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Comparison of pullulan production performances of air-lift and bubble column bioreactors and optimization of process parameters in air-lift bioreactor

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ABSTRACT

The production of pullulan from synthetic medium by *Aureobasidium pullulans* DSM-2404 in air-lift and bubble column bioreactors was investigated. The kinetics of polysaccharide, pullulan and biomass production were determined. Maximum effective yield (g pullulan produced g^{-1} initial sugar) obtained in air-lift bioreactor ($Y_{P/S}$ = 0.402) was found to be higher than that obtained in bubble column bioreactor ($Y_{P/S}$ = 0.342). Response surface methodology was used to investigate the effects of three factors (initial sugar concentration, aeration rate and incubation time) on the concentration of pullulan in air-lift bioreactor for batch cultures of *A. pullulans*. No previous work has used statistical analysis in determining the interactions among these variables in pullulan production in an air-lift bioreactor. Results of the statistical analysis showed that the fit of the model was good in all cases. Initial sugar concentration, aeration rate and incubation time had a strong linear effect on pullulan concentration. Moreover, pullulan concentration was significantly influenced by the negative quadratic effects of the given variables and by their positive or negative interactions except the interaction between initial sugar concentration and incubation time ($P > 0.05$). Maximum pullulan concentration of 38.77 g/L was obtained at the optimum levels of process variables (initial sugar concentration 95.2 g/L, aeration rate 1.93 vvm and incubation time 5.36 days)

1. Introduction

Pullulan is an extracellular water-soluble microbial exopolysaccharide produced by strains of yeast like fungus *Aureobasidium pullulans*. Pullulan consists mainly of linear maltotriose subunits connecting each other with α-(1 → 6) linkages [1].

Pullulan is a non-immunogenic, non-toxic and biodegradable material having excellent mechanical properties such as oxygen-impermeability, film and fiber forming capacity, adhesive ability and high viscosity in solutions [1,2]. Pullulan has wide range applications including food, pharmaceutical and biomedical industries. Typical industrial uses of pullulan are as thin, oxygen-impermeable food coatings and packaging material due to its good film-forming properties; as an ingredient of low calorie foods and as a starch substitute; as an adhesive and thickener in the form of pastes and sauces with water, as a construction material (after esterification) with fibers similar in strength and elasticity to those in nylon; and as a bulking agent and stabilizer for tablets in the pharmaceutical industry [2]. Typical biomedical uses of pullulan are as targeted drug/gene delivery agent; as scaffold or artificial extracellular matrix for tissue engineering; as plasma expander and as molecular chaperon after some chemical modifications [3]. Despite of the wide range applications of pullulan, the major constraint prevailing on the use of pullulan is its cost, which is three times higher than the price of other polysaccharides such as dextran and xanthan [4]. There are some solutions to compensate the cost of the pullulan production such as using agricultural wastes as substrate or different bioreactor configurations increasing the productivity of pullulan.

Pullulan is synthesized by the polymorphic fungus *A. pullulans* which has a complex life cycle involving different morphological types depending on culture conditions and incubation time [5]. *A. pullulans* produces various polysaccharides other than pullulan and it has been shown that the rate of the pullulan in total polysaccharides produced may vary according to the substrate used [6–8].

One of the undesired features associated with the production of pullulan by *A. pullulans* is the light-induced synthesis of melanin pigment which is removed by treatment with activated

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charcoal, solvent/solvent blends or by solvent/salt combinations after fermentation. Alternatively for purification of melanin after fermentation, some minerals such as $MnCl_2$ added to the medium could decrease the melanin production during fermentation [9]. Also some mutant *A. pullulans* strains deficient in melanin production can be used for pullulan production [2,10].

The production of pullulan from different sources of carbohydrate (sucrose, olive oil, beet molasses, deproteinized whey, brewery wastes, Asian palm kernel, bio-ethanol byproducts) by *A pullulans* has been described [11–18].

Aeration and agitation are two of the major factors for bioreactors. Aeration provides both aeration and agitation in pneumatically agitated bioreactors. Less energy is consumed and shear stress originated from agitator/blades in mechanically stirred tank bioreactors is eliminated in pneumatically agitated bioreactors [21]. No reports were found in literature about comparison of pneumatically agitated bioreactors (air-lift and bubble column bioreactors) in pullulan production.

The statistical planning of experiments is a useful tool to optimize the processes where many variables are involved, enabling a better manipulation of the parameters, and a more representative analysis of the results [22]. The classical one variable at a time (OVAT) optimization is a time-consuming methodology that does not allow evaluation of the combined effects of all the factors involved in the process. Drawbacks of OVAT can be prevented by the use of statistical experimental design combined with response surface methodology (RSM) [23]. RSM is a collection of statistical techniques for designing experiments, building models that relate process parameters (factors) as independent variable and response as dependent variable, evaluating the effects of factors and searching optimum conditions of factors for desirable responses. RSM also reduces the number of experiments to be carried out [24].

Some reported process parameters used for optimization of pullulan production by RSM are media components, temperature, initial pH, incubation time, agitation speed, aeration rate [10,25–27]. However no previous work has used RSM or other statistical techniques to optimize pullulan production and to determine the interactions among initial sugar concentration, aeration rate and incubation in pullulan production in an air-lift bioreactor.

The aims of this study were comparison of pullulan production performance of air-lift and bubble column bioreactors, and the optimization and determination of the interaction among fermentation parameters in pullulan biosynthesis in an air-lift bioreactor by RSM. The optimized fermentation parameters were initial sugar concentration, aeration rate and incubation time. This work is the first article on optimization of pullulan production in a pneumatically agitated bioreactor (air-lift bioreactor) by RSM.

2. Materials and methods

2.1. Organism and culture media

A pullulans DSM-2404 was maintained on potato dextrose agar slants at 4 °C and subcultured every 3weeks. Cells for inoculation of the culture medium were obtained from cultures grown on potato dextrose agar slants at 28 °C for 48 h. Two loops of *A. pullulans* cells were transferred to 250 cm^3 conical flasks containing 50 cm^3 of culture medium (pH 5.5) of the following composition (g/L): sucrose 30.0, $(NH_4)_2SO_4$ 0.6, yeast extract 0.4, K_2HPO_4 5.0, $MgSO_4{\cdot}7H_2O$ 0.2, NaCl 1.0 and $MnCl_2$ 0.01. The flasks were incubated at 28 °C for 48 h in a rotary shaker incubator at 200 rpm. These cultures were used to inoculate the production medium at a level of 5% (v/v).

2.2. Fermentation conditions

The production medium used had the following composition (g/L): sucrose 50.0, $(NH_4)_2SO_4$ 0.6, yeast extract 0.4, K_2HPO_4 5.0, $MgSO_4{\cdot}7H_2O$ 0.2, NaCl 1.0 and $MnCl_2$ 0.01 (pH 7.5). After adjusting the pH to 7.5 with 5 M HCl, the substrate was sterilized at 121 °C for 20 min. The fermentation temperature was 28 °C. Two different types of pneumatically agitated bioreactors which are bubble column and air-lift bioreactor were used. Conical bottom internal loop air-lift bioreactor was made of glass with 49 mm internal diameter (ID) and 380 mm height. The draft tube (riser) was located axially in the center of the column and fixed at 17.2 mm distance from the sinter glass plate sparger with 29 mm ID. The ratio of riser crossectinal (A_r) to downcomer crossectinal area (A_d) is 0.54 and the bioreactor column was expanded with an angle at the disengagement zone. The bioreactor was jacketed to control the temperature by using circulating bath. The total volume and working volume of the air-lift bioreactor are 1300 ml and 1100 ml, respectively. The bubble column bioreactor also has a conical bottom and it was made of glass with 35 mm ID and 320 mm height equipped with 35 mm ID sinter glass plate sparger. The bioreactor was jacketed to control the temperature and it has a 300 ml of total volume and 260 ml of working volume.

To compare pullulan production performance of air-lift and bubble column bioreactors, different aeration rates (0.2, 1.1 and 2 vvm for bubble column and 1, 2 and 3 vvm for air-lift bioreactor) at fixed initial sugar concentration and different initial sugar concentration (70, 90 and 110 g/L) at fixed aeration rates were investigated.

To determine the effect of initial sugar concentration, aeration rate and incubation time on pullulan production in air-lift bioreactor; initial sugar concentration of 70, 90 and 110 g/L; aeration rates of 1, 2 and 3 vvm (vol air/vol medium/min) and incubation time of 3, 4 and 5 days were used. The levels of initial sugar concentration, aeration rate and incubation time used in the optimization studies by RSM are given in Table 1.

Table 1
Levels of factors used in the experimental design.

Factor	Name	Level		
		−1	0	+1
X_1	Initial subs. conc. (g/L)	70	90	110
X_2	Aeration rate (vvm)	1	2	3
X_3	Incubation time (day)	3	4	5

2.3. Analytical techniques

Biomass dry weight was determined by centrifuging 5 ml (for bubble column bioreactor) or 10 ml (for air-lift bioreactor) of fermentation broth at 9000 rpm for 10 min and washing with distilled water followed by drying at 80 °C. Polysaccharide content was determined by adding the first supernatant from biomass dry weight determination to the washings, and the polysaccharide was precipitated with two volumes of ethanol at 4 °C for overnight. The precipitate was centrifuged at 7000 rpm for 10 min followed by drying at 80 °C overnight and then weighed. To determine the pullulan content of the polysaccharide, the precipitate was resuspended in 0.05 M sodium acetate (pH 5.0) at a concentration of 10 mg/ml. 10 μl of pullulanase (Promozyme D2, Novozymes A/S, Bagsuaerd, Denmark) was added to 1 ml of this sample. The mixture was incubated at 25 °C for 21 h according to the procedure of Leathers et al. [28]. The enzyme was also added to a pure sample of pullulan (Hayashibara Biochemical Co., Okayama, Japan) of the same concentration as described earlier. Using a reducing sugar assay [29], the glucose reducing equivalents were determined and the actual pullulan content was derived. Total sugar was determined according to the phenol sulfuric acid method using sucrose as the standard [30]. The data reported are the average values of three replicate experiments.