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Evaluation of different alkali treatments of bagasse pith for cultivation of *Cellulomonas* sp.

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Biomass production of *Cellulomonas* was optimal with 1% (w/v) bagasse pith pre-treated with either 0.2 M NaOH for 1 h at 80°C or 0.4 M NaOH for 40 h at 28 to 30°C. Growth was similar to that obtained with a more severe treatment used as control and compared well with other reports for cellulolytic bacteria cultivated on pre-treated bagasse pith.

Key words: Bacteria, biomass, lignocellulosic, pre-treatment, single cell protein, sodium hydroxide.

One of the most important improvements that can be made in single cell protein (SCP) production from lignocellulosic substrates is to lower the cost of the substrate pre-treatment. Several authors have increased the digestibility of different lignocellulosic materials using NaOH (Dunlap 1969; Molina *et al.* 1984). In general, the best treatment depends on the nature of the substrate and the microbial system employed. The aim of this work was to develop a simple alkali pre-treatment of bagasse pith for the production of SCP by *Cellulomonas* sp. IIbc, using statistically designed experiments.

Materials and Methods

Microorganism and Cultivation Conditions

Cellulomonas sp. IIbc (Enríquez 1978) was cultivated on a shaker for 48 h at 100 rev/min and 32°C, in 500-ml Erlenmeyer flasks containing 50 ml of 'MO' medium (Rodriguez *et al.* 1983) and 1% (w/v) sugar-cane bagasse pith as the carbon and energy source.

Alkali Treatment

Bagasse pith (400 g) was mixed with 8 l of 0.2 or 0.4 M NaOH solution for a final NaOH/pith ratio of 10% or 20% (w/w), respectively. The mixture was kept at various controlled temperatures and either stirred at 800 rev/min or left unstirred. The pith was squeezed with a linen cloth, washed with 8 l of tap water, neutralized with 2 M HCl and dried at 80°C. The treatment described by Dunlap (1969) (NaOH/pith ratio of 100% for 1 h at 180°C) was used as the control.

Statistical Design Methods

To evaluate the effects of temperature and time of treatment (X_1 and X_2, respectively), a 3^2 factorial design was used (Bacon & Henson 1971). A 2^2 factorial design was used to assay the effect of NaOH concentration and washing of the treated material, in treatments carried out at 80°C for 1 h. Finally, the effect of increasing the time of treatment at room temperature (28–30°C) was evaluated in separate experiments, together with the effect of stirring. The optimization criterion was the bacterial biomass concentration obtained after cultivation in the pre-treated pith.

Analysis

Growth was followed turbidimetrically at 600 nm, after allowing the bagasse particles to settle for 10 min. At the end of growth, the culture was filtered through a sintered glass crucible (pore diameter 80 μm). The retained pith was washed with distilled water and dried to constant weight to determine the residual substrate. The filtrate was used for dry biomass determination (Enríquez 1978). Cellulose, hemicellulose and lignin were determined as described previously (Enríquez 1978). Sodium was determined by atomic absorption.

Results and Discussion

Effects of Temperature and Time of Treatment

The results of the 3^2 plan are shown in Table 1. Treatment variant 9 gave the best results with similar bacterial growth and lower losses than the more severe

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Table 1. Effect of temperature and time of treatment of bagasse pith on its biodegradability by *Cellulomonas* sp. IIbc.

Treatment variant*	Temperature (°C)	Time (min)	Biomass (g/l)	Pith consumption (%)	Yield†		Material loss (%)
					Y_1	Y_2	
1	30	10	1.3	39	0.12	0.34	3
2	55	10	1.6	41	0.13	0.35	6
3	80	10	2.2	65	0.21	0.35	14
4	30	35	2.5	62	0.24	0.39	12
5	55	35	2.9	71	0.27	0.38	24
6	80	35	3.1	72	0.28	0.38	21
7	30	60	3.2	76	0.27	0.39	33
8	55	60	3.2	77	0.30	0.40	38
9	80	60	3.5	78	0.33	0.42	40
10	180	60	3.6	75	0.33	0.46	58

* Variants 1 to 9 had a NaOH/pith ratio of 20% (w/w) while that of variant 10 (control) was 100% (w/w).
† Y_1—g biomass/g fed pith; Y_2—g biomass/g consumed pith.

treatment used as control (variant 10). By applying the regression matrix system for a 3^2 factorial design (Bacon & Henson 1971), the relationship of bacterial biomass yield (Y) and both experimental variables was determined. After eliminating the non-significant coefficients (by means of a Fisher test), the equation was:

$$Y = 2.79 + 0.815X_1 + 0.3X_2 - 0.33X_1^2$$

i.e. both variables significantly affected biomass production (Table 1). From this equation, a value of 3.3 g biomass/l was calculated to be the theoretical maximum biomass production. Therefore, variant 9, in which 3.5 g/l of biomass were obtained, can be considered the optimum treatment.

A direct relationship between the degree of delignification and the biodegradability of the material was observed (data not shown). The lignin concentration decreased from 24% (w/w) in the untreated pith to 10% (w/w) in variant 9; the corresponding changes in cellulose and hemicellulose were increases from 43% to 50% (w/w), and from 24% to 32% (w/w), respectively. Variations in the content of Na^+ and ash, probably as a result of the manual washing operation, were also observed.

Effects of NaOH Concentration and of Washing

Neither washing nor the decrease in NaOH concentration from 0.4 M to 0.2 M affected bacterial growth, as supported by the calculation of the polynomial equation of the 2^2 plan (data not shown) and the significance of the coefficients. Furthermore, lower losses of material occurred with the lower NaOH concentration. Good results using unwashed treated bagasse have been also reported by Han & Callihan (1974) for a mixed bacterial culture.

Treatments at Room Temperature

Although the best variant from the 3^2 plan was the treatment at 80°C, good results with the treatments carried out at room temperature were also achieved (Table 1). Increasing the time of treatment at 28 to 30°C led to an increase in all growth parameters (Table 2). However, all treatments of >4 h gave similar results. Nevertheless, the

Table 2. Growth of *Cellulomonas* sp. IIbc on pith treated at room temperature (28 to 30°C) for different times, with and without stirring.

Treatment variant	Treatment conditions	Biomass (g/l)	Substrate consumption (%)	Yield*		Material loss (%)
				Y_1	Y_2	
11	1 h, with stirring	2.5	65	0.24	0.36	20
12	2 h, with stirring	2.9	66	0.25	0.38	20
13	4 h, with stirring	3.2	71	0.27	0.36	17
14	24 h, with stirring	3.4	71	0.29	0.37	31
15	1 h, without stirring	2.8	65	0.25	0.37	12
16	2 h, without stirring	3.0	65	0.28	0.39	12
17	4 h, without stirring	3.3	71	0.30	0.39	12
18	24 h, without stirring	3.4	72	0.29	0.38	20

* Y_1—g biomass/g fed pith: Y_2—g biomass/g consumed pith.

results obtained with the 4-h treatments were similar (according to a Student's *t*-test with 95% reliability) to those from variant 9 (80°C, 1 h) and to the control, from the 3 × 3 plan (Table 1). Molina *et al.* (1984) also reported good growth of a mixed bacterial culture on bagasse pith treated at room temperature with a NaOH/pith ratio of 10%, but their optimum treatment time was 24 h.

Treatments without stirring were equally efficient as the stirred ones. Therefore, variant 17 appears an attractive procedure with low material losses, no heating requirements and good biodegradability.

In further experiments, when the NaOH concentration for treatments at room temperature for 4 h was reduced to 0.2 M, a decrease in the biodegradability of the material was observed (data not shown).

Conclusions

The best treatment conditions for good bacterial growth correspond to variants 9 and 17. They constitute simpler and much cheaper procedures than the traditional treatment (variant 10; Dunlap 1969) previously employed in studies with this strain (Enríquez 1978; Rodríguez *et al.* 1983), comparing well with results reported for other bacteria grown on treated bagasse pith (Molina *et al.* 1984).

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