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Monitoring structure and activity of nitrifying bacterial biofilm in an automatic biodetector of water toxicity

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ABSTRACT

Automatic biodetector of water toxicity is a biosensor based on monitoring of catalytic activity of the nitrifying bacteria. To create a standardized biosensing system, development of the biofilm must be characterized to determine the prerequisites for its biological (biocatalytic) stability. In this paper, growth of biofilm comprising ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) in the open cellular polyurethane material polyurethane sponge bioreactor has been investigated.

Dynamics of the biofilm formation was estimated using AOB and NOB metabolic activity and the volume occupied by these two types of bacteria in the biofilm. Spectrophotometry liquid ion chromatography and image cytometry were used, respectively, for these measurements. A mathematical model of the dynamics of biofilm formation was established. These data indicate that open cellular polyurethane material is a good basis for the immobilization of nitrifying bacteria. Moreover, growth of the biofilm leads to its stable structural form, whose biocatalytic activity (12.29 for AOB and 6.84 μmol min^{-1} for NOB) is constant in the long term.

1. Introduction

A release of anthropogenic toxins to the environment results in a growing need for environmental monitoring. The use of biosensing for that purpose permits simultaneous detection of diverse chemical components that contribute to overall toxicity level in water. There are several commercially available biosensing systems, based on luminescent marine bacteria (Microtox), plankton organisms (Daphtoxkit F, Protoxkit F, Algaltoxkit F) (Christensen et al., 1992; Pessala et al., 2004; Kahru et al., 2008), molluscs (*Unio tumidus*, Symbio) and fishes (*Gnathonemus petersii*). Despite their obvious usefulness, these systems have certain limitations. First, their response time is usually long (from 24 h to several days), with the exception of systems using bacteria as the sensing element. Second, they may require a number of samples (with the exception of systems based on higher organisms) and therefore do not permit in situ measurements. In order to solve these problems and create system for fast, continuous estimation of water toxicity, in situ may apply nitrifying bacteria as a sensing element.

Nitrification is a multi-stage biological transformation of reduced forms of nitrogen to nitrate. The nitrification reactions are catalyzed by two groups of ubiquitous lithoautotrophic bacteria. The first group comprises ammonium-oxidizing bacteria (AOB), and the second group comprises nitrite-oxidizing bacteria (NOB) (Hovanec et al., 1998; Bothe et al., 2000; Gieseke et al., 2001; Koops and Pommerening-Röser, 2001; Kowalchuk and Stephen, 2001; Mota et al., 2005). In aquatic environments, genera of both AOB and NOB have been observed exclusively in the form of flocs or biofilms.

Use of nitrifying bacteria for biosensing is motivated by their high sensitivity (Ilzumi et al., 1998; König et al., 1998; Cui et al., 2005) to a wide range of toxins, including phenols (Ilzumi et al., 1998; König et al., 1998), cyanides (Kim et al., 2008) and heavy metals (Chandran and Love, 2008; Park and Ely, 2008). Moreover, the nitrifying bacteria have natural ability to form biofilms (Liu and Tay, 2001). Thus, unlike other bacteria used as active elements of biodetectors (Bang et al., 2001; de Ory et al., 2004) and biosensors (Blum, 1997; Bettaieb et al., 2007), they do not require artificial immobilization. Therefore, we present a biosensor based on consortia of nitrifying bacteria immobilized on a porous carrier (polyurethane) to detect the presence of toxins in flowing water. The bacteria were immobilized during growth by the formation of complexes of surface-associated cells enclosed in a matrix of extracellular polymeric substances (EPS) (Wimpenny et al., 2000). The biofilm is usually populated together by the two groups

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of bacteria (catalyzing two stages of nitrification), linked by mutualistic interactions. Composition and spatial architecture of such biofilms are, in general, non-uniform and may vary in time. Therefore, to create a standardized biosensing system, the development of the biofilm must be characterized to determine the prerequisites for its biological (biocatalytic) stability. In other words, conditions in which the biofilm stability will be maintained by its natural homeostasis have to be established. We have applied spectrophotometry, ion chromatography and image cytometry complemented by computed X-ray tomography (Al-Raoush and Willson, 2005; Xi et al., 2006). Furthermore, we described the biofilm development using a mathematical model (Diaz et al., 1999; Lokshina et al., 2001; Tsoularis and Wallace, 2002; Fujikawa et al., 2004; van Impe et al., 2005; Liu, 2006; Peleg et al., 2007).

2. Materials and methods

In our experiment, biofilm growth in the automatic biodetector of water toxicity (ABTOW) bioreactor with high ammonium level was examined. Non-buffered mineral medium was used for that purpose. Ammonium concentration and pH were corrected daily to 7.6 mM and 7.5 units, respectively. The medium was continuously aerated (4 L min^{-1}). Velocity of medium laminar flow was 0.0037 m s^{-1} in order to simulate natural slow flow of the surface water. In the OCPM, we observed process of biofilm maturation on form growing on the polyurethane arm or caught in the node of sponge.

2.1. Bacterial culture

Consortia of nitrifying bacteria were used. Bacteria were isolated from activated sludge from water treatment plant Klimzowiec, Katowice, Silesia (Woznica et al., 2006). Consortia of nitrifying bacteria had grown in 2 L laboratory scale reactor as a fed-batch culture in Braun Melsulgen Biostat in 1.5 L mineral liquid medium (MacDonald and Spokes, 1980). Reactor was aerated continuously 2 L min^{-1}, and the oxygen concentration was about 5 mg L^{-1}, pH was 7.5, and temperature was 20 °C. Biological filter in the ABTOW was fed on the modified liquid medium (g L^{-1}): $(NH_4)_2SO_4$ 0.5, KH_2PO_4 0.2, $CaCl_2$ 0.016, $MgSO_4 \cdot 7H_2O$ 0.04, $NaHCO_3$ 0.12.

2.2. Bacteria immobilization in the calcium alginate

Nitrifying bacteria were harvested by centrifugation (9000 rpm, 50 min) of 0.5 L of the bacterial culture. The pellet was mixed with 100 mL of 2% sodium alginate for bacteria dispersion. The suspension was instilled into the 4% solution of $CaCl_2$ (4 °C). In this way, bacteria were trapped in the calcium alginate spheres (±3 mm diameter). Material was stored in 4 °C, and the nitrification activity was maintained for at least 14 d.

2.3. AOB and NOB nitrification kinetics

Consortia of nitrifying bacteria immobilized in the calcium alginate were used for the determination of standard Michaelis-Menten kinetic parameters of nitrification (Nakhla et al., 2006). Oxygen consumption by bacteria was used as an estimator of the nitrification rate. The consumption was measured in 100 mL closed chamber on the magnetic stirrer and the oxygen electrode dipped in medium with immobilized biomass. Rate of nitrification was defined as first derivative (taken at 0) of a square function of time, describing the magnitude of oxygen consumption. On the basis of those data, half-saturation constants $K_{m(AOB)}$, $K_{m(NOB)}$ and nitrification rates V_{AOB}, V_{NOB} were determined using iteration method with quasi-Newton algorithm (Statistica 8.0 Statsoft, US).

2.4. Biofilm bioreactor system

Biofilms have grown in the cylindrical-shaped OCPM (length 100 mm, diameter 25 mm) in the 120 mm long polystyrene tube of 25 mm diameter. Bioreactor was placed in a horizontal position (Fig. 1a). The constant laminar flow velocity (u) equal to 0.0037 m s^{-1} (100 mL min^{-1}) was maintained with a laminar pump. Biofilm activity was monitored using two oxygen sensors on both ends of the bioreactor (as described later). The system in-

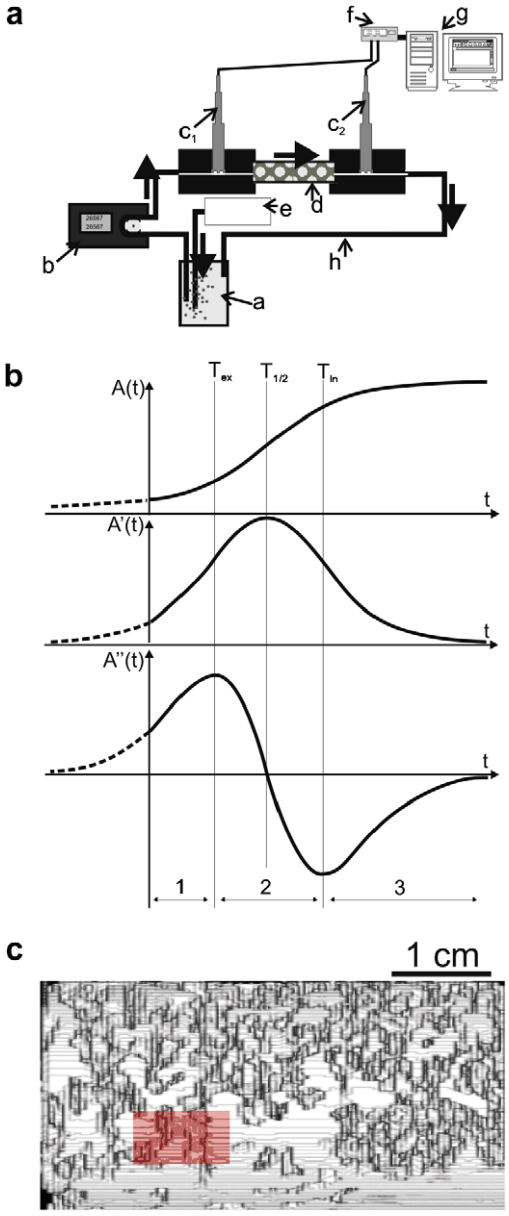

Fig. 1. (a) Scheme of ABTOW bioreactor. The main components, described with thin arrows, as following: a – nutrient and mixing chamber, b – peristaltic pump, c – oxygen electrode, d – foam reactor, e – air pump, f – transducer, g – data registrating computer, h – recirculation loop. Direction of the flow (air and water) is marked with thick arrows, (b) Verhulst model: $A(t)$ – changes in bioreactor activity, $A'(t)$ – rate of bioreactor activity changes, $A''(t)$ – acceleration of bioreactor activity changes section (1) is the exponential stage increasing an activity, section (2) is the linear stage, section (3) is the logarithmic stage. X-ray tomography image of 28 d biofilm. (c) Red box shown in the region of probe collection.