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The Effects of Agitation and Aeration on the Production of Gluconic Acid by *Aspergillus niger*

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ABSTRACT

The effects of agitation and aeration in the production of gluconic acid by *Aspergillus niger* from a glucose medium were investigated. Experiments were conducted at aeration rates of 5.0 and 10.0 L/min. Four different agitation speeds were investigated for each aeration rate. Gluconic acid concentration and biomass concentration were analyzed, and the rate of consumption of substrate by *A. niger* was noted. The main purpose of this work was to find the optimal conditions of agitation and aeration for the growth of *A. niger* and production of gluconic acid in submerged culture in a batch fermentor at a bench-top scale. The oxygen-transfer rates at different agitation and aeration rates were calculated. The gluconic acid concentration and rate of growth of *A. niger* increased with increase in the agitation and aeration rates.

Index Entries: *Aspergillus niger*; aeration; agitation; gluconic acid.

Nomenclature: N, oxygen uptake rate, mmol/L/min; y, yield, g dry cells produced/100 g glucose used; U, growth rate, g dry cells/L/min.

INTRODUCTION

Submerged aerobic fermentations are used in industry for production of antibiotics, chemicals, yeast cells, and vitamins, and for bioremediation and waste treatment. Such fermentations require large amounts of oxygen

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to support microorganism growth (*1*). An intensive yeast culture will utilize all dissolved oxygen in only a few seconds at oxygen uptake rates of 2–3 g/m^3/s (*2*). The process of making oxygen available to a growing culture involves bringing gas into contact with liquid, dissolving the gas into the liquid, and then transferring the dissolved gas in the bulk liquid to the organisms (*3,4*). This process requires effective gas–liquid mass transfer, typically with large interfacial area, and adequate liquid-phase mixing (*5,6*). Thus, in aerated systems, oxygen supply and agitation are virtually inseparable.

The effects of mixing and gas–liquid mass transfer can be important in aerobic fermentations that utilize filamentous microorganisms. As the biomass concentration increases, the rheology of the fermentation broth can become viscous and non-Newtonian with adverse effects on mixing and oxygen transfer rates within the reactor (*4*). The factors that must be considered when analyzing the mixing needs include gas–liquid mass transfer, liquid–particle mass transfer, the rheological properties of the broth, and the shear sensitivity of the microorganism (*5*). It has been suggested that the production of gluconic acid by *Aspergillus niger*, which is a filamentous fungi, was an ideal system to study the effect of mixing on oxygen transfer in bioreactors (*6*).

METHODS

The microorganism used in this study, *A. niger* ATCC 9029, was obtained from the American Type Culture Collection. This microorganism produces gluconic acid and biomass when suspended in a aerated glucose medium.

Equipment

The fermentor used in the studies (L&H Fermentation) was of conventional design, consisting of a 20-L glass vessel, a pair of six-bladed Rushton impellers, four baffles, and a heating and cooling element immersed in the broth. The pH was controlled using a glass electrode system immersed in the fermentation broth. A solution of 2.0*N* sodium hydroxide was used to control the pH at a preset value. The dissolved oxygen was measured using a polarographic electrode manufactured by Ingold Inc.

Experimental Procedure

The microorganism *A. niger* was maintained on potato dextrose agar (PDA) slants. To prepare for an experiment, a fresh, well-grown culture was selected. Spores from two slants were washed from the agar surface with 150 mL of sterile 1*M* phosphate buffer solution of pH 5.5. The suspension was then poured into a 1.5-L flask containing sterile growth

media. The growth media consisted of 100 g/L glucose, 0.2 g/L KCl, 0.4 g/L $MgSO_4 \cdot 7H_2O$, 3.0 g/L yeast extract, and 3.0 g/L malt extract. This culture was then shaken at an agitation rate of 80 rpm at 30°C for 24 h to obtain a filamentous morphology. Increased shaking rates led to pelletized growth.

The production media consists of 100 g/L glucose, 0.10 g/L urea, 0.20 g/L KH_2PO_4, 0.15 g/L $MgSO_4$ $7H_2O$, and 0.40 g/L $(NH_4)_2$ HPO_4. The fermentor was filled with 15 L of production media and then sterilized at 120°C and 1.5 atm for 30 min. The fermentor was subsequently cooled to 30°C and maintained at this temperature throughout the experiment. The growth medium was then transferred to the fermenter under aseptic conditions, and the air flow, agitation, and pH control were started. The level of sodium hydroxide was recorded every hour. The biomass (dry weight) was determined by taking samples during the fermentation at regular intervals of 12 h, and by filtering a known volume of broth (usually 100 mL) on a filter paper and drying the sample at 50°C for 24 h. The supernatant was used for analysis of gluconic acid and residual glucose.

The oxygen electrode was standardized after sterilizing the fermenter by passing pure nitrogen gas through the fermenter for approx 25 min to establish a zero-level reading. Air was then passed through the fermenter until the medium was saturated in order to establish the reading for complete saturation.

Analysis

The glucose consumption was analyzed using the dinitro salicylic acid (DNSA) method as suggested by Miller (*7*). One milliliter of the supernatant solution was withdrawn and diluted to 10 times. One milliliter of this diluted solution was added to 5 mL of distilled water. One milliliter of DNSA reagent was added, and the mixture was heated in boiling water for 5 min and cooled. One milliliter of Rochelle's salt was added to stabilize the color developed, and the sample was analyzed at 540 nm in a Perkin Elmer UV/VIS spectrophotometer Lambda 3B. Gluconic acid was analyzed using a diagnostic kit supplied by Boehringer Mannheim Cat. No. 428 191. A sample of 0.10 mL was mixed with the diagnostic solutions and the absorbance measured at 340 nm. The oxygen uptake rate of the fermentations was obtained by the following equation taken from the literature (*8*):

$$N = [3333 / (y) - 41.8] U \quad (1)$$

Here, N is the oxygen uptake rate (mmol/L/min), y is the cell yield (g dry cells produced/100 g glucose used), and U is the growth rate (g dry cells/L/min). This equation was derived from material balance considerations assuming that glucose is the only carbon source, the cells have same composition as yeasts, and cells, carbon dioxide, and water are the only products of metabolism. The solubility of oxygen at 30°C was taken to be 0.88 mmol/L atmosphere at 30°C from data reported in the literature (*8*).

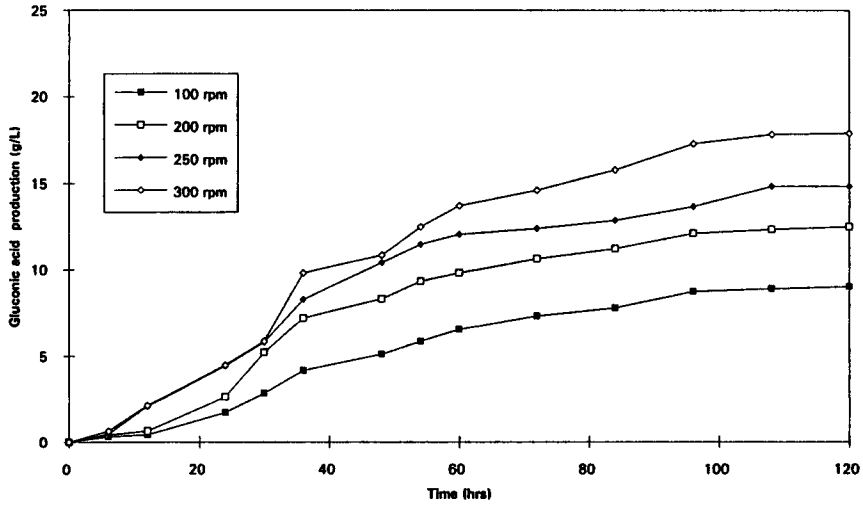

Fig. 1. Gluconic acid production at various agitation rates at 5.0 L/min air flow.

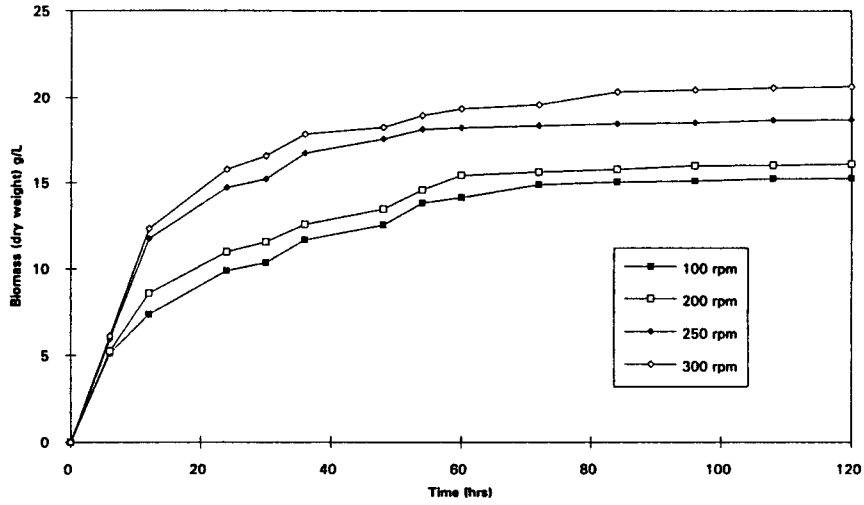

Fig. 2. Gluconic acid production at various agitation rates at 10.0 L/min air flow.

RESULTS AND DISCUSSION

Experiments were carried out to determine the effect of agitation and aeration on the production of gluconic acid. Effects of agitation were investigated in the range of 100–300 rpm at air flow rates of 5 and 10 L/min at 30°C. Changes in the gluconic acid concentration, biomass concentration, and glucose concentration were noted as functions of time. Since the cells produced gluconic acid during the fermentation, the pH continually decreased. The rate of base addition was proportional to the rate of acid production and, hence, to the rate of oxygen utilization. The results

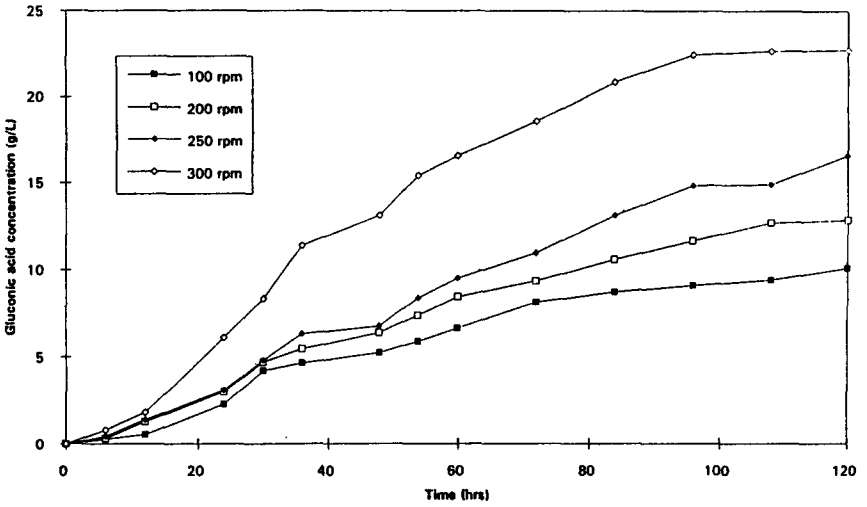

Fig. 3. Biomass (dry weight) production at various agitation rates at 5.0 L/min air flow.

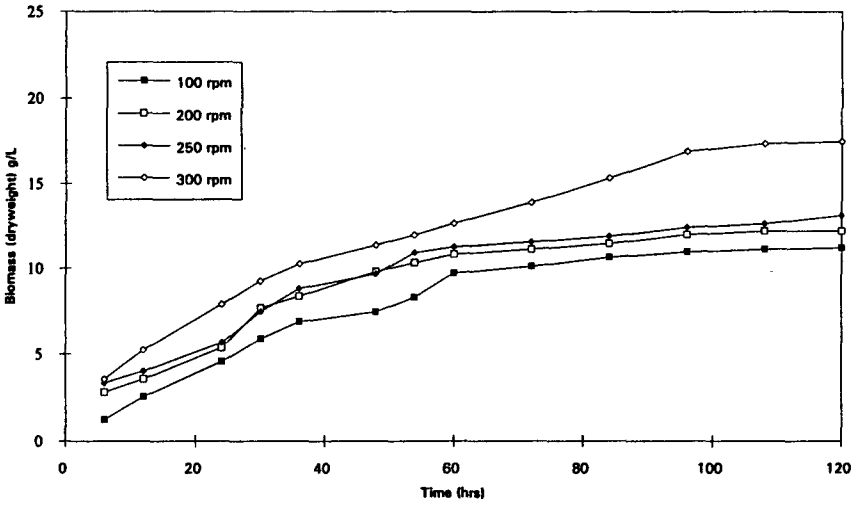

Fig. 4. Biomass (dry weight) production at various agitation rates at 10.0 L/min air flow.

at 5 and 10 L/min are presented in Figs. 1 and 2. These data indicate that the gluconic acid production increased with increasing agitation rate. The maximum concentration of gluconic acid obtained for 10 L/min and 300 rpm was 22.72 g/L after 120 h of fermentation. The concentration of gluconic acid at 100 rpm and 10 L/min was 10.08 g/L. Similarly, the gluconic acid concentration increased with increased agitation rate for an air flow rate of 5 L/min, with the maximum concentration of gluconic acid reaching 17.86 g/L. In each experiment, the biomass concentration was also noted with respect to time. The biomass concentrations at different agitation rates are presented in Figs. 3 and 4 for aeration rates of 5 and 10 L/min,

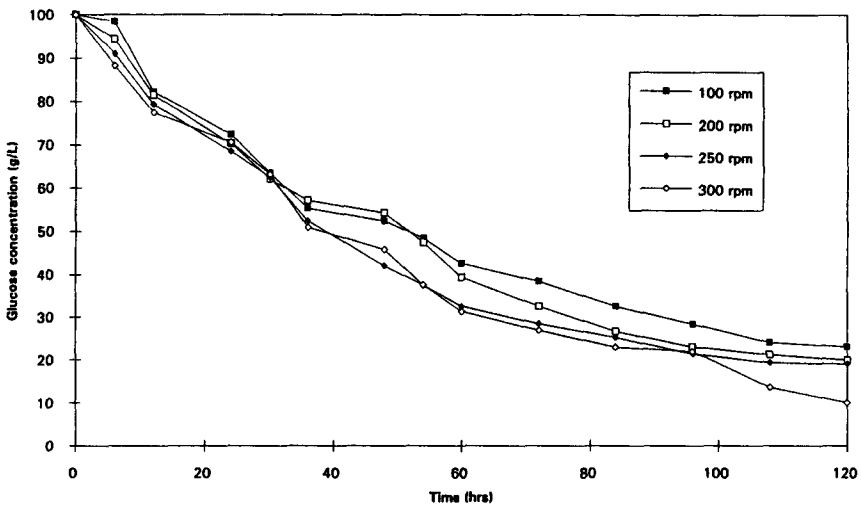

Fig. 5. Glucose consumption at various agitation rates at 5.0 L/min air flow.

respectively. The increase in gluconic acid production at increased agitation rates may be the result of the fact that the increased agitation speed leads to an increase in the dissolved oxygen concentration of the fermentation broth. Similar behavior has been reported for gluconic acid fermentations with *A. niger* in air lift and stirred-tank fermentations (*9*) and for other fermentations involving filamentous microorganisms (*10,11*). It has been reported that cell growth and activity of glucose oxidase (I) per gram of mycelium (*A. niger*) increases with increased agitation and increased concentration of dissolved oxygen in the fermentation broth (*12*). The fluctuations in the concentrations might be in connection with insufficient oxygen supply of the culture. In the course of fungal growth, the distribution of oxygen becomes uneven, the size of the bubble increases, and as a consequence, the oxygen supply decreases. The oxygen absorption rate is also influenced by viscosity of the culture. Possible accumulation of CO_2 and of products of enzyme reaction as glucose-δ- lactone and H_2O_2 may also cause an inhibition in the activity of glucose oxidase and hence gluconic acid production (*13*).

The consumption of glucose also increased with increasing agitation rate. Glucose concentration profiles for aeration rates of 5 and 10 L/min are presented in Figs. 5 and 6, respectively. The rate of oxygen transfer, as shown in Figs. 7 and 8, increased with increasing agitation and aeration, which led to a increase in the biomass concentration and gluconic acid concentration.

CONCLUSIONS

The studies on the effects of agitation and aeration show that the rate of production of gluconic acid increases with increased aeration and agitation. To obtain increased yields of gluconic acid, increased aeration

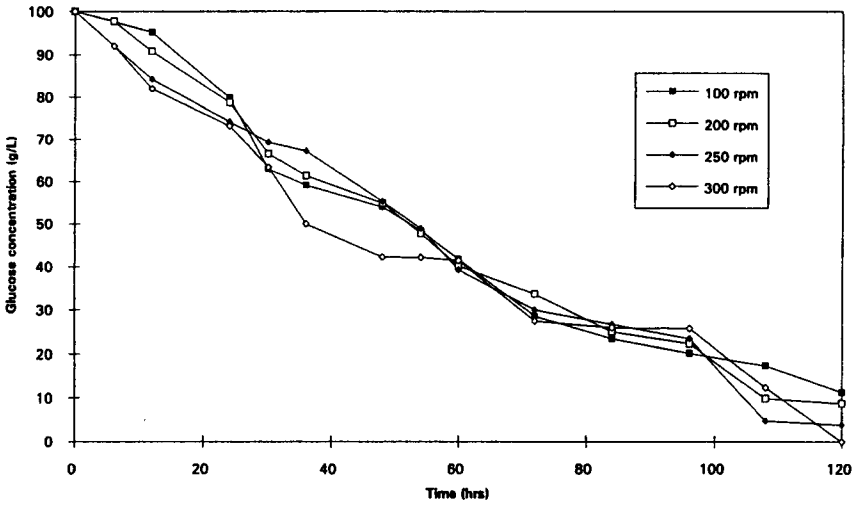

Fig. 6. Glucose consumption at various agitation rates at 10.0 L/min air flow.

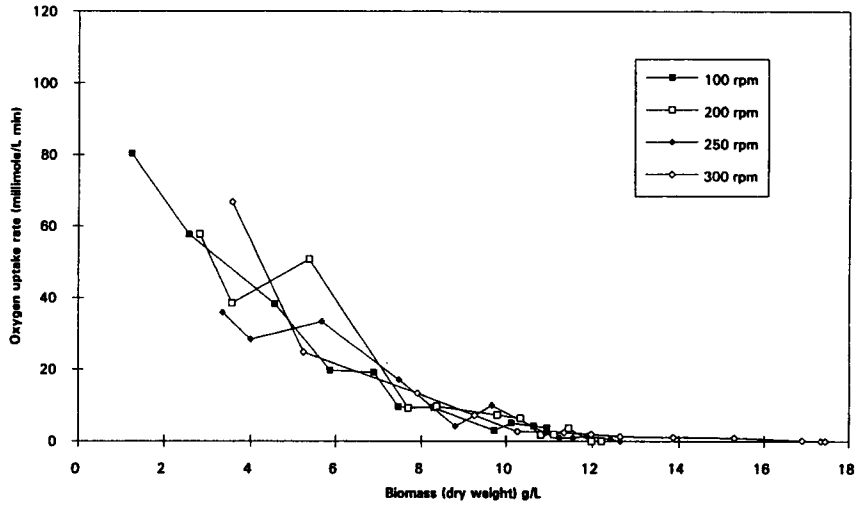

Fig. 7. Oxygen uptake rate at various agitation rates at 5.0 L/min air flow.

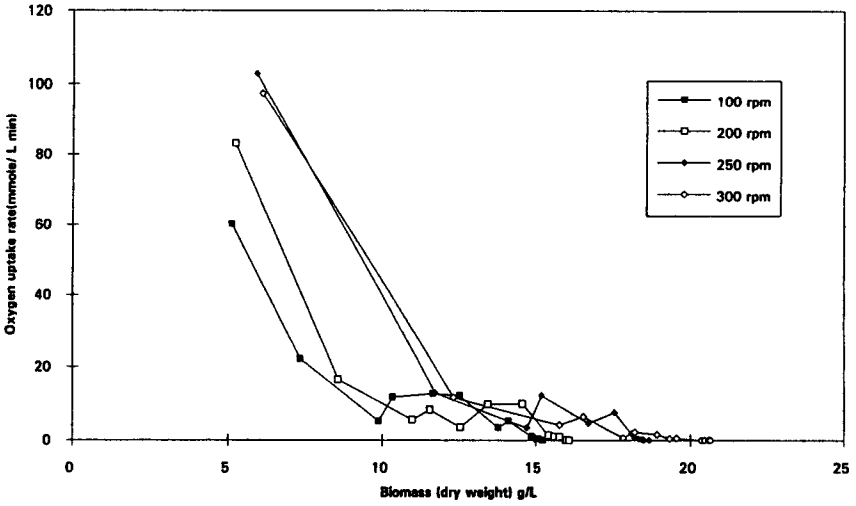

Fig. 8. Oxygen uptake rate at various agitation rates at 10.0 L/min air flow.

and aeration rates are to be maintained in the bioreactor. The increase in biomass concentration and gluconic acid production is owing to the fact that oxygen-transfer rate increases with increase in agitation and aeration rate.

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