STARTER CULTURE PRODUCTION IN FLUIDIZED BED REACTOR WITH A FLOCCULENT STRAIN OF *L.plantarum*

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SUMMARY

A lactic starter culture of a flocculent *Lactobacillus plantarum* was produced in a fluidized bed reactor with higher cell volumetric productivities than in a continuous stirred tank reactor. The fluidized bed reactor was operated at optimised parameters obtained in batch reactor performed with and without pH control.

INTRODUCTION

Food and feed industries make an increasing demand for lactic acid starter cultures. Lactic starter cultures can be used in the production of milk-derived products (cheese, yoghurt, butter, butter-milk) and fermentation of cereals, vegetables and meats. They have important characteristics such as production of flavours, bacteriocins and antibiotics, and they are known to have nutritional and therapeutical benefits (Chassy, 1986; Day,1987).

Lactobacillus plantarum is a facultative heterofermentative lactobacillus, which means that it produces mainly lactic acid from hexoses, but can produce lactic acid and acetic acid depending on the level of substrate and oxygen in the medium (Condon, 1987; Sandine, 1987). Some of its industrial applications are production of crackers, sauerkraut, pickles, green olives, cured ham, sausages, meats, silage starters.

Traditionally these starter cultures are produced by batch or continuous stirred tank reactors (CSTR) followed by freezing or freeze-drying. To obtain a lactic starter culture more efficiently we are exploring the use of a fluidized bed reactor (FBR) for biomass generation, using a flocculating strain. Fluidization keeps the particles separated and individually surrounded by nutrients and, at the same time, reduces inhibitory final products, thus mass transfer conditions and volumetric and biomass production rates can be several times higher than in conventional submerged stirred fermentors. Also, at low Reynolds numbers (higher than, aproximately, 1) this reactor type has the highest energetic efficiency (transport efficiency/energy dissipated) in comparison with CSTR or packed bed reactors (Cheetham, 1983).

The optimal operational parameters allowing maximum cell production in batch system, with or without pH control, and in CSTR were studied before running the fluidized bed reactor with the flocculating strain.

MATERIAL AND METHODS

Culture used was *Lactobacillus plantarum* 7, a flocculating strain supplied by Profs. V. Bottazzi and S. Cocconcelli (Istituto di Microbiologia, Univ. Cattolica del Sacro Cuore, Piacenza, Italy). Culture was kept at 4°C in MRS medium (Man, 1960) and transfered monthly.

Experiments were performed with MRS medium which contained (per liter of deionized water): tryptone (Difco), 10g; beef extract (Difco), 1g; yeast extract (Fould Springer), 5g;K_2HPO_4, 2g; sodium acetate, 5g; di-ammonium hydrogen citrate, 2g; $MgSO_4.7H_2O$, 0.2g; $MnSO_4.H_2O$, 0.05g; Tween 80, 1ml. Glucose concentration varied according to experiments (20-85g/l). For FBR, Tween 80 was omitted and tap water utilized throughout.

Dry weights of biomass were determined using 10ml samples of broth culture filtered through a membrane (0.2µm) and dried for 72h at 105°C. Turbidities of broth cultures were expressed as optical densities (O.D.) at 540nm. A calibration curve was constructed by plotting optical densities against dry weights.

Glucose, ethanol, lactic and acetic acids were determined by HPLC with a Shodex Sugar SH-1011 column (Macherey-Nagel), using 0.01N H_2SO_4 as the eluent, and a flow-rate of 1.0ml/min at 50°C; a refraction index detector was used (ERC - 7511, Erma Inc., Japan).

Batch studies without pH control were carried out in 100ml flasks in a shaker bath.

Batch fermentations with pH control and chemostat studies (CSTR) were performed in a 2 L Setric fermentor (SGI, France).

The fluidized bed system (FBR) was a cylindrical column, 80cm in height and 4cm wide with an external settler allowing recirculation (fig.1), similar to the column utilised by Adisasmito to produce fungal starter cultures (Adisasmito, 1987). Temperature was controlled with a water jacket along the column and the pH value was maintained, aproximately, at the desired value by a pH control system, with a pH electrode placed at the top of the column.

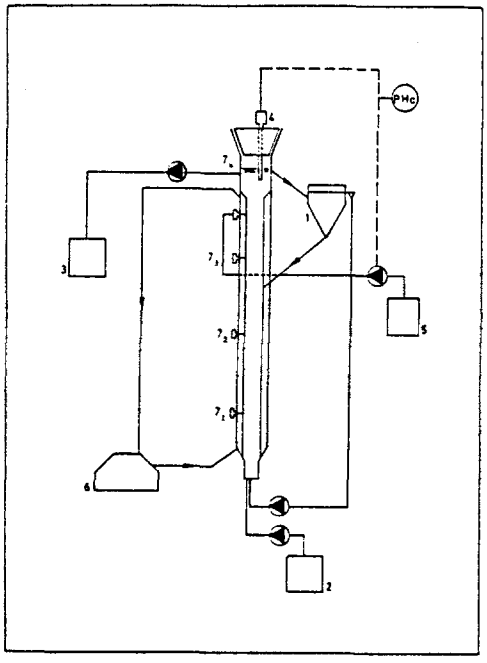

Fig.1. Fluidized bed reactor
1 - Settler
2 - Feed vessel
3 - Overflow vessel
4 - pH electrode
5 - Base vessel
6 - Temperature control
7 - Sampling ports (1,2,3 and 4)

RESULTS AND DISCUSSION

L. plantarum 7 was used in batch experiments with and without pH control. Tests without pH control indicated 31°C as the ideal temperature. Under these conditions, the maximum cell concentration obtained was 4.2g/L, maximum cell and acid volumetric productivities were, respectively, 0.23g/L.h and 1.1g/L.h .

In batch tests with pH control, the pH was controlled with an ammonia solution at values between 4.5 to 6.5. Initial glucose concentration was 85g/L and temperature 31°C. From the results the pH chosen was 5.5. Under these conditions maximum cell concentration, maximum cell and acid volumetric productivities were respectively, 11.9g/L, 0.56g/L.h, 2.2g/L.h .

In chemostat studies, temperature and pH were set at 31°C and 5.5, respectively, and the system was operated at different dilution rates (D). Studies were done with MRS medium and the initial glucose concentration was 70g/L. Results are shown in Fig.2 and Table 2. At high dilution rates wall growth could be observed, similar to reports refering to other lactic acid bacteria (Keen, 1972; Rogers, 1978), making the variation of cell concentration with dilution rate appear anomalous.

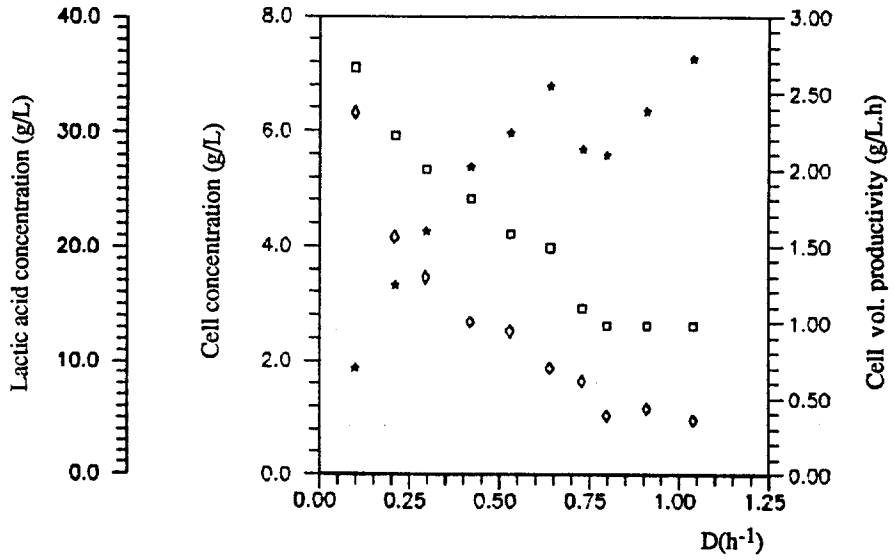

Fig.2. Cell (▫) and lactic acid (◊) concentrations, and cell volumetric productivity (*) in CSTR with *L. plantarum 7*.

The FBR was operated with a total system volume of 1740ml. The temperature was maintained at 31°C and the pH was controlled at 5.5. Tween 80 was omitted from MRS medium so that it would not interfere with the flocculation process. Medium was pumped into the fermentor at the desired dilution rate (D). The recirculation rate was 10 to 60 times higher than the feed rate. Samples were taken at three sampling ports (1,3 and 4 in Fig.1). Flocs appeared soon after the column entered in continuous operation, and they had different sizes along the column, from very small at the top to an average of 10mm wide and 4mm height at the bottom.

Since these tests were carried out without biomass purge, apart from that occurring naturally in the effluent, we considered a steady state on the lactic acid production. Constant lactic acid concentrations were

obtained after 25 to 50 residence times. Results are shown in Fig.3. Effluent stream samples coincided with sampling port 4.

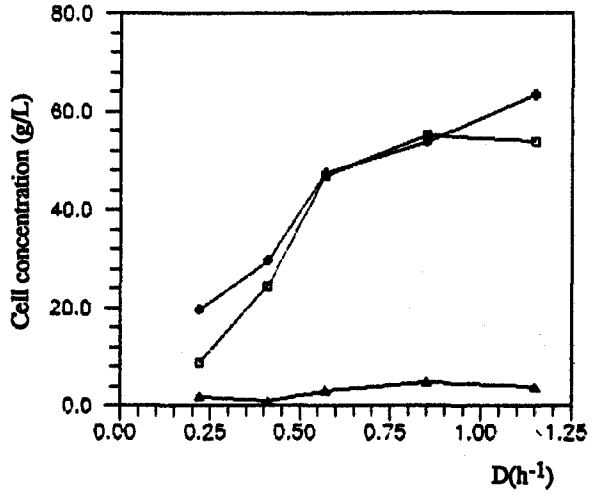

Fig.3. Cell concentration in FBR, sampling ports 1 (♦), 3 (▫) and 4 (▵).

The efficiency of flocculation in the system can be assessed by a flocculation factor:

$$\text{Flocculation factor} = \frac{\text{Cell concentration in the column samples}}{\text{Cell concentration in the effluent stream}}$$

This flocculation factor was calculated for every dilution rate tested. Results are shown in Table 1. It should be noticed that after the initial jump in flocculating activity with an increase in D, the values of the flocculation factor became approximately constant. This might be interpreted as if at higher D a limitation to floc growth appears; thus there is an increase in free cells that are allowed to escape in the effluent.

Simultaneously, at higher D, there was higher addition of ammonia; this contributes to diminuish the average floc size as ammonia seems to interfere with floc stability.

Table 1 - Efficiency of flocculation in FBR.

	Flocculation factor	
D (h^{-1})	Sampling port 1	Sampling port 3
0.22	9.94	4.49
0.41	36.39	29.93
0.57	15.17	14.96
0.85	11.12	11.38
1.15	16.72	14.45

Cell volumetric productivities for FBR were calculated using the following mass balance:

$$\frac{dx}{dt} = D.x_{out} + \frac{dx_{reactor}}{dt} \qquad (1)$$

where,

x - cell concentration, $g.L^{-1}$

t - time, h

D - dilution rate, h^{-1}

The term $D.x_{out}$ is given by the free cells leaving the reactor, while the term $dx_{reactor}/dt$ is the contribution of the growth of flocs during the time the system was kept at the same dilution rate.

The equation 1 does not account for the accumulation of biomass in the settler. This term was accounted for only at the end of each run, assuming constant rate of accumulation with time, representing approximately 0.3 g/L.h for the high dilution rates and lower values for the lower dilution rates.

The values for the productivities thus calculated are presented in Table 2.

Table 2- Comparative results between CSTR and FBR (sampling port 1)

Nominal dilution rate	D(h-1)				
	0.2	0.4	0.5	0.8	1.0
Effective dilution rate					
CSTR	0.21	0.42	0.53	0.8	1.04
FBR	0.22	0.41	0.57	0.85	1.15
Cell vol.productiv.(g/L.h)					
CSTR	1.24	2.02	2.24	2.1	2.72
FBR	0.91	1.21	2.75	4.4	4.9
Specific prod.(gacid/gcell.h)					
CSTR	0.74	1.18	1.59	1.59	1.92
FBR	0.22	0.24	0.29	0.44	0.42

Comparative results between CSTR and FBR show that cell volumetric productivities, at low dilution rates are higher for CSTR, while at high dilution rates they are higher for FBR as expected.

At low D, in FBR, the flocs are larger than at high D. Inside these flocs, diffusional problems inhibit cell growth and lactic acid production, so less amonia is added to the system, as mentioned before, and less free cells appear in the effluent stream. As the dilution rate increases, the smaller flocs formed, work in a "virtuous cycle", as the diffusional limitations are then reduced allowing the cell volumetric productivity of the FBR to clearly overtake that of CSTR. These results indicate that continuous cell purge strategy should be applied at high dilution rates, when cell volumetric productivities are high and could then be improved.

The diffusional limitations inside the flocs, mentioned before,could also explain the lower values of specific productivities in FBR, in comparison to CSTR.

As the aim of this work is the production of a starter culture, viability of the cells produced is a very important parameter. So, different types of methods for its determination are being tried, like CFU (colony

forming units) and a kinetic method based on the measurement of rate of change in pH at the start of a new fermentation.

The mechanism of flocculation of this *L. plantarum* is not yet known. Scanning electronic microscopy seems to indicate that there is extracellular material responsible for the adhesion of cells. As the mechanism of adhesion of other lactic bacilli is dependent on superficial carbohydrates, this hypothesis can be put forward (Bottazzi, 1987). Another important role in some mechanism of adhesion comes from the presence of cations in the medium, that could form bridges with the negative surface of bacteria and so help in the adhesion process (that is why tap water, which has calcium, was utilised). Experiments of flocculating activity with different cationic solutions are being carried out and, so far, the results are not very conclusive but seem to indicate no effects at ion concentration below 0.1 M. Ammonia has a definite effect in destroying or reducing the size of the flocs, as indicated earlier, and might thus indicate protein bridge being destroyed in the floc.

These preliminary results in utilisation of a flocculent strain in a fluidized bed reactor will be completed with more experiments, at different recirculation rates and cell purge in order to verify the cell productivity values at real steady state; viability will also be measured by the above mentioned methods.

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