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THE EFFECT OF YEAST EXTRACT ON THE FERMENTATION OF GLUCOSE TO 2,3-BUTANEDIOL BY BACILLUS POLYMYXA[+]

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Abstract

The fermentation of glucose to 2,3-butanediol by Bacillus polymyxa was improved by increasing the amount of yeast extract in the culture medium. A level of 1.5% (w/v) resulted in optimal 2,3-butanediol production. A comparable fermentation could be achieved with 0.5% yeast extract if the phosphate level of the medium was increased from 0.0026 to 0.078 M and the medium was supplemented with 40 µM iron and 1.7 µM manganese.

Introduction

The current instability of the petrochemical industry has generated a renewed interest in the microbiological production of 2,3-butanediol (diol), a potential chemical feedstock. Although the diol fermentation was studied extensively in the past using various organisms, including Bacillus polymyxa, it was never applied and many aspects of the process require clarification.

Yeast extract stimulates diol production by B. polymyxa from wheat (Fratkin and Adams, 1946; Ledingham et al., 1945), sugar beet molases (Simpson and Stranks, 1951), starch (Katznelson, 1946), xylose (Laube et al., 1984) and glucose (Groleau et al., 1984). However, the literature contains conflicting reports regarding the concentration of yeast extract required for good diol yields and concerning the components of yeast extract responsible for the stimulation. Thus, in an attempt to optimize the diol yields from glucose by this bacterium and to find an inorganic replacement for at least a portion of the yeast extract, the effects of yeast extract and some of its components on substrate utilization and product formation were examined.

Materials and Methods

Organism, medium and culture conditions: Bacillus polymyxa (NRCC 9035), the organism used in these experiments, was checked for purity and stored as previously outlined (Laube et al., 1984). Inocula were grown in 125 mL Erlenmeyer flasks containing 50 mL of the medium of Stanier and Adams (1944) without $CaCO_3$ and with yeast extract present in specified concentrations. Experimental cultures were grown similarly but with various supplementary nutrients. Glucose, prepared as a 50% filter-sterilized solution (pH 7.2), was added, before inoculation, to autoclaved medium to give a final sugar concentration of 5% (w/v). Cultures were incubated at 30°C with shaking at 125 rpm.

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Analytical methods: Aliquots of 1 mL were withdrawn from cultures at intervals during growth and the pH was measured. The aliquots were then centrifuged for 4 min. at 13 to 15,000 X g. The supernatant fluids were either stored at -20°C for future analysis or were analyzed immediately for 2,3-butanediol, acetoin and ethanol using gas chromatography according to the method of Ackman (1972) with 1,2-butanediol as internal standard and for glucose by the dinitrosalicylic acid method of Miller (1959) with corrections for acetoin (Murphy et al., 1951).

Results and Discussions

By increasing the concentration of yeast extract in the culture medium, diol yields were increased to optimal levels of 18 g/L with 1.3 and 1.5% yeast extract (Figure 1A). Also, with increased yeast extract levels, increased rates of glucose consumption were observed (Figure 1B). With such high levels of yeast extract required, it was of interest to replace yeast extract with known components, where possible, and to obtain comparable high yields of diol. Since yeast extract is an excellent source of nitrogen and other growth factors, an attempt was made to improve the low yields of diol and the slow rate of glucose utilization with medium containing 0.5% yeast extract (Figure 1) by adding extra nitrogen in the form of urea (1% and 0.5%, w/v), $(NH_4)_2SO_4$ (0.3% and 0.1%, w/v) or cysteine monohydrate (0.2%, w/v) or by adding a mixture of vitamins (Khan et al., 1979). These additions did not stimulate the fermentation. However, if a mixture of trace minerals known to be present in yeast extract (Grant and Pramer, 1962) was added to medium containing 0.5% yeast extract at levels found in 1.5% yeast extract, a significant improvement in the yields of diol and the rate of substrate consumption was observed (Figure 2A).

To determine which minerals from yeast extract were responsible for the stimulatory effect, they were divided into two groups, each of which was tested. The mixture containing Al, Co, Cu, Fe, Mg, Mn, Mo and Zn resulted in the stimulation of diol production and when this group was further divided, the mixture containing Mn and Mg gave the greatest stimulation. The mixture of Fe, Mo and Zn resulted in some stimulation whereas the Al, Co and Cu mixture was without effect. Some stimulation of diol production occurred if only Fe was added to the medium (Figure 2B), a result that agrees with the work of Roberts (1947) which showed that the addition of Fe resulted in faster glucose utilization and greater diol production. Increasing the level of Fe above 40 μM, the level originally present in the mineral mixture, did not result in further improvements of the fermentation. The principal stimulatory factor proved to be Mn (Figure 2B) with an optimal concentration of 1.7 μM. Although Mn has been shown to activate a number of enzymes, the stimulation demonstrated was probably linked to the Mn requirement of the enzyme acetolaetate decarboxylase in the pyruvate to diol pathway (Sokatch, 1969).

The effect of phosphate, another component of yeast extract, was also examined. Since Murphy et al. (1951) found that increasing the level of phosphate with molasses resulted in increased yields of diol, the effect of increased phosphate in medium containing 0.5% yeast extract was investigated. The pH profiles of the control cultures (0.5% and 1.5% yeast extract) were initially similar with the pH falling from 6.5 to 5.0 (Figure 3A). However, in cultures containing 1.5% yeast extract, the pH rose to 6.0 once the substrate was used. Such a rise after the completion of the active diol forming stage has been previously reported (Long and Patrick, 1963). In contrast, the pH did not fall as drastically with phosphate as with the control cultures and the pH did not rise after substrate utilization (Figure 3A). The addition of increasing amounts of K phosphate resulted in a progressive improvement in the rate of formation and the yield of diol

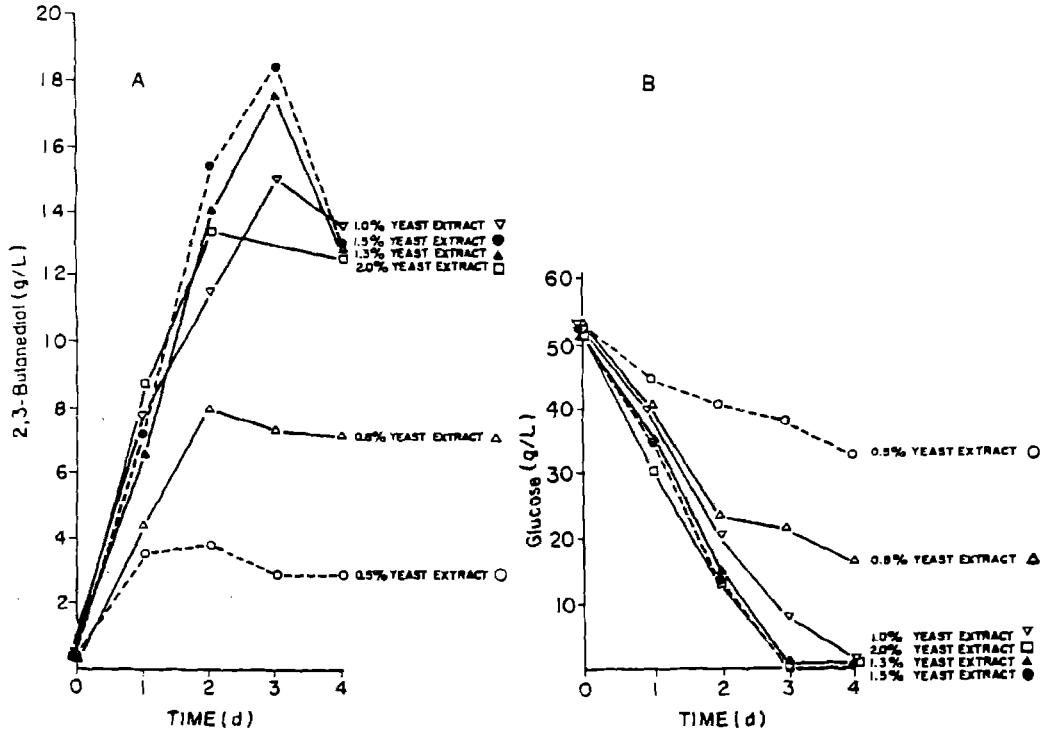

FIGURE 1: THE EFFECT OF YEAST EXTRACT ON (A) 2,3-BUTANEDIOL PRODUCTION AND (B) GLUCOSE UTILIZATION.

AL BA CD* CO CU CR FE MG MN MO NI PB TI V ZN

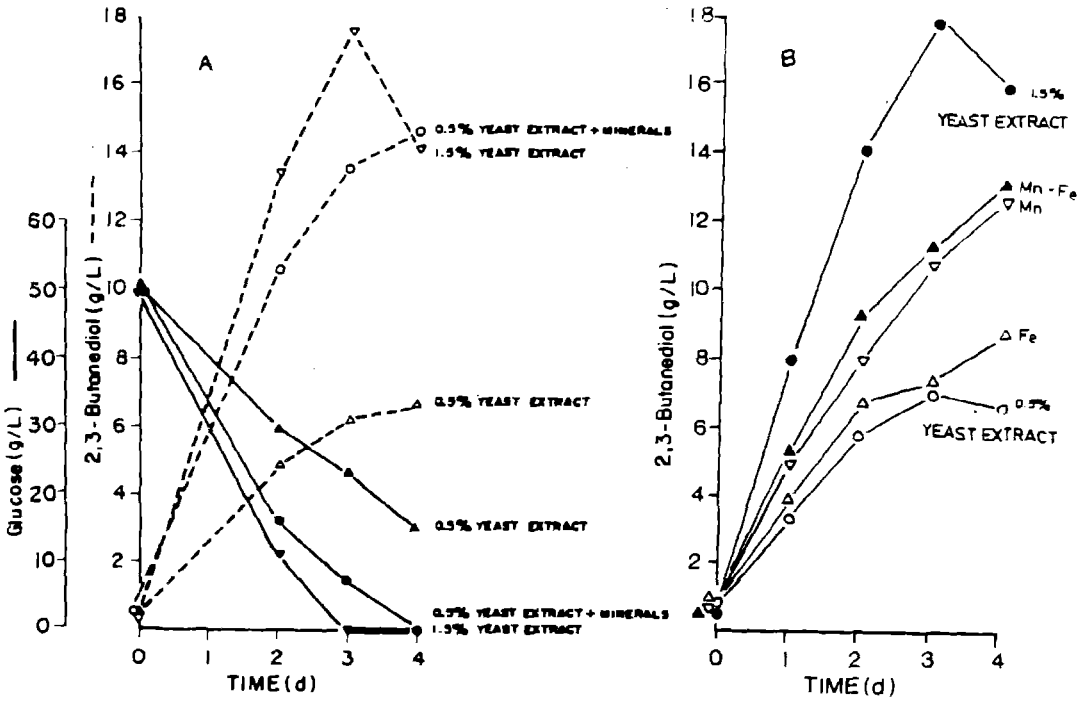

FIGURE 2: THE EFFECT OF (A) TRACE MINERALS ON 2,3-BUTANEDIOL PRODUCTION (---) AND GLUCOSE UTILIZATION (——) AND THE EFFECTS OF SPECIFIC TRACE MINERALS (B) ON THE PRODUCTION OF 2,3-BUTANEDIOL.

*CD WAS NOT INCLUDED IN SUBSEQUENT TESTS SINCE IT IS RARELY REQUIRED FOR GROWTH.

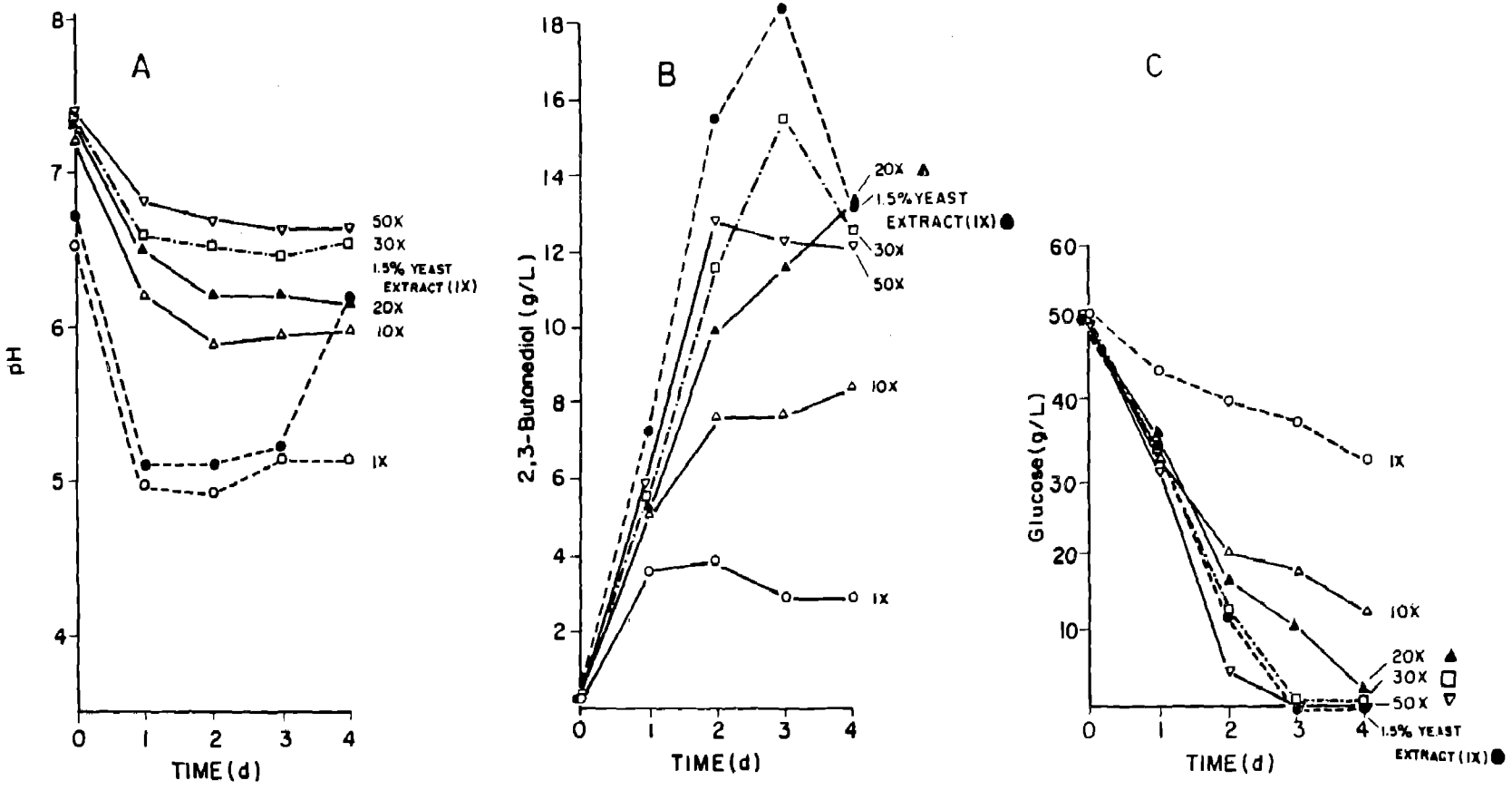

FIGURE 3: THE EFFECT OF K PHOSPHATE ON (A) PH, (B) 2,3-BUTANEDIOL PRODUCTION AND (C) GLUCOSE UTILIZATION.

(YEAST EXTRACT CONCENTRATION, WHERE NOT INDICATED =0.5% (W/V); STANDARD (1X) PHOSPHATE LEVEL =0.0026 M).

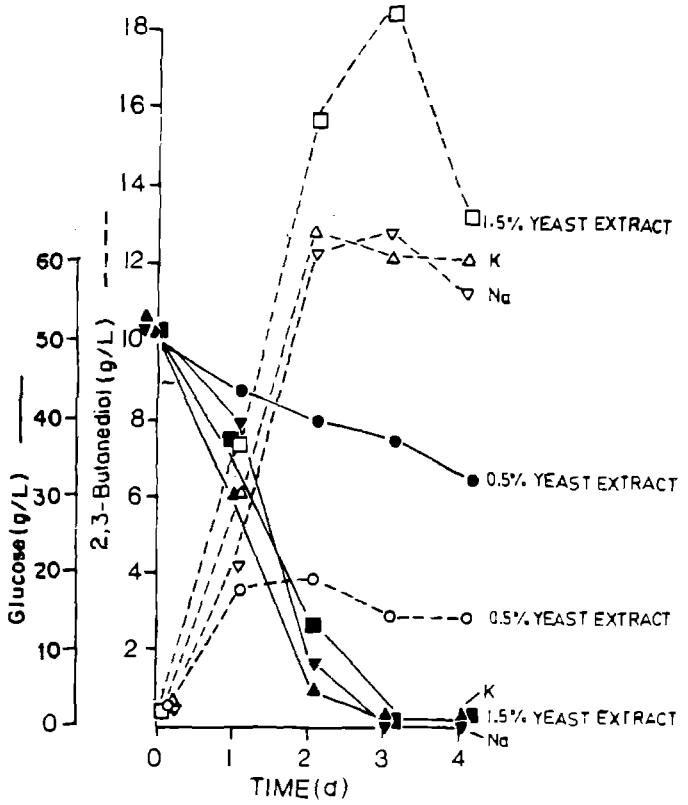

FIGURE 4: A COMPARISON OF THE EFFECT OF 0.078 M Na PHOSPHATE AND K PHOSPHATE ON 2,3-BUTANEDIOL PRODUCTION. (YEAST EXTRACT CONCENTRATION, WHERE NOT INDICATED =0.5%, (W/V : 1.5% AND 0.5% YEAST EXTRACT CONTROLS CONTAINED 0.0026 M ADDED K PHOSPHATE).

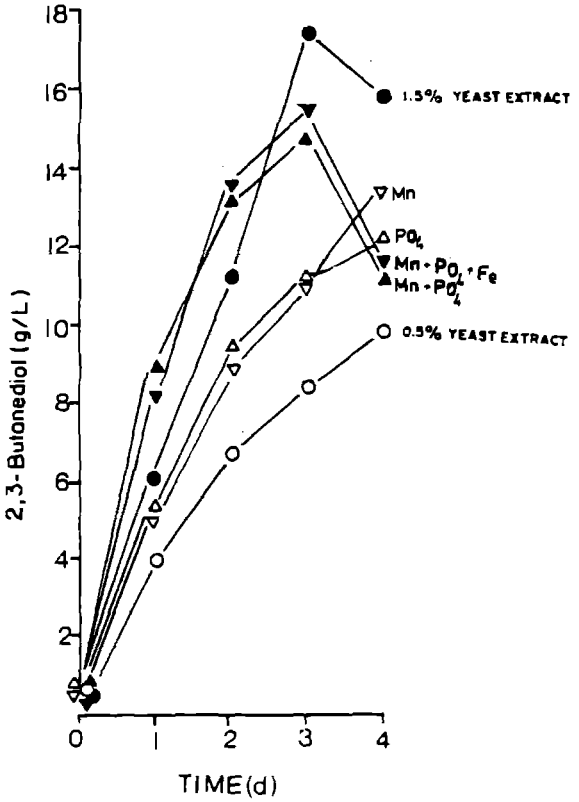

FIGURE 5: THE COMBINED EFFECT OF Mn, Fe AND K PHOSPHATE ON 2,3-BUTANEDIOL PRODUCTION.

(Figure 3B), with the highest yield, a value approaching the diol yield obtained with 1.5% yeast extract being obtained with 0.078 M phosphate. These results are similar to those of Taha et al. (1971) who found, while studying the effects of pH on the B. polymyxa fermentation , that maximum diol yields were achieved with phosphate concentrations of 0.070 M. At 0.078 M phosphate, the rate of glucose utilization also approached that obtained with 1.5% yeast extract (Figure 3C).

Although Murphy et al. (1951) showed that phosphate stimulated the fermentation of B. polymyxa, Katznelson (1947) observed that K was stimulatory. Thus, the question arose of whether K or phosphate was the stimulatory factor in our experiments. Altering the phosphate cation from K to Na had no effect on the fermentation profiles (Figure 4). Thus, the positive effects on diol yields and glucose metabolism were not due to a cationic effect but were truly an effect of phosphate, with phosphate most likely functioning to stimulate the complete metabolism of the bacterium.

By combining all the stimulatory minerals, phosphate plus Mn plus Fe, and adding these to medium containing 0.5% yeast extract, the rate of diol evolution was initially higher than the rate found with 1.5% yeast extract (Figure 5). This rate, however, leveled off at day 3 and by day 4 diol levels dropped off. Maximum yields, achieved at day 3, approached maximum yields found with 1.5% yeast extract. Thus, considering yields and rates, low yeast extract medium, supplemented with phosphate, Mn and Fe, could adequately replace 1.5% yeast extract medium.

Presently, conditions to further decrease the yeast extract requirement of this fermentation and to improve diol yields are being studied.

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