UTILIZATION OF ADDITIVES TO INCREASE FLOCCULATION BIOREACTOR PERFORMANCE

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ABSTRACT

The capacity of several flocculating additives - BPA 1000, Polyoxyethylene bis-amine 20.000 and Magna Floc LT25 - to increase the performance of flocculation bioreactors was evaluated. A membrane biorector was used to measure the maximum specific glucose consumption rate of a flocculating strain of S. cerevisiae in the presence of the several additives.
A significant increase in specific glucose consumption rate was observed, confirming that flocculation additives may be able to reduce significantly floc internal diffusional limitations, thus allowing for an increase in bioreactor performance.

INTRODUCTION

Flocculation bioreactors are one of the most important technologies to be used in high cell density fermentations [1,2,3,4,5], since they present low investment costs (simple construction) and low running costs (the agitation energy is low).
However flocculation fermentors present some problems namely floc internal diffusional limitations. In spite of its relevance, little has been done to overcome this problem [6]. Under internal diffusional limitation the substrate diffusion rate towards the intrafloc cells is lower than the cell metabolic rate and therefore substrate cannot be metabolized at the maximum rate.
As cell walls are negatively charged, diffusional limitations may be reduced by the utilization of cationic polymer additives. They act by replacing calcium ions, which are the usual ions present in the bridges between yeasts' cell walls [7,8,9]. Due to its larger size, they may increase intrafloc void volume, thereby reducing floc internal diffusional limitations.
In this work the substrate consumption rate for a flocculating strain of S. cerevisiae in the presence of several cationic polymer additives - using a previously described method [10], is measured.

MATERIALS AND METHODS

Strains - two strains of Saccharomyces cerevisiae were used: the first was a flocculating one and the other - S. cerevisiae sake - was non flocculating.

Culture medium - the culture medium had the following composition, per liter of tap water: glucose, 5g; KH_2PO_4, 5g; $(NH4)_2 SO_4$, 2g; $MgSO_4.7H_2O$, 0.4; yeast extract 1g. Ca^{2+} concentration was 1mM.

Operating conditions - the pH was adjusted to 4.0 by the addition of H_3PO_4; the temperature was controlled at 30°C; the dilution rate in all experiments was $2h^{-1}$; and the agitation speed of the magnetic stirrer 600 r.p.m..

Glucose determination - glucose was determined by the DNS method [11].

Additives - the cationic polymers added to medium at a concentration of 0.01‰ (w/v) were the following:

BPA 1000   (cationic resin)
Polyoxyethylene bis-amine 20000
Magna Floc LT 25   (cationic resin)

Experimental Device - it consisted of a 5 mm thick plexiglass microreactor (Fig. 1) formed by two chambers separated by a 0.45 μm filter, to which fresh medium was pumped. The total working volume was 40 ml.

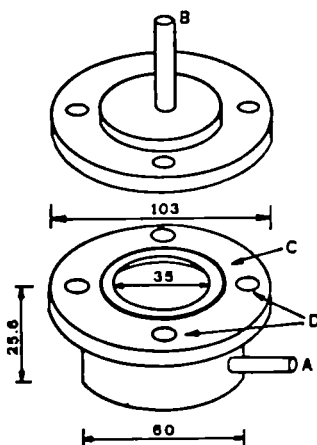
Figure 1. Exploded view of the membrane microreactor :   A - feed inlet;   B - medium outlet;
C - O-ring;D - butterfly screw holes. All dimensions are in mm.

Cells of the flocculating strain of *S. cerevisiae* were deflocculated with a 1.5% aquous solution of NaCl, pH2 and washed several times with ultra-pure water. The cells from the non flocculating strain were washed with ultra-pure water, only.Then the cells were placed in contact with the culture medium and put in the lower chamber of the microreactor. After sealing the system, feeding was started. Samples for glucose analysis were collected at regular intervals from the outflow. The cell concentration was determined after each experiment by measuring the biomass dry weight.

RESULTS AND DISCUSSION

If X is the cell dry weight concentration (g/l) and S the glucose concentration in the medium (g/l) at time t, the specific glucose consumption rate is defined as

$$q_s = -1/X * dS/dt \qquad (1)$$

If the biomass concentration X was constant, the specific glucose consumption rate can be calculated by the slope of the straight line obtained by plotting S/X against time.Considering the short sampling time (maximum 40 minutes) as well as the high dilution rate, the cells operated at the maximum consumption rate and no cell division occurred during the experiments. Even assuming a maximum specific growth rate of 0.4 h^{-1}, the cells would present a minimum doubling time of 1.7 h which validates the former assumptions.Table 1 displays the conditions of each experiment, as well as the values obtained for the specific substrate uptake rate and the correlation coefficient for each each straight line.On Fig.2 it is represented the variation of S/X with time for the flocculating strain, in the presence of the various additives.

TABLE 1
Operating conditions and results for the diffusion experiments for the flocculating strain

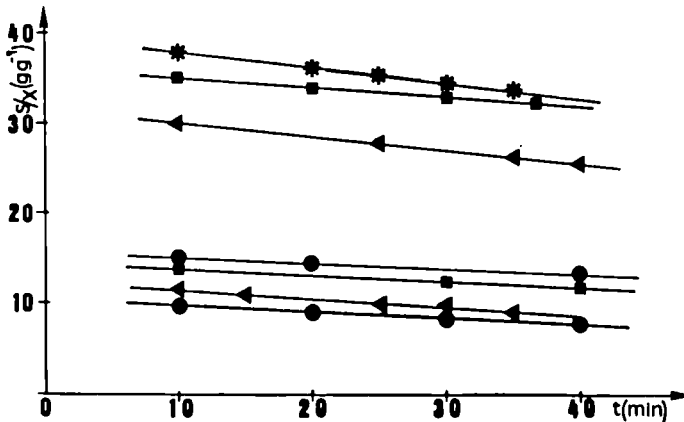| Strain	Additive	Initial glucose (gl^{-1})	Dry wt. (g)	$-q_s$	$-q_s$ (av) ($gg^{-1}min^{-1}$)	Correl. coeff.
S.cerevisiae	Ca^{2+}	5.81	0.342	0.089		0.999
					0.088	
		5.04	0.450	0.086		0.999
	BPA1000	4.98	0.383	0.126		0.999
					0.132	
		5.62	0.174	0.137		0.998
	Polyoxyethy lene bis-amine 20000	5.67	0.152	0.101		0.999
					0.105	
		4.58	0.286	0.108		0.999
Floc$^+$	Magna Floc LT 25	5.48	0.146	0.169		0.997
					0.172	
		5.54	0.142	0.176		0.999

Figure 2 . Rate of change of the normalized glucose concentration   (S/X)   for the flocculating strain.●- Ca^{2+} ;◄ - BPA1000 ; ●- Polyoxyethylene bis-amine; ▲- Magna Floc LT 25

For the flocculating strain, it may be seen that the straight lines for the calcium and the polyoxyethylene bis-amine experiments were parallel, demonstrating that this additive had a negligible effect on the reduction of floc internal diffusional limitations.On the other hand, the straight lines for the other additives were convergent with the calcium straight line. For BPA 1000, the increase in specific glucose uptake rate is of 25% and for Magna Floc LT25 there was a 2 fold increase. Such an increase suggested that there might be some influence of Magna Floc on the intrinsic metabolic activity of the yeast. In order to eliminate any possible doubt, the influence of the additive in a non flocculating strain of *S. cerevisiae* was tested. Table 2 and Fig. 3 indicate that specific glucose uptake rate has an identical value either in the presence of Ca^{2+} or the additive.

TABLE 2
Operating conditions and results for the diffusion experiments for the non flocculating strain

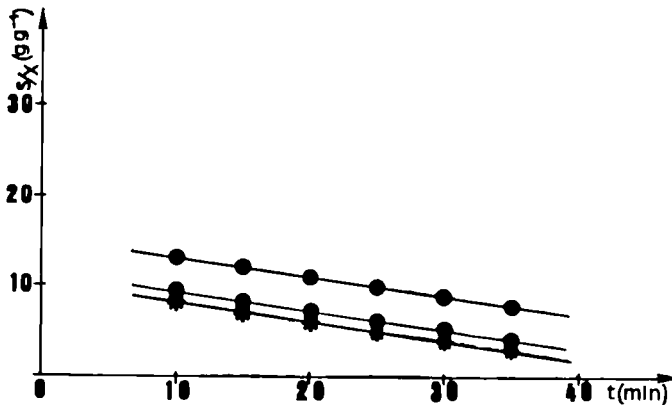| Strain	Additive	Initial glucose (gl^{-1})	Dry wt. (g)	$-q_s$ $(gg^{-1}min^{-1})$	$-q_s$ (av)	Correl. coeffic.
S. cerevisiae	Ca^{2+}	5.78	0.533	0.213		0.998
					0.216	
		5.78	0.386	0.219		0.999
Floc⁻	Magna Floc LT 25	5.22	0.528	0.232		0.999
					0.223	
		4.09	0.427	0.214		0.999

Figure 3 . Rate of change of the normalized glucose concentration (S/X) for the non flocculating strain. ● - Ca^{2+} ; ✳ - Magna Floc LT 25

This confirms that the observed increase in specific substrate uptake rate for the flocculating strain is due to a reduction in floc internal diffusional limitations, and not to an increase of the intrinsic metabolic activity.

CONCLUSIONS

The obtained results clearly demonstrated that the additives BPA1000 and Magna Floc LT25 were effective in the reduction of floc internal diffusional limitations, thus allowing for the increase of the performance of flocculation bioreactors.

However, floc internal diffusional limitationswere still present , as it can be seen by comparing the substrate uptake rates for both non flocculating and flocculating strains.

The additive polyoxyethylene bis-amine had a similar behaviour to calcium, and there it did not show the same positive effect as BPA1000 and Magna Floc LT25.

It may also be concluded that this method can be used to compare the relative efficiency of different flocculation additives.

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