Biotechnol Lett (2006) 28:1347–1350
DOI 10.1007/s10529-006-9098-3

ORIGINAL PAPER

Improvement in production and quality of gellan gum by *Sphingomonas paucimobilis* under high dissolved oxygen tension levels

R. M. Banik · A. Santhiagu

Received: 14 February 2006 / Accepted: 2 May 2006 / Published online: 4 July 2006

Abstract The effect of agitation rate and dissolved oxygen tension (DOT) on growth and gellan production by *Sphingomonas paucimobilis* was studied. Higher cell growth of 5.4 g l^{-1} was obtained at 700 rpm but maximum gellan (15 g l^{-1}) was produced at 500 rpm. DOT levels above 20% had no effect on cell growth but gellan yield was increased to 23 g l^{-1} with increase in DOT level to 100%. Higher DOT levels improved the viscosity and molecular weight of the polymer with change in acetate and glycerate content of the polymer.

Keywords Aeration · Agitation · Exopolysaccharide · Gellan · *Sphingomonas paucimobilis*

Introduction

Gellan is an exopolysaccharide (EPS) produced by *Sphingomonas paucimobilis*. It has applications as a gelling, binding and thickening agent in food and pharmaceutical industries due to its ability to form thermoreversible gels (Morris 1990). Gellan is composed of tetrasaccharide repeating units of D-glucose, L-rhamnose and D-glucuronic acid in the ratio of 2:1:1 with *O*-acetyl and L-glyceryl groups on the D-glucosyl residue as the side chain (O'Neill et al. 1983). The viscous and non-toxic nature of gellan places it as a vehicle for ophthalmic and other sustained release dosage forms (Calfors et al. 1998; Fattah et al. 1996). Gelrite, a commercial form of gellan, is used as a replacement for agar in solidifying microbial media, especially those used for the isolation of thermophiles (Lin and Casida 1984) and only 0.3–0.5% of gellan is needed to form gels. Agitation and dissolved oxygen (DO) control are important in aerobic fermentation processes particularly in polysaccharide production, since the broth becomes highly viscous and limits mass and oxygen transfer which influence the cellular activities and secondary metabolite production. Studies related to the effect of agitation and dissolved oxygen tension (DOT) on the production and quality of gellan are scare. The present study reports the effect of varying agitation rate and DO levels on growth of *Sphingomonas paucimobilis*, gellan production and polymer quality to identify most suitable aerobic condition that favours gellan fermentation.

R. M. Banik · A. Santhiagu (✉)
School of Biochemical Engineering, Institute of Technology, Banaras Hindu University, Varanasi 221 005, India
e-mail: asanthiagu@rediffmail.com

Materials and methods

Media and culture conditions

Sphingomonas paucimobilis ATCC-31461 was grown in medium (g l^{-1}): 40 sucrose, 1.25 monosodium glutamate, 1 Casaminoacid, 3 KH_2PO_4, 5 Na_2HPO_4, 1 K_2SO_4, 0.35 $CaCl_2 \cdot 2H_2O$, 0.45 $MnSO_4 \cdot 7H_2O$, 1 NaCl. Inoculum was developed in 50 ml of the medium in 250 ml Erlenmeyer flasks and incubated in an orbital shaker at 30 ± 1 °C and 200 rev/min for 24 h.

Production of gellan

Gellan production was carried out in a 5 l fermentor with 3 l of production medium with 5% (v/v) inoculum and at 30 ± 1 °C, pH 6.5 and 4.5 l air min^{-1}. The fermentor was equipped with two Rushton turbines (diameter 7 cm). To study the effect of agitation speed, fermentations were carried out at different agitator speeds and the DO was maintained above 20% by adjusting air flow rate. The effect of DOT was analysed by carrying out experiments at different DOT levels. Dissolved O_2 was measured as % pO_2 by a Ingold DO probe (Ingold, Switzerland). Standard deviation of the readings was lower than 5% in oxygen tension.

Determination of maximum specific growth rate, K_La and oxygen transfer rate

Maximum specific growth rate (μ_{max}) was calculated from the slope of the plot of ln (X/X_0) versus time as per the equation:

$$\ln (X/X_0) = \mu_{max} t$$

Determination of K_La was done by gassing out method (Rainer 1990). The culture medium was degassed by sparging nitrogen followed by aeration and agitation as per given conditions. Oxygen absorption was measured by using a DO probe by recording the change in DO concentration with time. Then K_La was calculated by taking the inverse slope of the semi logarithmic plot of ($1 - pO_2$) versus time according to equation given below.

$$\ln (1 - pO_2) = K_L a \times t$$

where t = time in hours and pO_2 = % DO concentration in broth. The oxygen transfer rate (OTR) from gas phase into the broth was calculated by

$$\mathrm{OTR} = K_L a (C^* - C)$$

Analytical methods

To isolate the polymer, the fermented broth was heated for 15 min, cooled and the pH was adjusted to 10 and heated for 10 min in a constant water bath, cooled and the pH was brought down to 7. Then the broth was centrifuged at $10{,}000 \times g$ for 45 min to separate the cells. The cells were treated with dimethyl sulphoxide (2×10 ml) to remove any adhering polymer and centrifuged ($10{,}000 \times g$ for 45 min) and dried at 80°C to express biomass (cell dry weight). The supernatant was added with thrice the volume of 2-propanol to precipitate the polymer and kept overnight at 4°C, centrifuged at $10{,}000 \times g$ for 45 min, dried at 80°C and weighed. Viscosity of the polymer (0.1% in water, 30°C) was measured by using Brookfield viscometer (RVTDV) using spindle No. 51 at 10 s^{-1} shear rate. Glucose content was estimated by using GOD-PAP enzymatic kit (Human, India) after hydrolyzing gellan with 2 M H_2SO_4 and the glucose formed was oxidized by glucose oxidase to hydrogen peroxide which turns red–violet with phenol and 4-aminophenazone. The absorbanc was measured at 500 nm. The content of rhamnose, glucuronic acid, glycerate and acetate of the polymer was determined by methods described by Dische and Shettles (1948), Bitter and Muir (1962), Martins and Serra-Correia (1994), and McComb and McReady (1957) respectively. Molecular weight of the polymer was determined by applying Mark–Houwink–Sakurada (MHS) relation (Rudin 1982) and the constants for the MHS relation were estimated by plotting intrinsic viscosities and molecular weights available from the literature.

Results and discussion

Effect of agitation on growth and gellan production

Growth of *Sphingomonas paucimobilis* increased up to 5.4 g dry cells l^{-1} with an agitation rate up of 700 rpm (Table 1). Specific growth rate (μ_m) was high at 700 rpm (0.38 h^{-1}) and was comparatively low at 1000 rpm (0.29 h^{-1}). This was in contrary to the report given by Giavasis et al. (2006), in which the authors reported higher cell growth at 1000 rpm. High agitation rate particularly with high airflow rate used in this case could be toxic to cells (Toma et al. 1991). Gellan production increased up to 500 rpm (14 g l^{-1}) due to increased mass and oxygen transfer and decreased at 700 rpm (13 g l^{-1}) because of stimulation of cell growth than gellan production. Sucrose and nitrogen were consumed at a faster rate (data not shown) with increase in agitation speed due to enhanced mass transfer.

Effect of DOT on growth and gellan production

DOT levels above 10% showed no significant effect on growth of *Sphingomonas paucimobilis* (Table 2). At 10% DOT level, oxygen limitation occurred as evidenced from low biomass (3.2 g l^{-1}) obtained at this level. At optimum agitation rate, DOT level act as a driving force to increase oxygen uptake rate by the cells, which resulted in higher gellan production. This was in agreement with Dreventon et al. (1996) who suggested that higher oxygen transfer conditions favoured gellan production. Growth and gellan production by *Sphingomonas paucimobilis* at 500 rpm agitation speed and 40% DOT level is given in Fig. 1. Decrease in gellan productivity occurred depending upon DOT levels at broth gellan concentration above 8 g l^{-1} (Fig. 2) due to mass and oxygen transfer limitation. Analysis of the gellan chemical composition produced by fermentation with higher DOT levels suggested a decrease in acetyl and increase in glycerate content of the polymer (Table 3) with no significant variation in glucose, rhamnose, glucuronic acid levels. Polymer with higher viscosity and molecular weight was obtained from fermentation at higher DOT levels since degree of acetylation and glycerate level play a significant role in determining the viscosity of gellan (Jay et al. 1998). Gellan fermentation using *Sphingomonas* is known for its low yield (40–50% of sugar) (Thorne et al. 2000, but, in this present study, by maintaining highly aerated conditions, a higher gellan yield (58% of sugar) was achieved. This is

Table 1 Effect of different agitation rates on gellan fermentation by *Sphingomonas paucimobilis*

Agitation rate (rpm)	K_La (h^{-1})	OTR (mol l^{-1} h^{-1})	μ_{max} (h^{-1})	Biomass (g l^{-1})	Gellan (g l^{-1})
200	24.7	2.7	0.21	2.8	7.5
300	44.2	5.6	0.24	3.6	10.4
500	81.6	10.3	0.32	4.2	14.2
700	117.4	11.6	0.38	5.4	13.2
1000	140.8	12.1	0.29	3.8	11.7

Table 2 Effect of different DOT levels on gellan fermentation by *Sphingomonas paucimobilis*

DOT level (%)	OTR (mol l^{-1} h^{-1})	μ_{max} (h^{-1})	Biomass (g l^{-1})	Gellan (g l^{-1})
10	3.2	0.20	3.3	9.4
20	7.8	0.26	5.6	12.4
40	12.3	0.30	5.6	15.7
60	16.7	0.33	5.6	18.5
80	19.8	0.38	5.6	20.4
100	24.6	0.42	5.6	23.2

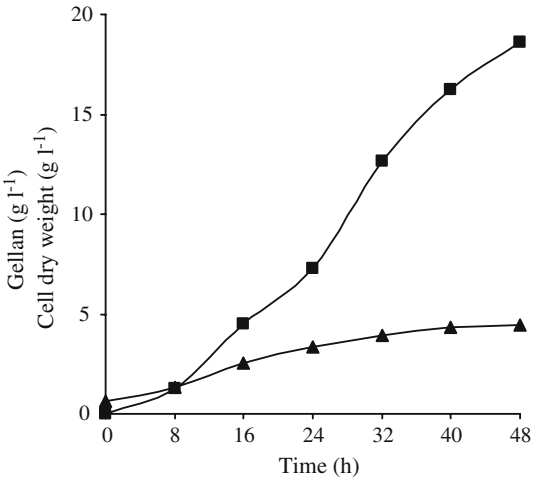

Fig. 1 Growth and gellan production by *Sphingomonas paucimobilis* at 500 rpm agitation speed and 40% DOT level. (▲) Cell dry weight (g l^{-1}); (■) Gellan (g l^{-1})

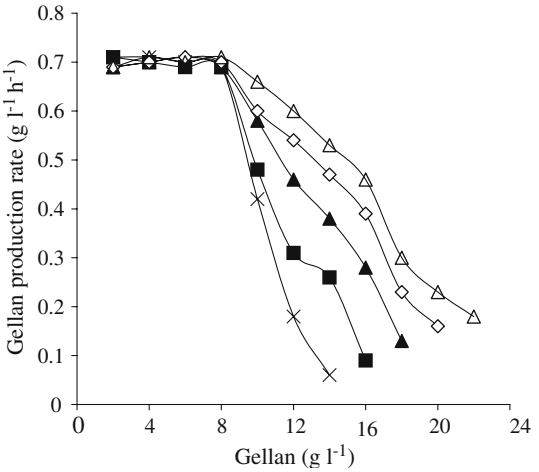

Fig. 2 Variation of gellan production rate with broth gellan concentration at different DOT levels. (×) 20% DOT; (■) 40% DOT; (▲) 60% DOT; (□) 80% DOT; (Δ) 100% DOT

Table 3 Analysis of gellan produced by *S. paucimobilis* at different DOT levels

DOT level (%)	Glycerate[a]	Acetate[a]	Viscosity (cP)	Molecular weight (Da)
10	0.55	0.58	191	285,000
20	0.63	0.52	280	330,000
40	0.70	0.44	352	382,000
60	0.81	0.32	382	446,000
80	0.88	0.25	462	522,000
100	0.96	0.16	546	607,000

[a]Molar ratio with respect to glucose

the first report about enhanced gellan production and quality with higher DOT levels.

References

Bitter T, Muir HM (1962) A modified uronic acid carbazole reaction. Anal Biochem 4:330–334

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

Dische Z, Shettles LB (1948) Specific colour reaction of methyl pentoses and a spectrophotometric micromethod for their determination. J Biol Chem 175:595–603

Dreventon E, Monot F, Lecortier J, Ballerini D, Choplin L (1996) Influence of fermentation hydrodynamics on gellan gum physico-chemical characteristics. J Fermen Bioeng 82:272–276

Fattah EA, Grant DJ, Gabe KE, Meshali MM (1996) Physical characteristics and release behaviour of salbutamol sulphate beads prepared with different ionic polysaccharide. Drug Dev Ind Pharm 24:541–547

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

Jay AJ, Colquhoun IJ, Ridout MJ, Brownsey GJ, Morris VJ, Fialho AM, Leitao JH, Sa-Correia I (1998) Analysis of structure and function of gellans with different substitution patterns. Carbohydr Polym 35:179–188

Lin CC, Casida LE (1984) Gelrite as a gelling agent in media for the growth of thermophilic microorganisms. Appl Environ Microbiol 47:427–429

Martins IO, Serra-Correia I (1994) Temperature profiles of gellan gum synthesis and activities of biosynthetic enzymes. Biotechnol Appl Biochem 20:385–395

McComb EA, McReady RM (1957) Determination of acetyl in pectin and in acetylated carbohydrate polymers. Anal Chem 29:819–820

Morris VJ (1990) Biotechnically produced carbohydrate with functional properties for use in food system. Food Biotechnol 4:45–47

O'Neill MA, Selvendran RR, Morris VJ (1983) Structure of the acidic extracellular gelling polysaccharide produced by *Pseudomonas elodea*. Carbohydr Res 124:123–133

Rainer BW (1990) Oxygen transfer in bioreactors. Chem Biochem Eng 4:185–196

Rudin A (1982) The elements of polymer science and engineering. Academic Press. London

Thorne L, Mikolajczak MJ, Armentrout RW, Pollock TJ (2000) Increasing the yield and viscosity of exopolysaccharides secreted by augmentation of chromosomal genes with multiple copies of cloned biosynthetic genes. J Ind Microbiol Biotechnol 25:49–57

Toma MK, Ruklisha MP, Vanags JJ, Zeltina MO, Leite MP, Galonina NI, Viesturs UE, Tendergy RP (1991) Inhibition of microbial growth and metabolism by excess turbulence. Biotechnol Bioeng 38:552–556