DETERMINATION OF MINERAL ION CONSUMPTIONS BY IONIC HPLC

Garrido-Sanchez L.E., Dantigny P., Pons M.-N.*

Laboratoire des Sciences du Génie Chimique CNRS-ENSIC-INPL
1, rue Grandville F-54042 NANCY Cedex FRANCE

SUMMARY
Ionic HPLC is a novel technique used to determine more easily mineral ions consumptions during fermentations.

INTRODUCTION
The purpose of fedbatch cultures is to achieve high productivities of biomass and metabolites. Maintaining optimal culture condition in the broth requires not only proper feeding of the carbon source but also of the nitrogen source, oxygen and mineral ions. A lot of work has been and is still done concerning carbon source. Oxygen source is not really a problem (Yano, 1981). Nitrogen source addition is generally related to pH control. Minerals ions feeding is seldom examined and taken care of, probably because of the lack of information about the mineral requirements by the microorganism.

Few methods are in fact available to determine the mineral ions concentrations. Some specific methods exist for some of them (PO$_4^{3-}$, SO$_4^{2-}$, NH$_4^+$). Ion selective electrodes are not used because of high interference with the other ions. Atomic absorption spectrophotometry has been used (Suzuki, 1985) but is limited to cations. Furthermore each ion must be analyzed separately. New ionic HPLC sounds attractive to resolve the problems as both anions and cations can be analyzed with minimal manipulation.

This paper deals with the use of such an equipment to evaluate the mineral requirements of a *Saccharomyces cerevisiae* strain. The work is focused on basic ions closely related to metabolism: K$^+$, Na$^+$, NH$_4^+$, Mg$^{2+}$, Ca$^{2+}$, Cl$^-$, PO$_4^{3-}$ and SO$_4^{2-}$.

MATERIALS AND METHODS

*Saccharomyces cerevisiae* dry yeast (Fermipan™ commercial strain from Société des Produits du Maïs, Clamart, France) has been used. The growth medium composition is the
following: \((\text{NH}_4\text{)}_2\text{SO}_4\) 6g/l, \(\text{KH}_2\text{PO}_4\) 3g/l, \(\text{MgSO}_4\cdot7\text{H}_2\text{O}\) 1.5 g/l, \(\text{NaCl}\) 1 g/l, \(\text{CaCl}_2\cdot2\text{H}_2\text{O}\) 0.1 g/l, iron citrate 0.03 g/l, yeast extract 3 g/l, m-inositol 0.05 g/l, nicotinic acid 0.01 g/l; 10 ml/l of mineral elements solution (content: \(\text{CuSO}_4\cdot5\text{H}_2\text{O}\) 0.05 g/l, \(\text{ZnSO}_4\cdot7\text{H}_2\text{O}\) 0.4 g/l, \(\text{MnSO}_4\cdot\text{H}_2\text{O}\) 0.4 g/l, \(\text{NaMoO}_4\cdot\text{H}_2\text{O}\) 0.3 mg/l, \(\text{CoSO}_4\cdot7\text{H}_2\text{O}\) 0.2 mg/l); 1 ml/l of vitamin solution (content: biotine 0.08 g/l, Ca-pantothenate 4 g/l, thiamine 6 g/l, pyridoxine 2 g/l, p-aminobenzoïc acid 2 g/l, thalic acid 0.02 g/l). For the fedbatch experiment the feed contains 250 g glucose /l (without any salts).

The experiments were run in a 20-liter fully instrumented fermenter (Chemap) connected to a Macsym 120 (Analog Devices) microcomputer for data logging and dissolved oxygen control. pH was regulated by automatic ammonia solution addition (pH set-point 3.9). A load-cell, connected to the microcomputer, was used to monitor the nitrogen source consumption. Dissolved oxygen level was controlled by automatic manipulation of agitation speed and air flowrate. The setpoint was fixed at 50% for batches and 10% for the fedbatch.

Cell concentration was estimated by optical density measurement. Ethanol and acetic acid were determined by a chromatographic method after filtration and acidification of the samples. A glass column packed with Porapak Q 80/100 mesh on a Delsi 121FL (FID detector) was used. Glucose was analyzed enzymatically (hexokinase method) by means of a Boehringer kit.

Ions were analyzed on a ionic HPLC fitted with an eluent suppressor (Dionex 4000 from Dionex Corporation, Sunnyvale, CA, USA). For the monovalent cations (K⁺, Na⁺ and NH₄⁺), a Dionex CS2 Column was used with a 25 HCl mM/l eluent; for the divalent cations (Mg²⁺ and Ca²⁺) the same column was used but the eluent contained 25 HCl mM/l and 2 histidine mM/l; finally for the anions (Cl⁻, PO₄³⁻ and SO₄²⁻) a Dionex AS4A column was used and the eluent contained 2.2 Na₂CO₃ mM/l and 0.75 NaHCO₃ mM/l. In eluent suppressor the eluent enters as a salt and by exchange of Na⁺ and H⁺ ions (anions case) or Cl⁻ and OH⁻ ions (cations case) the acid species is formed, which has a very low conductivity. The detector responds only to the ions of interest, which exhibit a higher conductivity than the eluent acid species.

RESULTS AND DISCUSSION

Figure 1 presents the kinetics of batch growth with initially 27 g glucose/l for glucose, ethanol, acetate and biomass and the ions kinetics. The ammonium concentration is constant due to ammonia addition for pH control and a complete ammonia balance taking into account the ammonium ions added is required to estimate nitrogen consumption. The calcium kinetics seem to be erratic but it was at a low concentration with respect to the other ions. Figure 2 presents the kinetics of the fedbatch for biomass, broth volume and the various ions. The growth stops after 800 min, at a time where no more phosphate was found in the broth, which induced growth limitation.

Table 1 gives the relative consumption of the ions for two batches at different initial glucose concentration and for the fedbatch. The consumption increases with the initial glucose concentration, which is coherent with the fact that the biomass produced depends