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 [3-6].

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): K$_2$HPO$_4$, 1.5;
NaH$_2$PO$_4$, 0.25; MgSO$_4$.7H$_2$O, 0.1; glucose, 8.8 and a variable amount of
(NH$_4$)$_2$SO$_4$, at pH 7.0. S. cerevisiae was maintained and grown on a
modified medium from [12] and had the following composition (g/L):
KHP0$_4$, 1.0; MgSO$_4$.7H$_2$O, 0.42; CaCl$_2$.2H$_2$O, 0.1, NaCl, 0.1; yeast extract
(Diffco), 1.0; glutamic acid, 0.75; FeCl$_3$.6H$_2$O, 0.012; ZnSO$_4$.7H$_2$O, 0.008;
CuSO$_4$.5H$_2$O, 0.0002; biotin, 0.0016; inositol, 0.020; nicotinic 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):
Na$_2$HPO$_4$, 6.0; KH$_2$PO$_4$, 3.0; (NH$_4$)$_2$SO$_4$, 2.64; MgSO$_4$.7H$_2$O, 0.5; CaCl$_2$
0.015; glucose, 5.0; thiamine HCl, 1mg/L; CuSO$_4$, 3 mg/L; FeCl$_3$, 3 mg/L;
MnSO$_4$, 3 mg/L and ZnSO$_4$, 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 (Chemag
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 N$_2$, and recording
the reoxygenation of cell-free medium with an IL530 polarographic oxygen