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SEMICONTINUOUS LACTIC FERMENTATION OF WHEY BY *Lactobacillus bulgaricus*.

I. EXPERIMENTAL RESULTS

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SUMMARY. A Monod-like equation correlates the lactic acid productivity and the volume fraction of inoculum in semicontinuous fermentation of whey by *Lactobacillus bulgaricus*. The volume of the inoculum varied from 10% to 80% of the reactor working volume.

INTRODUCTION

Several papers were published on batch and continuous lactic fermentation of whey by *L.bulgaricus*, using ammonia as the neutralising agent, with the purpose to produce a ruminant feed supplement high in crude protein (Arnott at al., 1958; Gerhardt and Reddy, 1978; Keller and Gerhardt, 1975; Marshall, 1972; Reddy at al. 1976; Stieber and Gerhardt, 1979; Stieber and Gerhardt, 1981). No information were found about the application of semicontinuous technique to the above fermentation. This is the main purpose of the present paper.

MATERIALS AND METHODS

Dehydrated whey (lactose, 73%; protein, 13%; minerals, 9%; fat, 1%; moisture < 4%) was dissolved in tap water in order to obtain the desired lactose concentration (39 to 49 $g.L^{-1}$) and was then pasteurized ($70^{o}C$; 15 min). Yeast extract was added to the fermenter (2.0 $g.L^{-1}$)at the beginning of each fermentation test.

The first inoculum of each semicontinuous test was prepared adding 80 mg of lyophilized *L.bulgaricus* to a 1-L erlenmeyer flask containing 400 ml of a sterilized ($115^{o}C$; 15 min) culture medium (40g of skim milk powder and 360g of tap water) and then incubating the flask (40-$42^{o}C$; 15h). The first fermentation of each semicontinuous test was carried out in a non-sterilized 15-L Biolafitte fermenter under the following conditions: volume of pasteurized whey, 4.8L; volume of inoculum, 1.2L; temperature, $45^{o}C$; pH 5.6 (automatically controlled by the addition of 266.1 $g.L^{-1}$ NH_4OH solution); impeller speed, 250 min^{-1}.

When the first fermentation was completed, a certain volume of the fermented medium was used as inoculum of the second fermentation, and pasteurized whey was added to the reactor in order to fill up the reactor (working volume = 6.0 L). The other experimental conditions were as described above.

This technique was successively repeated using, in each serie of tests, always the same volume of recently fermented medium as inoculum of the next fermentation cycle. Several series of semicontinuous tests were carried out using the following volumes of fermented medium as inoculum: 0.6 L(β = 0.10 or 10%), 1.2 L(β = 0.20), 2.4 L(β = 0.40), 3.6 L (β = 0.60) and 4.8 L(β = 0.80).
Total sugars concentrations (calculated as lactose) were determined by the method described by Montgomery (1961). The lactic acid productivities were calculated from the ammonium hydroxide consumption because it was observed (Baralle and Borzani, 1987) that the concentration of lactic acid produced is proportional to the volume of NH_4OH solution added to the reactor in order to control the fermenting medium pH.

RESULTS

Tables 1 and 2 show the results obtained using a fast-fermenting and a slow-fermenting *L.bulgaricus*, respectively.

TABLE 1 - Results obtained in semicontinuous tests using a fast-fermenting *L.bulgaricus*.

Test Nº	N	V_i (L)	V_w (L)	β	S_w ($g.L^{-1}$)	V_a (mL)	T (h)	P ($g.L^{-1}.h^{-1}$)
1	9	0.6	5.4	0.10	49 ± 3	331 ± 6	12 ± 2	2.98
2	10	1.2	4.8	0.20	42 ± 2	309 ± 11	8 ± 1	4.19
3	9	2.4	3.6	0.40	45 ± 3	219 ± 4	4.6 ± 0.5	5.23
4	6	3.6	2.4	0.60	45 ± 1	146 ± 5	3.9 ± 0.5	4.16
5	14	4.8	1.2	0.80	43 ± 4	70 ± 4	1.5 ± 0.1	5.26

TABLE 2 - Results obtained in semicontinuous tests using a slow-fermenting *L.bulgaricus*.

Test Nº	N	V_i (L)	V_w (L)	β	S_w ($g.L^{-1}$)	V_a (mL)	T (h)	P ($g.L^{-1}.h^{-1}$)
6	8	1.2	4.8	0.20	39 ± 6	292 ± 5	15 ± 2	2.12
7	11	2.4	3.6	0.40	39 ± 4	218 ± 7	9 ± 1	2.66
8	9	3.6	2.4	0.60	43 ± 2	146 ± 6	6.1 ± 0.3	2.66

Figure 1 shows that, as was theoretically expected, a good proportionality (V_a = 61.6 V_w) was observed between V_a and V_w. Considering that the average value of S_w was 43 $g.L^{-1}$, and assuming that all the sugar was converted into lactic acid, the following correlation between V_a and V_w would be obtained: V_a = 66.2 V_w. In other words, the average fermentation yield was 93.0% of the theoretical value.

Figure 2 shows that the following empirical equations represent the influence of β on P:

a) fast-fermenting bacteria: $P = 6.37\ \beta/(0.111 + \beta)$ (1)

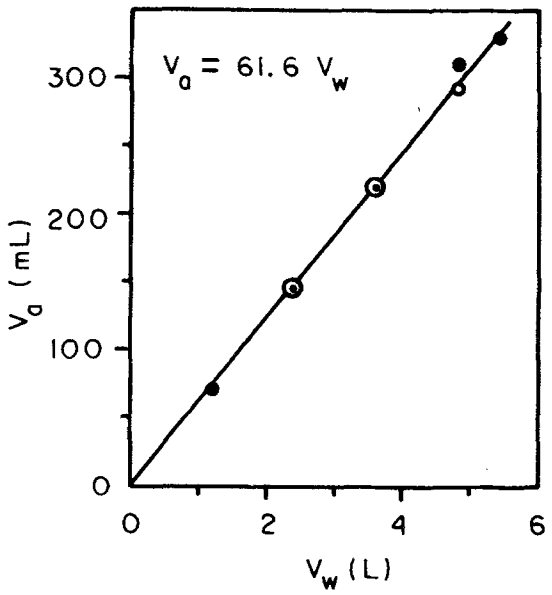

Fig. 1 - Proportionality between the volume of NH_4OH solution used to control the pH (Va) and the volume of whey added to the reactor (V_w). Tests were carried out with fast-fermenting (•) and with slow-fermenting (o) bacteria.

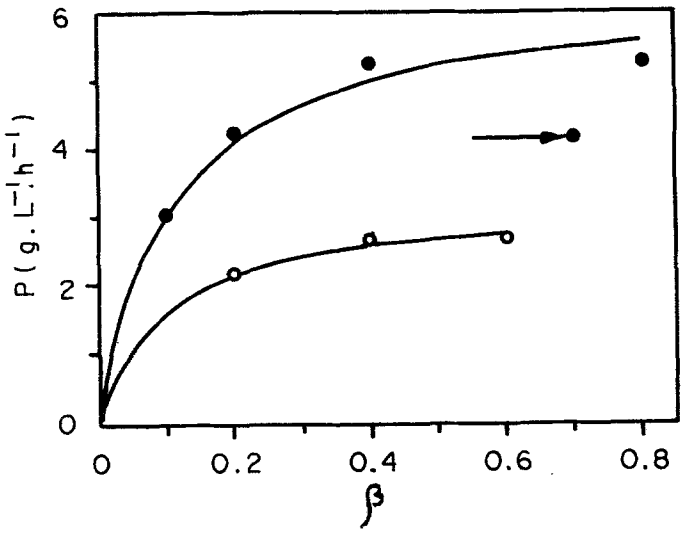

Fig. 2 - Influence of the volume fraction of inoculum (β) on the lactic acid productivity (P) when fast-fermenting (•) and slow-fermenting (o) bacteria were used. The point indicated by an arrow was not considered when equation (1) (see text) was obtained.

b) slow fermenting bacteria: $P = 3.19\beta/(0.099+\beta)$ (2)

When the value of β approaches 1.0, a continuous fermentation with no cell recycle is attained. In this case, equations (1) and (2) permit to evaluate the correspondent lactic acid productivities: 5.73 and 2.90 $g.L^{-1}.h^{-1}$.

NOMENCLATURE

N : number of fermentation cycles after the first fermentation.

P : lactic acid productivity.

S_W : average total sugars concentration of the whey as lactose (the standard deviation is indicated).

T : average fermentation time (the standard deviation is indicated).

V_a : average total consumption of NH_4OH solution (the standard deviation is indicated).

V_i : volume of recently fermented medium used as inoculum of the next fermentation cycle.

V_W : volume of whey added to the reactor at the beginning of each fermentation cycle.

β : volume fraction of inoculum = $V_i / (V_i + V_W)$.

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