Journal of Industrial Microbiology, 13 (1994) 63–69

Published by The Macmillan Press Ltd

Influence of operational parameters in the flocculation and production of *Lactobacillus plantarum*

M.T. Barreto Crespo[1] and M.J.T. Carrondo[1,2]

[1]*Instituto de Biologia Experimental e Tecnológica (IBET)/Instituto de Tecnologia Química e Biológica (ITQB), Apartado 12, 2780 Oeiras, Portugal and* [2]*Lab. Engenharia Bioquímica, Fac. de Ciências e Tecnologia, Universidade Nova de Lisboa, 2825 Monte de Caparica, Portugal*

(Received 21 May 1993; revision received 1 October 1993; accepted 21 October 1993)

Key words: Flocculation; Fluidized bed reactor; Lactic starter cultures; *Lactobacillus plantarum*; Viability; Dilution rate; Bleed rate

SUMMARY

The influence of different operational parameters, such as the dilution rate (D) and the bleeding rate (B), in the production of a flocculent strain of *Lactobacillus plantarum* was studied. The effect of the dilution rate was demonstrated to be related to the lactic acid concentration inside the reactor. The effect of the bleeding rate was shown to be critical in the stabilization of the operation (due to a better pH control). It also allowed a continuous recovery of cells outside the reactor. Viability testing of the lactic starter cultures showed that operation with cell purge increased the viability of the starter cultures obtained.

INTRODUCTION

Starter cultures are used in the industrial production of an increasing number of fermented products. The use of these starter cultures evolved from the inoculation of substrates with wild strain(s) to the actual utilization of pure and controlled strains. The industry depends on the efficiency of production of the cultures and on their viability. The starter cultures most frequently used are lactic starters which are utilized to produce and preserve milk-derived and bakery products, fermented vegetables, fermented fruit and meat products as well as silage for animal feed [5,11].

In order to keep its costs low, the production process of these starter cultures must be carefully optimized. Low price is even more critical for silage or vegetable utilization as the cost of the final product has to be kept low. Thus, *Lactobacillus plantarum*, often used for such fermentations, was chosen as a model microorganism. To increase the cell volumetric productivity it was decided to produce the starter culture under high cell concentration in a reactor with 'free immobilized' cells in aggregates. Therefore, a flocculent strain was chosen to be used in this work.

Flocculation followed by sedimentation is one of the oldest methods of recovering cells. Although there are other, more mechanical, techniques of separation, a large number of industrial processes like beer production, waste water treatment and anaerobic digestion often still depend on flocculation and sedimentation given its inherent simplicity [1,16].

Cellular aggregation or flocculation can be defined as a process where cells come together to form stable associations. This definition implies the existence of two phenomena: physical movement and multicellular stable contacts. Physical movement includes the Brownian collisions (perikinetic flocculation) and mechanically induced turbulence that promotes the collision of particles and subsequent adhesion (orthokinetic flocculation).

The physical contact between the units of the aggregate must be intercellular and multicellular. Ten cells is the minimum, although arbitrary, number usually required for a group of cells to be considered as an aggregate [6]. Several factors can influence the formation of an aggregate. Atkinson [2] described microbiological and environmental factors that affect the living organism and so influence the flocculation process:

- The microbiological factors include genetics, nutrition, physiological age, as well as cell wall structure.
- The environmental factors can be divided into physical, chemical and biological. The hydrodynamic properties, namely, intensity of agitation, dilution rate, aeration rate as well as shape and size of the fermentor (as these influence the type of flux) are included amongst the physical factors. The biological factors include the inoculum size, the presence of other microorganisms or strains and floc concentration.

Thus, the choice of the reactor to produce flocculent microorganisms and its conditions of operation has to be

Correspondence to: M.J.T. Carrondo, Inst. de Biologia Experimental e Tecnológica (IBET), Instituto de Tecnologia Química e Biológica (ITQB), Apartado 12, 2780 Oeiras, Portugal.

carefully thought over. Usually tower fermentors and fluidized bed reactors are preferred as they allow the accumulation of flocs and so higher cell concentrations and cell volumetric productivities can be achieved [8,13,14].

The authors have previously established that fluidized beds can be used to produce lactic starter cultures [3]. In this paper, the use of bleeding rate (B) to continuously produce and extract the cells, as well as the assessment of the starter culture viability are described and correlated to two operational parameters, the dilution rate (D) and the recirculation velocity (R).

MATERIALS AND METHODS

Microorganism

The microorganism used throughout this work was *Lactobacillus plantarum* 7, a flocculent strain belonging to the collection of Professor V. Bottazzi (Istituto di Microbiologia, Univ. Cattolica del Sacro Cuore, Piacenza, Italy).

Maintenance and fermentation media

The culture was kept in MRS medium [9] at 4 °C and transferred monthly. The fermentation medium was a slightly modified MRS which contained: tryptone 10 g; beef extract 1 g; yeast extract 5 g; K_2HPO_4 2 g; sodium acetate 5 g; diammonium hydrogen citrate 2 g; $MgSO_4 \cdot 7H_2O$ 0.2 g; $MnSO_4 \cdot H_2O$ 0.05 g in 1 L of tap water. Glucose was used as carbon source. Changes in the medium carried out during the work will be referred to in the text.

Reactor

The fluidized bed reactor (FBR) used in this work was a cylindrical column with an external settler allowing recirculation (Fig. 1). Details of the system were described elsewhere [3]. As soon as appropriate a cell bleed was imposed to the system. This cell bleed was controlled by a peristaltic pump (9 in Fig. 1). The fermentation broth was collected in a sterile vessel that was immersed in ice to avoid cell growth. The vessel was changed periodically, the volume obtained was measured and the cell concentration determined. The reactor was operated at 31 °C and the pH controlled at 5.5 with addition of an ammonia solution.

Analytical methods

The cell concentration was determined by filtration of 5 ml of cell suspension through a membrane with 0.2 μm pore diameter. The membrane and the retained cells were dried at 105 °C until constant weight was achieved. Glucose, lactic and acetic acids were determined by HPLC with a Shodex Sugar (SH-1011) column (Showa Denko K.K., Japan), using 0.01 N H_2SO_4 as eluent and a flow rate of 1.0 ml min^{-1} at 50 °C. A refraction index detector was used (ERC-7511, Erma Inc., Japan).

Determination of the viability of the cells

The method used to determine the viability of the cells being produced is a kinetic method, based on the inoculation of a new fermentation with the sample of the starter culture taken out of the reactor [4].

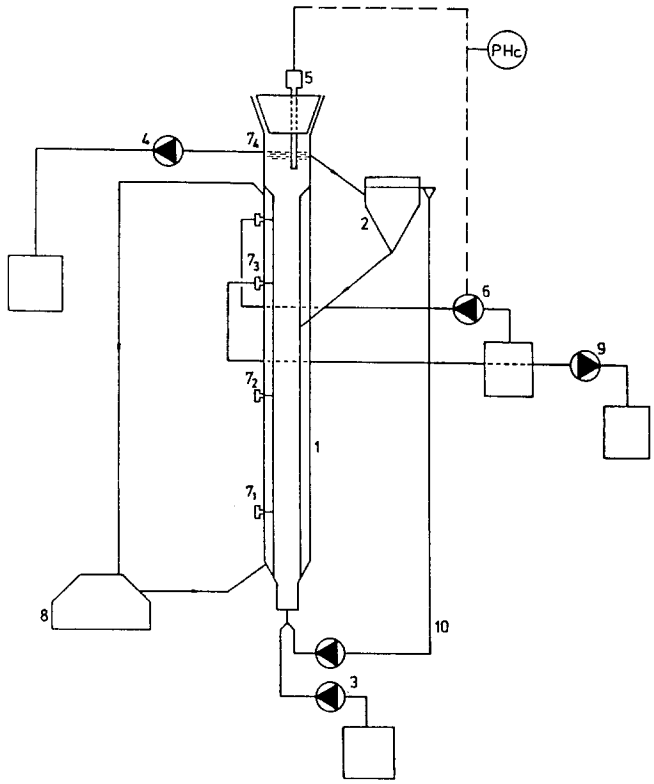

Fig. 1. Fluidized bed reactor: (1) Column; (2) Settler; (3) Feed system; (4) Overflow system; (5) pH electrode; (6) pH control system; (7) Sampling ports (1, 2, 3 and 4); (8) Temperature control system; (9) Cell bleed system; and (10) Recirculation system.

Reactor homogeneity test

This test was performed to assess whether the reactor behaved like a continuous stirred tank reactor or if, on the contrary, some of it constituted a dead volume. The method used was described by Mota [12]. It consisted of filling the reactor with a glucose solution (90 g L^{-1}), adding water at a known flow rate (corresponding to a desired dilution rate) and changing the recirculation velocity according to the experimental design envisaged.

Samples were taken every 15 min for 6 h at the sampling port number 4 of the reactor (overflow of the reactor). The glucose concentration was determined in each sample. The change in glucose concentration could be obtained by a material balance:

$$V \cdot \frac{dS}{dt} = -F \cdot S \qquad (1)$$

where S is the glucose concentration leaving the system and F is the feed flow rate. Integrating and representing ln S as a function of t, a straight line can be obtained, whose slope corresponds to the actual dilution rate (D). The closest the value of D obtained experimentally is to the dilution rate

imposed to the system, the more the reactor behavior approximates a continuous stirred tank regime.

RESULTS AND DISCUSSION

Study of the homogeneity of the reactor

These tests were performed prior to working with cells in the reactor, to assess whether the reactor had a behavior close to the continuous stirred tank reactor. The agitation inside the reactor is mainly caused by the recirculation flow rate, as its value is much higher than the feed flow rate. The tests were performed at the same feed flow rate used throughout most of the work described here (F = 646 ml h^{-1}) and at four different recirculation flow rates (3770 ml h^{-1}, 10640 ml h^{-1}, 19702 ml h^{-1} and 26760 ml h^{-1}) (see Materials and Methods).

Table 1 summarizes the results. Representing the logarithm of the substrate concentration as a function of time, linear relationships were obtained. The slopes obtained were only slightly different; thus, the percentage of dead volume does not vary considerably with the recirculation flow rate in the range tested. This small dead volume can probably be due to the lower part of the settler since the liquid flows out from the top and not from the bottom of the settler.

Considering these results it is acceptable to assume that the main body of the reactor behaves like a chemostat. This fact will ease the analysis of the results obtained when the cells are present in the fermentor.

Floc formation and lactic starter culture production with flocculent cells

Orthokinesis (mechanical agitation) is very important when particles with a diameter superior to 1 μm interact [7,15], as it happens with bacteria (1–5 μm). In the reactor used in this work the mechanical agitation is caused by the recirculation flow. The effect of the recirculation velocity in the flocculation process and in the production of a lactic starter culture is described elsewhere [3].

Among several other factors described in the introduction as affecting floc formation, this work will discuss the influence of the dilution rate (upon concentration of lactic acid and micronutrients); the effect of cell bleed and the viability of the cells obtained, will also be covered.

1. Influence of the dilution rate

The dilution rate is an environmental factor of the physical type. Three dilution rates were tested D = 0.24 h^{-1}, 0.76 h^{-1} and 1.27 h^{-1}. The recirculation velocity was kept at 8.46 m h^{-1}. Preliminary results have already been published showing that the dilution rate has a strong effect in the starting of the flocculation process: increased dilution rates fasten the flocculation process [3].

As the total ascensional velocity does not vary more than 10% between the run performed at the lower dilution rate and the run at the highest dilution rate, the change in speed of the flocculation process cannot be attributed to the mechanical agitation inside the system. Thus, two causes can be put forward to explain the effect of the dilution rate: a) concentration of the lactic acid present in the system as this compound is inhibitory of cell growth, or b) the level of micronutrients that the cells receive in their surroundings per unit of time ($g_{micronut.}$ L^{-1} h^{-1}).

Concentration of lactic acid. If the first explanation is considered it can be hypothesized that the accumulation of the acid in the system can inhibit the metabolism of the cells and consequently the flocculation process. The concentration of lactic acid, P obtained in level 1 of the fluidized bed reactor, during operation at three different dilution rates, is represented in Fig. 2.

The lactic acid volumetric productivities were calculated using a mass balance:

$$\nu \cdot X = D \cdot P_{out} + \frac{dP}{dt} \qquad (2)$$

Equation 2 assumes that there is a small accumulation of lactic acid, P, during each run. This accumulation is accounted for by the term dP/dt. The term $D \cdot P_{out}$ is, nevertheless, the largest contribution to the calculation of lactic acid volumetric productivity.

Figure 3 represents the lactic acid volumetric productivities. It can be seen that, before the flocculation process, the lactic acid concentration in the system is higher at the lower dilution rates. The lactic acid volumetric productivity at D = 0.24 h^{-1} has lower values than those obtained in the other two runs (Fig. 2). It can be concluded that the lactic

TABLE 1

Results of the homogeneity studies

Recirculation flow rate (ml h^{-1})	Initial substrate concentration, S_0 (g L^{-1})		Dilution rate, D (h^{-1})		Reactor volume, V (ml)		Dead volume (%)
	Theoretical	Experimental	Theoretical	Experimental	Theoretical	Experimental	
3770	91.8	93.2	0.37	0.396	1735	1630	6.0
10640	94.2	95.9	0.37	0.388	1735	1665	4.1
19702	92.9	96.7	0.37	0.396	1735	1631	6.0
26760	43.9	46.9	0.37	0.403	1735	1603	7.7

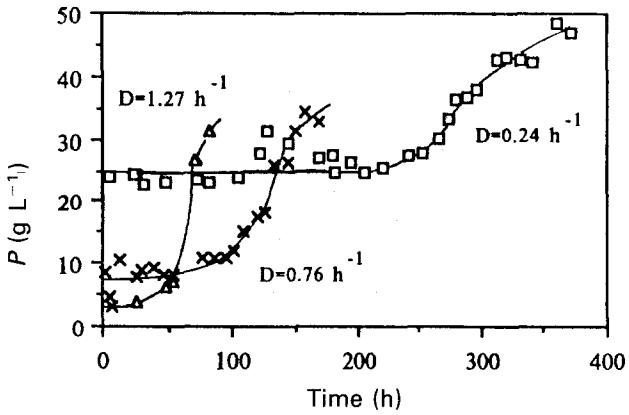

Fig. 2. Lactic acid concentration, P, obtained during operation of the FBR at R = 8.46 m h^{-1} and different dilution rates.

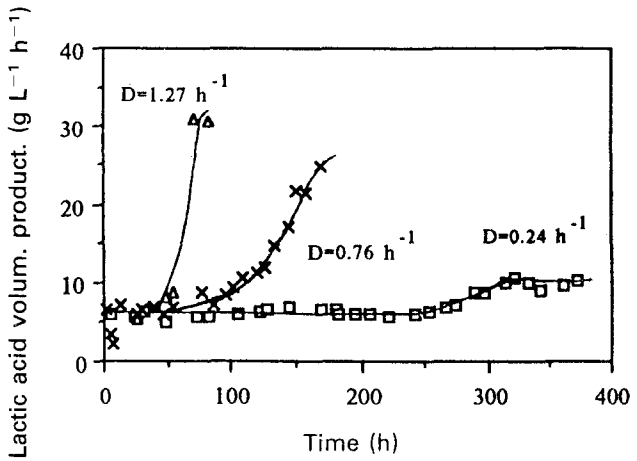

Fig. 3. Lactic acid volumetric productivities obtained during operation of the FBR at R = 8.46 m h^{-1} and different dilution rates.

acid concentration present in the system seems to have a strong influence in the flocculation process of *Lactobacillus plantarum*.

Concentration of micronutrients. This hypothesis could be stated as follows: at low dilution rates the residence time, T_r in the system is higher and, consequently, the quantity of micronutrients that the cells receive per unit of time can be insufficient to ensure the cell growth and the lactic acid production without introducing limitations to growth.

To verify whether this hypothesis was correct another run, conducted at concentrated MRS (named MRS_{conc}), was performed. In comparison with the medium used before and described in Materials and Methods, MRS_{conc} has double the concentration of all nutrients except for glucose. The dilution rate at which the reactor was operated was approximately half of the one used for comparison. Operational conditions are summarized in Table 2.

The growth curves obtained in two levels of the column (level 1 and level 3) are represented in Fig. 4. The process of flocculation can be seen to start sooner in the experiment performed with MRS and D = 0.76 h^{-1}. The cell concentration obtained in both runs is similar but the lactic acid concentration is much larger when MRS_{conc} is used (Fig. 5).

As the concentration of cells is similar in both cases, it does not seem that any kind of micronutrient limitation took place. It was also verified that there was no glucose limitation (Fig. 6). Thus, it can be concluded that the effect of the dilution rate in the flocculation process is caused by the lactic acid concentration inside the system. The lactic acid is washed faster from the system at higher dilution rates, the inhibition problems being avoided in this way.

The dilution rate cannot be considered as an environmental factor of the physical type, with influence in the flocculation process, when a reactor of this kind is utilized. The dilution rate must then be considered a microbiological factor in accordance with Atkinson's classification of factors affecting flocculation [2].

2. Influence of the cell bleed

If the fluidized bed reactor is operated with increasing cell concentration with flocculent cells, stability problems can occur. In fact, cells not only adhere to each other but also adhere to the walls of the reactor and to the pH electrode, rendering it more difficult to accurately control the pH.

One way of obtaining a continuous stream of cells outside the reactor and also of minimizing the stability problems referred to above is to establish a cell bleed. This cell bleed will be established when the column has already a large number of flocs; in this case the concentration of the flocs will change. In accordance with Atkinson's definition [2] cell bleed can be considered an environmental factor of the biological type, affecting the floc formation. This cell bleed strategy can be predicted with the help of a few mathematical manipulations; first a mass balance is performed on the reactor:

$$\mu \cdot X = D \cdot X_{out} + B \cdot X + \frac{dX}{dt} + \left(\frac{\Delta X}{\Delta t}\right)_{set} \quad (3)$$

In Eqn 3, X represents the cellular concentration in the level of the column where the purge will be established (Fig. 1). $D \cdot X_{out}$ quantifies the cells that continuously come out of the system, dX/dt the cells that accumulate in the column and $(\Delta X/\Delta t)_{set}$ the cells that accumulate in the settler; B represents the bleeding rate (h^{-1}) which can be defined as:

$$B = \frac{Q_b}{V} \quad (4)$$

Since it is desired that the cell mass formed in the column leave the reactor, Eqn 3 reduces to:

$$B \cdot X = \mu \cdot X - D \cdot X_{out} \quad (5)$$

After obtaining the values of μ, the highest value was selected and then the cell bleed value was calculated. The

TABLE 2

Operational conditions for the FBR with different substrate concentrations

Experiment	Dilution rate, D (h^{-1})	Residence time, T_r (h)	Feed velocity, I (m h^{-1})	Recirculat. velocity, R (m h^{-1})	Time of ascens.flow, T_p (h)
MRS	0.76	1.3	0.57	15.7	0.049
MRS_{conc}	0.34	2.9	0.26	15.8	0.05

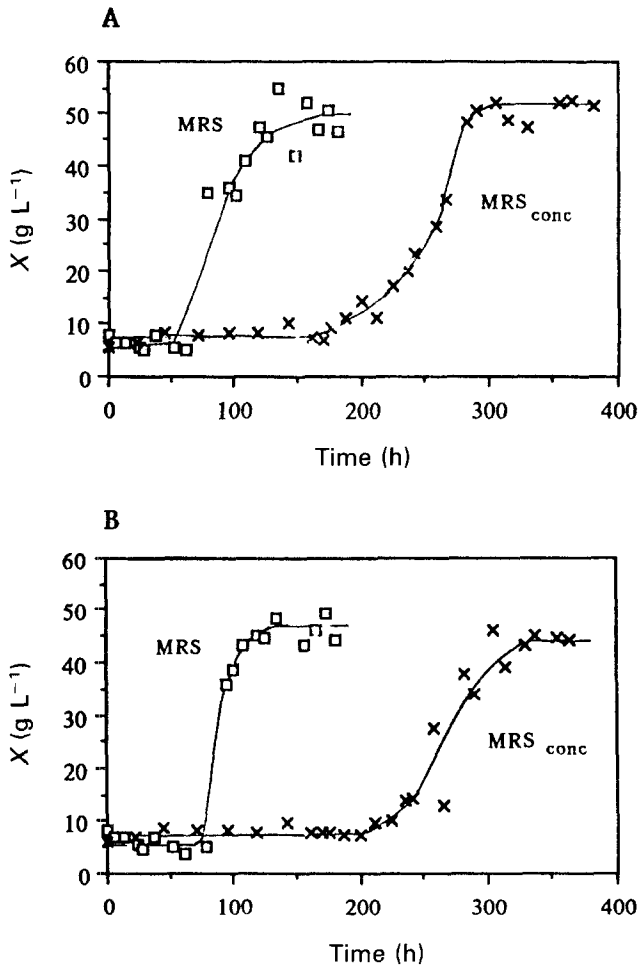

Fig. 4. Growth curves obtained in the experiments performed in the FBR with MRS and MRS_{conc}. (A) at level 1 in the column; and (B) at level 3 in the column.

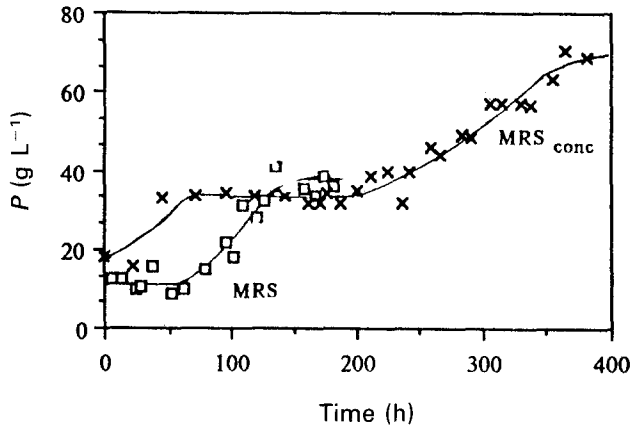

Fig. 5. Lactic acid concentration, P, obtained in the experiments performed in the FBR with MRS and MRS_{conc} (at level 1 in the column).

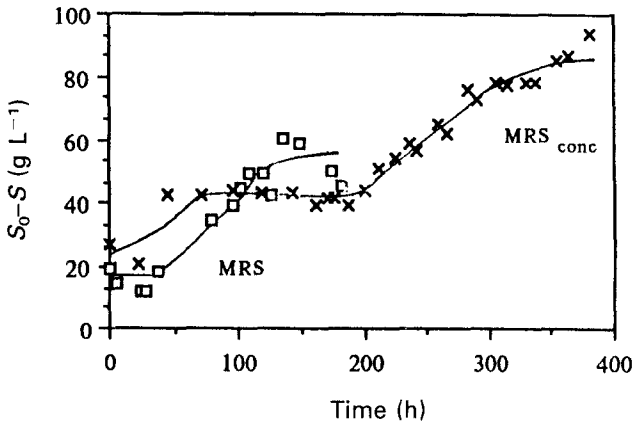

Fig. 6. Glucose consumption, S_0–S, obtained in the experiments performed in the FBR with MRS and MRS_{conc} (at level 1 in the column).

operational conditions of the experiment with cell bleed are summarized in Table 3.

The cell concentrations obtained in levels 1 and 4 are represented in Fig. 7. It can be seen that after cell purge is started there is a decrease in cell concentration in level 1. Approximately 50 h later the system became stable and was operated without instabilities for another 100 h, after which it was decided to discontinue the test. The flocs do not reach the top of the column and thus, the effect of the interference of the cells with the pH electrode is sharply reduced.

Another experimental observation was that the flocs coming out of the reactor through the cell bleed system were smaller than before the flocculation process. The cell bleed affects the size of the flocs formed but allows the formation of a larger number of flocs, as the same cell concentration is attained at the end of the run. Given the

TABLE 3

Operational conditions utilized in FBR during operation with cell bleed

Dilution rate, D (h^{-1})	0.72
Bleeding rate, B (h^{-1})	0.14
Recirculation velocity, R (m h^{-1})	15.7

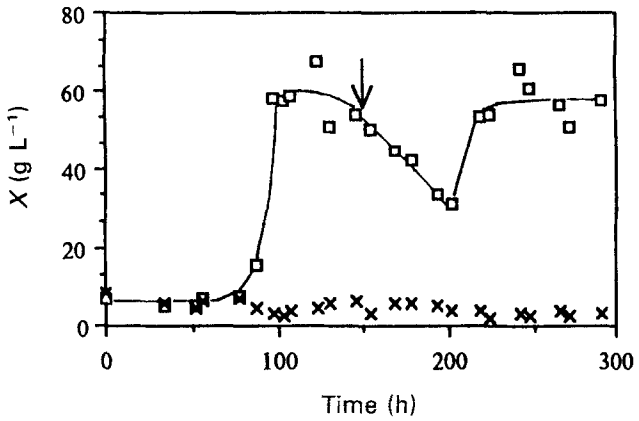

Fig. 7. Cellular concentration obtained in the FBR operated with cell bleed. The arrow indicates the moment when the cell bleed started. The symbols (□) and (×) represent, respectively the concentration in levels 1 and 4 of the column.

smaller diameter of the flocs formed, mass transfer problems should be reduced and cell viability should be improved (see below).

Furthermore these flocs desegregate almost completely when they reach the outside vessel, i.e. the intercellular sticking forces are low. This is an important aspect from the technological point of view as it shows that a process of floc breaking can be avoided prior to the processes of concentration and lyophilization of the starter culture.

3. Viability of the cultures produced

For production purposes, the viability of the cells is a fundamental aspect to consider. Cultures with very low or no viability have no capacity to start new fermentations or to lower the pH of the new media, thus do not act as real starter cultures and so endanger the production of the fermented product.

During a process of cell production, one can obtain viable cells, non-viable cells (cells that cannot duplicate but can still do some oxidation of the substrates) and dead cells (cells that neither respire nor replicate) [10]. In the case of the production of a starter culture using aggregates this problem is even more critical, as inside the flocs there can be an accumulation of non-viable and dead cells, if size is not controlled, and mass transport limitations take place.

The viability of the cultures obtained throughout this work was measured by a kinetic method [4]. Some viability results obtained in the FBR without cell bleed are represented

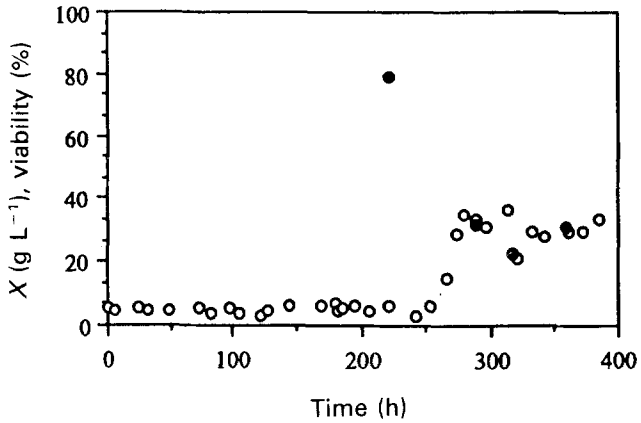

Fig. 8. Cellular concentration (○), viability (●) of the lactic starter cultures produced in the FBR.

in Fig. 8. It can be seen that soon after the flocculation process stops, the viability drops to values near 25%.

The specific productivity (g lactic acid g $cell^{-1}$ h^{-1}) can be used as a measure of the loss of activity of the cells, i.e. the capacity to produce lactic acid. Fig. 9 shows the comparison between the specific productivity and the viability of the cells obtained in the FBR without cell bleed. The shape of the curves are similar. It can be concluded that while producing cells in the FBR the loss of viability is always accompanied by a loss of activity.

The immobilization of cells in aggregates might cause a chemical and an osmotic stress to the cells due to diffusional problems, not only in terms of substrate concentration but also in terms of lactic acid accumulation. This stress is mainly responsible for the loss of viability and, eventually, the death of the cells.

Thus, if it is desired to produce starter cultures in a fluidized bed reactor without using a cell bleed to the system, the ideal phase to stop the production is soon after the end of the flocculation phase. At that moment a high cell concentration and good viability are still present (Fig. 9).

In the tests performed with cell bleed, the viability of

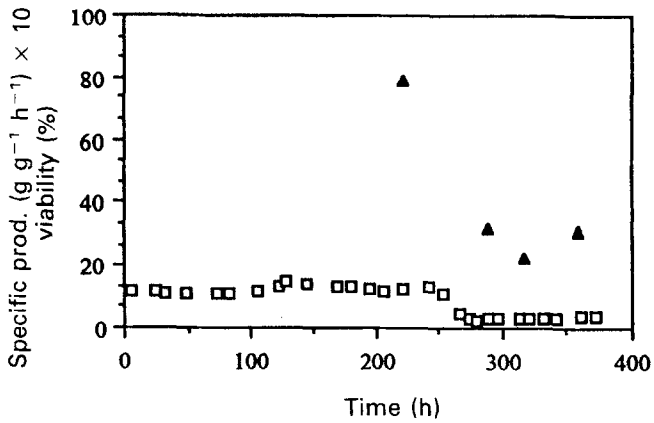

Fig. 9. Specific productivity (□) and viability (▲) of the lactic starter cultures obtained in the experiments performed in the FBR.

the cultures produced was also determined. The values obtained were around 35%. These values indicate that, once the cell bleed is stabilized the starter cultures obtained have a higher viability than those obtained in the runs realized without cell bleed. This change is possibly due to the smaller size and looser aggregation of the flocs formed, as referred to above.

CONCLUSIONS

During operation in FBR it was verified that at high dilution rates, the lactic acid was washed away faster, thus not causing strong inhibition problems to the growth of the cells. A faster flocculation process was obtained. On the other hand, it was demonstrated that it was not the limitation of micro nutrients that influenced the flocculation process. From these studies it can be concluded that the dilution rate, described as an environmental factor of the physical type [2] affecting the flocculation process, acts as a microbiological factor.

Furthermore, it can be concluded that the operation of the system must be a compromise between the recirculation velocity [3] and the dilution rate – which should be as high as possible – and the costs of energy, fermentation media and sterilization.

The cell bleed, an environmental factor of the biological type affecting the floc formation was another factor to be studied. The operation with cell bleed allowed the obtention of cell mass in a continuous fashion, keeping the cell volumetric productivity high. Operation under cell bleed also decreased the problems of pH control, thus allowing a better operational stability.

Finally it should be stressed that when operating the FBR with a cell bleed, the viability of the starter cultures produced was higher. This fact, the experimental observation that the flocs coming out of the reactor desegregate spontaneously and the high cell productivities obtained support the conclusion that this process of producing lactic starter cultures represents a very good alternative to the classical methods.

NOMENCLATURE

B	Bleeding rate, h^{-1}
D	Dilution rate, h^{-1}
F	Feed flow rate, L h^{-1}
I	Feed velocity, m h^{-1}
μ	Specific growth rate, h^{-1}
ν	Lactic acid specific productivity, g g^{-1} h^{-1}
P	Product concentration (lactic acid), g L^{-1}
P_{out}	Product concentration leaving the system, g L^{-1}
Q_b	Bleeding flow rate, L h^{-1}
R	Recirculation velocity, m h^{-1}
S	Substract concentration, g L^{-1}
t	Time, h
T_p	Time of ascensional flow (length of the column/ total ascensional velocity), h
T_r	Residence time ($1/D$), h
V	Volume of the reactor, L
X	Cell concentration, g L^{-1}
X_{out}	Cell concentration leaving the system, g L^{-1}

ACKNOWLEDGEMENTS

Thanks are due to Professors V. Bottazzi and P.S. Cocconcelli for the gift of the strain *Lactobacillus plantarum* 7. We acknowledge the technical assistance of Mrs Maria de Fátima Cunha. The work was supported by grant no. 3.3/P248 from Fundação Luso Americana para o Desenvolvimento. The support of Investimentos e Participaçoes do Estado and the funding of NATO Science for Stability Program through project NATO Po FeedChem are also acknowledged.

REFERENCES

1 Aiba, S. and M. Nagatani. 1971. Separation of cells from culture media. In: Advances in Biochemical Engineering (Ghose, T.K. and A. Fiechter, eds), Vol. 1, pp. 31–54, Springer-Verlag, New York.

2 Atkinson, B. and I.S. Daoud. 1976. Microbial flocs and flocculation in fermentation process engineering. In: Advances in Biochemical Engineering (Ghose, T.K., A. Fiechter and N. Blakebrough, eds), Vol. 4, pp. 41–124, Springer-Verlag, New York.

3 Barreto, M.T.O., E.P. Melo, J.L. Moreira and M.J.T. Carrondo. 1991. High cell density reactor for the production of *Lactobacillus plantarum*. J. Ind. Microbiol. 7: 63–70.

4. Barreto, M.T.O., E.P. Melo, J.S. Almeida, A.M.B.R. Xavier and M.J.T. Carrondo. 1991. A kinetic method for calculating the viability of lactic starter cultures. Appl. Microbiol. Biotechnol. 34: 648–652.

5 Bottazzi, V. 1988. An introduction to rod-shaped lactic-acid bacteria. Biochimie 70: 303–305.

6 Calleja, G.B. 1984. Microbial Aggregation. CRC Press Inc., Boca Raton, Florida.

7 Kihn, J.C., L.C. Masy, M.M. Mestdagh, P.G. Rouxhet. 1988. Yeast flocculation: factors affecting the measurement of flocculence. Can. J. Microbiol. 34: 779–781.

8 Kwok, K.H. and I.G. Prince. 1989. Flocculation of *Bacillus* species for use in high-productivity fermentation. Enzyme Microbiol. Technol. 11: 597–603.

9 Man, J.C de, M. Rogosa and M.E. Sharpe. 1960. A medium for the cultivation of *Lactobacilli*. J. Appl. Bacteriol. 23: 130–135.

10 Mason, C.A., G. Hammer and J.D. Bryers. 1986. The death and lysis of microorganisms in environmental processes. FEMS Microbiol. Rev. 39: 373–401.

11 Moon, N.J. 1984. A short review on the role of Lactobacilli in silage fermentation. Food Microbiol. 1: 333–338.

12 Mota, M. 1985. Inhibition et fermentation alcoholique: quelques concepts non conventionelles. Thesis. Inst. National des Sciences Appliquées, Toulouse, France.

13 Prince, I.G. 1985. The use of flocculated cells for high productivity fermentations. Biotech. 85 Asia(Singapore) 3: 409–412.

14 Schügerl, K. 1989. Biofluidization: application of the fluidization technique in biotechnology. Can. J. Chem. Eng. 67: 178–184.

15 Stratford, M. and P.D.G. Wilson. 1990. Agitation effects on microbial cell–cell interactions. Lett. Appl. Microbiol. 11: 1–6.

16 Whittington, P.N. 1990. Fermentation broth clarification techniques. Appl. Biochem. Biotechnol. 23: 91–121.