

# Immobilisation of *Acinetobacter johnsonii* cells within alginate beads

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## Abstract

The growth and distribution of *A. johnsonii* cells, immobilised within alginate beads suspended in an aerated activated sludge mixed liquor medium, were assessed by viable cell counts on nutrient agar and scanning electron microscope (SEM). Both techniques indicated that *A. johnsonii* cells did survive and grow within alginate beads. *A. johnsonii* immobilised cells were metabolically active as they removed phosphate from the activated sludge mixed liquor medium. While cells were expected to occur preferably in the outer layer after a few hours of incubation, beads entrapping bacterial cells showed a random distribution of cell colonies 24 h and 2 weeks after incubation. This constant random distribution might be attributed to constant aeration (that would have facilitated mass transfer) added to extracellular substances which maintained daughter cells in the colonies close to one another, thus preventing them from moving to the outer layer.

## Introduction

Several polyphosphate (polyP)-accumulating bacteria, especially from the genus *Acinetobacter*, have been isolated from activated sludge in which enhanced biological phosphate removal (EBPR) has been observed (Fuhs and Chen, 1975; Streichan et al., 1990). Currently attention is being drawn to immobilisation of these bacterial cells in order to get an insight into the mechanism of biological phosphate removal. One of these bacteria, namely *A. johnsonii* has been reported to be an efficient polyP-accumulating species (Van Groenestijn et al., 1989; Van Veen et al., 1993), therefore an ideal model organism to illustrate the possible use of the alginate immobilisation technique for EBPR. The efficiency of an immobilised system for EBPR will depend on a number of factors e.g. the bacterial strains being used, the properties of the support material, and the immobilisation method itself. The extent of phosphate-accumulation depends on growth rate of the polyP-accumulating bacteria (Van Groenestijn et al., 1989) which in turn is restricted by the spatial distribution of the cells within the gel bead.

Some electron microscopic observations of different bacteria entrapped in gels have been reported by different investigators (Garde et al., 1981). Structural details about organelles or cellular integrity, matrix architecture and cell distribution within the matrix have been enlightened by electron microscopic observations. Electron microscopic studies have shown alginate to have a spongy-like structure consisting of filaments and cavities and also bulges on the surface and inside the beads (Garde et al., 1981; Bashan 1986). Bashan (1986), using scanning electron microscopy, has indicated that alginate beads at various stages of production revealed a rounded structure with shallow grooves every 10 to 15  $\mu\text{m}$  and a few cavities on its rough surface.

Growth within the matrix has been reported to be limited to the outer 50  $\mu\text{m}$  layer of gel beads. Just before the start of incubation, a homogeneously distributed bacterial population is observed throughout the gel lattice. After a few hours of incubation, the cells in the central part of the bead disappear, while the cells in the layer near to the gel surface multiply (Wada et al., 1980; Shinmyo

et al., 1982).

The aim of this study was to determine the growth and distribution of *A. johnsonii* immobilised cells within alginate beads. P-uptake ability of the immobilised cells was also determined as an example of how the alginate immobilisation technique could be used.

## Material and methods

### *Bacterial culture and inoculum used in this study*

A culture of *A. johnsonii* strain 105 was obtained from the culture collection at the Environmental Biotechnology Laboratory, Department of Microbiology and Plant Pathology, University of Pretoria. An aliquot of the bacterial stock culture was incubated in 100 ml Biolab nutrient broth on an Edmund Buhler TH 10 rotary shaker, 160 r/min at 28°C for 72 h.

### *Activated sludge mixed liquor medium for growth and distribution studies*

Activated sludge mixed liquor was collected from the anaerobic tank at the Daspoort activated sludge plant, Pretoria. It was filtered twice on Whatman filter paper (0 185 mm) and enriched with 5 mg/l  $\text{CH}_3\text{COONa}$ , 0.18 g/l  $\text{KNO}_3$  and 0.5 g/l  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , respectively before autoclaving. The nutrient additions were adapted from Bosch and Cloete (1993).

### *immobilisation technique*

Entrapment of bacteria within alginate beads was performed according to a procedure of Bashan (1986) modified by Cloete et al. (1994). A sterile 20 ml syringe using a 26G needle was used to add the alginate-bacterial cell mixture dropwise into 1.1%  $\text{CaCl}_2$  solution for cross-linking. Beads of approximately 2 mm in diameter were immediately formed in the  $\text{CaCl}_2$  solution. 3.5% and 4% sodium alginate (BDH) bead concentrations were used.

Alginate beads entrapping bacterial cells were washed with sterile distilled water and suspended in activated sludge mixed liquor medium and incubated on a rotary shaker, 160 r/min at 28°C. The above cultural conditions, aeration by shaking and temperature were maintained throughout the experimental procedure.

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