AMMONIUM CONCENTRATION CONTROL IN FED-BATCH FERMENTATIONS FOR THE PRODUCTION OF BIOMASS AND ENZYMES

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ABSTRACT

A control system has been devised to maintain stable ammonium concentrations throughout a fed-batch fermentation. The control system is based on an ammonium gas-sensing electrode that monitors a pH adjusted effluent stream from the fermentor. The ammonium electrode (Orion 95-12) was stable throughout the fermentation period. This control system was used to study the growth of Escherichia coli and Saccharomyces cerevisiae at controlled ammonium concentrations. Apparent specific growth rates, biomass and protein production, and glucose and ammonium yield were determined.

The effect of controlling ammonium concentration on growth and protease production by Bacillus subtilis in fermentors was also studied. Protease production was optimum when ammonium concentration was controlled at 5 mM. Protease production in fermentations controlled at this ammonium concentration was 1.5 times greater than in uncontrolled batch fermentations. Simultaneous control of ammonium and glucose concentrations using controllers based on an ammonium electrode and an oxygen electrode doubled protease production compared with fermentations having only ammonium control and tripled protease production compared with uncontrolled batch fermentations. The protease yield per mole of glucose and ammonium was greatest in simultaneous glucose and ammonium controlled fermentations.

INTRODUCTION

The biosynthesis of cellular protein and other nitrogenous products is dependent on the availability of nitrogen sources in the fermentation medium. Ammonium can be used as the source for virtually all nitrogen...
requirements in many microorganisms. Ammonium sulphate is a commonly used nitrogen source in industrial fermentation media because it is easily available and is inexpensive [1]. High ammonium concentrations inhibit the synthesis of glutamine synthetase and stimulate the synthesis of glutamate dehydrogenase [2]. Both glutamine and glutamate are key metabolites in amino acid biosynthesis and their availability affects the flow of assimilated ammonium into protein.

An enzyme producing organism such as Bacillus can be affected by the availability of nitrogen and carbon source in the medium. Either an excess or a low level of nitrogen may cause an inhibition of biosynthesis of protease by species of Bacillus [3-5].

In order to study the effects of ammonium concentration on its assimilation by E. coli and S. cerevisiae, Kole and co-workers [6-9] examined growth and ammonium assimilation under controlled ammonium concentrations. To control ammonium concentration in the fermentation medium, the ammonium control system described by Hill and Thommel [10] was modified. The control system described here maintains a much more stable ammonium concentration, allowing careful study of the effects of controlled ammonium concentration on growth and protease production.

In this communication, we will review some of the experimental results obtained with the ammonium control system in fermentations of S. cerevisiae and E. coli, and in fermentations for the production of protease by Bacillus.

**MATERIALS AND METHODS**

The organisms used in these studies were E. coli B ATCC 11303, S. cerevisiae NCYC 1018 and Bacillus subtilis NCIB 8054. The basal medium used for E. coli fermentations was the modified Davis and Mingioli medium [11] having the following composition (g/L): KH2PO4, 1.5; NaH2PO4, 0.25; MgSO4.7H2O, 0.1; glucose, 8.8 and a variable amount of (NH4)2SO4 at pH 7.0. S. cerevisiae was maintained and grown on a modified medium from [12] and had the following composition (g/L): KH2PO4, 1.0; MgSO4.7H2O, 0.42; CaCl2.2H2O, 0.1; NaCl, 0.1; yeast extract (Difco), 1.0; glutamic acid, 0.75; FeCl3.6H2O, 0.012; ZnSO4.7H2O, 0.008; CuSO4.5H2O, 0.0002; biotin, 0.0016; inositol, 0.020; nicotinamide acid, 0.008; calcium pantothenate 0.0077; pyridoxine, 0.00038 and thiamine hydrochloride, 0.00038. The basal medium used for protease production by Bacillus subtilis was synthetic M9 medium with some modification. The medium had the following composition (g/L unless otherwise noted): Na2HP04, 6.0; KH2PO4, 3.0; (NH4)2SO4, 2.64; MgSO4.7H2O, 0.5; CaCl2, 0.015; glucose, 5.0; thiamine HCl, 1 mg/L; CuSO4, 3 mg/L; FeCl3, 3 mg/L; MnSO4, 3 mg/L; and ZnSO4, 3 mg/L. In all cases, the glucose was sterilized separately. The trace metals and vitamins were filter sterilized and added to cool medium. The ammonium concentration was varied from batch to batch according to the controlled conditions of the batch. E. coli was grown at 37°C, S. cerevisiae at 25°C and Bacillus at 35°C.

Fermentations were performed in a standard design fermentor (Chemap A.G., Volketswil, Switzerland) with a 14L vessel containing 10L of medium. Temperature was controlled at 35°C. Mixing was with three, 6-bladed flat-blade impellers operating at 500 rpm with an aeration rate of 3L air/min. The oxygen transfer rate under these conditions was found to be 50 mM/L/h by purging the tank with oxygen-free N2 and recording the reoxygenation of cell-free medium with an IL530 polarographic oxygen