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Effect of the Shear Rate on Pullulan Production from Beet Molasses by *Aureobasidium pullulans* in an Airlift Reactor

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Abstract

The effect of the shear rate on pullulan production from beet molasses by *Aureobasidium pullulans* P56 in an airlift reactor was investigated. A maximum polysaccharide concentration (18.5 g/L), biomass dry weight (14.0 g/L), polysaccharide yield (38.5%), and sugar utilization (96%) was achieved at a shear rate of 42 s^{-1}. *A. pullulans* grown on beet molasses produced a mixture of pullulan and other polysaccharides. The highest value of pullulan proportion (30% of total polysaccharide) was obtained at a low shear rate (42 s^{-1}). The apparent viscosity of the fermentation broth increased as the shear rate increased up to 42 s^{-1} and then decreased. On the other hand, the dissolved oxygen concentration and the volumetric mass transfer coefficient increased with the increase of the shear rate from 21 to 84 s^{-1}. The external addition of L-glutamic acid, olive oil, and Tween-80 improved significantly the production of crude polysaccharide (27.0 g/L), but the pullulan content of the polysaccharide was low (20%).

Index Entries: Beet molasses; pullulan; *Aureobasidium pullulans*; batch culture; airlift reactor.

Introduction

Pullulan is an extracellular water-soluble microbial polysaccharide produced by strains of *Aureobasidium pullulans*. It is a linear mixed linkage α-D-glucan consisting mainly of maltotriose units interconnected via α-(1→6) linkages. A number of potential applications have been reported for this biopolymer as a result of its good film-forming properties; pullulan can form thin films that are transparent, oil resistant, and impermeable to

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oxygen. Pullulan may be used as a coating and packaging material, as a sizing agent for paper, as a starch replacer in low-calorie food formulations, in cosmetic emulsions, and in other industrial and medicinal applications *(1)*.

The production of pullulan from a chemically defined medium by different strains of *A. pullulans* has been described *(2–4)*. However, utilization of sucrose or glucose as a carbon source is not economical, and a less expensive carbohydrate source would be beneficial. LeDuy and Boa *(5)* and Leathers and Gupta *(6)* utilized peat hydrolyzate and fuel ethanol by-products, respectively, as fermentation substrates for pullulan production. Recently, considerable interest has been focused on using agricultural crops such as Jerusalem artichoke tubers and carob pods for polysaccharide production *(7,8)*. Molasses is a by-product of the sugar industry readily available at relatively low cost. However, little published information is available on the utilization of molasses as a carbohydrate raw material to produce pullulan *(9)*.

Airlift fermentor is an elongated nonmechanically stirred fermentor with an aspect ratio of height/diameter through which there is a unidirectional flow of gases. Airlift reactor compared with other fermentors has several advantages. The fermentation process can be controlled more easily. In addition, the required bulk mixing and mash transfer is more cost-effective and requires less energy cost.

The aim of this investigation was to examine the production of pullulan from treated molasses by a nonpigmented strain of *A. pullulans* in an airlift reactor as well as to study the effect of the shear rate on kinetic parameters of molasses fermentation.

Materials and Methods

Microorganism and Culture Conditions

A. pullulans P56, a strain deficient in melanin production, was kindly supplied by Prof. A. Mersmann of the Technical University of Munich. The microorganism was maintained on potato dextrose agar plates at 4°C and subcultured every 3 wk. Cells for inoculation of the culture medium were obtained from cultures grown on potato dextrose agar plates at 28°C for 48 h. From the Petri dish, two loops of *A. pullulans* cells were transferred to a 500-mL conical flask containing 100 mL of culture medium (pH 5.5) of the following composition: 30.0 g/L sucrose; 0.6 g/L $(NH_4)_2SO_4$; 0.4 g/L yeast extract; 5.0 g/L K_2HPO_4; 0.2 g/L $MgSO_4 \cdot 7H_2O$; and 1.0 g/L NaCl. The flask was incubated at 28°C for 48 h in a rotary shaker incubator (Lab Line Orbit-Environ Shaker, Lab-Line, Melrose Park, IL) at 200 rpm. This culture was used to inoculate the production medium at a level of 5% (v/v).

Pretreatment of Molasses

Beet molasses was obtained from the Greek sugar factory (Platy, Thessaloniki). One hundred and thirty-five grams of molasses were mixed

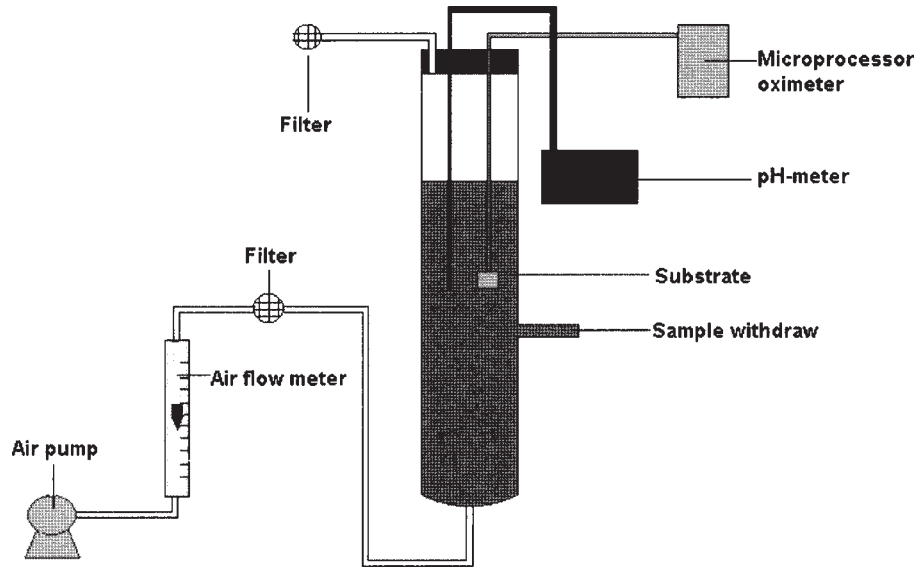

Fig. 1. Schematic diagram of airlift reactor used for pullulan production from beet molasses.

with 1.3 L of distilled water, and the solution was adjusted to pH 3.0 with 1 *N* H_2SO_4. The liquid was allowed to stand for 24 h to encourage the precipitation of heavy metals. The molasses solution was then centrifuged at 5000*g* for 15 min, and the pH of the supernatant was adjusted to 6.5 with 20 *N* NaOH. The solution containing 5% (w/v) total sugar concentration was sterilized at 121°C for 20 min. Samples of molasses prepared this way (production medium) were used for the production of pullulan by *A. pullulans*.

Fermentation Conditions

Fermentation was carried out in a 2-L glass bioreactor (height 65 cm, diameter 6.3 cm) with a working volume of 1.4 L (Fig. 1). The fermentor was sterilized at 121°C for 15 min. After cooling, 1.4 L of production medium was added. The medium was inoculated with 70-mL of inoculum. The fermentor was incubated at 28°C in a controlled temperature chamber. The air was supplied from the bottom of the column at rates of 1, 2, 3, and 4 vvm (1.4, 2.8, 4.2, and 5.6 L/min). To study the effect of nutrients on polysaccharide production, the molasses solution (treated with 1 *N* H_2SO_4, 50 g/L initial sugar) was supplemented with 1% L-glutamic acid (w/v), 2.5% olive oil (v/v), and 0.5% Tween-80 (v/v). The pH of the solution was adjusted to 6.5 with 20 *N* NaOH and the medium sterilized at 121°C for 20 min. The fermentation was carried out at an aeration rate of 2 vvm. All other fermentation conditions were the same as previously described.

Analytical Techniques

At specific time intervals, fermentation broth was removed from the reactor and analyzed. Total biomass (mycelial and yeast cells) dry weight was determined by centrifugation of the broth at 10,000*g* for 20 min and then washing the sediment with distilled water and drying at 105°C overnight. The first supernatant was combined with the washings, and the polysaccharide was precipitated with 2 vol of ethanol at 4°C for 1 h. The precipitate was filtered through a preweighed Whatman GF/A filter and dried at 105°C overnight. Residual sugars as glucose were determined in the filtrate according to Dubois et al. *(10)*. pH was measured using a Knick 646 pH meter (Funke Gerber, Berlin, Germany) equipped with a glass electrode. Dissolved oxygen concentration was determined with a microprocessor oximeter (OXI 96, WTW, Weilheim, Germany). The values of the readings were expressed as percentage of the initial level of saturation. The shear rate (γ, expressed in s^{-1}) was calculated using the following equation *(11)*:

$$\gamma = 2800U_g$$

where U_g is the air velocity (m/s) supplied into the column. The air velocity was given by the following equation:

$$U_g = 4Q/\pi d^2$$

in which Q is the air supply (m^3/s) and d is the diameter of the column (m). Based on this equation, the shear rate values corresponding to air supply of 1.4, 2.8, 4.2, and 5.6 L/min were 21, 42, 63, and 84 s^{-1}, respectively. The volumetric mass transfer coefficient (K_{La}, expressed as s^{-1}) was determined by the equation *(12)*:

$$K_{La} = 8.35 \times 10^{-4} U_g^{0.44} \cdot n_a^{-1.01}$$

in which U_g is the air velocity (m/s) and n_a is the apparent viscosity (Pa · s).

The viscosity of fermentation broth was measured with a Brookfield viscometer DV-II (Brookfield Engineering Laboratories, Stoughton, MA) fitted with a small samples adaptor SC4-18/13R. Determinations were made at 25°C and shear rate of 15.8 s^{-1}. Polysaccharide yield and sugar utilization were expressed as grams of polysaccharide/100 g of sugar utilized and grams of sugar utilized/100 g of initial sugar, respectively. Microscopic observations were made on a hemocytometer using a standard 14 Zeiss microscope, equipped with phase contrast. All experiments were repeated three times, and the reported data are averages. Statistical evaluation of the data was carried out through analysis of variance using the randomized block design. Comparison of the means was assessed using the least significant difference test. Variability was also expressed by coefficient of variation (cv) values.

When the maximum concentration of polysaccharide was observed, 100 mL of the fermentation broth was centrifuged at 10,000*g* for 20 min. The polysaccharide was precipitated from the supernatant with 2 vol of ethanol. After drying the precipitate, it was resuspended in 0.05 *M* sodium

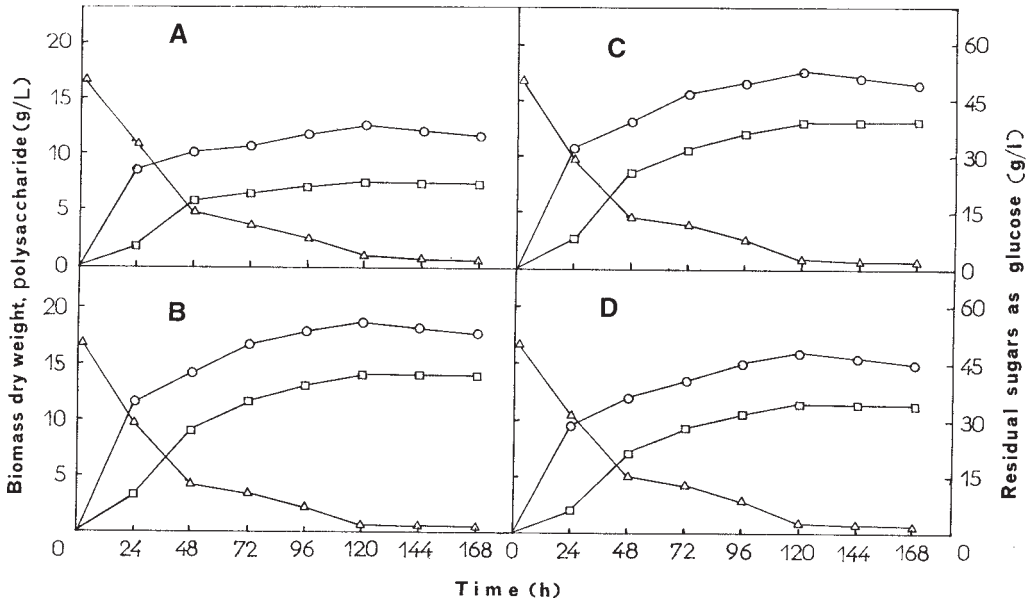

Fig. 2. Fermentation kinetics of *A. pullulans* during polysaccharide production from beet molasses in an airlift reactor at different shear rates **(A–D)** Shear rates 21, 42, 63, and 84 s^{-1}, respectively. ○, polysaccharide; □, biomass dry weight; △, residual sugars as glucose. Each point is the mean of three repetitions. cv values for all measured parameters did not exceed 5.5% in all cases.

acetate (pH 5.0) at a concentration of 1 mg/mL. To a 1-mL sample, 0.1 U/mL of Pullulanase (Sigma, St. Louis, MO; EC 3.2.1.41) was added and then incubated for 21 h at 25°C *(13)*. Enzyme was also added to a pure sample of pullulan (Sigma; P-4516) of the same concentration as previously described. The glucose equivalents were quantitated using the reducing sugar assay of Dygert et al. *(14)*. From this data, the pure pullulan content of the crude polysaccharide was determined.

Results and Discussion

Effect of Shear Rate on Polysaccharide, Biomass, and Residual Sugars Concentration

One important factor that influences the production of polysaccharide in an airlift reactor is the shear rate. The purpose of this experiment was to determine the optimum shear rate that would result in the highest pullulan concentration. As shown in Fig. 2, the polysaccharide concentration increased significantly when the shear rate was increased from 21 to 42 s^{-1}. This was probably owing to the increase in biomass, dissolved oxygen concentration, and volumetric mass transfer coefficient responsible for the accumulation of polysaccharide (Figs. 2, 3, and 4). Aeration could be beneficial to the growth and performance of microorganism cells by

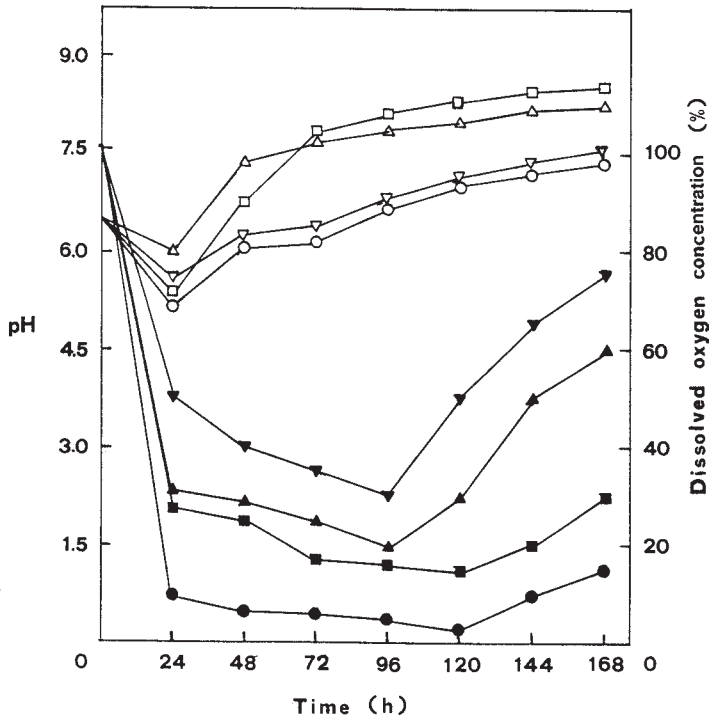

Fig. 3. Effect of the shear rate on pH and dissolved oxygen concentration during polysaccharide production from beet molasses by *A. pullulans* P56 in an airlift reactor. ○, □, △, ▽: Shear rates 21, 42, 63, and 84 s^{-1}, respectively (pH); ●, ■, ▲, ▼: shear rates 21, 42, 63, and 84 s^{-1}, respectively (dissolved oxygen concentration). Each point is the mean of three repetitions. cv values for all measured parameters did not exceed 4.7% in the case of pH and 8.5% in the case of dissolved oxygen concentration.

improving the mass transfer characteristics with respect to substrate, products/by-products, and oxygen. Aeration results in better mixing of the production medium, thus helping to maintain a concentration gradient between the interior and the exterior of the cells. This concentration gradient works in both directions; through better diffusion it helps maintain a satisfactory supply of sugars and other nutrients to the cells, while it facilitates the removal of gases and other by-products of catabolism from the microenvironment of the cells.

Finally, moderate air supply favors oxygen supply to the cells; this is especially important for high biomass concentrations. On the other hand, a further increase of the shear rate at values over 42 s^{-1} resulted in a decrease in biomass and polysaccharide concentration and a significant increase in dissolved oxygen concentration and volumetric mass transfer coefficient (Figs. 2, 3, and 4). *A. pullulans* is not a strict aerobic microorganism. Thus, high shear rates do not improve the growth of microorganism. The highest concentration of polysaccharide (18.5 g/L) was obtained at a shear rate of 42 s^{-1} after 120 h of incubation, and then decreased. A glucoamylase activ-

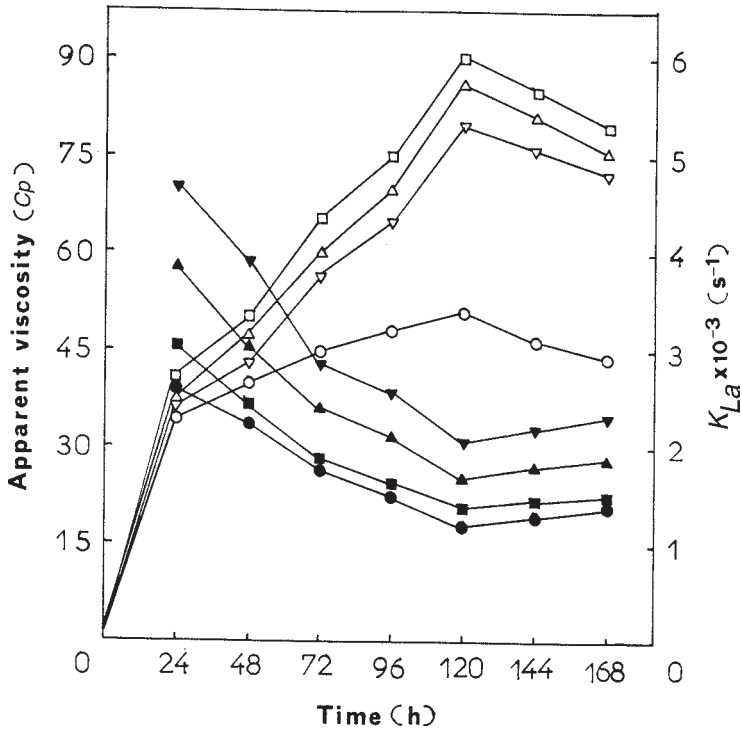

Fig. 4. Effect of the shear rate on apparent viscosity and volumetric mass transfer coefficient during polysaccharide production from beet molasses by *A. pullulans* P56 in an airlift reactor. ○, □, △, ▽: shear rates 21, 42, 63, and 84 s^{-1}, respectively (apparent viscosity); ●, ■, ▲, ▼: shear rates 21, 42, 63, and 84 s^{-1}, respectively (volumetric mass transfer coefficient). Each point is the mean of three repetitions. cv values for all measured parameters did not exceed 7.5% in all cases.

ity, named glucoamylase B, was recently detected in fermentation broth of *A. pullulans* at the late stages of fermentation *(15)*; this enzyme is capable of degrading not only starch but also pullulan. At shear rates of 21, 63, and 84 s^{-1}, the maximum polysaccharide concentration was 12.5, 17.5, and 16.0 g/L, respectively.

In our previous work (unpublished results), a maximum pullulan concentration (31 g/L) was obtained when *A. pullulans* P56 was grown in a chemically defined medium in a stirred tank fermentor after 168 h of incubation. Wecker and Onken *(16)* and Gibbs and Seviour *(17)* reported a maximum polysaccharide concentration of 9–20 g/L when different strains of *A. pullulans* were grown in synthetic medium in an airlift fermentor. Israilides et al. *(9)* reported a very little amount of polysaccharide produced from untreated molasses by *A. pullulans* NRRLY-6220 in shake flask culture. Roukas and Biliaderis *(8)* have reported a 6 g/L polysaccharide concentration from carob pod extract in shake flask culture, whereas in similar experiments by LeDuy and Boa *(5)*, maximum polysaccharide levels of 12–14 g/L were found for various strains of *A. pullulans* grown in peat

hydrolyzates. Leathers and Gupta *(6)* reported that a maximum concentration of polysaccharide (4.5 g/L) was obtained when *Aureobasidium* sp. strain NRRL Y-12,974 was grown in fuel ethanol by-products in batch culture. With a mixed culture of *A. pullulans* and *Kluyveromyces fragilis*, Shin et al. *(7)* obtained pullulan concentrations of 17.5 and 15.5 g/L using inulin and Jerusalem artichoke extracts as carbon sources, respectively. There are several reasons for such variability in pullulan production, including the strain of microorganism, the chemical composition of the substrate, the fermentation system, and generally the conditions utilized during fermentation.

The biomass concentration followed a pattern similar to polysaccharide concentration with maximum biomass concentration observed at the same time as the maximum concentration of polysaccharide was observed (Fig. 2). In all cultures the biomass concentration increased up to 120 h and then remained constant. The maximum biomass concentration (14.0 g/L) was obtained in culture grown at a shear rate of 42 s^{-1}.

As expected, the concentration of residual sugars decreased during the fermentation, coinciding with an increase in biomass and polysaccharide production (Fig. 2). The concentration of residual sugars fell rapidly during the first 48 h of fermentation, after which it decreased slowly. This was owing to a rapid increase of biomass and polysaccharide concentration observed at the same time. When the maximum concentration of polysaccharide was achieved, 26.3, 38.5, 36.4, and 33.7% of the sugar consumed was converted to polysaccharide in cultures grown at shear rates of 21, 42, 63, and 84 s^{-1}, respectively (Fig. 5). In this case, the total amount of sugar utilized was 96% in all cultures grown at different shear rates (Fig. 5).

The proportion of pullulan (as a percentage of total polysaccharide) decreased as the shear rate increased over 42 s^{-1} (Fig. 5). As shown in Fig. 5, there were significant differences (at the 5% level) in pullulan proportion of the crude polysaccharide between cultures grown at shear rates of 21, 42, 63, and 84 s^{-1}. The highest value of pullulan proportion (30% of total polysaccharides) was obtained in culture grown with a shear rate of 42 s^{-1}, whereas in cultures grown at shear rates of 21, 63, and 84 s^{-1}, the maximum pullulan proportion of the crude polysaccharide was 23, 25, and 18%, respectively. This may be explained by the fact that *A. pullulans* grown on molasses medium produced other polysaccharides.

In relevant work from our laboratory (unpublished results), it was found that *A. pullulans* P56 produced only pullulan from synthetic medium. In a previous study *(8)* on the production of pullulan from carob pod extract by *A. pullulans* SU-M18, it was found that the pure pullulan was 70% of the total polysaccharide. West and Reed-Hamer *(13)* reported that the pullulan content of the crude polysaccharide was 54 and 100% when *A. pullulans* ATCC 42023 was grown on sucrose or corn syrup as a carbon source, respectively. When *A. pullulans* QM-3092 was grown on 5% sucrose as a carbon source, the pullulan content of the polysaccharide synthesized was 89%, whereas when the same strain was grown on 5% glucose, the pullulan

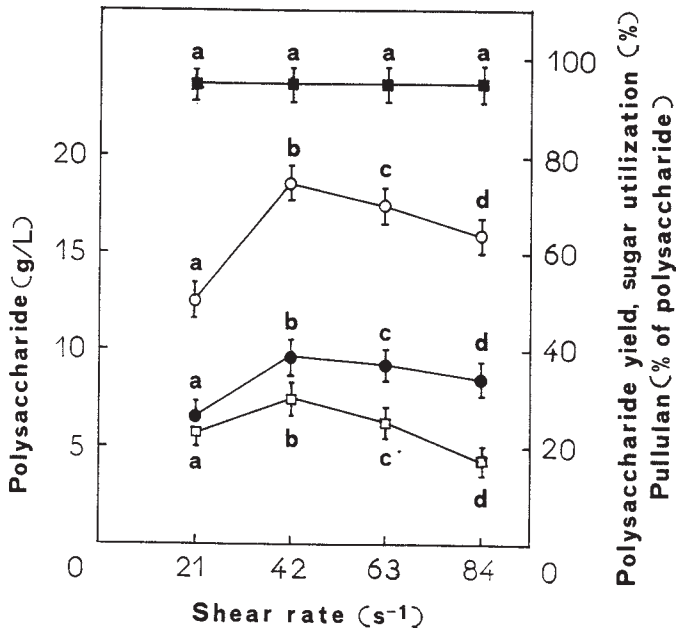

Fig. 5. Kinetic parameters of beet molasses fermentation by *A. pullulans* P56 in an airlift reactor at different shear rate values. ○, polysaccharide; □, pullulan (% of polysaccharide); ●, polysaccharide yield; ■, sugar utilization. Each point is the mean ± SD of three repetitions. Means with different letters in the same curve are significantly different at the 5% level by the least significant difference test (values are reported at 120 h of fermentation).

content of the polysaccharide was reduced to 65% *(18)*. In conclusion, these results show that the pullulan content of the crude polysaccharide depends on the strain of microorganism used and the chemical composition of the substrate.

The decrease of polysaccharide concentration and the pullulan content of the crude polysaccharide (Figs. 2 and 5) at shear rates higher than 42 s^{-1} was owing to the change in morphology of the microorganism during fermentation. Microscopic examination of the fermentation broth indicated that at an increase of shear rate greater than 42 s^{-1}, the number of yeastlike cells was decreased, whereas the number of mycelial forms increased. Generally, we observed that the amount of yeastlike cells was distinctly higher at low shear rates, whereas the amount of mycelial forms was higher at high shear rates. Roukas and Biliaderis *(8)*, Keely and Catley *(19)*, and Heald and Kristiansen *(20)* reported that pullulan was produced primarily by the yeastlike forms of *A. pullulans*. In conclusion, our results clearly show that low shear rates improved the production of polysaccharide and increased the pullulan content of the crude polysaccharide. These results agree with those of Wecker and Onken *(16)* but are not in agreement with those of McNeil and Kristiansen *(21)*, who reported that pullulan production increased with an increase in agitation speed.

Effect of Shear Rate on pH and Dissolved Oxygen Concentration

In all culture systems, the pH decreased during the first 24 h of fermentation and then increased until the end of the fermentation (Fig. 3). This was owing to the deamination of amino acids of molasses by *A. pullulans* and the production of ammonia, which increased the pH of the fermentation broth. The highest pH value was obtained in culture grown at a shear rate of 42 s^{-1}.

The concentration of dissolved oxygen increased with the increase of the shear rate (Fig. 3). In all cultures the concentration of dissolved oxygen fell rapidly during the first 24 h of fermentation and then decreased slowly. In the cultures grown with shear rates of 21, 42, 63, and 84 s^{-1}, the concentration of dissolved oxygen from 48 to 120 h fluctuated at 5, 15, 25, and 38% of the initial saturation level, respectively. At shear rates of 21–63 s^{-1}, only within the last period of the fermentation (120–168 h) did the dissolved oxygen concentration start to increase. On the other hand, in culture grown at a higher shear rate (84 s^{-1}), the dissolved oxygen concentration increased after 96 h of fermentation.

Effect of Shear Rate on Apparent Viscosity and Volumetric Mass Transfer Coefficient

During the fermentation, the broth consists of the liquid medium, in which the microorganism grows, the biomass, and any product that is produced by the microorganism. Thus, the rheology of the fermentation broth is affected by the composition of the original medium and its modification by the growing culture, the concentration and morphology of the biomass, and the concentration of microbial products. As shown in Fig. 4, the apparent viscosity increased as fermentation progressed up to 120 h, and then decreased. This was owing to the change in biomass and polysaccharide concentration during fermentation (Fig. 2). *A. pullulans* is a polymorphic microorganism that has a complex life cycle involving yeastlike cells and mycelial forms. The yeastlike cells in the polysaccharide fermentation made a minimal contribution to the high culture viscosity. Thus, the apparent viscosity of the fermentation broth was primarily owing to the mycelial and polysaccharide formation. The viscosity increased with the increase of the shear rate from 21 to 42 s^{-1} and decreased as the shear rate was further increased. The maximum apparent viscosity (90 c_p) was obtained in culture grown at a shear rate of 42 s^{-1} after 120 h of incubation. These results show that the apparent viscosity of the fermentation broth followed a pattern similar to that of biomass and polysaccharide concentration during fermentation (Figs. 2 and 4). In general, the changes in biomass, polysaccharide concentration, and viscosity with increasing shear rate indicate that the shear rate is the major determinant of the rheological properties of the fermentation broth.

In all culture systems, the volumetric mass transfer coefficient decreased with the increase of fermentation time up to 120 h, and then

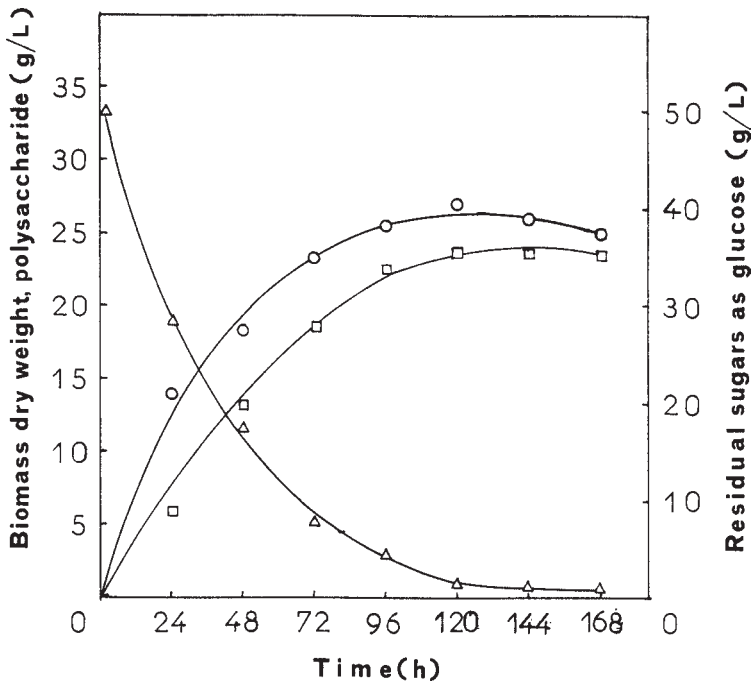

Fig. 6. Fermentation kinetics of *A. pullulans* P56 during polysaccharide production from beet molasses supplemented with nutrients in an airlift reactor. ○, polysaccharide; □, biomass; △, residual sugars as glucose. Each point is the mean of three repetitions. cv values for all measured parameters did not exceed 5% in all cases.

increased (Fig. 4), owing to the increase/decrease of apparent viscosity of the fermentation broth. This finding shows that the biomass and the polysaccharide concentration present problems in oxygen transfer and bulk mixing during fermentation. As shown in Fig. 4, the volumetric mass transfer coefficient increased as the shear rate increased from 21 to 84 s^{-1}. The volumetric mass transfer coefficient is a measure of the aeration capacity of bioreactor; the larger the K_{La} the higher the aeration capacity of the system. The maximum K_{La} (4.6 s^{-1}) was obtained in culture grown at a shear rate of 84 s^{-1} after 24 h of incubation.

Effect of Nutrients on Kinetic Parameters of Beet Molasses Fermentation by A. pullulans

As shown in Fig. 6, the polysaccharide concentration increased significantly with the addition of nutrients. The maximum polysaccharide concentration (27.0 g/L) was 46% higher than that obtained in culture grown in medium without nutrients (18.5 g/L) (Fig. 2B). Shabtai and Mukmenev *(3)*, who studied the production of pullulan by *A. pullulans* ATCC 42023 in a two-stage fermentation with a shift from soybean oil to sucrose, found that the addition of glutamic acid, soybean oil, and Tween-80 suppressed the morphogenetic shift from yeastlike cells to filamentous growth. They

observed yeastlike cells, blastospores, and chlamydospores during fermentation. Simon et al. *(22)* reported that chlamydospores are responsible for the synthesis of the extracellular pullulan. The swollen cells may in fact be responsible for the synthesis of other polysaccharides with a possible further transformation to pullulan when cell develops into a chlamydospore, depending on culture conditions.

According to our observations by microscopic examination, yeastlike cells formed during the exponential phase. These cells, after a certain time (about 24 h of incubation), produced free chlamydospores. Thus, we observed mainly chlamydospores and a low number of mycelial forms in the fermentation broth after 24 h of incubation. These observations agree with those of Dominguez et al. *(23)*, who described the direct production of chlamydospores from yeastlike cells; this seems to be the case with the strain we have studied. Our results showed that the addition of glutamic acid, olive oil, and Tween-80 increased significantly the production of polysaccharide, whereas the pullulan proportion of the crude polysaccharide was low (20%). This means that chlamydospores and mycelial forms produced mainly other polysaccharides and low concentrations of pullulan. These results are not in agreement with Simon et al. *(22)*, who reported that chlamydospores are the major producers of pullulan.

The maximum biomass concentration (22.5 g/L) was obtained after 120 h of incubation and then remained constant. At the highest concentration of polysaccharide obtained, 55.6% of sugar consumed was converted to polysaccharide, and the total amount of sugar utilized was 97%.

Conclusion

Our results showed some important aspects of pullulan production from beet molasses by *A. pullulans* in an airlift reactor. *A. pullulans* grown on beet molasses produced a mixture of pullulan and other polysaccharides. Chlamydospores were mainly responsible for the synthesis of other polysaccharides. Shear rate influenced the morphogenetic changes of the microorganism and the rheological properties of the fermentation broth. The maximum polysaccharide concentration and the highest pullulan proportion of crude polysaccharide were obtained at low shear rate. Research should be focused on the structure of the other polysaccharides. Further screening of a number of strains and optimization of the respective fermentations would be desirable to identify strains producing high yields of pure pullulan.

References

1. Deshpande, M. S., Rale, V. B., and Lynch, J. M. (1992), *Enzyme Microb. Technol.* **14,** 514–527.
2. Schuster, R., Wenzig, E., and Mersmann, A. (1993), *Appl. Microbiol. Biotechnol.* **39,** 155–158.
3. Shabtai, Y. and Mukmenev, I. (1995), *Appl. Microbiol. Biotechnol.* **43,** 595–603.
4. Gibbs, P. A. and Seviour, R. J. (1996), *Appl. Microbiol. Biotechnol.* **46,** 503–510.

5. LeDuy, A. and Boa, J. M. (1983), *Can. J. Microbiol.* **29,** 143–146.
6. Leathers, T. D. and Gupta, S. C. (1994), *Biotechnol. Lett.* **16,** 1163–1166.
7. Shin, Y. C., Kim, Y. H., Lee, H. S., and Cho, S. J. (1989), *Biotechnol. Bioeng.* **33,** 129–133.
8. Roukas, T. and Biliaderis, C. G. (1995), *Appl. Biochem. Biotechnol.* **55,** 27–44.
9. Israilides, C., Scanlon, B., Smith, A., Harding, S. E., and Jumel, K. (1994), *Carbohydr. Polymers* **25,** 203–209.
10. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956), *Anal. Chem.* **28,** 350–356.
11. Suh, I. S., Schumpe, A., and Deckwer, W. D. (1992), *Biotechnol. Bioeng.* **39,** 85–94.
12. Young, M. M., Helard, B., Allen, D. G., Burrell, R., and Kawase, Y. (1987), *Biotechnol. Bioeng.* **30,** 746–753.
13. West, T. P. and Reed-Hamer, B. (1993), *Microbios* **75,** 261–268.
14. Dygert, S., Li, L. H., Florida, D., and Thoma, J. A. (1965), *Anal. Biochem.* **13,** 367–374.
15. West, T. P. and Strohfus, B. (1996), *J. Basic Microbiol.* **36,** 377–380.
16. Wecker, A. and Onken, U. (1991), *Biotechnol. Lett.* **13,** 155–160.
17. Gibbs, P. A. and Seviour, R. J. (1992), *Biotechnol. Lett.* **14**, 491–494.
18. Catley, B. J. (1971), *Appl. Microbiol.* **22,** 641–649.
19. Keely, P. J. and Catley, B. J. (1977), *J. Gen. Microbiol.* **102,** 249-254.
20. Heald, P. J. and Kristiansen, B. (1985), *Biotechnol. Bioeng.* **27,** 1516–1519.
21. McNeil, B. and Kristiansen, B. (1987), *Biotechnol. Lett.* **9,** 101–104.
22. Simon, L., Caye-Vaugien, C., and Bouchonneau, M. (1993), *J. Gen. Microbiol.* **139,** 979–985.
23. Dominguez, J. B., Goni, F. M., and Uruburu, F. (1978), *J. Gen. Microbiol.* **108,** 111–117.