Effects of Different Agitator Blades on the Production Process of Biopolymer WL Gum by *Sphingomonas* sp. WG

Hui Li[1], Wanlong Zhou[1], Jiqian Wang[1,*] and Hu Zhu[1,2,*]

[1]*Centre for Bioengineering and Biotechnology, China University of Petroleum (East China), 66 Changjiang West Road, Qingdao 266580, People's Republic of China*

[2]*Engineering Research Centre of Industrial Biocatalysis, Fujian Province Higher Education Institutes, College of Chemistry and Materials Science, Fujian Normal University, 32 Shangsan Road, Fuzhou 350007, People's Republic of China*

Abstract: The effects of three agitators with different blade shapes, including the commonly-used six-flat-blade disc turbine, six-curved-blade disc turbine, and six-arrow-blade disc turbine on the production of biopolymer WL gum by *Sphingomonas* sp. WG was investigated in detail. The experiments were performed at the agitation speeds ranging from 100 to 500 r/min. The results showed that the moderate agitation speed was conducive to cell growth and WL gum production when using the agitators with curved-blade and arrow-blade. The maximal cell growth and WL gum were obtained at 300 r/min and 400 r/min, respectively. The six-arrow-blade disc turbine yielded the highest cell growth (11.74 g/L), WL gum production (40.89 g/L), and broth viscosity (91.62 Pa·s) among the three agitators. A comparison of the mixing characteristics showed that the power consumption of a six-arrow-blade disc turbine was the lowest. Overall, the six-arrow-blade disc turbine is suitable for WL gum fermentation with low energy consumption and high WL gum yield and viscosity. This work provided valuable information for large-scale industrial production of biopolymer WL gum.

Keywords: WL gum, *Sphingomonas* sp. WG, Agitator type, Mixing characteristics.

INTRODUCTION

Microbial exopolysaccharides (EPS) are natural biopolymers which can be widely used in many industries due to their remarkable rheological properties, thermostability, thickening and suspension properties, and emulsibility. WL gum is a kind of EPS that was secreted by the strain of *Sphingomonas* sp. WG [1]. It belongs to the EPS sphingans containing gellan gum, welan gum, diutan gum, etc. [2]. WL gum has a similar composition to welan gum, and the molar ratio of the neutral sugars mannose, glucose, and rhamnose is 1:2.28:2.12 [1]. It also presents good rheological properties such as high viscosity, typical shearing-thinning behavior, good viscoelasticity, and great stability to high salinity [3], which is attracting much attention for its special structure and function. Therefore, it has great potential to be applied in the enhanced oil recovery as an environmentally benign polymer [3]. Besides, it can also be used in food, ink, concrete additives as suspending, stabilizing, and thickening agents. However, low productivity severely limits the application of WL gum in the industry. The nutritional and environmental conditions showed a significant effect on the production of many microbial EPS [4-7]. In our previous work, the fundamental culture conditions such as the carbon source, nitrogen source, salt, pH, loading volume, and shaking speed were optimized by statistical methods. The WL gum production reached 39.95 g/L, 2.37-fold of the initial production (16.82 g/L) in shake flask cultivation [8]. However, how to realize its scale-up production is still a great challenge. One problem to solve is the oxygen supply, which is an essential factor in aerobic fermentation processes. During the fermentation production process, the accumulation of polysaccharides such as xanthan gum and welan gum led to viscous Non-Newtonian broth, and further limited mass and oxygen transfer, influenced cellular activities and metabolite production [9]. There is only a little available information about the effects of oxygen supply on sphingan production, such as gellan gum, welan gum, and some findings were even controversial [10]. For example, Banik *et al*. found that the increase in dissolved oxygen (DO) tension increased gellan gum yield [11], while Giavasis *et al*. found that oxygen limitation preceded the phase of maximum gellan production and probably enhanced polysaccharide biosynthesis [12]. Similarly, a moderate DO concentration (approximately 20%) was beneficial for welan gum production [9]. Besides, too high agitation in the fermentation process might cause mechanical damage to a biopolymer and result in low biopolymer molecular weight [13]. Therefore, finding a proper oxygen supply strategy in WL gum fermentation

*Address correspondence to this author at the Centre for Bioengineering and Biotechnology, China University of Petroleum (East China), 66 Changjiang West Road, Qingdao 266580, People's Republic of China;
Tel: +86-532-86981563; E-mail: jqwang@upc.edu.cn; zhuhu@fjnu.edu.cn.

E-ISSN: 2311-8717/20

process is very necessary. Agitator is one of the essential factors to influence the oxygen supply and to realize high efficient and viscous fermentation. However, to our knowledge, there was little information about the suitable agitator type for WL gum production. Therefore, the suitable agitator were chosen from three different types of disc turbines for efficient polysaccharide production with high yield and high viscosity.

Rushton turbine (flat-blade disc turbine) is one of the most popular agitators used in the common aerobic fermentation process with low viscosity [14]. However, the dead zone may exist when the broth was viscous and shear-thinning, resulting in high power consumption and decreased overall fermentation [14]. Many modified agitators such as curved-blade and semi-circular-blade agitators with low power consumption were developed [15] to solve the questions mentioned above. In this work, besides the commonly-used six-flat-blade disc turbine, the other two modified disc turbines, including six-curved-blade disc turbine and six-arrow-blade disc turbine (Figure **1**), were selected to investigate their effects on biopolymer WL gum production.

2. MATERIALS AND METHODS

2.1. Strains, Culture Medium, and Inoculum Preparation

Sphingomonas sp. WG (CCTCC M2013161) was screened from sea mud samples of Jiaozhou Bay, China, and was maintained on LB agar slants. The composition of the seed medium was: glucose 10 g/L, yeast extract 1 g/L, tryptone 5 g/L, KH_2PO_4 2 g/L, and $MgSO_4$ 0.1 g/L. The fermentation medium was the optimized medium in our previous work [8] and contained 72.40 g/L of glucose, 3.58 g/L of yeast extract, 3.00 g/L of K_2HPO_4, 0.10 g/L of $MgSO_4$, and 0.10 g/L of $ZnSO_4$ and pH was adjusted to 7.0. At first, the strain was activated in 50 mL of seed medium in a 250-mL flask at 30°C and 150 r/min for 16 h-20 h. Ten milliliters of the activated cells were then transferred into 200 mL seed medium in a 1-L flask and cultured at 30°C, 150 r/min for 16 h-20 h to obtain the inoculums. Finally, batch fermentation was performed in a multi parallel bioreactor (Labfors 7.5, Infors, Bottmingen, Switzerland). Each bioreactor contained 4 L of medium and was equipped with a double-layer identical agitator (six-flat-blade disc turbine, six-curved-blade disc turbine, or six-arrow-blade disc turbine). The fermentation parameters were maintained: tank diameter: paddle diameter = 2: 1; inoculum size, 5% (v/v); temperature, 32.5°C; aeration rate, 0.5 vvm; incubation time, 72 h. The agitator speed was set at 100, 200, 300, 400, 500 r/min, respectively, to obtain the optimal speed of agitator for biopolymer WL gum production.

2.2. Analytic Methods

Samples were taken at 8-h intervals, and cell growth, viscosity, WL gum production, and pH were analyzed. The growth of *Sphingomonas* sp. WG was determined as the dry cell weight (DCW) after the cell mass was separated from the broth by centrifugation and dried [8]. The fermentation broth viscosity was detected on a Brookfield Viscometer DV-III with an ultra-low adaptor (Brookfield Engineering Laboratories), and No. 3 rotor spindle was chosen, and the shearing rate was set at 0.5 r/min at 20°C [1]. The EPS concentration was measured using the phenol-sulfuric acid colorimetric method, as described previously [16]. pH and dissolved oxygen (DO) were recorded by the pH electrode and DO electrode. The residual glucose was detected on an SBA-40C biosensor analyzer equipped with a glucose oxidase electrode[17] (Shandong Academy of Sciences, China) after the broth was diluted 100 times.

Figure 1: Three agitators with different blade shapes. (**A**) Six-flat-blade disc turbine. (**B**) Six-curved-blade disc turbine. (**C**) Six-arrow-blade disc turbine.

2.3. Calculation of the Mixing Characteristic

The value for the shear stress is directly proportional to impeller tip speed (ITS) $\mu_{t,}$ which has been calculated using the formula (1) involving the agitator diameter (d) and the impeller ratio (N) and is very close to the maximal shear stress γ_{max}.

$$\mu_t = \pi \times d \times N \quad (1)$$

The flow pattern changes can be characterized by the Reynolds number *R*e, calculated from the stirring speed N, d, the density ρ, and the apparent viscosity μ using the formula (2).

$$Re = ,N, d\text{-}2.\rho\text{-}\mu. \quad (2)$$

According to the value of *R*e, the flow pattern in a bioreactor with baffles can be classified as laminar flow (*R*e > 10) or turbulent mixing (*R*e > 300 for Non-Newtonian fluid). The mixing power is not only related to the flow pattern but also related to the structure of the reactor. Therefore, the mixing power for one impeller P_0 and multiple impellers P_m were calculated as follows:

$$P_0 = N_P \times N^3 \times d^5 \times \rho \quad (3)$$

$$P_m = P_0\,(0.4+0.6M) \quad (4)$$

Where Np is power number, N is agitation speed, d is the diameter of the impeller, ρ is the density, and M is the layer number of the impeller. Since the ratio of the diameter of the fermenter (D) to the diameter of the impeller (d) is different, the mixing power should be corrected by the correction factor f using the formula (5) and (6).

$$P_m^* = f \times P_m \quad (5)$$

$$f = ,1\text{-}3.\times [,D\text{-}d.\times ,H_L\text{-}d.]^{0.5} \quad (6)$$

Where Pm* is the corrected mixing power, f is the correction factor, D and d is the diameter of the bioreactor and impeller, respectively, H_L is the height of the fluid. When ventilating in the fermentation process, the gassed power Pg is related to the ventilation coefficient Na and is calculated in a formula (7) to (9).

$$Na = {}_{,}Q_{g}\text{-}Nd^3{}_{.} \quad (7)$$

$$P_g = P_m \times (1\text{-}12.6Na)\ (Na \le 0.0035) \quad (8)$$

$$P_g = P_m \times (0.62\text{-}1.85Na)\ (Na \ge 0.0035) \quad (9)$$

Where Na is the ventilation coefficient, Pm is the mixing power without ventilation.

3. RESULTS AND DISCUSSION

3.1. Effects of Agitçators with Different Blade Shapes on the Cell Growth

The agitation speed was set from 100 r/min to 500 r/min for the WL gum fermentation, and it showed a great influence on cell growth. As the agitation speed increased from 100 r/min to 300 r/min, the cell growth increased significantly. However, when the agitation speed beyond 300 r/min, the cell growth changes were different when agitators with different blade shapes were used. As shown in Figure **2A**, for the fermentation using the six-flat-blade disc turbine, the DCW still increased in the range of 300 r/min to 500 r/min. However, when six-curved-blade disc turbine and six-arrow-blade disc turbine were used (Figure **2B** and **2C**), the DCW were decreased at 400 r/min and 500 r/min, which meant that the too high agitation speed is not very suitable for cell growth. The effects of different impellers on cell growth at the same agitation speed were also compared. Among the three impellers, the

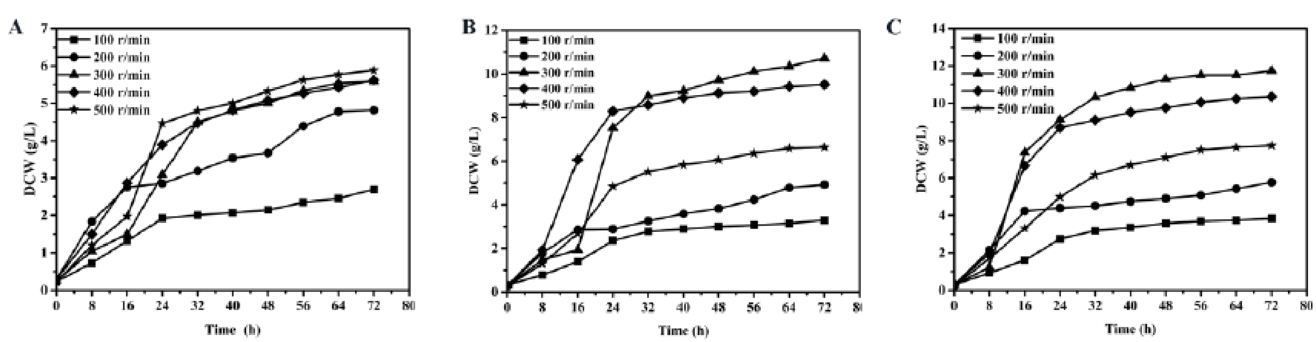

Figure 2: Time profiles of cell growth during fermentation with different impellers at different agitation speeds. (**A**) Time profiles of cell growth during fermentation with a six-flat-blade disc turbine. (**B**) Time profiles of cell growth during fermentation with a six-curved-blade disc turbine. (**C**) Time profiles of cell growth during fermentation with a six-arrow-blade disc turbine.

six-arrow-blade disc turbine was most suitable for cell growth, and the DCW reached 11.74 g/L, which was much higher than that of the six-curved-blade disc turbine (10.71 g/L) and six-flat-blade disc turbine (5.58 g/L) at 300 r/min. These data implied that the six-arrow-blade disc turbine at moderate agitation speed is better for cell growth.

3.2. Effects of Agitators with Different Blade Shapes on WL Gum Production

The six-flat-blade disc turbines are often chosen for their gas-handling capacity. For the WL gum fermentation with the six-flat-blade disc turbine, the agitation speed also greatly impacted WL gum production (Figure **3**). When flat-blade turbines were used, low agitation speed showed weak mixing performance in mass transfer and oxygen transfer. It formed a nearly unyielding region far from the rotating impeller, especially for highly viscous fluids [18]. The heterogeneity of the fermentation broth caused hypoxia or insufficient substrate concentration in the local region; this restricted the biosynthesis of WL gum. Therefore, the WL gum production increased as the agitation speed increased from 100 r/min to 400 r/min, and was 7.05 g/L, 10.02 g/L, 19.82 g/L, and 28.11 g/L when cultured for 72 h, respectively. However, when the agitation speed was 500 r/min, higher agitation speed might cause mechanical damage and hindered the polymerization of polysaccharides. Therefore, WL gum production was reduced and was 21.82 g/L when cultured for 72 h. The highest WL gum production and viscosity of the fermentation broth was 28.11 g/L and 44.86 Pa·s (Figure **4**), which was much lower than that in the shake flask (39.95 g/L and 76.91 Pa·s) [8]. Therefore, the six-flat-blade disc turbine might not be suitable for WL gum production.

Similar to the flat-blade disc turbines, curved-blade disc turbines generated primarily radial flow. But the shape changes of the blades reduced the impeller power requirements and enhanced the gas-handling capacity. Rotation with the concave side forward hindered trailing vortices formation behind the blades, and no large ventilated cavities generated on the convex surfaces with sparging. Curved-blade disc turbines can handle gas flow rates several times higher than those that caused flooding of flat-blade disc turbine [19]. Furthermore, during the gas-liquid dispersion processes, the mass transfer of curved-blade impeller was 20-30% higher than that of the Rushton turbine without significant loss of power [15]. In the process of WL gum fermentation, the maximal WL gum production was also reached at the agitation speed of 400 r/min and much higher (38.77 g/L) than that of the flat-blade disc turbine when cultured for 72 h. It indicated that the mixing performance at 400 r/min is the most sufficient for curved-blade disc turbine. The mass transfer and oxygen transfer are relatively high and thus favored the synthesis and accumulation of WL gum. Besides, the maximal viscosity of the fermentation broth is 83.21 Pa·s, higher than the viscosity obtained in shake flask, indicating that the shear force generated by the impeller has little effect on the viscosity quality of WL gum.

Differently from flat-blade disc turbines and curved-blade disc turbines, arrow-blade disc turbines generated radial flow and axial flow. They showed higher mixing efficiency and gentle shear force. Therefore, they are more suitable for the fermentation process with highly viscous broth. A comparison of biopolymer WL gum production obtained from different agitators at the same speed showed that the arrow-blade shape was most beneficial to WL gum production. The WL gum production was 12.03, 17.86, 38.20, 40.89, and 29.98 g/L at the agitation speed of 100 r/min to 500 r/min.

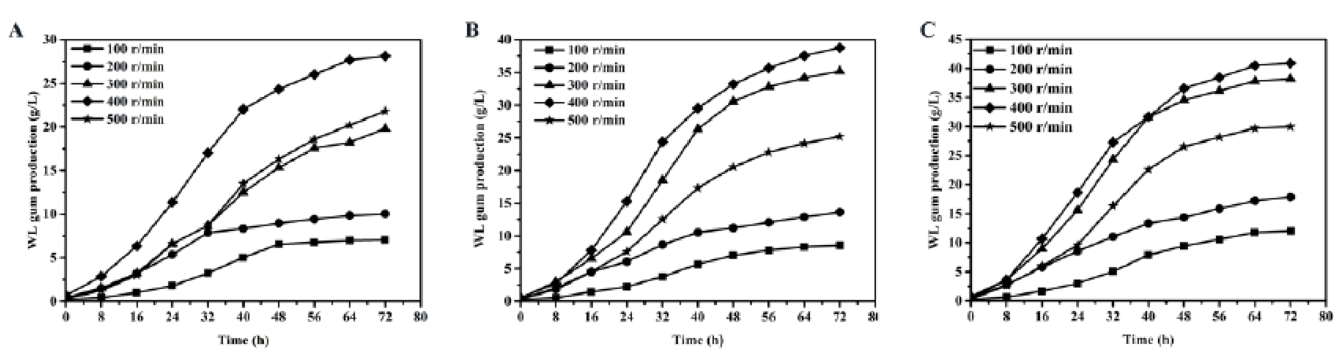

Figure 3: The effects of different impellers on WL gum production at different agitation speeds. (**A**) The effect of the six-flat-blade disc turbine on WL gum production. (**B**) The effect of a six-curved-blade disc turbine on WL gum production. (**C**) The effect of six-arrow-blade disc turbine WL gum production.

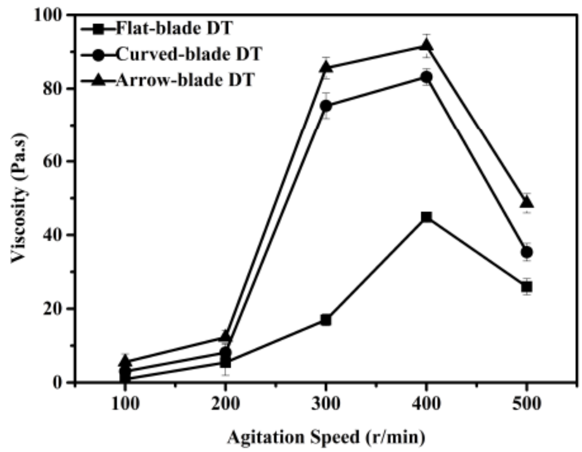

Figure 4: The effects of different impellers on fermentation broth viscosity at different agitation speeds. DT means disc turbine.

Furthermore, fermentation viscosity was an important parameter for WL gum quality. For all the impellers, as the good bulk mixing and dissolved oxygen increased caused by the increased agtitation speed during 100 ro 400 r/min, the viscosity of fermentation broth increased. However, when the agitation speed beyond 400 r/min, the viscosity decreased, which might be attributed to the mechanical damage caused by the very high agitation to WL gum. Li *et al*. [20] found that the molecular weight of welan gum increased from 400 r/min to 600 r/min and decreased from 600 r/min to 1000 r/min, which might suggest the mechanical damage of high agitation to welan gum. Thus, it indicated that the high agitation speed was not helpful for a high viscosity. The blade shape of the impeller also showed a great influence on the fermentation broth viscosity (Figure **4**). Like the cell growth and WL gum production, the broth viscosity using an arrow-blade disc turbine was also the highest. The viscosity significantly The maximal broth viscosity reached 91.62 Pa·s at 400 r/min when cultured for 72 h, which was nuch higher than that of the flask cultivation. All results suggested that the six-arrow-blade disc turbine is the best impeller among the three agitators.

3.3. The Comparison of Mixing Power of Different Impellers

In the actual fermentation process, the mixing process accounts for the majority of the total energy consumption because it transformed mechanical energy into kinetic energy of the fluid. Stirring power consumption is, therefore, one critical factor to be considered for microbial fermentation due to its possible effect on the fermentation production costs. The mixing efficiency is related to many factors, such as mixed phases, viscosity, and density of liquids, temperature, stirrer type, etc. Among these factors, the most critical property is the shape of the impeller [21]. Therefore, the mixing power characteristic of the three different impellers was compared (Table **1**). All the calculated *Re* of three impellers at different agitation speeds were ＞300, indicating that the fermentation

Table 1: The Parameters in Bioreactor

Agitation (r/min)	*N* (r/s)	μ_t (m·s^{-1})	Agitator Shape	*Re*	P_m (kW)	Na	P_g (kW)
100	1.67	0.42	Flat-blade	129587.54	0.1476	0.0390	0.0808
			Curved-blade	114551.82	0.1124		0.0616
			Arrow-blade	45507.50	0.0937		0.0513
200	3.33	0.84	Flat-blade	161320	1.1699	0.0196	0.8817
			Curved-blade	74479.26	0.8914		0.6718
			Arrow-blade	44004.70	0.7429		0.5599
300	5.00	1.26	Flat-blade	20536.98	3.9603	0.0130	3.3104
			Curved-blade	4629.43	3.0173		2.5220
			Arrow-blade	4073.29	2.5145		2.1019
400	6.67	1.68	Flat-blade	8482.19	9.4015	0.0098	8.2453
			Curved-blade	5591.60	7.1630		6.2822
			Arrow-blade	5130.03	5.9692		5.2351
500	8.33	2.09	Flat-blade	18754.87	18.3127	0.0078	16.5107
			Curved-blade	12815.38	13.9526		12.5797
			Arrow-blade	10445.63	11.6271		10.4830

broth is a turbulent fluid. Therefore, the power number Np value of six-flat-blade disc turbine, six-curved-blade disc turbine, and six -arrow-blade disc turbine were 6.0, 4.7, and 3.7, respectively.

The power input of the three types of the agitator was measured under conditions of no ventilation. The results showed that the Pm of all three impellers increased as agitation speed increased, but the power consumption of the six-arrow-blade disc turbine was the lowest at a given speed. In the actual fermentation process, ventilation might reduce the stirring power, and therefore the gas power was also calculated. Similar to Pm, the Pg of all impellers increased as the agitation speed increased, and the Pg value of the agitator with an arrow-blade shape was lower than that of the agitator with a curved-blade shape than that of agitator with a flat-blade shape. These results also confirmed that the change of Rushton turbine shape from flat-blade to arrow-blade reduced power consumption. The six-arrow-blade disc turbine is most suitable for WL gum production among the three impellers with different shapes.

CONCLUSION

The effects of different disc turbines with flat-blade, curved-blade, and arrow-blade on the fermentation process of WL gum were investigated at different agitation speeds ranging from 100 r/min to 500 r/min. The agitation speed greatly impacted cell growth and WL gum production, and the moderate agitation speed is conducive to cell growth and WL gum production. The six-arrow-blade disc turbine yielded the highest cell growth, WL gum production, and broth viscosity among the three impellers. A comparison of the mixing characteristics also proved that the power consumption of a six-arrow-blade disc turbine was the lowest. Overall, the six-arrow-blade disc turbine is suitable for WL gum fermentation with low energy consumption and high WL gum yield and viscosity. This work provided valuable information for WL gum scale-up production.

ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (31800075 and U1805234), 863 Program (2015AA020925), 100 Talents Program of Fujian Province, Natural Science Foundation of Fujian Province of China (2019J01264), Program for Innovative Research Team in Science and Technology in Fujian Province University, Fundamental Research Funds for the Central Universities (18CX02124A), and the Development Fund of State Key Laboratory of Heavy Oil Processing (20CX02202A).

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Received on 15-9-2020 Accepted on 20-10-2020 Published on 25-10-2020

DOI: https://doi.org/10.12974/2311-8717.2020.08.6