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AN EXPLANATION FOR THE INTERACTION MECHANISM OF AN ANIONIC POLYMERIC ADDITIVE ON YEAST FLOCCULENT CELLS

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SUMMARY

Magna Floc LT25 is a high molecular weight anionic polymer that has been described as increasing reaction rates inside flocs of yeast cells. However, no clear indication has been given on how this anionic polymer interacts with flocculent cells. Flocculation experiments made with a strain of *Saccharomyces cerevisiae* corroborate that it bridges calcium ions bound to flocculent yeast cell walls, thus enlarging the available flux area for the transport of solutes inside the flocs.

INTRODUCTION

The utilization of yeast flocculent cells in bioprocess technologies is of great interest. Its application in industries such as brewing and continuous ethanol fermentation is well known (Atkinson and Daoud, 1976; Bu'Lock *et al.*, 1984; Fontana *et al.*, 1992; Sousa *et al.*, 1994).

However a major disadvantage is presented when using these aggregated cells. Mass transfer limitations occur inside the flocs resulting in reduced reaction rates (Libicki *et al.*, 1988; Logan and Hunt, 1988; Teixeira and Mota, 1990a, 1990b) and, most probably, in changes in metabolic activities (Fontana *et al.*, 1991). As suggested by Lima *et al.* (1992), one way to circumvent these limitations is the utilisation of polymeric additives, that cause an increase in floc porosity with a consequent improvement in reaction rates. Reports on the utilization of either anionic (Sousa and Teixeira, 1991; Lima *et al.*, 1992) or cationic (Teixeira and Mota, 1990b; Lima *et al.*, 1992) polymeric additives have been presented.

Since yeast cell walls are negatively charged (Calleja, 1994), it is easily understandable the utilization of cationic additives. For negatively charged polymers Lima *et al.* (1992) present an hypothesis explaining the interaction mechanism of these macromolecules with flocculent yeast cells. However, such a mechanism has not been demonstrated, so far.

In this work, evidence of this mechanism is reported. Flocculation experiments made with a strain of *Saccharomyces cerevisiae* confirm that anionic polymers act by bridging calcium ions bound to flocculent yeast cell walls, thus contributing to the reported reduction of mass transfer limitations.

MATERIALS AND METHODS

Strain: the strain used was a highly flocculent *Saccharomyces* strain - *S. cerevisiae* NRRL Y265.

Growth conditions: Cells, transferred directly from the agar slant, were grown in 250 mL Erlenmeyer flasks containing 100 mL of a semi-synthetic medium with the following composition, per liter of tap water: glucose - 50 g; KH_2PO_4 - 5 g; $(NH_4)_2SO_4$ - 2 g; $MgSO_4 \cdot 7H2O$ - 0.4 g; yeast extract - 1 g. The medium contained also 0.01 % (w/v) of Magna Floc LT25 whenever the cells were to be suspended in solutions containing the additive (solutions C and D bellow). The pH was initially adjusted to 4 ± 0.1 by the addition of H_3PO_4. Cells were grown at 30°C in an orbital shaker, at 120 r.p.m.. After 15 hours of growth, the cultures were harvested and washed 3 times with an excess of ultra-pure water. To ensure floc dispersion, cells were vigorously washed (3 times) with an EDTA solution (250 mM). Again cells were washed 3 times with ultra-pure water.

Additive: Magna Floc LT25 is a high molecular weight anionic polyacrylamide, gently provided by ALLIED COLLOIDS, Ltd..

Measurement of flocculation ability: the flocculation ability was determined using a modification of the Helm sedimentation test (Stewart, 1975). One mL of the concentrated cell suspension was pipetted into a 25 mL cylinder, containing 24 mL of one of the following solutions:

A) ultra-pure water

B) an aqueous solution of Ca^{2+} (1 mM)

C) an aqueous solution of Magna Floc LT25 (0.01% w/v)

D) an aqueous solution of Ca^{2+} (1 mM) and Magna Floc LT25 (0.01% w/v)

Immediately after this, the suspension was homogenized by inverting the cylinder 20 times. Then, at defined intervals, samples were taken from a fixed position (level corresponding to 20 mL) and dispersed in a 15 g/L NaCl solution at pH 2, to promote deflocculation. Cell concentration was determined, spectrophotometrically, at 620 nm, by reference to a dry weight calibration curve. The obtained biomass concentrations were normalized against the initial biomass concentration and the sedimentation profile was plotted as the percentage biomass in suspension with time. The initial cell dry weight concentration in the cylinder was about 1 g/L. The results presented are the average of at least two independent experiments.

All the solutions were prepared with ultra-pure water. All the glassware was washed in an ultrasound bath, treated with sulfa-chromic mixture, washed with HCl 1M and rinsed three times with ultra-pure water.

RESULTS AND DISCUSSION

As previously described, the flocculation ability of yeast cells was measured in four different conditions. The obtained flocculation profiles are represented in Fig 1.

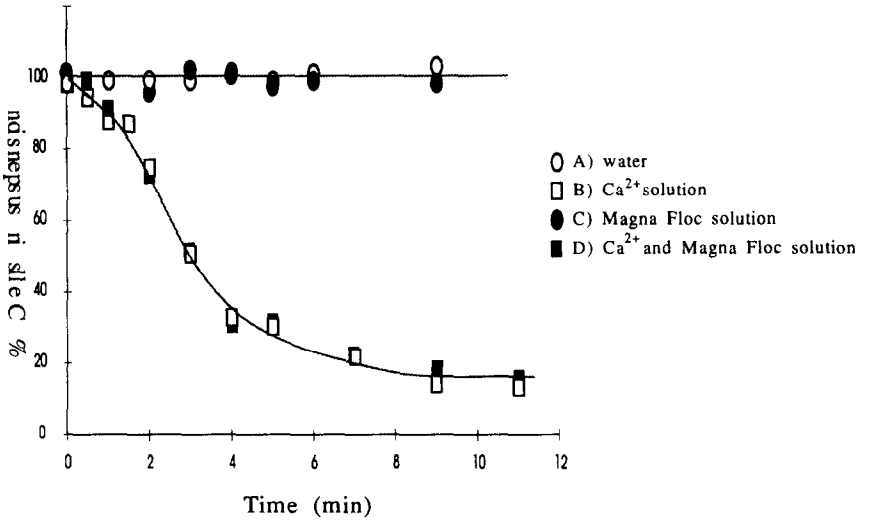

Fig. 1 - Sedimentation profiles of yeast cells in four different solutions

From the analysis of the plots, the following observations can be made:

- no flocculation occurs when ultra-pure water is used, as expected;
- cells strongly flocculate in the presence of calcium ions; this result is in agreement with the generally accepted mechanism for yeast flocculation, by which the role of calcium is considered crucial, as revised recently by Calleja (1994); calcium ions are supposed to promote flocculation by ensuring the correct conformation of lectin like proteins located in the yeast cell wall (Miki *et al.*, 1981);
- in the presence of Magna Floc LT25 and when no calcium is in the solution, flocculation does not occur, indicating that the anionic polymer, on its own, is not responsible for the flocculation of yeast cells; the Magna Floc macromolecule does not bound, directly, to cell walls;
- when calcium and Magna Floc LT25 are both present, flocculation is very intense.

The similarity between the flocculation profiles of yeast cells in the presence of either calcium only, or calcium and Magna Floc LT25, and the absence of flocculation when Magna Floc only is present, might suggest that no cell-cell interaction mediated by the anionic polymer occurs. In such circumstances, calcium would be the sole responsible for the formation of flocs, even when Magna Floc LT25 is present.

However, if these results are coupled with the values for floc porosity obtained by Lima *et al.* (1992) for the same strain, it is clear that Magna Floc LT25 must be involved in the cell-cell interaction process. Otherwise, it would not possible to explain the observed increase in void volume.

The only possible way for Magna Floc LT25 to interfere in the flocculation mechanism is by linking the positive charged particles - calcium ions - that are bound to yeast cell walls, when flocculation occurs.

With this simple set of experiments the model proposed by Lima *et al.* (1992) is validated. Fig. 2 illustrates this model, depicting Magna Floc bridging calcium ions (at least, some of them) bound to yeast cell walls and enlarging the distance between cells. Thus the available flux area for the transport of nutrients inside the flocs is increased.

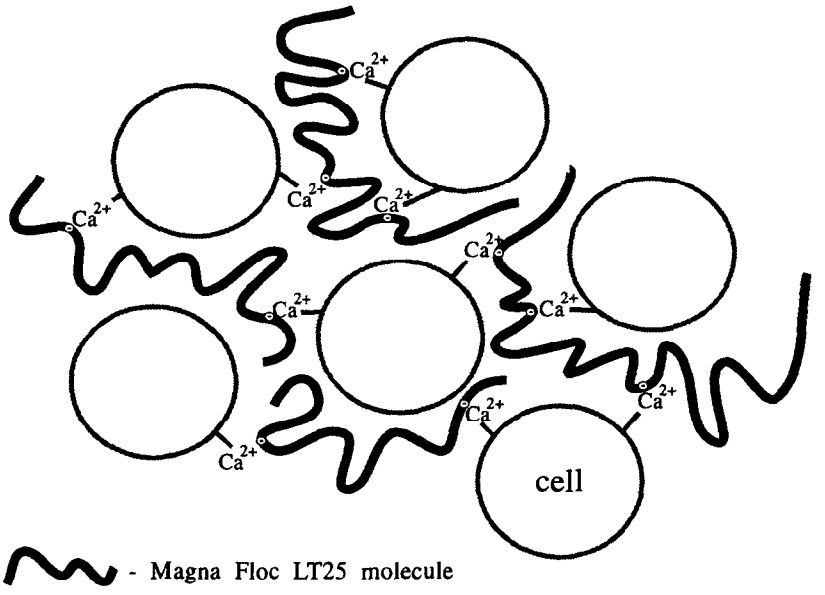

Fig. 2 - Mechanism of interaction between Magna Floc LT25 and yeast cells

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