Immobilisation of *Acinetobacter calcoaceticus* using natural carriers

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Abstract

There is a growing interest to immobilize desired bacteria using inexpensive materials in order to improve the wastewater treatment process. Three different types of carriers namely natural zeolite, magnesium-exchanged natural zeolite and quartz sand of different particle size were used to immobilize the phosphate-accumulating bacteria *Acinetobacter calcoaceticus* and to determine which one was the most effective. Bacteria were cultured for 24 h in various reactors containing different particle sizes of each of the carriers. The majority of the cultured bacterial population was immobilised onto the different carriers by means of adsorptive growth while a minority of free cells was observed in the supernatant. The number of immobilised viable cells (CFU) depended on the type of carrier and the particle size. The highest loading rate of immobilised cells (68.61±1.11 x 10^8 CFU/g) was observed with the smallest particle size (<0.125 mm) of magnesium-exchanged natural zeolite.

Keywords: *Acinetobacter calcoaceticus*, immobilisation, phosphate, quartz sand, wastewater, zeolite

Introduction

Enhanced biological phosphorus removal (EBPR) from wastewater, a biological alternative to chemical phosphate (P) precipitation, is based on the activity of P-accumulating bacteria. Bacteria from the genus *Acinetobacter* have been reported to be the most efficient P-accumulating species (Muyima and Cloete, 1995; Sidat et al., 1999; Hrenović et al., 2003a; Hrenović et al., 2003b).

Currently attention is being drawn to the immobilisation of bacteria in order to achieve a higher cell density in bioreactors; based on this, smaller reactors, shorter residence/retention time or higher flow rates can be employed. Immobilisation of *Acinetobacter* spp. has been investigated using alginate (Muyima and Cloete, 1995) or ceramic (Kariminiaae-Hamedaani et al., 2003) carriers. Besides the synthetic carriers, natural zeolite (NZ) has been shown as a promising material for the immobilisation of micro-organisms (Shindo et al., 2001) due to its high porosity and large surface area. The extent of bacterial colonization depends on the chemical properties and particle size of NZ.

It has been suggested that available magnesium ions (Mg^{2+}) are important for the stable EBPR and efficient P-removal (Seviour et al., 2003). The negative charge of the NZ, which is attributed to the tetrahedrally co-ordinated aluminium, is balanced by exchangeable cations. Mg^{2+} is one of the most common cations in some NZ. Based on the presence of Mg^{2+}, the use of NZ as carrier of immobilised P-accumulating bacteria may improve the P-uptake capacity of the system.

The aim of this study was to determine the capacity of NZ and quartz sand for the immobilisation of P-accumulating bacteria *A. calcoaceticus*. In addition, the influence of Mg^{2+} present in the NZ on the P-uptake ability of immobilised cells was also determined.

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Material and methods

Micro-organism

A culture of a P-accumulating bacterium *A. calcoaceticus* (DSM, 1532) was obtained from the *Deutsche Sammlung von Microorganismen und Zellkulturen GmbH* (Hrenović et al., 2003a).

Carriers

Natural zeolite (NZ): The zeolitised tuff from Donje Jesenje, Croatia contained more than 50% of zeolite of the heulandite group (clinoptilolite), some quartz and plagioclase and accessory minerals from the mica group (illite-celadonite and biotite). Among the exchangeable cations, potassium was the dominant one in the sample. The NZ (10 g of each particle size) was washed three times with demineralised water (300 mℓ) and then dried at 105°C in oven for 16 h before the experiments were to commence.

Magnesium-exchanged natural zeolite (NZMg): The NZMg was prepared by treating 10 g of the particular fraction of original NZ with 250 mℓ of neutral 1 M $MgCl_2$ (Kemika, Croatia) solution. Erlenmeyer flasks were agitated on a mechanical shaker (Inko SP17) at 200 r/min for 48 h at room temperature (24±2°C). The supernatant was decanted and particles were washed with demineralised water (1 ℓ) until a negative chloride ion test with 1% silver nitrate solution was obtained. The NZMg was air-dried for 7 d, followed by drying at 105°C in an oven for 16 h before the experiments were to commence.

Quartz sand (QS): The QS from Vrtlinska, Croatia contained dominantly quartz as well as minor quantities of feldspars and micas. The QS (10 g of each particle size) was washed three