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Determination of the energy maintenance coefficient of *Zymomonas mobilis*

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SUMMARY

Continuous culture experiments with *Zymomonas mobilis* Z-1-81, indicated the existence of linearity between specific substrate uptake rates and dilution rates. The value of the energy maintenance coefficient was estimated at 2,521 g/g.h. The percentage of carbon used for maintenance energy was shown to increase with the decrease in the dilution rate.

INTRODUCTION:

It is well known that microorganisms need energy for growth and for maintenance functions, such as: transporting cell material, osmotic activity and cell mobility. Energy maintenance has as much relevance to basic kinetic studies as it has to the development of a fermentation process. In continuous fermentation systems the energy consumed by cells for maintenance varies according to the dilution rate. At low dilution rates, that is, lower than 25% of the critical dilution rate, a significant proportion of the carbon and energy source is used for maintenance. If, however, the cells are growing at their maximum dilution rate, the carbon uptake used for maintenance represents only 5-10% of the total demand (10).

Traditionally, the evaluation of the energy maintenance coefficient (**m**) has been based upon data obtained from non-cell recycling homogeneous chemostats. This is possible when linearity exists between the specific substrate uptake rate (q_s) and the dilution rate (**D**), bearing in mind that, in this particular case, these parameters are inter-related by the equation: $q_s = D/Y_G + m$. Therefore, the energy maintenance coefficient is determined by the extrapolation of the specific substrate rate to a zero dilution rate.

MATERIALS & METHODS:

Organisms: *Zymomonas mobilis* Z-1-81 was kindly provided by the Department of Antibiotics of the Federal University of Pernambuco - Brasil. **Growth & Fermentation media:** The Schereder-modified medium was used for inoculum propagation and fermentation experiments. Its composition was as follows: commercial sugar, 100 g/L; yeast extract, 5.0 g/L; K_2HPO_4, 1.5 g/L; $MgSO_4$. 7 H_2O, 0.5 g/L. The pH was adjusted at 6.0 +/- 0.1 and the media were sterilized at 120 ^oC/15 min. **Analyses & Experimental Procedures:** Cell concentration was determined by dry-weight measurements at 90 ^oC/24 hrs. Reduced total sugars were determined by the colourimetric assay of the 3,5 dinitrossalicylic acid (2), after hydrolysis of sucrose (HCl 2N - 65 ^oC/10 min). The specific substrate uptake rates were evaluated according to the following expression: $q_s = D\,(S_i - S)/X$; where S_i is the substrate concentration in the feeding medium, S and X are the substrate and cell concentration in the effluent, respectively. The fermentation experiments were carried out in a 14 L - CSTR/New Brunswick fermenter, model FS-314, adjusted in order to be operated continuously. The fermenter working volume was 10 L, and the feeding and discharging operations were done by two pumps (Sigma motor - model T-6S). The working volume was kept constant through a U-shaped tube, which was connected to the discharge pump. The continuous operation was initiated after a batch fermentation in its final exponential growth phase, and the dilution rates varied from 0.048 to 0.206 h^{-1}. Steady state was achieved when the bacterial culture had undergone 4 - 5 residence times. Once steady state was established, the system was allowed to operate for a further 24 hours in order to observe its stability. Thereafter the dilution rate was changed so as to reach another steady state. During transient and steady state conditions, samples were collected from the effluent at intervals of 2 or 3 hours for analyses of cell and substrate concentrations.

RESULTS & DISCUSSION:

Figure 1 shows the data for the specific substrate uptake rate for each dilution rate in its respective steady-state. It can be seen, within the limits of dilution rate variation, that there exists a linear correlation between q_s and D, which allowed us to determine the energy maintenance coefficient by linear regression. A similar trend was observed by several researchers (1,5,6,8), who also verified minor variations between those parameters when glucose or sucrose were used, or even when the concentrations of these substrates were varied. The determined value of 2.521 g/g.h is greater than the ones obtained by other reseachers using media containing glucose in the same concentration as the one used in this work, but with different strains of *Zymomonas mobilis*. Lee *et al* (4), working with *Zymomonas mobilis* ATCC 10988 obtained 1.6 g/g.h. However, when the concentration of glucose increased to 150 g/L the energy maintenance coefficient was 3.1 g/g.h. Beyeler *et al* (1), using *Zymomonas*

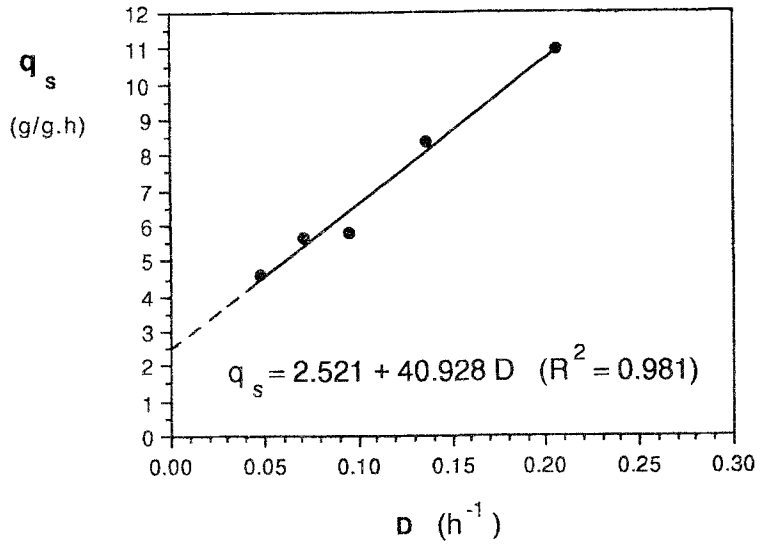

Fig. 1: Graphical determination of the energy maintenance coefficient (m) of *Zymomonas mobilis* Z-1-81.
q_s = specific substrate uptake rate, g/g.h
D = dilution rate, h^{-1}

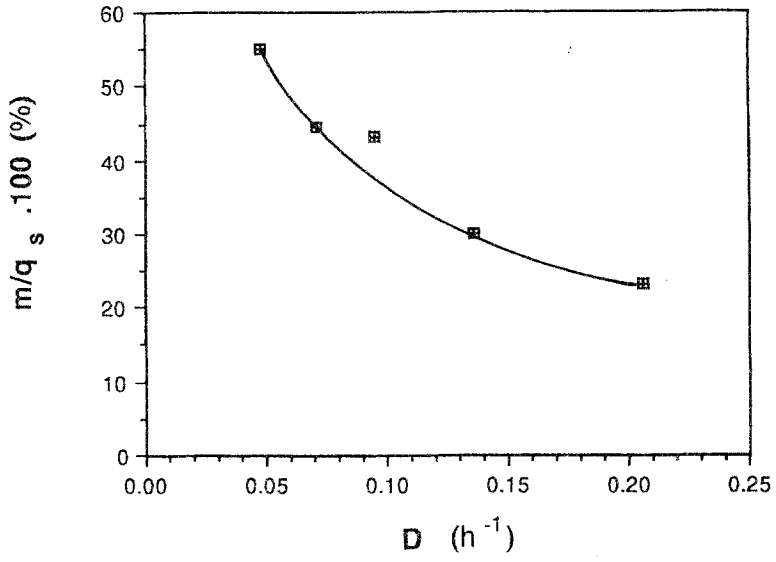

Fig. 2: Effect of the dilution rate [D] on the percentage of carbon used for energy maintenance [m/q_s . 100 (%)]. *Zymomonas mobilis* Z-1-81.

mobilis ATCC 31821, obtained 1.46 g/g.h, employing the same technique adopted in the present work, and 1.59 g/g.h, working with cell recycling. The graphical correlation between the ratio m/q_s and D under steady-state conditions is displayed in Figure 2. It also corroborates other researchers' findings that the carbon consumption for energy maintenance is significantly high, when the system is operated at low dilution rates. It can be observed that at D = 0.05 h^{-1} more than half of the substrate (55.2%) used by cells is for energy maintenance, while at the highest dilution rate (D = 0.206 h^{-1}) it is only 23.1%. Lee *et al* (4) also reported similar results, working with *Zymomonas mobilis* ATCC 10988 in a medium containing glucose in a concentration of 100 g/L. However, with 150 g/L of glucose, the ratio m/qs was significantly higher, which indicated an increase in carbon consumption for energy maintenance. This was explained to be due to the increase in osmotic pressure and ethanol concentration in the medium.

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