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GLYCEROL PRODUCTION FROM GLUCOSE IN ALKALINE MEDIUM

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ABSTRACT

Glycerol production from glucose using an osmophilic yeast *Pichia farinosa* (ATCC 20210) in an alkaline medium has been investigated in shake flasks. The amount, form and mode of sodium carbonate addition have been found to affect the yields of glycerol, ethanol and biomass. These effects are explained in terms of the critical parameters of pH and dissolved oxygen levels in the medium. Relatively high glycerol yields and concentrations coupled with rapid fermentations have been obtained.

INTRODUCTION

Glycerol production by yeasts has been studied for many decades. Neuberg first reported that relatively high glycerol yields can be obtained in a modified yeast fermentation of hexoses in the presence of sulfites (Prescott and Dunn, 1949). Eoff *et al* (1919) found that glycerol production can also be stimulated in the presence of alkaline agents such as hydroxides and carbonates of sodium and potassium with non-osmophilic yeasts. This method is referred to as an 'alkaline fermentation process'. The yield of glycerol was influenced by the alkalinity of the fermenting solutions and with optimum concentration of alkali they could get maximum glycerol yields in the range of 20-25% based on sugar. Onishi (1960) found that osmophilic yeasts allowed high substrate concentrations to be used and gave good glycerol yields but the fermentations were slow. Our studies have shown that higher glycerol yields are obtained when osmophilic yeasts are used in alkaline conditions. This paper reports on the effect of the dosage and the mode of addition of sodium carbonate on glycerol, ethanol and biomass yields in shake flask experiments.

MATERIALS AND METHODS

Culture: The yeast culture *Pichia farinosa* (ATCC 20210) obtained from American Type Culture Collection, USA, was maintained on MGYP-Agar slants.

Inoculum preparation : A loopful of culture from the stock agar slant was transferred to a boiling tube containing 10ml of MGYP media and grown for 24h on a rotary shaker at 180 rpm. This was then transferred to a 500ml shake flask containing 90ml of MGYP media and was grown on the shaker for 24h, and then used for the inoculum after centrifugation.

Fermentation: The fermentation medium consisted of the following in g/l - glucose, 300; Yeast extract, 1.0; Urea, 0.5; $MgSO_4.7H_2O$, 0.5; KH_2PO_4, 1.0 and $CaCl_2.2H_2O$, 0.1. Wet cells corresponding to 0.5g dry cell weight (DCW) were suspended in 5ml of the medium and inoculated into 500ml shake flasks containing 80ml of the above medium and incubated at 30°C on a rotary shaker at 180 rpm.

Analytical procedure : Glucose concentration was measured with glucose oxidase using a glucose analyser (Yellow Springs Instruments, USA). Glycerol concentration was measured by the method of Neish (1952). Ethanol concentration was measured by butanol extraction followed by gas chromatography as described by Varma et al (1983). The cell concentration was measured by the absorbance of the broth at 660nm in a UV-visible spectrophotometer (Shimadzu model 240).

RESULTS AND DISCUSSION

Mode of addition of sodium carbonate: Eoff et al (1919) recommended the addition of alkali in five unequal portions, as the addition of all the alkali at once drastically inhibits the fermentation. We investigated the addition of sodium carbonate (AR grade, Glaxo make) according to a number of policies involving the addition of equal or unequal portions at equal or unequal intervals of time in solution as well as in solid form. The results in terms of the yields of different products are summarized in Table 1. It was found that the addition of solid sodium carbonate (fine powder form) in five unequal portions (15.0%, 15.0%, 27.5%, 27.5% and 15.0% of total 30 g/l in the medium) at unequal time intervals (4,7,9,12 and 24h after the start of fermentation) was best, giving good glycerol yields of 38% in 96-120 hrs. Corresponding ethanol and biomass yields were 8% and 1.03% respectively. Solid carbonate added in five equal portions at 2h intervals or in ten equal portions at 1h intervals gave somewhat lower glycerol yields. However, when the carbonate was added in a solution form with the same addition policies as the solid form, significantly lower glycerol and higher biomass and ethanol yields were always obtained(table 1). A similar observation was made by Eoff et al (1919). This effect is linked to pH changes and cell growth effects. When the solid sodium carbonate was added (portionwise, but irrespective of the precise pattern of the additions) the pH rose initially, from 4.8 to 8.0 over 24h, and then fell slowly to pH 6.8 to 7.5; under these conditions glucose was consumed steadily at about 2.5g/lh over 120h. When the same additions were made using the sodium carbonate solution of concentration of 30g/l, the initial pH rise was followed by a faster fall, to pH 5.0-5.5, accompanied by a more rapid consumption of glucose at about 3.1g/lh over 96h. Thus there is a faster respiration which may well demand more oxygen than can be provided on the shaker and under such oxygen-starved conditions the metabolic pathway shifts more towards ethanol formation. This explanation is supported by the observations of Spencer et al(1968) who reported that the presence of ethanol was always associated with decreased glycerol yields; that this condition was brought about by sub-optimal aeration levels and that the optimum aeration in turn varies with the extent of cell growth. A clearer proof of this explanation will need experiments in an instrumented fermentor with monitoring and control of pH and dissolved oxygen.

Table 1

Effect of mode of addition of sodium carbonate

on the glycerol, ethanol and biomass yields

Initial glucose concentration - 300 g/l; total carbonate added 30g/l

Form and mode of sodium carbonate addition	Interval of addition after start of fermentation	Yield Glycerol %	Yield Ethanol %	Yield Biomass(DCW) %
Solid, 5 equal portions	2h	33	8	1.0
Solution, 5 equal portions	2h	26	14	1.6
Solid, 5 unequal portions	4,7,9,12 and 24h	38	8	1.1
Solution, 5 unequal portions	4,7,9,12 and 24h	28	14	1.6
Solid, 10 equal portions	1h	31	10	1.4
Solution, 10 equal portions	1h	25	18	1.8

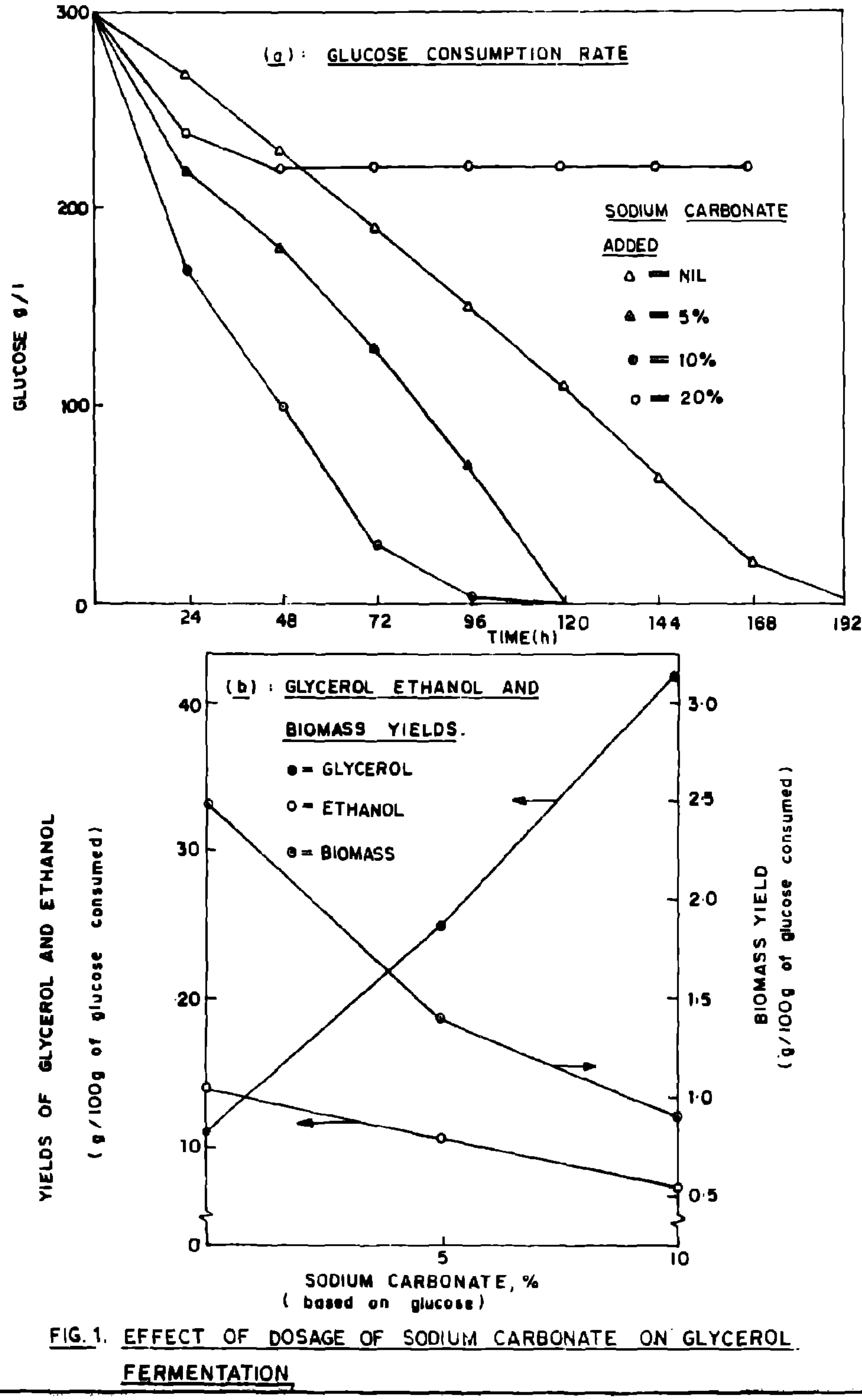

FIG. 1. EFFECT OF DOSAGE OF SODIUM CARBONATE ON GLYCEROL FERMENTATION

Dosage of sodium carbonate: The effect of the total carbonate added during the course of the whole fermentation was investigated by varying its concentration in the fermenting media from 0 to 20% based on glucose and the results are shown in fig.1. The carbonate was added in solid form at unequal intervals of time according to the policy determined earlier. It was observed that the glycerol yield was directly proportional to the amount of carbonate added upto a concentration of about 10% beyond which the fermentation was severely inhibited. As seen in fig.1 the glycerol yield increased from 11.3 to 42% as the carbonate dose increased from 0 to 10%. Corresponding ethanol and biomass yields decreased from 14 to 7.2% and 2.5 to 0.9% respectively. However, carbonate at a level of 20% gave very poor glucose consumption rates, perhaps due to high alkalinity (pH 10 and above) of the fermenting medium. From our studies it was found that for a 30% glucose medium a concentration of 10% carbonate based on glucose was the best.

CONCLUSIONS

Although alkaline fermentation has been reported to give good glycerol yields using non-osmophilic yeasts, it was not tried earlier with osmophilic yeasts. Our investigations have resulted in higher glycerol yields and concentrations coupled with rapid fermentations, using an osmophilic yeast *Pichia farinosa* in the presence of sodium carbonate. The mode of addition of the carbonate influenced the course of fermentation and yields. Higher glycerol yields were obtained when carbonate was added in solid rather than in solution form which is explainable in terms of pH and aeration effects.

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