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RELATIONSHIP BETWEEN MAINTENANCE ENERGY REQUIREMENT, MINERAL SALTS AND EFFICIENCY OF GLUCOSE TO ETHANOL CONVERSION BY ZYMOMONAS MOBILIS

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SUMMARY

Minimizing the usage of glucose carbon for growth and cell maintenance energy requirement, specific glucose uptake rates, specific ethanol production rates were increased 5-fold. At 0.2 hr^{-1} and Y_g = 0.007-0.009, ethanol production rates of 7.99 - 8.46 g/ltr/hr, Q_p values of 14.85 g/g/hr were obtained. This relationship is discussed in regard to glucose fermentation efficiency.

INTRODUCTION

The efficiency of ethanol production depends on the ability of the microorganism to fully utilize its carbon source or raw material into the desired product. This means that as little as possible of the carbon and energy source should be diverted into growth and cell maintenance (Pirt, 1975). It is known that growth of Zymomonas mobilis can be restricted owing to its growth factor requirement (Swings and De Ley, 1977) and that high amounts of yeast extract increase cell yields but not necessarily ethanol productivity (Cromie and Doelle, 1980, submitted for publication). Since maintenance energy requirements depend very much on the nutrient supply (Tempest, 1978; Doelle and Hollywood, 1978), Zymomonas mobilis is an ideal microorganism for studying metabolic activity. Zymomonas mobilis obtains only 1 mole ATP/mole glucose owing to its glucose utilization via the Entner-Doudoroff pathway and the aim should therefore be to minimize growth and cell maintenance energy requirement in an effort to channel as much glucose carbon as possible towards ethanol production.

Previous reports have claimed (Lee et al., 1979) that this microorganism can efficiently convert glucose concentrations up to 25 per cent. However, the results obtained from batch (Rogers et al., 1979) and continuous

cultures (Lee et al., 1979) clearly demonstrated that at a dilution rate of 0.2 hr^{-1}, only 80% of the 10% glucose were converted to the product ethanol with the rest, ~20%, left in the fermenter vessel. The high maintenance energy coefficients, high yields together with the increasing specific growth rate certainly indicate unfavourable nutrient conditions for the catabolic activity of the organism.

In the presented investigations, continuous culture experiments were carried out at different dilution rates, different inorganic nitrogen and magnesium concentrations in a 10% glucose system with a 0.25% yeast extract in an effort to study the relationship between maintenance energy requirement, cell yield, glucose uptake and utilization rate and ethanol productivity.

MATERIALS AND METHODS

The organism used was Zymomonas mobilis Z 10, a laboratory strain developed from Zymomonas mobilis UQM 410 (ATCC 10988). The strain was maintained on a slope containing 0.1% K_2HPO_4, 0.1% $(NH_4)_2SO_4$; 0.05% $MgSO_4 \cdot 7\ H_2O$; 0.05% yeast extract and 10% glucose at pH 7.0.

Continuous culture experiments were carried out in a non-aerated 3 ltr fermenter (Reichelt and Doelle, 1971) controlled at pH 5.0 and 30°C with gentle stirring. Mineral salts-yeast extract (0.25%) media were sterilized separately from the glucose and aseptically mixed.

Biomass was determined by measuring the absorption at 540 nm and the corresponding dry weight was obtained from a standard curve. Glucose was determined using a YSI Model 27 Industrial Analyzer and ethanol using gas chromatography (Shimadzu Model GC-4A), Poropak Q as column material operated isothermally at 160°C, detector temperature at 250°C, injection port temperature at 150°C, nitrogen as carrier gas (75 ml/min) and flame ionization.

All kinetic parameters were determined as described by Lee et al. (1979).

RESULTS

Kinetic data for the different steady states of a 10% glucose system are presented in table 1. In varying not only the dilution rate but also the ammonium sulfate (nitrogen source) and magnesium sulfate concentrations, the maintenance energy coefficient, m was successfully reduced to zero at all dilution rates (0.05 to 0.2 hr^{-1}) with 0.45% magnesium sulfate and 0.05 or 0.1% $(NH_4)_2SO_4$. Further increases in the nitrogen source resulted

Table 1: Kinetic parameters of continuous culture experiments with Zymomonas mobilis Z 10

N	Mg	D	S_1	Eth	GUR	Q_g	Y_g	EPR	Q_p	Y_p	m
0.0	0.15	0.05	9.9	35.4	4.5	6.9	0.007	1.8	2.7	78.6	5.9
		0.1	39.1	26.0	6.1	8.0	0.012	2.6	3.4	85.4	
		0.2	63.3	10.3	7.3	18.7	0.011	2.1	5.3	56.4	
	0.30	0.05	0.3	43.7	4.9	5.2	0.010	2.2	2.3	87.7	2.9
		0.1	41.7	28.2	5.8	7.5	0.013	2.8	3.6	96.8	
		0.2	54.9	17.6	9.0	15.8	0.013	3.5	6.2	78.3	
	0.45	0.05	2.8	43.0	4.8	5.6	0.009	2.1	2.5	88.5	3.2
		0.1	33.2	29.5	6.7	7.9	0.013	2.9	3.5	88.5	
		0.2	61.9	19.3	7.6	14.4	0.014	3.6	6.9	96.5	
0.05	0.15	0.05	8.2	40.1	4.6	7.9	0.006	2.0	3.4	87.4	3.9
		0.1	24.6	35.1	7.5	12.2	0.008	3.5	5.6	93.0	
		0.2	57.3	21.4	8.5	16.4	0.012	4.3	8.2	100.0	
	0.30	0.05	5.2	47.4	4.7	8.1	0.006	2.4	4.1	100.0	5.8
		0.1	13.5	43.3	8.6	9.9	0.010	4.3	4.9	100.0	
		0.2	58.5	20.8	8.3	20.2	0.010	4.1	10.1	100.0	
	0.45	0.05	2.7	48.6	4.9	5.7	0.009	2.4	2.8	100.0	0.0
		0.1	13.7	42.1	8.6	11.8	0.009	4.2	5.7	97.5	
		0.2	55.1	21.7	9.0	19.5	0.010	4.3	9.4	96.6	
0.10	0.15	0.05	0.3	42.3	4.9	9.4	0.005	2.1	3.9	84.8	1.8
		0.1	8.9	45.5	9.1	16.9	0.006	4.5	8.4	100.0	
		0.2	44.4	27.8	11.1	22.2	0.009	5.6	11.1	100.0	
	0.30	0.05	4.1	47.2	4.8	8.7	0.006	2.3	4.3	98.4	1.0
		0.1	19.2	38.4	8.0	16.7	0.006	3.8	7.9	95.1	
		0.2	31.6	34.2	13.7	26.0	0.008	6.8	13.0	100.0	
	0.45	0.05	0.8	46.3	4.9	6.4	0.008	2.3	3.0	93.3	0.0
		0.1	6.3	42.6	9.4	14.4	0.007	4.2	6.5	90.9	
		0.2	34.2	32.9	13.1	29.7	0.007	6.6	14.8	100.0	
0.20	0.30	0.1	4.2	43.3	9.5	12.9	0.008	4.3	5.8	90.4	3.0
		0.2	29.2	35.4	14.1	22.8	0.009	7.1	11.4	100.0	
	0.45	0.1	5.3	47.3	9.5	14.5	0.007	4.7	7.3	100.0	5.4
		0.2	20.1	39.9	15.9	24.0	0.008	8.0	12.0	100.0	
	0.60	0.2	15.4	42.3	16.9	23.2	0.009	8.4	11.6	100.0	

Abbreviations: N - $(NH_4)_2SO_4$ (%); Mg - $MgSO_4$ (%); D - dilution rate (hr^{-1}); S_1 - non-utilized glucose (g/ltr); Eth. - ethanol (g/ltr); GUR - glucose uptake rate (g/ltr/hr); Q_g - specific glucose uptake rate (g/g/hr); EPR - ethanol production rate (g/ltr/hr); Q_p - specific ethanol production rate (g/g/hr); Y_g - cell yield (g/g); Y_p - ethanol yield (%); m - maintenance coefficient (g/g/hr).

in significant increases of the maintenance energy coefficient. In Fig. 1 the effect of the nitrogen and magnesium source on the proportion of glucose used for cell maintenance compared to the total specific glucose uptake rate at a specific growth rate of 0.2 hr^{-1} are demonstrated. These results exhibit the importance of the mineral salt concentration on the energy requirement of the cell. Under conditions of m = 0 it should be expected that the efficiency of glucose to ethanol conversion would increase. Figure 2 represents the effect of magnesium sulfate on various kinetic parameters at a specific growth rate of 0.2 hr^{-1} and 0.1% ammonium sulphate. The values for Q_g and Q_p are increasing and consequently the residual glucose decreases rapidly between 0.15 and 0.3% magnesium sulfate, and increases again slightly towards 0.45 per cent. Consequently, the ethanol concentration increases up to 0.3% and also decreases slightly towards 0.45 per cent magnesium sulphate. If, however, one selects the 0.45% magnesium sulfate concentration, where the maintenance energy coefficient is zero, and compares the trend in relation to increasing ammonium sulfate concentrations (Fig. 3), it can be demonstrated that the metabolic activity of the microorganism is not as yet at its maximum. Despite the observation that no glucose is required for cell maintenance (table 1), residual glucose rapidly decreases and ethanol production increases. An interesting observation is the drop in Q_g and Q_p values, but increase in the glucose uptake rate (g/ltr/hr), which explains the increase in ethanol concentration. The drop in Q_g and Q_p did not affect the ethanol production rate, which was 7.99 g/l/hr at 0.2% ammonium sulfate and 0.45% magnesium sulfate.

If the magnesium sulfate concentration was further increased to 0.6%, the conversion was even better with only 15.4 g/ltr glucose left and the ethanol production rate increasing to 8.46 g/ltr/hr. The values for Q_g and Q_p further decreased, but ethanol concentration further increased to 42.29 g/ltr and the glucose uptake rate to 16.91 g/ltr. Apart from those experiments without ammonium sulfate, most of the conversions were between 95 and 100% of the glucose utilized. Y_g values varied only slightly between 0.005 and 0.009 at the higher ammonium sulfate concentrations.

Further increases in ammonium sulfate and magnesium sulfate concentrations led to reductions in all kinetic parameters. An increase in specific growth rate to 0.3 hr^{-1} at 0.2% ammonium sulfate and 0.45% magnesium sulfate resulted in an increase in non-utilized glucose, glucose uptake

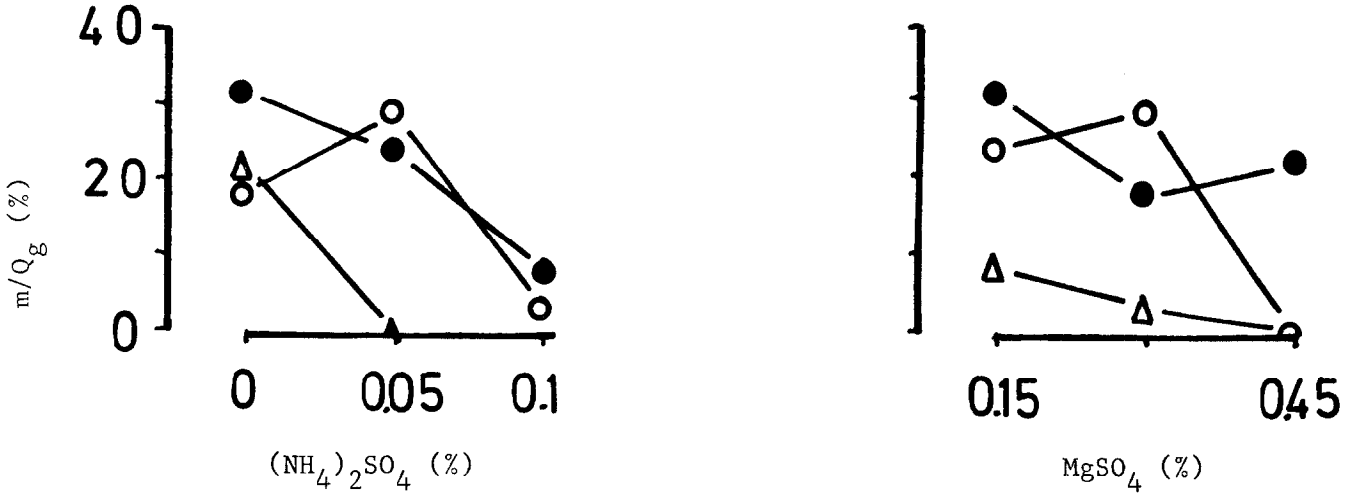

Fig. 1: The effect of the inorganic nitrogen and magnesium source on the ratio of maintenance energy coefficient to glucose uptake rate.
A: ●, 0.15%; o, 0.3%; △, 0.45% $MgSO_4$; B: ●, 0%; o, 0.05%; ▲, 0.1% $(NH_4)_2SO_4$

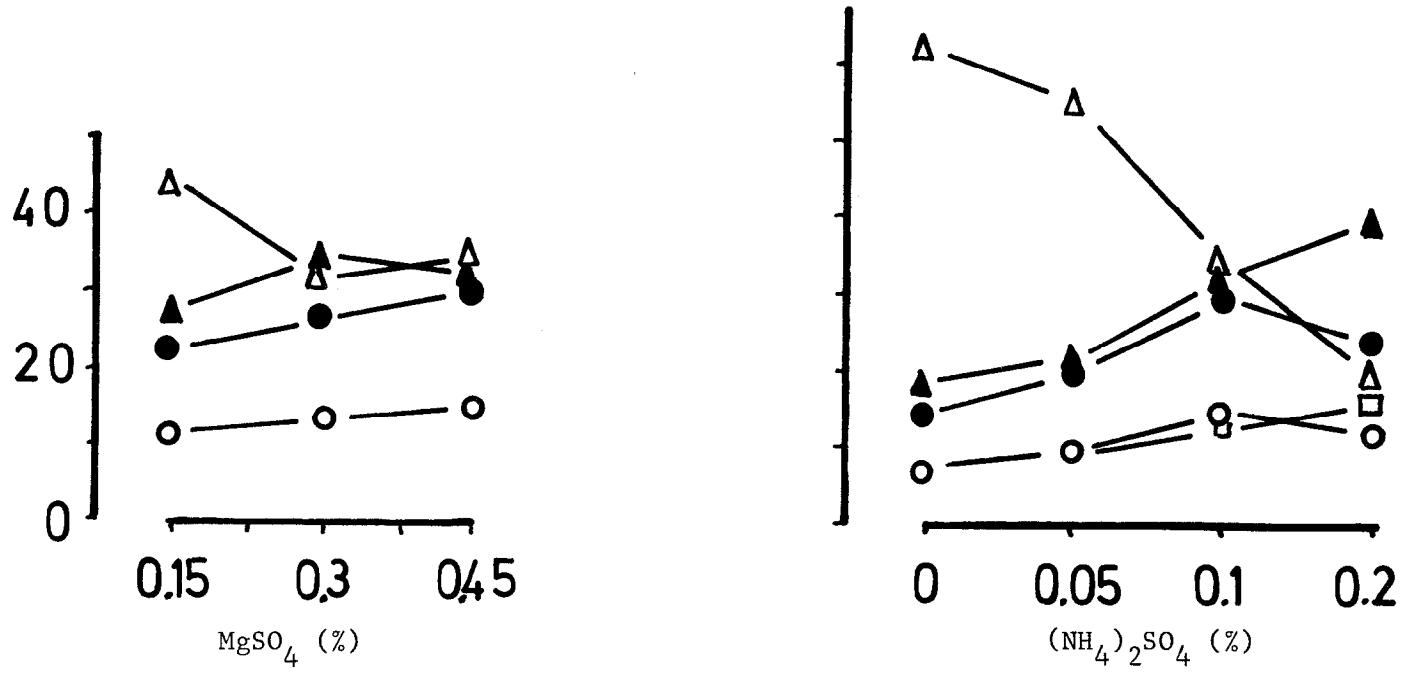

Fig. 2: The effect of increasing magnesium sulfate concentrations on different kinetic parameters at μ=0.2 hr^{-1} and 0.1% $(NH_4)_2SO_4$. △, S_1(g/ltr); ▲, ethanol (g/ltr); ●, Q_g; o, Q_p (g/g/hr); ■, glucose uptake rate (g/hr)

Fig. 3: The effect of increasing ammonium sulfate concentrations on different kinetic parameters at μ=0.2 hr^{-1} and 0.45 % $MgSO_4$. Legends as in Fig. 2.

rate (19.46 g/ltr), whereas ethanol production rate stayed constant at 8.26 g/ltr/hr and the ethanol concentration (g/ltr) decreased. The conversion of glucose to ethanol stayed at 97.8 per cent.

DISCUSSION

The data presented demonstrate, that a change in nutrient supply leads to a minimal cell maintenance energy requirement and that growth can be restrained by yeast extract. Under the conditions of no glucose requirement for cell maintenance and a biomass yield, Y_g, of 0.007-0.009, specific glucose uptake rates increased to 29.69 g/g/hr, almost 5-times higher than those reported by Lee et al. (1979). A corresponding increase was obtained with the specific ethanol production rate, Q_p. Although the ethanol yield deviated only slightly from 0.50, at a dilution rate of 0.2 hr^{-1}, this value always demonstrated 100 % conversion. Despite the increases in Q_g and Q_p and the ethanol yield of 0.50, it was still not possible to reduce the residual glucose below 15.4 g/ltr at μ = 0.2 hr^{-1} and the highest ethanol production rate of 7.99-8.46 g/ltr/hr was similar to those reported earlier (Lee et al. 1979). In minimizing growth and cell maintenance energy requirements it was therefore possible to obtain a four-fold increase in ethanol production and an almost 5-fold increase in specific glucose uptake and specific ethanol production rate. In order to improve the efficiency of complete glucose conversion further, studies are required into the metabolic activity and the optimal conditions of catabolic enzyme catalysis.

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