Carbohydrate Polymers 98 (2013) 829–834

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

A new strategy to enhance gellan production by two-stage culture in *Sphingomonas paucimobilis*☆

Guilan Zhu[a,b], Long Sheng[a], Qunyi Tong[a,c,*]

[a] School of Food Science and Technology, Jiangnan University, Wuxi 214122, China
[b] Department of Life Science, Hefei Normal University, Hefei 230061, China
[c] State Key Laboratory of Food Science and Technology, Jiangnan University, Jiangsu, Wuxi 214122, China

ARTICLE INFO

Article history:
Received 22 May 2013
Received in revised form 20 June 2013
Accepted 26 June 2013
Available online 4 July 2013

Keywords:
Two-stage culture
Gellan
Sphingomonas paucimobilis ATCC 31461

ABSTRACT

The effects of different initial sucrose concentrations and temperatures on gellan biosynthesis by *Sphingomonas paucimobilis* ATCC 31461 were investigated. Lower sucrose concentrations and higher temperatures were favorable for cell growth. Higher sucrose concentrations and lower temperatures promoted gellan production but retarded cell growth. Based on these results, a two-stage culture strategy was developed to improve gellan production. During the first 24 h, *S. paucimobilis* was cultured in a pulse fed-batch mode with an initial sucrose concentration 10 g/L. Ten grams per liter of sucrose were added at 12 h and 24 h, and the temperature was controlled at 33 °C. Batch culture was performed, and the temperature was reduced to 28 °C to achieve a high gellan accumulation. The two-stage culture strategy achieved the highest gellan production (22.61 g/L) at 60 h that was 35.71% higher than the result of the best conventional batch operation (16.66 g/L). Meanwhile, high gellan yield was related to high UDPG-pyrophosphorylase activity and glucosyltransferase activity.

1. Introduction

Gellan is an anionic extracellular bacterial polysaccharide composed of repeating tetrasaccharide units of two residues of β-D-glucose, one of β-D-glucuronate and one of α-L-rhamnose (Per Erik, Bengt, & Paul, 1983). With its novel physico-chemical properties, gellan has been widely applied in food, coating materials, medicine, and cosmetics (Banerjee, Ravi, & Bhattacharya, 2013; Banik, Kanari, & Upadhyay, 2000; Nuno et al., 2012; Prajapati, Jani, Zala, & Khutliwala, 2013).

Gellan is industrially produced from sucrose through fermentation by the nonpathogenic bacterium, *Sphingomonas paucimobilis*. To date, many attempts have been made to optimize the variables in gellan fermentation, such as the nutrient composition (Banik, Santhiagu, & Upadhyay, 2007; Fialho et al., 1999; Huang et al., 2012; Nampoothiri, Singhania, Sabarinath, & Pandey, 2003; Wang et al., 2006; West & Strohfus, 1998), temperature (Martins & Sa-Correia, 1994; West, 2003), pH (West & Fullenkamp, 2001), dissolved oxygen (Banik & Santhiagu, 2006), and adding surfactants (Arockiasamy & Banik, 2008). All of these approaches have resulted in some improvement.

A previous study found that carbon source concentration was one of the most important factors in the high-level production of gellan (Bajaj, Survase, Saudagar, & Singhal, 2007). It was observed that a lower carbon source concentration favored cell growth, but resulted in a lower gellan production. Temperature was also a critical parameter influencing the production of gellan (Fialho et al., 2008). Martins and Sa-Correia (1994) observed that gellan production showed a maximal yield at 20–25 °C, which was not coincident with the optimal range for cell growth (30–35 °C). To obtain a high specific cell growth rate and gellan biosynthesis rate, it is thus important to control cell growth and the production formation process through an appropriate carbon source concentration and temperature level. To date, however, very little attention has been paid to identifying a strategy that uses different carbon source concentrations and temperature conditions to optimize both cell growth and gellan biosynthesis.

In this study, the process of gellan fermentation using *S. paucimobilis* ATCC 31461 at different initial sucrose concentrations and different temperatures was examined. A two-stage culture strategy to enhance gellan production was then proposed based on the identified effects of sucrose and temperature on gellan biosynthesis.

* Corresponding author at: State Key Laboratory of Food Science and Technology, Jiangnan University, Jiangsu, Wuxi 214122, China. Tel.: +86 0510 85919170; mobile: +86 13861715020.
E-mail address: qytong@263.net (Q. Tong).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.06.060

2. Materials and methods

2.1. Microorganism and media

S. paucimobilis ATCC 31461 was used in this study. The strain was maintained by monthly subculture on nutrient agar slants. Seed culture medium contained (g/L): beef extract 3, peptone 10, NaCl 5 and pH 7.4. Fermentation medium contained (g/L): sucrose 10, 20, 30, or 40, yeast extract 1, peptone 2, KH_2PO_4 3, K_2SO_4 1, K_2HPO_4 1and $MgSO_4{\cdot}7H_2O$ 1. The pH was adjusted to 7.0.

2.2. Culture conditions

In the seed preparation process, the microorganism from fresh slant cultures was inoculated into 50 mL of seed medium in 250 mL flasks and cultivated for 24 h at 30 °C on a rotary shaker at 200 r/min.

Gellan fermentation was carried out in a 5 L fermentor (Ko Bio Tech Co., Ltd) with a working volume of 3 L. 10% (v/v) of the seed culture was inoculated the fermenter with uncontrolled pH and an agitation speed of 200 r/min.

To determine the effect of the initial carbon source on gellan fermentation, the sucrose concentration was controlled at 10, 20, 30, and 40 g/L, respectively, at a temperature of 30 °C. To determine the effect of temperature on gellan fermentation, the fermentation culture was incubated at 25, 28, 30, and 33 °C with an initial sucrose concentration of 30 g/L.

In the two-stage culture strategy experiment, a pulse type fed-batch culture was used to 10 g/L sucrose added for 12 h and 24 h, respectively. The batch culture had an initial sucrose concentration of 10 g/L. The first-stage control strategy was to realize fast cell growth within 24 h. The batch culture of *S. paucimobilis* ATTCC 31461 was initiated in the second stage. The temperature for the first-stage culture and the second-stage culture was 30 °C, and 28 °C, respectively.

2.3. Preparation of cell-free extract

The cells in 5.0 mL of the culture were collected by centrifugation at 10,000 × *g* for 30 min at 4 °C, washed 3 times with ice-chilled distilled water, and suspended in 1.0 mL of ice-chilled 1.0 M Tris–HCl (pH 7.0) to make a thick paste. The product was homogenized in an ultrasonic cell disruptor (JYD-900, Zhixin, Shanghai, China) for 16 min on an ice bath. The cell debris was removed by centrifugation at 10,000 × *g* at 4 °C for 30 min. The supernatant was the cell-free extract (and also the enzyme preparation). The protein concentration in the cell-free extract was determined by the Bradford method with bovine serum albumin as the standard (Pan, Yao, Chen, & Wu, 2013).

2.4. Analytical methods

Dry cell weight (DCW) was determined as follows: The broth was immersed in a boiling water bath for 15 min, cooled and the pH was increased to 10.0 using 2.0 mol/L NaOH. The broth was heated for 10 min in a boiling water bath, following which the pH was brought down to 7.0 using 2.0 mol/L HCl. The cultures were centrifuged at 10,000 × *g* for 30 min to separate the cells. The cell precipitate washed twice with distilled water, and dried at 105 °C to constant weight to determine dry cell weight (Jina et al., 2003).

The supernatant was added to 2 volumes of ethanol (95%, v/v) to precipitate the polysaccharide, and then kept overnight at 4 °C. The precipitate was recovered by centrifugation at 10,000 × *g* for 30 min at 25 °C and dried in a hot-air oven (60 °C, 24 h). The gellan production was obtained.

The sucrose concentration was measured using UV spectrophotometry (Zhan, 2005). 1 mL of sucrose sample was mixed with 3 mL of concentrated HCl. The solution was topped up with double-distilled water to 10 mL, and heated for 8 min in a boiling water bath. The sucrose concentration was obtained by determining the optical density (OD) of the cooled solution at 291 nm.

Glucosyltransferase activity and UDP-glucose pyrophosphorylase activity were determined according to the methods described by Duan, Chi, Wang, and Wang (2008).

3. Results and discussion

3.1. Effect of initial sucrose concentration on gellan fermentation

In the fermentation process, the concentration of the carbon source determined the C/N ratio in the medium, and directly affected the yields, compositions, structures, and the properties of the polysaccharide. Excessive quantities of the initial carbon source inhibited cell growth.

The effects of the initial sucrose concentration on cell growth, residual total sugar, and gellan yield were investigated in the 5 L fermenter (Fig. 1). As shown in Fig. 1A, the highest dry cell weight of 3.1 g/L was obtained at an initial sucrose concentration of 20 g/L, which was a similar result to that reported by Kanari, Banik, and Upadhyay (2002). It was obvious that a high initial sucrose concentration was not beneficial to cell growth. The gellan yield obtained was 5.7, 14.1, 16.6, and 18.2 g/L at sucrose concentration of 10, 20, 30 and 40 g/L, respectively (Fig. 1B). A higher gellan yield was achieved at higher initial sucrose concentrations up to a maximum value of 40 g/L. These results demonstrated that increasing the sucrose led to high gellan production. Consistent with increasing gellan production, the consumption of sucrose declined from 40 to 10 g/L (Fig. 1C).

As a carbon source, sucrose, is used not only in cell growth but also in gellan biosynthesis. UDP-glucose, UDP-glucuronic acid and UDP-rhamnose are the precursors of gellan biosynthesis. Glucose-1-P and Glucose-6-P, which are the precursors of UDP-glucose, are also the precursors of lipopolysaccharides (cell wall) (Sá-Correia et al., 2002). There was thus a competition between gellan biosynthesis and cell growth. A lower amount of carbon source was more favorable for cell growth, and thus gellan yield was low. Conversely, cell growth was inhibited at high sucrose concentration.

Alleviating of the competition between cell growth and gellan biosynthesis may be an efficient strategy for the enhancement of gellan production. As discussed, an appropriately low sucrose concentration was favored for cell growth, while a high sucrose concentration was favored for gellan biosynthesis. This study thus developed a two-stage culture strategy, in which a fed-batch culture was applied in the first stage for cell growth and a batch culture was used in the second stage for gellan production.

Fig. 2A shows the time-courses of the specific cell growth rate (μ_{cell}) with different initial sucrose concentrations. All of the μ_{cell} profiles showed a similar tendency. The maximum μ_{cell} value was attained with a sucrose concentration of 10 g/L. After 24 h of cultivation, gellan biosynthesis rate (q_{gellan}) increased and reached a maximum value (Fig. 2B). The higher the sucrose concentration that was used, the higher the q_{gellan} achieved.

In the first stage of gellan fermentation, the pulse type fed-batch culture with a lower initial sucrose concentration (e.g. 10 g/L) was used to maximize the μ_{cell}, whereas a higher sucrose concentration was employed to maintain a high q_{gellan} in the second stage.

3.2. Effect of temperature on gellan fermentation

Fig. 3A–C shows the time course of gellan production by *S. paucimobilis* at different temperatures (25, 28, 30, and 33 °C). The evolution of dry cell weight (DCW) at different temperatures is

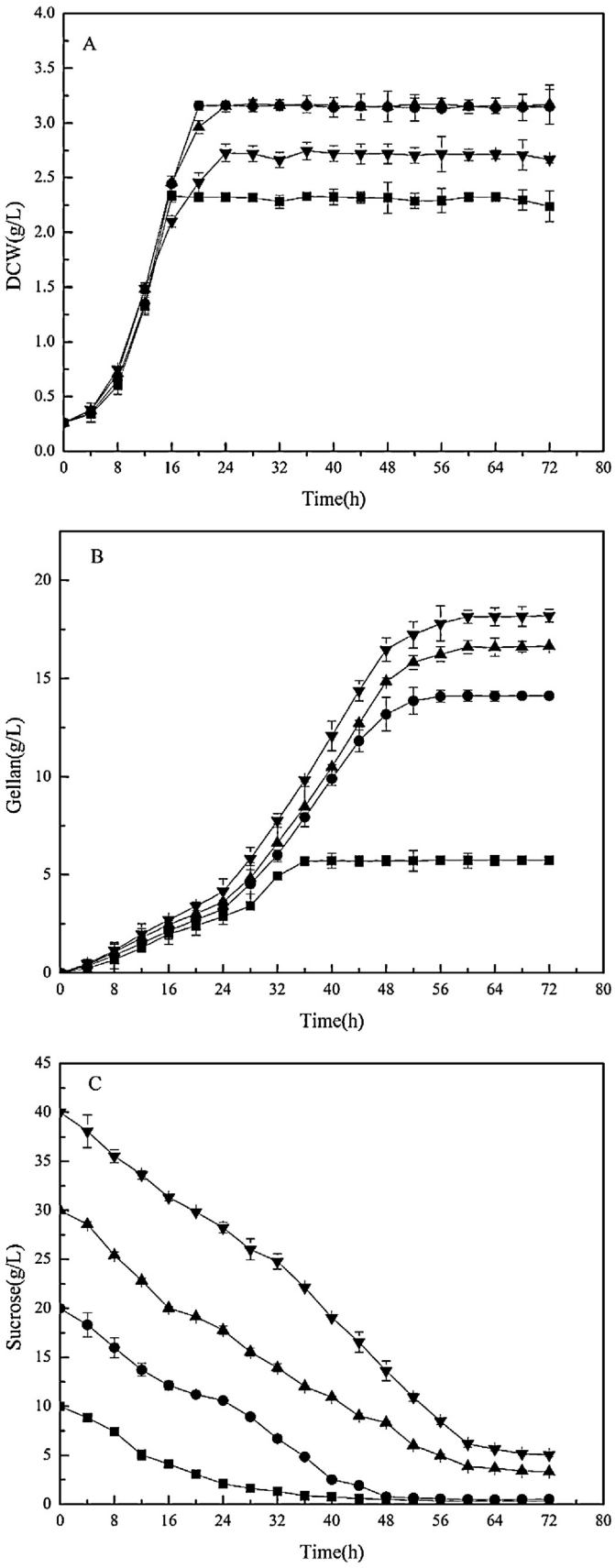

Fig. 1. Effect of initial sucrose concentration on DCW (A), gellan production (B) and residual sucrose concentration (C) during gellan fermentation by *S. paucimobilis*. ■, 10 g/L; ●, 20 g/L; ▲, 30 g/L; ▼, 40 g/L. Data are shown as mean ± S.D. ($n = 3$).

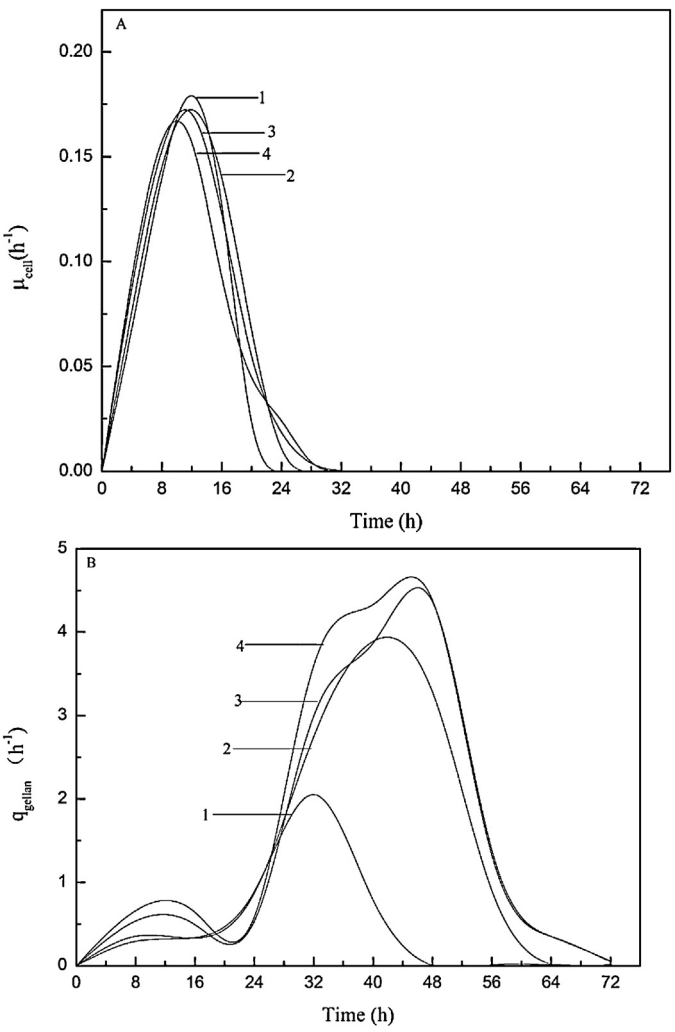

Fig. 2. Effect of initial sucrose concentration on μ_{cell}(A) and q_{gellan} (B) during gellan fermentation by *S. paucimobilis*. 1, 10 g/L; 2, 20 g/L; 3, 30 g/L; 4, 40 g/L.

shown in Fig. 3A. The results indicated that the DCW value increased as culture temperature increased. At lower temperatures (25 and 28 °C), cell growth took longer and gellan biosynthesis occurred at the same time as cell growth. At higher temperatures (30 and 33 °C), the lag phase was shorter, but gellan biosynthesis also started with cell growth. The maximum gellan production (16.68 g/L) was obtained at 28 °C (Fig. 3B), and the lowest gellan concentration (11.61 g/L) was observed at 25 °C. It took less time to reach the maximum DCW value at higher temperatures. Consistent with the increasing cell growth, the sucrose consumption declined as the temperature from 33 to 25 °C (Fig. 3C). A relatively high culture temperature was also favorable for sucrose consumption.

The results illustrate that the culture temperature plays an important role in cell growth and product biosynthesis during gellan fermentation. The results in Fig. 3 indicated that gellan production was not accompanied by an increasing DCW. Similar results have been observed by other researchers (Martins & Sa-Correia, 1994; West, 2003). The two-stage cultivation strategy could be applied to maximize the level of target metabolite. Therefore, optimal cell growth and metabolite production were achieved with separately optimized conditions.

With the two-stage strategy, it is important to determine the temperature-shift point, which involves, finding out the time profiles of the specific cell growth rate and gellan biosynthesis. The time profile for the specific cell growth rate (μ_{cell}) and gellan

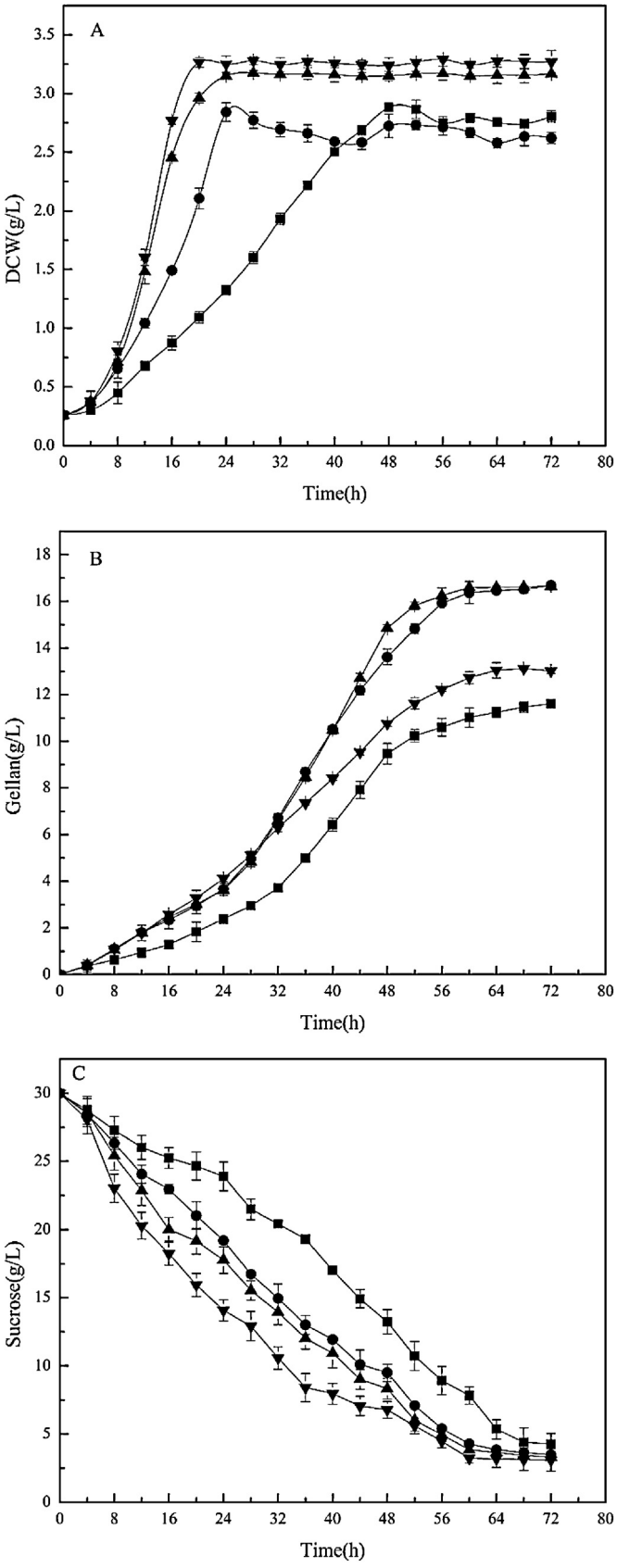

Fig. 3. Effect of temperature on DCW (A), gellan production (B) and residual sucrose concentration (C) during gellan fermentation by *S. paucimobilis*. ■, 25 °C; ●, 28 °C; ▲, 30 °C; ▼, 33 °C. Data are shown as mean ± S.D. ($n = 3$).

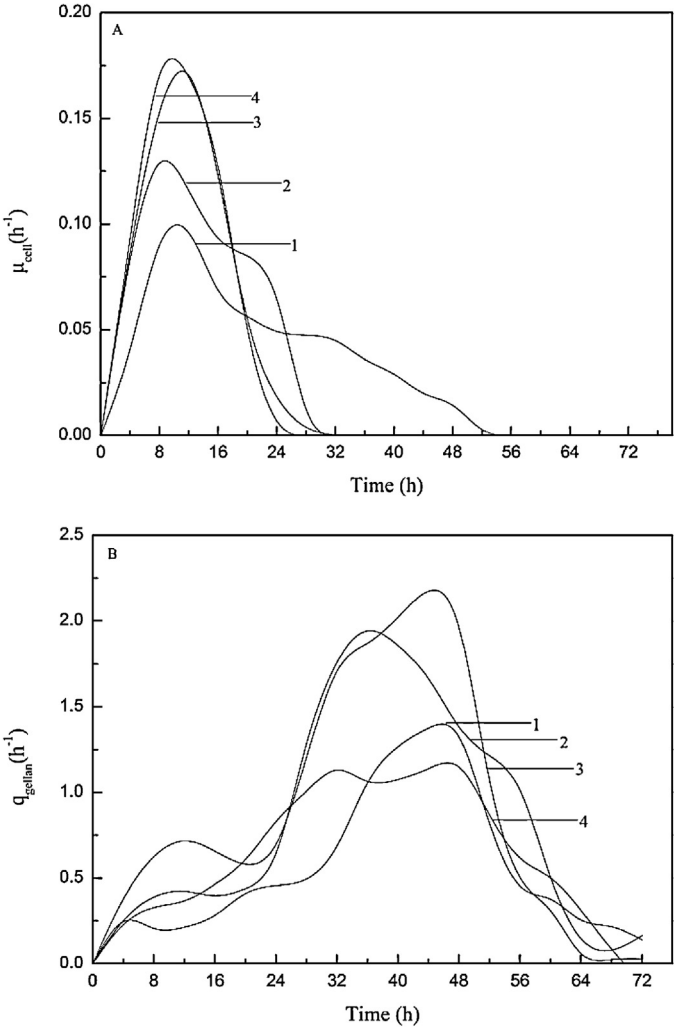

Fig. 4. Effect of temperature on μ_{cell} (A) and q_{gellan} (B) during gellan fermentation by *S. paucimobilis*. 1, 25 °C; 2, 28 °C; 3, 30 °C; 4, 33 °C.

biosynthesis rate (q_{gellan}) at different culture temperatures are presented in Fig. 4. The figure showed that, the μ_{cell} value (Fig. 4A) was higher at 33 °C than at any other temperatures. The time taken to reach the maximum μ_{cell} value at this temperature was short and the rate of μ_{cell} change was high. These results suggest that it was appropriate to use a relatively high temperature (e.g. 33 °C) for cultivation to shorten the lag phase of cell growth at the first stage of gellan fermentation.

The highest q_{gellan} was reached at 30 °C (Fig. 4B). However, the q_{gellan} value decreased faster from its maximum value at 30 °C than it did at 28 °C. Although the maximum q_{gellan} was relatively low at 28 °C, the rate of decrease from its maximum value was slow. Following both 24 h and 36 h of cultivation, q_{gellan} was higher at 28 °C than at 30 °C. Thus, a lower temperature should be used (e.g. 28 °C) to maintain a high q_{gellan} in the second stage.

3.3. *Effect of the two-stage strategy on gellan production*

The following two-stage strategy was developed based on the foregoing results. The initial sucrose concentration was 10 g/L, to which 10 g/L of sucrose was added at 12 h and 24 h, respectively. The pulse type fed-batch culture strategy achieved rapid cell growth. Batch culture was then performed for 24–72 h. A relatively high temperature (33 °C) will be needed for better cell growth in first 24 h stage of fermentation. Conversely, an appropriately high

Table 1
Comparison of parameters in gellan fermentation under different culture strategy.

	Initial sucrose concentration (g/L)				Temperatures (°C)				Two-stage strategy
	10	20	30	40	25	28	30	33	
DCW (g/L)	2.3	3.2	3.1	2.7	2.7	2.8	3.1	3.2	4.2
Gellan yield (g/L)	5.7	14.1	16.6	18.2	11.5	16.4	16.6	13.0	22.6
Total sugar concentration (g/L)	10	20	30	40	30	30	30	30	30
Cell yield on total sugar consumption (g/g)	0.23	0.16	0.10	0.07	0.09	0.09	0.10	0.11	0.14
Gellan yield on total sugar consumption (g/g)	0.57	0.71	0.55	0.46	0.38	0.55	0.55	0.43	0.75
Gellan productivity (g/L/h)	0.16	0.27	0.28	0.30	0.17	0.27	0.27	0.22	0.38

Table 2
Comparison of enzyme activity in gellan fermentation under different culture strategy.

	Initial sucrose concentration (g/L)				Temperatures (°C)				Two-stage strategy
	10	20	30	40	25	28	30	33	
UDP-glucose pyrophosphorylase activity (U/mg)	12.06 ± 0.15	18.77 ± 0.21	23.64 ± 0.16	20.67 ± 0.14	18.34 ± 0.11	19.06 ± 0.23	21.64 ± 0.13	12.03 ± 0.17	42.33 ± 0.32
Glucosyltransferase activity (U/mg)	1.87 ± 0.06	2.11 ± 0.14	2.14 ± 0.05	2.06 ± 0.23	2.06 ± 0.11	2.19 ± 0.07	2.14 ± 0.12	1.53 ± 0.05	5.42 ± 0.08

Data are shown as mean ± S.D. (n = 3).

concentration of sucrose and a lower temperature (28 °C) would facilitate gellan biosynthesis.

As shown in Fig. 5, the use of the two-stage strategy, led the DCW to increase continuously until it reached 4.21 g/L at 24 h. The highest gellan production (22.61 g/L) was achieved at 60 h with 30 g/L of total sucrose, and was 35.71% higher than the result of the best conventional batch operation (16.66 g/L) with an initial sucrose concentration of 30 g/L. The fermentation parameters are listed in Table 1. It was obvious that the two-stage culture strategy considerably improved both the DCW and the gellan yield. The values of the gellan yield on total sugar consumption and gellan productivity with different fermentation process are also shown in Table 1. By applying the two-stage strategy, the value of the gellan yield on total sugar consumption and gellan productivity were 0.75 g/g and 0.38 g/L/h, whereas the highest values using the traditional approach were 0.71 g/g and 0.28 g/L/h. The proposed two-stage strategy was thus proven to successfully improve gellan production.

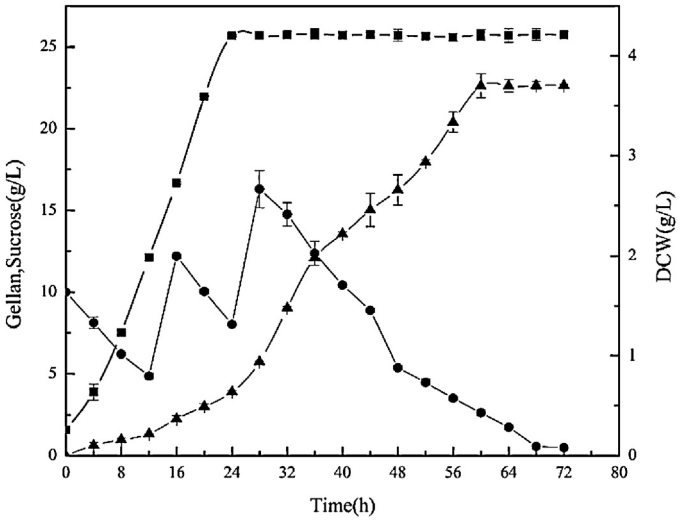

Fig. 5. Effect of two-stage strategy on DCW (■), gellan concentration (▲) and residual sucrose concentration (●) during gellan fermentation by *S. paucimobilis*. Data are shown as mean ± S.D. (n = 3).

3.4. Effects of the two-stage strategy on the glucosyltransferase activity and UDP-glucose pyrophosphorylase activity

UDP-glucose pyrophosphorylase, which is the key enzyme involved in the sugar metabolism and is potentially involved in the production of the precursors of gellan biosynthesis in *S. paucimobilis* ATCC 31461, was grown at different cultures and analyzed. The results in Table 2 demonstrated that UDP-glucose pyrophosphorylase activity in *S. paucimobilis* ATCC 31461 grown using the two-stage strategy was higher than that in cultures grown on other cultures.

Glucosyltransferase, which can sequentially transfer sugar donors to an activated lipid carrier to generate gellan polymerization, plays an important role in the biosynthesis of gellan with a high yield in *S. paucimobilis* ATCC 31461 (Sá-Correia et al., 2002). Thus, the activity level of glucosyltransferase, the key enzyme potentially involved in the production of gellan at different culture, was determined. The results (Table 2) showed that glucosyltransferase activity was higher with two-stage strategy than with the traditional approach.

These results demonstrated that both UDPG-pyrophosphorylase activity and glycosyltransferase activity were enhanced in cells cultivated using the two-stage strategy, which was strongly correlated with gellan production.

4. Conclusions

This study analyzed the effect of the different initial sucrose concentrations and temperatures on the gellan fermentation process. The results show that low sucrose concentrations and relatively high temperatures at the early stage of fermentation are advantageous to cell growth and sucrose consumption (cell growth stage). However, moderately high sucrose concentrations and low temperatures may improve the gellan production (gellan production stage). This phenomenon indicates that the best sucrose concentration and temperature for cell growth and gellan accumulation differ, and that single sucrose concentration and temperature control experiments will only produce one set of conditions suitable for either cell growth or product formation. It is beneficial to control sucrose concentration and culture temperature simultaneously in gellan fermentation, and a two-stage strategy that achieves this was thus developed.

The results demonstrate that gellan biosynthesis is considerably improved by using the proposed two-stage strategy. Meanwhile, high gellan yield was related to high UDPG-pyrophosphorylase activity and glucosyltransferase activity. The strategy is particularly attractive for large-scale gellan fermentation, because the key variables are easily controlled and manipulated.

Acknowledgements

The authors gratefully acknowledge the financial support from The provincial natural science research project of China (No. KJ2013B215).

References

Arockiasamy, S., & Banik, R. M. (2008). Optimization of gellan gum production by *Sphingomonas paucimobilis* ATCC 31461 with nonionic surfactants using central composite design. *Journal of Bioscience and Bioengineering*, *105*, 204–210.

Bajaj, I. B., Survase, S. I., Saudagar, P. S., & Singhal, R. S. (2007). Gellan gum: Fermentative production, downstream processing and applications. *Food Technology and Biotechnology*, *45*, 341–354.

Banerjee, S., Ravi, R., & Bhattacharya, S. (2013). Textural characterisation of gellan and agar based fabricated gels with carrot juice. *LWT – Food Science and Technology*, *53*, 255–261.

Banik, R. M., Kanari, B., & Upadhyay, S. N. (2000). Exopolysaccharide of the gellan family: Prospects and potential. *World Journal of Microbiology and Biotechnology*, *16*, 407–414.

Banik, R. M., & Santhiagu, A. (2006). Improvement in production and quality of gellan gum by *Sphingomonas paucimobilis* under high dissolved oxygen tension levels. *Biotechnology Letters*, *28*, 1347–1350.

Banik, R. M., Santhiagu, A., & Upadhyay, S. N. (2007). Optimization of nutrients for gellan gum production by *Sphingomonas paucimobilis* ATCC-31461 in molasses based medium using response surface methodology. *Bioresource Technology*, *98*, 792–797.

Duan, X. H., Chi, Z. M., Wang, L., & Wang, X. H. (2008). Influence of different sugars on pullulan production and activities of a-phosphoglucose mutase, UDPG-pyrophosphorylase and glucosyltransferase involved in pullulan synthesis in *Aureobasidium pullulans* Y68. *Carbohydrate Polymers*, *73*, 587–593.

Fialho, A. M., Martins, L. O., Donval, M. L., Leitão, J. H., Ridout, M. J., Jay, A. J., et al. (1999). Structures and properties of gellan polymers produced by *Sphingomonas paucimobilis* ATCC 31461 from lactose compared with those produced from glucose and from cheese whey. *Applied and Environmental Microbiology*, *65*, 2485–2491.

Fialho, A. M., Moreira, L. M., Granja, A. T., Popescu, A. O., Hoffmann, K., & Sá-Correia, I. (2008). Occurrence, production, and applications of gellan: Current state and perspectives. *Applied Microbiology and Biotechnology*, *79*, 889–900.

Huang, J., Jiang, S., Xu, X., Wu, H., Zhu, X., Ke, Z., et al. (2012). Effects of carbon/nitrogen ratio, dissolved oxygen and impeller type on gellan gum production in *Sphingomonas paucimobilis*. *Annals of Microbiology*, *62*, 299–305.

Jina, H., Lee, N. K., Shinb, M. K., Kimc, S. K., Kapland, D. L., & Lee, J. W. (2003). Production of gellan gum by *Sphingomonas paucimobilis* NK2000 with soybean pomace. *Biochemical Engineering Journal*, *16*, 357–360.

Kanari, B., Banik, B. B., & Upadhyay, S. N. (2002). Effect of environmental factors and carbohydrate on gellan gum production. *Applied Microbiology and Biotechnology*, *102*, 129–139.

Martins, L. O., & Sa-Correia, I. (1994). Temperature profiles of gellan gum synthesis and activities of biosynthetic enzymes. *Biotechnology and Applied Biochemistry*, *20*, 385–395.

Nampoothiri, K. M., Singhania, R. R., Sabarinath, C., & Pandey, A. (2003). Fermentative production of gellan using *Sphingomonas paucimobilis*. *Process Biochemistry*, *38*, 1513–1519.

Nuno, A. S., Micheal, J. C., Roger, Y. T., Nuno, S., Antonio, J., Salgado, R. L., et al. (2012). The effects of peptide modified gellan gum and olfactory ensheathing glia cells on neural stem/progenitor cell fate. *Biomaterials*, *33*, 6345–6354.

Pan, S., Yao, D., Chen, J., & Wu, S. (2013). Influence of controlled pH on the activity of UDPG-pyrophosphorylase in *Aureobasidium pullulans*. *Carbohydrate Polymers*, *92*, 629–632.

Per Erik, J., Bengt, L., & Paul, A. S. (1983). Structural studies of gellan gum, an extracellular polysaccharide elaborated by *Pseudomonas elodea*. *Carbohydrate Research*, *156*, 135–139.

Prajapati, V. D., Jani, G. K., Zala, B. S., & Khutliwala, T. A. (2013). An insight into the emerging exopolysaccharide gellan gum as a novel polymer. *Carbohydrate Polymers*, *93*, 670–678.

Sá-Correia, I., Fialho, A. M., Videira, P., Moreira, L. M., Marques, A. R., & Albano, H. (2002). Gellan gum biosynthesis in *Sphingomonas paucimobilis* ATCC 31461: Genes, enzymes and exopolysaccharide production engineering. *Journal of Industrial Microbiology and Biotechnology*, *29*, 170–176.

Wang, X., Xu, P., Yuan, Y., Liu, C., Zhang, D., Yang, Z., et al. (2006). Modeling for gellan gum production by *Sphingomonas paucimobilis* ATCC 31461 in a simplified medium. *Applied and Environmental Microbiology*, *72*, 3367–3374.

West, T. P. (2003). Effect of temperature on bacterial gellan production. *World Journal of Microbiology and Biotechnology*, *19*, 649–652.

West, T. P., & Fullenkamp, N. A. (2001). Effect of culture medium pH on bacterial gellan production. *Microbios*, *105*, 133–140.

West, T. P., & Strohfus, B. (1998). Effect of complex nitrogen sources upon gellan production by *Sphingomonas paucimobilis* ATCC 31461. *Microbios*, *94*, 145–152.

Zhan, D. D. (2005). Ultraviolet spectrophotometric determination of sucrose content. *Physical Testing and Chemical Analysis Part B: Chemical Analysis*, *41*, 363–365.