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Factors influencing adhesion of *Pseudomonas putida* on porous clay ceramic granules

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Abstract

The aim of the study was to determine factors affecting adhesion of bacteria *Pseudomonas putida* on porous clay ceramic granules. The number of colony-forming units (CFU) of *P. putida* on Devonian clay granules sintered at temperature of 1100 °C after 4-h-long incubation was estimated. The level of the adhesion significantly ($P < 0.05$) depended on temperature, pH value, ionic strength of the medium and initial bacterial concentration. The optimum pH for bacterial adhesion was about pH 6. Temperature of 30 °C was more appropriate for the adhesion than temperature of 20 °C. Negative correlation was observed between the number of adhered bacteria and ionic strength ($r = -0.84$) in the medium and between the number of bacterial CFU in the suspension and ionic strength ($r = -0.77$) in the medium over the range from 0 to 200 mM NaCl. Investigated concentrations of bacterial suspension up to 11 log CFU mL^{-1} did not reach saturation of bacteria on the surface of granules. The adhesion intensity appears to be sufficient for subsequent development of bacterial colonies in adequate environmental conditions.

Key words: adhesion, *Pseudomonas putida*, porous clay ceramic granules.
Abbreviations: CFU, colony-forming units; PCA, Plate Count Agar.

Introduction

Adhesion of microorganisms is a common ecological feature. Surface adhesion and biofilm development is a survival strategy employed by virtually all bacteria and refined over millions of years (for review, see Dunne 2002). Nowadays, the study and application of adhesion have taken great importance for biotechnology as one of the methods of cell immobilization for practical needs.

Various organic and inorganic materials and also methods are used for immobilization of microorganisms. Microbial adhesion occurs on both rough and smooth surfaces, including glass (Mitik-Dineva et al. 2009). Porous clay ceramic is one of materials suitable for microbial adhesion. Several studies have shown that porous ceramic carriers can be used in wastewater treatment (Kariminiaae-Hamedaani et al. 2003), in environmental bioremediation (Grundmann et al. 2007; Muter et al. 2011) and other biotechnological applications.

Microbial adhesion is a complex process involving interactions at the cell surface level. Primary adhesion between bacteria and abiotic surfaces is generally mediated by non-specific, e.g., acid-base hydrophobic interactions (Pezron et al. 2004; Rochex et al. 2004). Adhesion depends on factors such as ionic composition and strength, pH, temperature, contact time and concentration of cells (Palmel et al. 2007; Hori, Matsumoto 2010) as well as surface charge, hydrophilicity/ hydrophobicity, porosity, roughness and microtopography of the surface (Van Loosdrecht et al. 1989).

In order to study factors affecting microbial adhesion on porous clay ceramic granules, bacterium *Pseudomonas putida* was used as a model organism. *P. putida* is a saprophytic, Gram-negative, mesophilic, aerobic bacterium commonly present throughout the environment, with an ability to degrade various natural and synthetic compounds (Timmis 2002), and it readily colonizes biofilms (Tolker-Nielsen et al., 2000). It is common in most soil and water habitats. The objective of this investigation was to study factors (temperature, pH, ionic strength, and initial bacterial concentration) affecting adhesion of *P. putida* on porous clay ceramic granules for further biotechnological application of immobilized microorganisms in environmental bioremediation.

Materials and methods

Organisms and materials

Pseudomonas putida strain MSCL 650 was cultivated on Plate Count Agar (PCA; Bio-Rad, France) at 20 °C for 48 h in aerobic conditions.

Ceramic granules from Devonian clay sintered at 1100 °C were prepared and characterized in the Institute of Silicate Materials, Riga Technical University. The main raw material was clay obtained from Liepa clay deposits, Latvia. The diameter of granules was 1.2 cm and the bulk density was 1.33 g cm^{-3} (Fig. 1).

Adhesion experiments

Bacterial adhesion was carried out in glass bottles (Simax, Czech Republic) with a volume of 100 mL. Ceramic granules were weighed and a batch of 15 g of granules was placed in each bottle and sterilized at 121 °C for 15 min in an autoclave (0.1 MPa). In the beginning of the experiment, 50 mL of sterile distilled water or appropriate 100 mM Na phosphate buffer or NaCl solution was added to each bottle.

Suspension of fresh *P. putida* cells was prepared and added to the bottles containing granules at concentration from OD_{540} 0.05 – 0.06 to 1.45 (Ultrospec 3100 Pro, Amersham Biosciences, UK).

Inoculated bottles were incubated in an incubator (Binder KB 53, Germany) at 20 °C or 30 °C with manual shaking twice per hour. After 4 h, the incubation liquid was decanted from granules. Samples of suspensions of 0.1 mL were subjected to microbiological analyses. The granules were carefully rinsed two times with phosphate buffered saline solution (137 mM NaCl, 2.7 mM KCl, 10 mM KH2PO4, pH 7.4). Three gram of granules were scrubbed and ground in a sterile mortar with a pestle in 3 mL of phosphate buffered saline to recover the bacteria adhered to the granules. The number of colony-forming units (CFU) of detached bacteria in the final liquid was determined.

The experiment was repeated three times.

Microbiological analyses

The amount of viable bacteria was determined by serial dilution of the bacterial suspension by spreading of obtained dilutions on agar plates and counting of bacterial CFU after incubation. Serial dilutions were plated in duplicate on the PCA. For each spread plate 0.1 mL of each dilution was used. PCA plates were incubated at 20 °C for 48 h. The results were expressed as CFU per millilitre of suspension or per gram of dry ceramic granules for bacteria recovered from granules. The respective detection limits were 10 CFU mL^{-1} and 2 CFU g^{-1}.

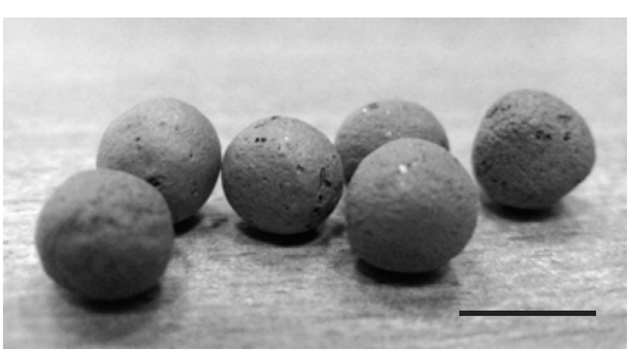

Fig. 1. Ceramic granules used in the experiments. Bar =10 mm.

Statistical analysis

Means, standard deviations and correlation coefficients were calculated. Analysis of variance (ANOVA) and the Student *t*-test was used to test differences among groups. $P < 0.05$ was considered statistically significant.

Results

Increase of incubation temperature from 20 to 30 °C led to increase in the amount of viable adhered and detached *P. putida* from 5.79 log CFU per gram to 5.90 log CFU per gram of ceramic granules after 4-h-long incubation (Fig. 2). The effect was statistically significant ($P < 0.05$).

The effect of pH on bacterial adhesion was studied in the range from 5.0 to 8.0. Maximal bacterial adhesion was at around pH 6 (Fig. 3). The amount of adhered bacteria decreased ($P < 0.05$) to 71% at pH 7, to 18% at pH 5, and to 3% at pH 8 in comparison with the maximum.

Adhesion was affected by ionic strength of incubation medium (Fig. 4). Negative correlation was observed between the number of adhered bacteria and ionic strength (r = –0.84) and between the number of bacterial CFU in the suspension and ionic strength (r = –0.77) in the medium

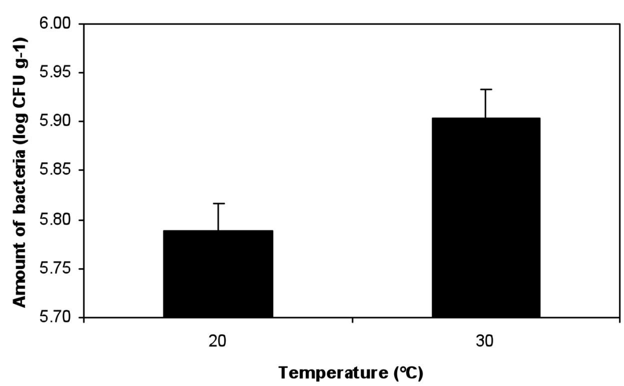

Fig. 2. Adhesion of bacteria on the porous clay ceramic granules at temperature 20 °C and 30 °C. Error bars represent the standard deviation.

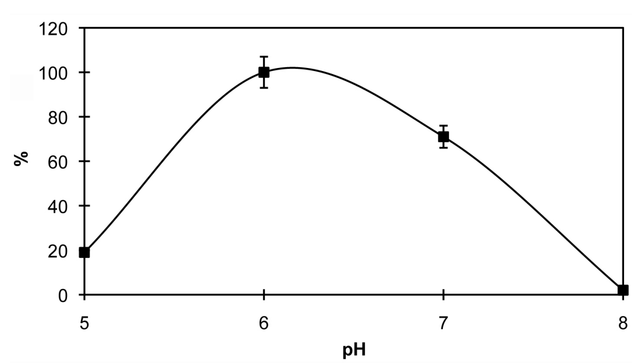

Fig. 3. Adhesion of bacteria on porous clay ceramic granules in relation to pH after 4 h incubation in 100 mM Na phosphate buffer at 30 °C. Maximal adhesion at pH 6.0 was assumed as 100%. Error bars represent the standard deviation

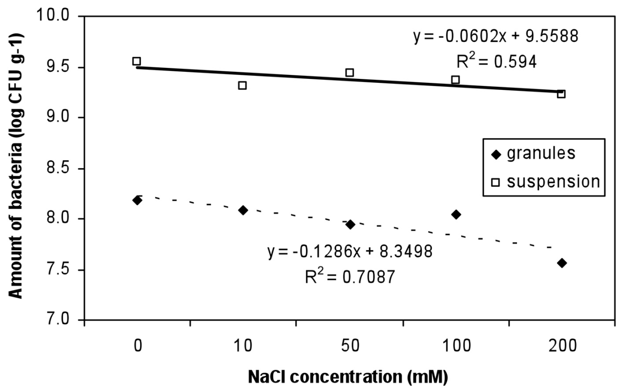

Fig. 4. Concentration of *P. putida* in suspension and on granules in relation to concentration of NaCl in the medium after 4 h incubation at 30 °C.

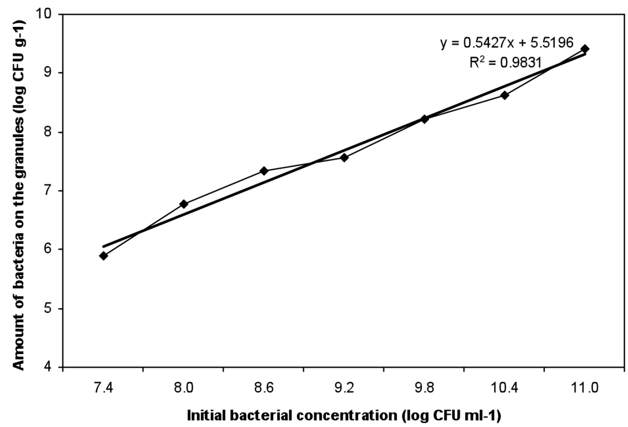

Fig. 5. Relationship between initial bacterial concentration and amount of bacteria on granules after 4 h incubation in 100 mM Na phosphate buffer, pH 7.0 and temperature 30 °C.

over the range from 0 to 200 mM NaCl.

Initial bacterial concentration affected adhesion of *P. putida*. A linear dependence of adhesion on initial bacterial concentration was evident (Fig. 5). It seems that initial concentration of bacterial suspension up to 11 log CFU mL^{-1} did not result in a saturation of bacteria on the surface of granules. It was calculated that about 3.0 ± 1.7 % of the total number of bacterial cells was adhered to the granules, with respect to initial concentration of bacteria.

Discussion

Porous clay ceramic is used in different practical applications, e.a., as a substrate in hydroponic cultivation systems in greenhouses (Pickens et al. 2009) and as a support material for microbial biofilm development in wastewater treatment and fermentation technologies (Barros et al. 2010). In these applications, ceramic comes into contact with living organisms. This study examined the impact of ceramic granules made from Devonian clays on adhesion of the widely distributed bacterial species *P. putida* in laboratory conditions.

It was previously shown that, in general, ceramic granules are suitable for adhesion and subsequent immobilization of microorganisms (Prieto et al., 2002; Yongming et al., 2002; Xiangchun et al., 2003; Nikolajeva et al. 2010; Muter et al. 2011). In particular, Devonian clay granules are appropriate for immobilization of viable organisms. However, the type of granules has important effect both on bacterial adhesion and their viability. Quaternary clay granules significantly diminish the number of viable bacteria on granules and in the surrounding suspension (Nikolajeva et al. 2011).

The present study demonstrated that bacterial adhesion on ceramic granules is significantly affected by temperature, pH, ionic strength and initial concentration of bacteria. The fact that the adhesion was larger at 30 °C than at 20 °C is in agreement with previous studies of *P. putida* adhesion (Robledo-Ortiz et al. 2010). However, the present results differ from those in other investigations, in respect to the effect of ionic strength and pH value on bacterial adhesion. Robledo-Ortiz et al. (2010) studied *P. putida* adhesion onto agave-fiber / polymer foamed composites and established that the adhesion increases with increasing ionic strength (NaCl concentration). They explained this effect with decrease of negative charge of the bacterial surface, as the polymer lacked functional groups and its surface was uncharged. It can be assumed that the surface of ceramic granules in the present experiments had positive charge and that addition of Na^+ diminished the negative charge on the bacterial surface. As a result, adhesion decreased on ceramic granules with Na^+ concentration.

Presently there is only indirect evidence regarding surface charge of clay ceramic granules. Previously, we showed that Devonian clay granules increase electrical conductivity in the aquatic environment of granules by 12 mS m^{-1} after 48 h incubation. In contrast, liquid medium of the Quaternary clay granules demonstrated approximately six times greater electrical conductivity (Nikolajeva et al. 2011). It is known that heating to 900 °C destroys the clay structure, leaving only oxide compounds of the clay (Haydel et al. 2008). Moreover, Sawai (2003) reported that metal oxides, especially ZnO, MgO and CaO, possess antibacterial activity. Oxides are readily hydrated and then dissolved and in such a way that increases electrical conductivity. Electrical conductivity characterizes the presence of soluble ions but can not estimate the exact concentration of ions (Helfferich 1995).

In our previous experiments with expanded clay ceramic granules, a relationship between electrical conductivity and pH value was stated (Nikolajeva et al. 2011). For *P. putida*, the isoelectric point occurs at pH 3 (Rochex et al. 2004). Below this value the net surface charge will be positive and above pH 3 will become negative. In our pH range from 5 to 8, as the pH decreased, the negative charge probably decreased. However, as we can see in Fig. 3, the pH-dependence of adhesion can not be described as a linear function. Evidently, this process is much more complicated and can not be explained only with electrostatic attraction between bacteria and ceramic surface.

Based on *P. putida* cell size from 0.5 to 0.6 µm in diameter and from 1.4 to 1.7 µm in length (Hwang et al. 2008), it was calculated that one bacterium could cover up to 1 µm2 of the surface. However, the surface allocated for one bacterial cell represents 2.2 μm^2 in the case of initial bacterial concentration of 11 log CFU mL^{-1}. Thus, the adhesion intensity attained in the present experiments is sufficient for subsequent development of bacterial colonies in adequate environmental conditions. Further studies are necessary to determine microbial biofilm formation on the surface of clay ceramic granules.

Acknowledgements

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