had similar tendencies and the maximum values appeared at approximately 10 h. Compared with the agitation speed of 600 rpm, μ and q_s were higher at the agitation speed of 800 rpm at the beginning of welan gum fermentation (approximately 22 h). These results indicate the agitation speed of 800 rpm was better for cell growth and glucose consumption at the beginning of the welan gum

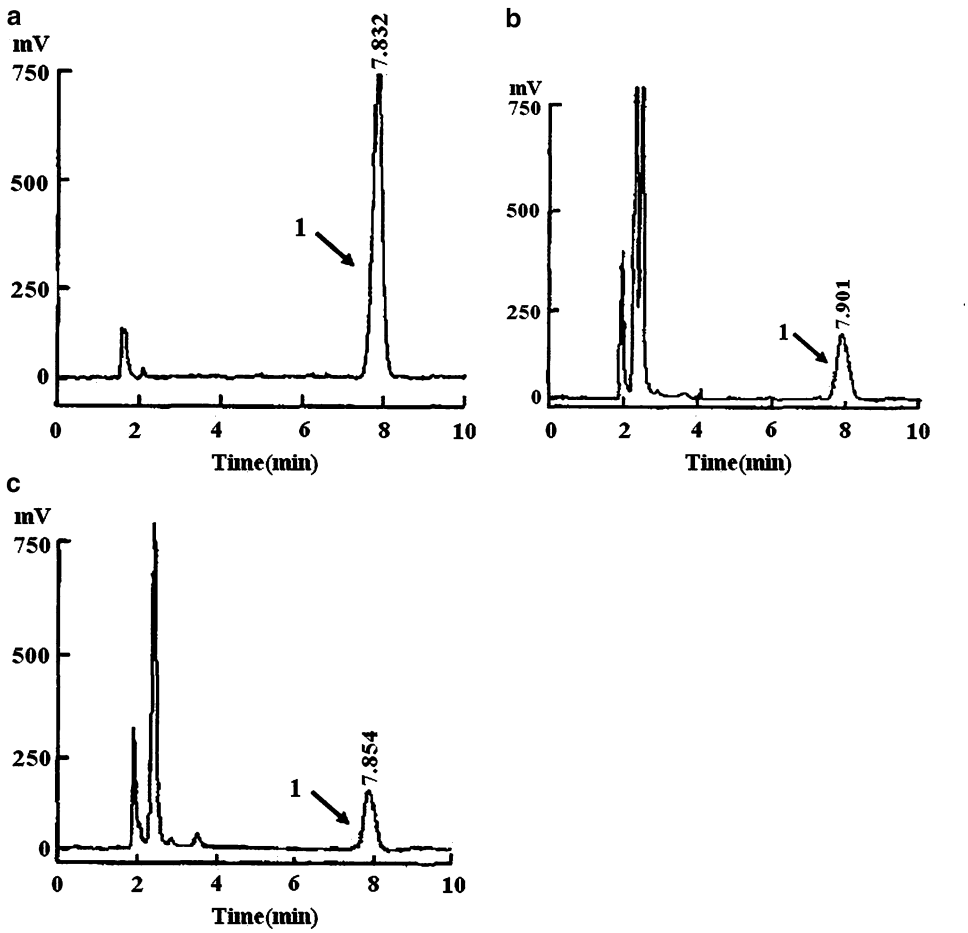

Fig. 4 Detection of D-glucuronic acid of welan gum chemical composition produced by fermentation at different agitation speeds (400, 1,000 rpm) using ion chromatography. **a** Standard sample of D-glucuronic acid, **b** polysaccharide hydrolyzate produced by fermentation with agitation speed of 400 rpm, **c** polysaccharide hydrolyzate produced by fermentation with agitation speed of 1,000 rpm. *1*, D-glucuronic acid

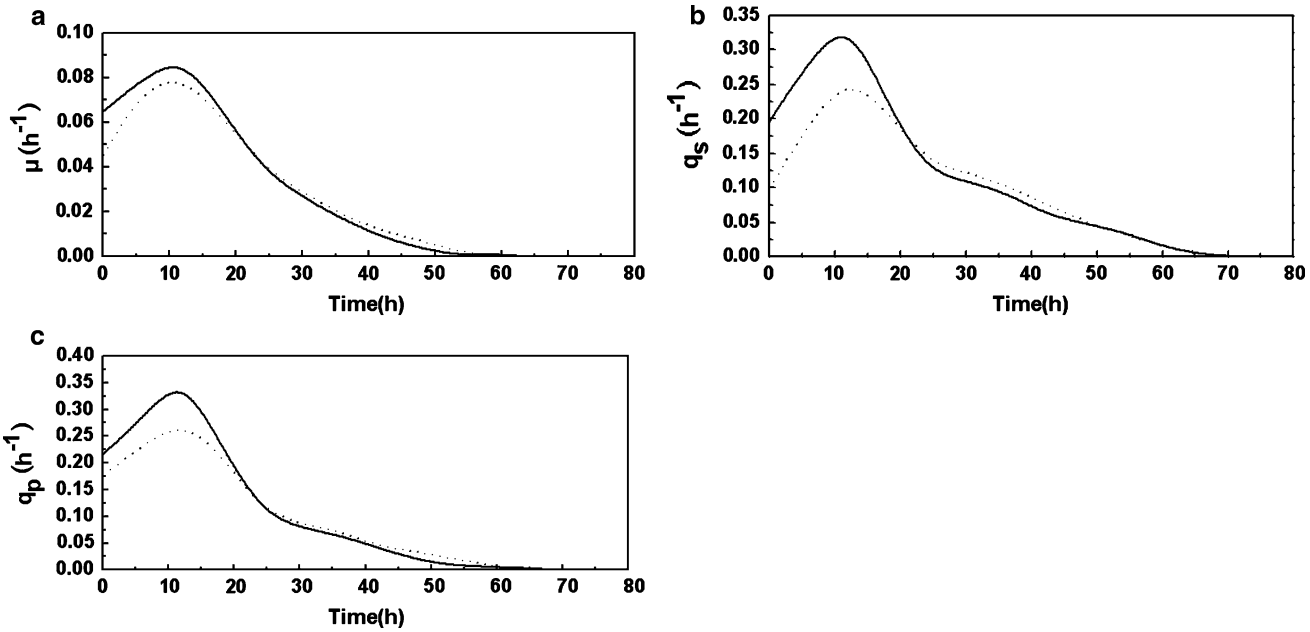

Fig. 5 Comparison of kinetic parameters in welan gum fermentation using *Alcaligenes* sp. CGMCC2428 at the agitation speeds of 600 rpm (*dashed line*) and 800 rpm (*solid line*): specific cell growth rate (μ) (**a**); specific glucose consumption rate (q_s) (**b**); specific welan gum formation rate (q_p) (**c**)

fermentation. After 22 h, however, the agitation speed of 600 rpm was beneficial for welan gum formation with a high value of q_p. A relatively lower agitation speed was also beneficial for high welan gum viscosity, which could help avoid damage to the polysaccharide in the late fermentation stage.

Based on the analysis of μ, q_p, and q_s, a two-stage agitation speed control strategy was proposed as follows. The agitation speed was controlled at 800 rpm in the first 22 h to maintain high μ and q_s for fast cell growth and glucose consumption, and then switched to 600 rpm after 22 h to maintain high q_p for the fermentation of welan gum with *Alcaligenes* sp. CGMCC2428.

Welan gum production with two-stage agitation speed control strategy

The time course of the proposed strategy for welan gum fermentation is shown in Fig. 6a. Table 1 lists the results of constant and two-stage agitation speed control strategy controlling experiments. Compared with the best results obtained in the cultivations at constant agitation speed, the maximal concentration of welan gum reached 26.3 ± 0.89 g L^{-1} with welan gum productivity of 0.40 ± 0.003 g L^{-1} h^{-1}. The welan gum viscosity and molecular weight were also highest [3.05 ± 0.10 Pa s (9.32 ± 0.13) × 10^5 Da]. Furthermore, using this two-stage agitation speed control strategy, the final welan gum yield achieved from glucose was up to 0.53 ± 0.003 g g^{-1}, which increased by an average of 15.2% higher than the best result controlled by single-agitation speed. The results were at a relatively high level compared with previously reported values [12, 17].

The changing patterns of DO concentration under different oxygen supply strategies are also described in Fig. 6b. A sharp decrease and rebound in DO concentration at the 600 rpm speed during the first 1–26 h was observed. In particular, the DO concentration dropping to 1.1% at approximately 24 h was harmful to the fermentation performance. The changing pattern of DO concentration under the proposed two-stage agitation speed control strategy was much stable or smooth compared with that of 600 rpm speed. DO concentration was maintained at approximately 10% saturation level from 34 h until the end of the fermentation. We can conclude that this proposed strategy not only can considerably improve welan gum concentration in the broth and welan gum yield from glucose but also increase welan gum productivity. The proposed strategy was proved successful in improving welan gum production.

Welan gum viscosity was improved after applying the proposed strategy. In the application of microbial polysaccharide, the viscosity of a product must meet industry standards [18, 19]. Thus, enhancing the viscosity of welan gum could be very important. In the late stage of welan gum fermentation, the broth became more viscous. At this period, a low agitation speed was unable to satisfy the oxygen demand of the cells, whereas a high agitation speed brought high shear stress that could shatter the biopolymers, which might lead to a decrease in molecular weight (Table 1). Therefore, the agitation speed of 600 rpm at the late phase of the process was relatively smooth and beneficial for high viscosity of welan gum in the proposed strategy.

Conclusions

This paper described a simple oxygen supply method, which comprised a two-stage agitation speed control strategy based on the kinetic analysis for efficient welan gum fermentation using *Alcaligenes* sp. CGMCC2428. This method was proved to be the better strategy for the

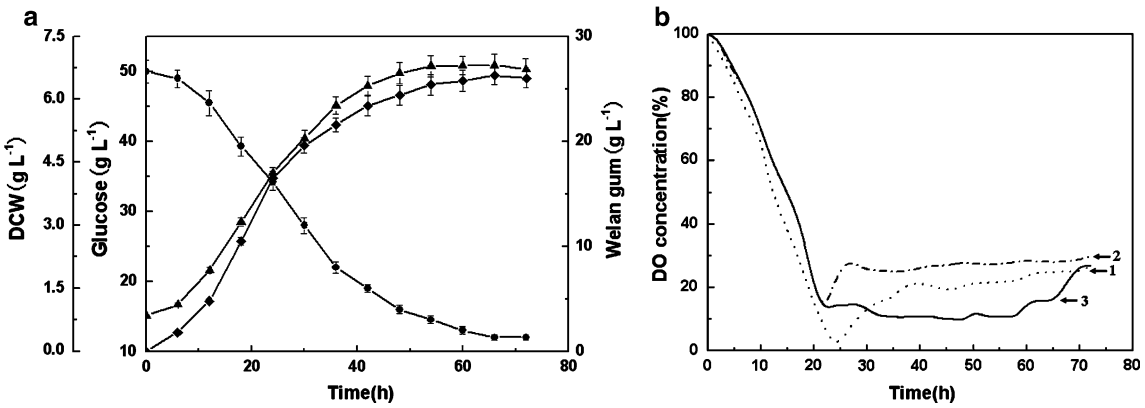

Fig. 6 Time course of welan gum fermentation by *Alcaligenes* sp. CGMCC2428 using two-stage agitation speed control strategy (**a**). Glucose (*filled circle*), DCW (*filled triangle*), welan gum (*filled diamond*). **b** DO concentration *1*, 600 rpm; *2*, 800 rpm; *3*, the two-stage agitation speed control strategy

enhancement of welan gum concentration, yield, productivity, and viscosity. This potential strategy might be useful to improve productivity of viscous biopolymers for industrial applications.

Acknowledgments This work was supported by the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (No. 08KJA180001) and Jiangsu Key Technology Research and Development Program (No. BE2009363).

References

1. O'Neill MA, Selvendran RR, Morris VJ, Eagles J (1986) Structure of the extracellular polysaccharide produced by the bacterium *Alcaligenes* (ATCC 31555) species. Carbohydr Res 145:295–313
2. Chandrasekaran R, Radha A, Lee EJ (1994) Structural roles of calcium ions and side chains in welan: an X-ray study. Carbohydr Res 252:183–207
3. Fialho AM, Moreira LM, Granja AT, Hoffmann K, Popescu A, Sá-Correia I (2007) In: Pereira MS (ed) A portrait of state-of-the-art research at the technical university of Lisbon, 6th edn. Springer, Netherlands
4. Kang KS, Veeder GT, Cottrell IW (1983) In: Bushell ME (ed) Microbial polysaccharides, 6th edn. Elsevier, New York
5. Banik RM, Santhiagu A (2006) Improvement in production and quality of gellan gum by *Sphingomonas paucimobilis* under high dissolved oxygen tension levels. Biotechnol Lett 28:1347–1350
6. Garcia-Ochoa F, Gomez Castro E, Santos VE (2000) Oxygen transfer and uptake rates during xanthan gum production. Enzyme Microb Technol 27:680–690
7. Giavasis I, Harvey LM, McNeil B (2006) The effect of agitation and aeration on the synthesis and molecular weight of gellan in batch cultures of *Sphingomonas paucimobilis*. Enzyme Microb Technol 38:101–108
8. Gao HJ, Chen J, Du GC, Zhang YF, Chen JC, Chen GQ (2003) Effect of agitation and mixing hyaluronic acid production by *Streptococcus zooepidemicus*. J Chem Ind Eng 54:350–356
9. Kim JH, Yoo SJ, Oh DK, Kweon YG, Park DW, Lee CH, Gil GH (1996) Selection of a *Streptococcus equi* mutant and optimization of culture conditions for the production of molecular weight hyaluronic acid. Enzyme Microb Technol 19:440–445
10. Schilling BM, Rau U, Maier, Fankhauser P (1999) Modeling and scale-up of the unsterile scleroglucan production process with *Sclerotium rolfsii* ATCC 15205. Bioproc Eng 20:195–201
11. Dreveton E, Monot F, Ballerini D, Lecourtier J, Choplin L (1994) Effect of mixing and mass transfer conditions on gellan production by *Auromonas elodea*. J Ferment Bioeng 77:642–649
12. Li S, Xu H, Shi N (2004) Production of a microbial polysaccharides by fermentation. Food Ferment Ind 30:6–9
13. Laurent TC, Ryan M, Pietruszkiewicz A (1960) Fraction of hyaluronic acid. The polydispersity of hyaluronic acid from the bovine virtreous body. Biochim Biophys Acta 42:476–485
14. Xu G, Chen R, Chang L (2000) Component analysis of monosaccharides in polysaccharides by capillary gas chromatography. J Instrum Anal 19:71–73
15. Mao XB, Zhong JJ (2004) Hyperproduction of cordycepin by two-stage dissolved oxygen control in submerged cultivation of medicinal mushroom *Cordyceps militaris* in bioreactors. Biotechnol Prog 20:1408–1413
16. Li Y, Hugenholtz J, Chen J, Lun SY (2002) Enhancement of pyruvate production by *Torulopsis glabrata* using a two-stage oxygen supply control strategy. Appl Microbiol Biotechnol 60:101–106
17. Li H, Li S, Feng X, Wang F, Xu H (2009) Production of welan gum by *Alcaligenes* sp. NX-3 with fed-batch fermentation. Food Ferment Ind 35:1–4
18. Sakata N, Maruyama K, Minami K (1996) In: Bartos PJM, Marrs DL (eds) Proceedings of the international RILEM conference production methods and workability of concrete, E & FN Spon, London
19. Calfors J, Edsman K, Peterson R, Journving K (1998) Rheological evaluation of gelrite in situ for ophthalmic use. Eur J Pharm Sci 6:113–119