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Effects of dissolved oxygen and shear stress on the synthesis and molecular weight of welan gum produced from *Alcaligenes* sp. CGMCC2428

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ABSTRACT

The effects of dissolved oxygen (DO) and shear stress on the synthesis and molecular weight (MW) of welan gum were systematically investigated in the batch fermentation of *Alcaligenes* sp. CGMCC2428 in a 7.5 L bioreactor with working volume of 4.5 L. The results showed that a high shear stress (1000 rpm) enhanced cell growth, but decreased MW and welan gum concentration. The welan gum concentration reached a maximum of 22.8 ± 0.61 g/L at 800 rpm, and the highest MW was $(9.01 \pm 0.12) \times 10^5$ Da at 600 rpm. Furthermore, a moderate DO concentration (20%) enhanced MW and welan gum concentration; the maximum values were $(9.20 \pm 0.11) \times 10^5$ Da and 24.5 ± 0.53 g/L, respectively. To further investigate the effects of DO concentration on the synthesis and MW of welan gum, this study also compared the metabolic flux distribution of *Alcaligenes* sp. CGMCC2428 at various DO concentrations. The results demonstrated that glucose-6-phosphate and acetyl-coenzyme A nodes were flexible. The flux of glucose-1-phosphate to welan gum was enhanced at DO concentration of 20%. Increased flux might also be a reason for the increased MW and welan gum concentration observed.

1. Introduction

Welan gum is an exopolysaccharide (EPS) produced by *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 is widely used in different industries thanks to its high MW and unique viscoelastic and rheological properties [3].

As with other biopolymers, welan gum synthesis and MW characteristics are likely to be affected by DO and agitation, given the fact that in such (polysaccharide) processes, the efficiency of nutrient and oxygen transfer is easily influenced by the high viscosity of the process fluid [4,5]. As the broth viscosity increases, there is a significant decrease in oxygen mass transfer rate due to the extracellular accumulation of the biopolymer. The DO concentration becomes the limiting nutrient, and the oxygen mass transfer rate is the controlling step rate for the overall process [6]. Therefore, it is necessary to study the characteristics of DO and mass transfer during welan gum fermentation.

Previous studies have shown that oxygen supply and shear stress are critical factors for the synthesis and MW of microbial polysaccharides, such as xanthan gum, gellan gum and hyaluronic acid. Different opinions exist regarding the effect of oxygen supply and agitation during microbial polysaccharide fermentation. Garcia-Ochoa found that an increase in oxygen supply could enhance xanthan gum production [6]. Giavasis observed that high agitation decreased the production of gellan gum, while moderate agitation yielded the highest gellan gum MW and concentration [7]. Gao observed that high agitation enhanced hyaluronic acid concentration, but decreased its MW [8]. Kim found that high agitation caused low biomass and hyaluronic acid production, and significantly increased MW [9]. Duan observed that, while high DO concentrations and mild shear stress enhanced hyaluronic acid MW, hyaluronic acid concentration was independent of agitation and DO [5]. The effects of agitation and DO on welan gum synthesis and MW have not been clearly studied. Research into the relationship between the environment within the bioreactor vessel and welan gum MW is important, because the MW of a biopolymer usually affects its quality and applications.

Metabolic flux analysis (MFA) is a powerful methodology for determining metabolic pathway fluxes. Comparing metabolic flux responses to environmental perturbations can further the current understanding of cellular physiology and metabolism [10,11]. The pathways of glucose metabolism in *Alcaligenes* sp. are very similar to those in *Alcaligenes faecalis* [12]. In a previous study, the

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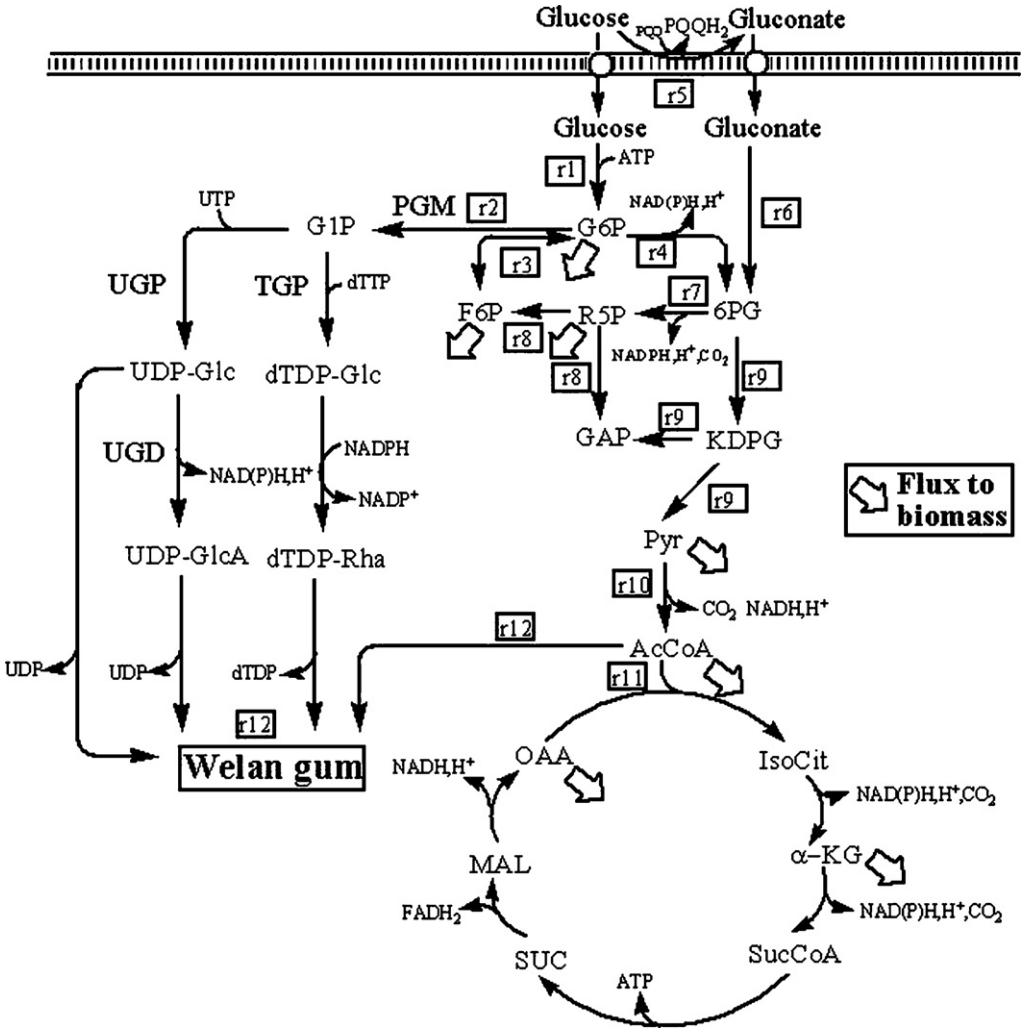

Fig. 1. The metabolic model of *Alcaligenes* sp. CGMCC2428 during welan gum production. Hollow arrows indicate the contribution of the metabolite to biomass synthesis.

biosynthetic pathway of welan gum by *Alcaligenes* sp. was examined, and the biochemical reactions involved in the network were determined [13]. In the literature, Gao et al. [14] used MFA to demonstrate that a low DO concentration favored hyaluronic acid yield.

In the present study, the effects of DO and shear stress on the physiology of *Alcaligenes* sp., welan gum synthesis and MW were systematically investigated in the batch fermentation of *Alcaligenes* sp. CGMCC2428. The flux distributions of different DO during welan gum fermentation were also calculated. The MFA results are expected to further the current understanding of the effect of DO on the synthesis and MW of welan gum.

2. Materials and methods

2.1. Microorganism and medium

Alcaligenes sp. CGMCC2428 used in this study was deposited at the General Microbiological Culture Collection Center in China. The seed medium contained (in g/L): 20 glucose, 1 yeast extract, 3 peptone, $2K_2HPO_4{\cdot}3H_2O$ and 0.1 $MgSO_4$ at pH 7.2–7.4. The fermentation medium was comprised of (in g/L): 50 glucose, 8 $(NH_4)_2SO_4$, $3K_2HPO_4{\cdot}3H_2O$ and 0.4 $MgSO_4$. The initial pH was adjusted to 7.0.

2.2. Cultivation conditions

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 bioreactor (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. The pH was automatically controlled at 7.0 by adding 3 M NaOH.

The standard fermentation conditions were as follows: the agitation speed increased stepwise from 200 to 800 rpm, which kept the DO concentration above 10%, and the aeration rate at 1.0 volume of air per volume of liquid per minute (vvm). To investigate the effects of different agitation speeds on welan gum production by *Alcaligenes* sp., the agitation speed was controlled at 200, 400, 600, 800 and 1000 rpm, with aeration rate fixed at 1.0 vvm. During fermentation at different DO concentrations, DO was controlled at 5%, 10%, 15%, 20% and 30% by adjusting aeration rates at 0.2–1.5 vvm and agitation speeds at 200–1000 rpm. All experiments were repeated three times.

2.3. Analytical methods

Dry cell weight (DCW) was determined from at least three 10 mL cell suspensions harvested by centrifugation, washed with distilled water, and dried at 105 °C to a constant weight. The glucose concentration was analyzed using a biosensor equipped with glucose oxidase electrode (SBA-40C, Shandong Academy of Sciences, China). The CO_2 and O_2 concentrations in the exhaust gas were measured using a gas analyzer (LKM2000A, LOKAS, Korea). The pH and DO were measured using inductors of the bioreactor. The concentration of welan gum was measured as previously reported [15].

The oxygen uptake rate (OUR), specific oxygen uptake rate (Q_{O_2}) and volumetric mass transfer coefficient (k_La) were measured using the dynamic gas-out/gas-in method reported by Bandyopadhyay [16]; the gassing out/in data were modified by taking the electrode response time into account [17]. Specifically, at low oxygen levels, the k_La was measured by the method of Na_2SO_3 flow feeding [18]. The concentration of Na_2SO_3 solution was 0.1–0.15 kM. Nitrogen gas was used to prevent the sulfite oxidation, and Co^{2+} (1 mM) was the catalyzator. For the operation time was within 100 s, the effects of microbial biological reaction and oxygen uptake rate