Optimization of Culture Medium Composition for Cellulolytic Bacteria by Mathematical Methods

H. RODRIGUEZA, A. ENRIQUEZB and O. VOLFOVÁB

a Department of Industrial Microbiology, National Center of Scientific Research, Apartado 6990, Havana, Cuba
b Institute of Microbiology, Czechoslovak Academy of Sciences, 142 20 Prague 4

Received July 13, 1982

ABSTRACT. The culture medium composition for cellulolytic bacteria growing on sugar cane wastes was optimized. A modified method of Rosenbrock was employed for shaker culture medium and a factorial plan design for fermenter culture medium optimization. A much more economical and productive medium was obtained for the production of single cell protein (SCP). A biomass concentration of 4.3 g/L was obtained in the optimized medium in batch fermentation, in comparison with 2.8 g/L previously obtained in the traditional medium under similar conditions.

One of the most important aspects of growing cellulolytic bacteria on cellulotic wastes for obtaining SCP is the formulation of the appropriate culture medium to be employed in the industrial process.

The analysis of the medium employed so far in our laboratories for this process shows an excess of phosphorus and potassium and a slight lack of nitrogen (Enriquez 1978). Besides that, other components have been reported to be important for the growth of cellulolytic bacteria, such as CaCl₂ and mineral trace elements (Srinivassan 1975; Schmid and Bomar 1975a, b).

Mathematical methods were recently employed for the optimization of microbiological processes, among them, that of Box and Wilson (1951) and Auden et al. (1967) and, especially in the optimization of culture media, the modified method of Rosenbrock (Votruba et al. 1975; Pilát et al. 1976a, b). The aim of our present work was to establish whether it was possible to apply modified Rosenbrock's method for the optimization of culture medium and to obtain a new medium composition more convenient for the industrial production of SCP from bagasse.

MATERIALS AND METHODS

Microorganisms. Cellulomonas sp. IIbc and Gill (Enriquez 1978; Rodriguez et al. 1982) were transferred in CMC-agar tubes at 30 °C and maintained at 4 °C.

Medium. We used traditional medium M9 (Miller 1972) with the following composition (g/L): Na₂HPO₄·12H₂O 15, KH₂PO₄ 3, NaCl 0.5, MgSO₄·7H₂O
0.25, NH₄Cl 1, thiamine 0.001, distilled water to 1 L. Pretreated bagasse (Dunlap 1969) was used at 1 % concentration. CMC-agar: 0.5 % carboxymethyl cellulose and 2 % agar were used in medium M₉.

Trace mineral solution (Schmid and Bomar 1975) had the following composition (mg/L): FeCl₃.6H₂O 10, CuSO₄·5H₂O 0.5, MnCl₂·4H₂O 0.45, CoCl₂·6H₂O 0.03, Na₂MoO₄·2H₂O 0.03, ZnSO₄·7H₂O 0.5.

Cultivation in flasks. The culture was grown on CMC-agar slopes at 30 °C for 2 d. The cells were then suspended in a sterile way and inoculated in 80 mL of medium with 1 % pretreated bagasse in 500-mL cultivation flasks. The cultivation was carried out on a reciprocal shaker at 30 °C.

Cultivation in laboratory fermentor. A Biolafitte glass fermentor was used under optimum cultivation parameters (Enriquez 1981): culture volume of 5 L, temperature 32 °C, pH 6.5, airflow 10 L/min and impeller frequency 10 Hz. The pH level was automatically regulated by adding 10 % NaOH solution. The medium was inoculated with 10 % (V/V) vegetative inoculum.

Vegetative inoculum. This was prepared in the fermentor. In the exponential growth phase the appropriate part of the culture was centrifuged and the biomass was resuspended in the same part of sterile medium.

Analyses. Growth was followed turbidimetrically at 600 nm after filtration of samples through a sintered glass filter (pore diameter 90-150 µm). This eliminates most of the residual bagasse.

During the cultivation the dry mass of the bacterial biomass was determined gravimetrically after filtering the sintered glass filtrates through a Synpor No. 6 membrane, washing and drying the cells at 105 °C to constant mass. The protein content of bacteria was determined by the biuret method (Herbert et al. 1971).

Mathematical methods of optimization. Rosenbrock's method. The modified method of Rosenbrock and Storey (1970) was employed for the optimization of the medium for cultivation in flasks. A detailed explanation of the method is given by Pilát et al. (1976a). The method is based on the evaluation of an optimization criterion in successive experimental runs, where each variable takes the value

\[ X = X_0 + k_1 \vec{v}_1, \]

where \( X \) is experimental value for the variable, \( X_0 \) start point, \( k_1 \) step length and \( \vec{v}_1 \) orthogonal unit vector.

In our case the optimization criterion was the final biomass concentration obtained.

Each new experiment is designed according to the results of the previous one and following equation (1). When increasing the nutrient concentration „success” is defined by the condition:

\[ F(X) > F(X_0). \]

When decreasing the concentration „success” is given by

\[ F(X) \geq F(X_0) \]