low concentrations in indoor air. The synthesis procedure offers cost-effective alternative to conventional adsorbent by virtue of single treatment for a wide range of VOCs. Also, it overcomes the problems associated with commercial adsorbents, namely activated carbon and Tenax, like regeneration, analyte catalytic degradation and polymerization of VOCs. The adsorption efficiency of SMZ was compared with activated carbon. This revealed that SMZ is more selective for adsorption of VOCs having a wide range of volatilities. Thus, with enhanced adsorption capacity for several VOCs, better stability and regeneration possibility, the use of SMZ-Y as adsorbent is an advancement for VOC monitoring.


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**Aerobic granular biomass: a novel biomaterial for efficient uranium removal**

Y. V. Nancharaiah, H. M. Joshi, T. V. K. Mohan, V. P. Venugopalan* and S. V. Narasimhan

Water and Steam Chemistry Division, BARC Facilities, Kalpakkam 603 102, India

Aerobic microbial granules, self-immobilized microbial consortia cultured in aerobically operated bioreactors, primarily consist of mixed species of bacteria ensconced in an extracellular polymeric matrix of their own creation. Such aerobically grown microbial granules have attracted considerable research interest in environmental biotechnology. In recent times, it has been demonstrated that the granules could be used for efficient degradation of recalcitrant organic compounds and for the treatment of a growing number of wastes. The objective of this study was to investigate whether aerobic granules could be used as novel biomass material for biosorption of uranium from aqueous solutions. The granular biomass for biosorption experiments was cultivated in a laboratory-scale sequencing batch reactor by feeding with synthetic wastewater. Biosorption of uranium [U(VI)] was studied at different initial pH values (1 to 8) and different initial uranium concentrations (6 to 750 mg l⁻¹). Biosorption was observed to be rapid (<1 h) in acidic pH range (1 to 6) compared to that at pH 7.0 or above. Almost complete removal of uranium was observed in the range 6–100 mg l⁻¹ in less than 1 h. Redlich–Peterson model gave the best fit when the experimental data were analysed using different adsorption isotherm equations. The maximum biosorption capacity of U(VI) was determined to be 218 ± 2 mg g⁻¹ dry granular biomass. Further, it was

*For correspondence. (e-mail: vpv@igcar.gov.in)
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observed that cations such as Na⁺, K⁺, Mg²⁺, and Ca²⁺ were simultaneously released into the bulk solution during U(VI) biosorption, indicating the involvement of ion exchange mechanism in radionuclide uptake. Live and dead biomass did not show significant difference in U removal, indicating the involvement of a passive sorption process. The study suggests that aerobic granular biomass has the potential to be employed as an effective biosorbent material for recovering/removing uranium (and probably other radionuclides) from dilute nuclear wastes.

Keywords: Aerobic granular sludge, biosorption, microbial granules, radionuclides, uranium.

HEAVY metal or radionuclide waste arises from activities like electroplating, mining, nuclear power generation and nuclear fuel reprocessing. Among the radioactive contaminants, uranium, neptunium and plutonium are the most problematic because they pose long-term environmental risks. Physico-chemical methods are often expensive to apply and not always suitable for cost-effective remediation of contamination. Biological approaches, on the other hand, offer the potential for removing metal/radionuclide pollutants from dilute solutions, where physico-chemical methods may not be feasible. Moreover, they can be employed using both in situ and ex situ methods. Such biological methods generally use microbial consortia, consisting of several species of microorganisms in the form of biofilms for removing/degrading the pollutants. In natural environments microbial communities predominantly (~99%) exist as biofilms, which often possess significant capability to immobilize metals. The different mechanisms by which biofilms immobilize metals or radionuclides include: (1) biosorption to cell components or extracellular polymeric substances (EPS), (2) bioaccumulation, (3) precipitation by reaction with inorganic ligands such as phosphate and (4) microbial reduction of soluble metal to insoluble metal. Aerobic granular biomass is considered a special form of biofilm formed without a carrier material (substratum) and is cultured using sequencing batch reactors. It basically consists of mixed species of bacteria held together by their own extracellular polymeric substances (EPS). Granular biomass has attracted a lot of research interest because of its characteristics such as superior settling ability, compact and dense microbial organization, good porosity, high biomass retention and the ability to withstand fluctuating shock or toxic loadings. Such characteristics of granular biomass implies that it could be considered as potential candidate for biosorption of toxic substances. Results of some preliminary studies on the metal immobilization capability of this novel biomass have been published for a few metals such as cadmium, copper, nickel and zinc. However, no data are available on biosorption of radionuclides by aerobic granular biomass. It is not clear whether it could remove metal pollutants from dilute solutions, where the metal concentration is less than ~20 mg/l. Such approach is necessary for developing cost-effective alternatives to other physico-chemical methods. The main objective of the study was to investigate whether aerobic granules could be used as novel biomass for biosorption of uranium from dilute aqueous solutions. In addition, experiments were carried out to study mechanistic aspects of uranium biosorption by aerobic granular biomass. The significance of the work is that techniques based on microbial granules could be used for the development of compact bioreactors for immobilizing uranium from low-level liquid nuclear waste generated during nuclear fuel cycle operations.

Aerobic granular biomass was cultivated in a 3.0 l working volume column-type sequencing batch reactor (SBR). Briefly, the SBR was inoculated with 800 ml of wastewater consisting of activated sludge flocs and operated at a cycle time of 6 h. Synthetic wastewater (SWW) was used as the feed. The chemical composition of SWW, prepared using tap water, is given in Table 1. Granular biomass was collected after two months of SBR operation and was used in uranium biosorption experiments. Before using, these granules were separated from coexisting flocs through sedimentation by simply allowing them to settle for 1 min and were washed thoroughly with deionized water. Morphology of the aerobic granular biomass was determined by capturing images using a digital camera (Olympus DP70) connected to a stereozoom microscope (Nikon SMZ1000). Average size of the granules was calculated using the image analysis freeware ImageJ 1.99 (http://rsb.info.nih.gov/ij). Gross microbial composition of the inoculum and internal microbial organization of the granules were analysed using a confocal laser scanning microscope (CLSM) (model Leica TCS SP2 AOBS, equipped with an inverted microscope Leica DMIRE2). For confocal imaging, the microbial granules were stained with 0.01% acridine orange for 15 min and thoroughly rinsed with phosphate-buffered saline for 15 min. A stained individual microbial granule was mounted on a cover slip and imaged using a 63 × 1.2 NA water immersion objective. The 488-nm line from an argon laser was used for excitation and the emission was collected by setting the detection bandwidth between 510 and 550 nm.

Uranium solutions for biosorption were prepared by suitably diluting a 10.0 g l⁻¹ stock solution. The uranium stock

Table 1. Composition of synthetic waste water used as feed for cultivation of granular biomass in the sequencing batch reactor (prepared in tap water)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>63</td>
</tr>
<tr>
<td>Nitritotriacetate</td>
<td>0.26</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>3.6</td>
</tr>
<tr>
<td>KCl</td>
<td>4.7</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>35.4</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>4.2</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>2.1</td>
</tr>
</tbody>
</table>

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was prepared by dissolving uranyl nitrate (UO$_2$(NO$_3$)$_2$) (eMerck, India) in deionized water. The effect of pH (ranging from 1 to 8) on uranium removal by granules was studied using 100 mg l$^{-1}$ solution. Subsequently, biosorption was studied at various initial U(VI) concentrations (ranging from 6 to 750 mg l$^{-1}$) at pH 4.0, in order to determine the maximum biosorption capacity of the granular biomass. All these experiments were performed in triplicate in 250 ml Erlenmeyer flasks containing 100 ml of U(VI) solution. After adjusting the pH with 1 M NaOH, the granules (wet weight: 5.0 g) were introduced into each flask. The Erlenmeyer flasks were incubated in a rotary shaker set at 100 rpm and 30°C. Samples of 0.5 ml aliquots were drawn from each flask at pre-defined time intervals for uranium analysis. The dry weight of the biomass was determined at the end of biosorption experiments by heating at 80°C for 24 h. Appropriate control experiments (without adding granular biomass) were also carried out simultaneously. In order to check whether U biosorption was mediated through active or passive processes, sorption experiments were carried out using live as well as dead biomass (granules). Dead biomass was obtained by heat-killing the granular biomass at 80°C for 1 h. This method did not affect the physical integrity of the granules.

Soluble uranium in the samples was analysed spectro-photometrically using arsenazo III method. The samples were centrifuged before analysis (8000 rpm, 5 min) to remove all suspended biomass. Solid-phase uranium associated with granules was analysed by X-ray photoelectron spectroscopy (XPS). A sample of granular biomass incubated with 100 mg l$^{-1}$ U solution for 24 h was freeze-dried using lyophilizer before analysis by XPS. During sorption experiments, it was observed that light metal (Na, K, Mg and Ca) ions were released from the biomass into solution during U(VI) uptake. Such metal ion release was analysed by flame photometry or atomic absorption spectrophotometry.

Experimental kinetic data were fitted using both first-order and second-order equations. Three equilibrium adsorption isotherm models (Langmuir, Freundlich and Redlich–Peterson) were used for analysing the experimental data obtained at pH 4.0 and describing the mechanisms of sorption.

Figure 1 a shows the gross morphology of the granular biomass obtained from the SBR and used as the biosorbent material. The granules were nearly spherical in shape and light brown in colour (Figure 1 b). The mean size of the granules used in the biosorption experiments was about 1.1 mm. Sludge volume index (SVI) of the granules was 40 ml g$^{-1}$, indicating excellent settling ability and compactness of the biomass. CLSM showed characteristic three-dimensional microbial organization in the granules.
Peripheral part of the granule was dominated by rod and cocci-shaped bacteria. Filamentous bacteria were not present in the mature granules, though they were abundant in the inoculum used for reactor-seeding. Bacterial cells appeared to be neatly organized into clusters or microcolonies within the granules, with several clearly defined, regular cell clusters consisting of uniform bacterial morphology, separated by void spaces in between (Figure 2). These cell clusters were enmeshed within EPS.

Figure 3a shows U(VI) uptake from 100 mg l⁻¹ U(VI) solution by granular biomass at different initial pH values, determined after 1 h incubation time. The U(VI) uptake was maximal between pH 3.0 and 5.0. Time course of U(VI) removal from 100 mg l⁻¹ U(VI) solution by granular biomass at pH 4.0 and 7.0 is shown in Figure 3b. Biosorption of U(VI) was faster at acidic pH (4.0) compared to that at neutral (7.0) or basic pH (8.0). However, the difference between specific U(VI) uptake at pH 4.0 and 7.0 was minimal after 24 h of contact time. Based on these results, all subsequent experiments were carried out at a pH of 4.0. Figure 3b shows soluble U(VI) removal by granular biomass from different initial U(VI) concentrations at pH 4.0. Almost complete removal of soluble U(VI) from 6 to 100 mg l⁻¹ U(VI) solution was observed within one hour of incubation (Figure 4). The U(VI) remaining in these solutions after biosorption was estimated to be less than 1 mg l⁻¹. In the case of 140 mg l⁻¹ U(VI) initial concentration, about 10 mg l⁻¹ U(VI) was remaining in solution even after 24 h of incubation (Figure 4). At the end of the experiments (pH 1 to 8; incubation time 24 h), physical appearance of the granular biomass used was intact and no granule disintegration was observed.

The experimental isotherm data were analysed using Langmuir, Freundlich and Redlich–Peterson isotherm equations to quantitatively express the relationship between the extent of sorption and the residual solute concentration. The amount of uranium associated with unit weight of the granular biomass (q) as a function of the residual equilibrium concentration ([e]) in solution was plotted. Among the three isotherm equations, the data fitted best (r² = 0.98) with the Redlich–Peterson isotherm (Figure 5). Uranium uptake increased initially in a linear manner and reached a plateau, representing the maximum uptake capacity of the granular biomass. The maximum uranium uptake capacity (q_max) of the granular biomass was estimated to be 218 ± 2 mg U g⁻¹ dry weight (0.92 mmol g⁻¹ dry granule biomass). The difference between U(VI) uptake by live and heat-killed biomass was not significant (Table 2). Analysis of the bulk solution indicated that cations such as Na⁺, K⁺, Mg²⁺, and Ca²⁺ were released from the aerobic granular biomass simultaneously during U(VI) uptake. Figure 6 shows the total amount of metal ions released into the solution during U(VI) uptake by the granular biomass from U(VI) solutions of 50 and 100 mg l⁻¹ initial concentrations (all experiments carried out at pH 4.0). The amount of metal ions released was in the order K⁺ < Ca²⁺ < Mg²⁺ < Na⁺.
release of metal ions was more in the case of 100 mg L\(^{-1}\) uranium solution (Figure 6). After the biosorption experiment, the divalent cation concentration in the 100 mg L\(^{-1}\) solution was almost double compared to that in the 50 mg L\(^{-1}\) U solution. Subsequent analysis of the granular biomass by XPS revealed that it was extensively loaded with uranium (Figure 7). This confirmed that uranium in the solution was immobilized onto the granular biomass during the course of biosorption.

In recent times, aerobic microbial granules have attracted a lot of research interest in view of their potential in developing viable biotechnological solutions for simultaneous degradation/removal of organic and inorganic pollutants from municipal and industrial wastewaters. A few studies on the metal immobilization capabilities of aerobic granular biomass for developing novel compact biosorbents have been reported. Immobilized microbial systems offer several advantages, including higher mechanical strength, easier biomass/liquid separation and increased metal tolerance. Immobilized living biomass has been mainly used in the form of bacterial biofilms developed on inert supports in a variety of bioreactor configurations. In aerobic microbial granules, the biomass is self-immobilized (without any substrate) into compact granules under aerobic conditions. For biotechnological applications, such granules are more promising than substratum-grown biofilms. Granules, because of their high microbial density and excellent settling ability, enable easy post-separation. For example, the settling velocity of the microbial granules used in the current study was about 70 m h\(^{-1}\).

Numerous microorganisms have been shown to remove soluble heavy metals and radionuclides, in particular uranium, to varying degrees. In the case of granular biomass, it is seen that uranium biosorption is pH-dependent, with optimal pH between 3.0 and 5.0 (Figure 2a). These results are in accordance with the observations of Sar et al. on uranium biosorption by Pseudomonas strain. The pH dependence of uranium biosorption is possibly related to the influence of pH on the functional groups present on the bacterial cell surface and EPS. Initial pH also influences the speciation of uranium. Monovalent uranyl species (UO\(_2\)\(^{+}\), (UO\(_2\)\(_2\)(_2\)(OH)\(_4\))\(^{+}\) dominate at higher pH (pH 4.0–5.0), while divalent species (UO\(_2\)\(^{2+}\)) dominates at low pH (2.0)\(^{23}\). However, amounts of uranium sorbed by the granular biomass at pH 4.0 and 7.0 after 24 h of incubation are not significantly different (Figure 3b), suggesting that

**Table 2.** Uranium uptake by live and heat-killed granular biomass

<table>
<thead>
<tr>
<th>Type of biomass</th>
<th>Uranium uptake (mg U g(^{-1}) dry wt)</th>
</tr>
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<tbody>
<tr>
<td>Live biomass</td>
<td>51.03 ± 0.75</td>
</tr>
<tr>
<td>Heat-killed biomass</td>
<td>48.2 ± 0.80</td>
</tr>
</tbody>
</table>

**Figure 5.** Biosorption of uranium from solutions of different initial uranium concentrations. Specific uptake of uranium (mg U g\(^{-1}\) dry biomass) was plotted as a function of uranium remaining in the solution after 24 h incubation time.

**Figure 6.** Release of metal ions during uranium removal by granular biomass. Concentration of metal ions released into the bulk solution from granules exposed for 24 h to 50 and 100 mg U L\(^{-1}\) (initial concentration) is shown.

**Figure 7.** XPS analysis of granular biomass-associated uranium. Granules retrieved after incubating with 100 mg U L\(^{-1}\) solution for 24 h were lyophilized and analysed by XPS.
initial pH influences the rate of uranium biosorption but not the total U sorption. The results indicated that uranium biosorption was efficient in the first few minutes. This is in accordance with heavy-metal biosorption by anaerobic and aerobic granular biomass reported by others. Most of the available uranium (6–100 mg L$^{-1}$ U) in the solution could be removed within the 1 h of contact (Figure 4). The time course of uranium removal from different initial U concentrations suggests that granular biomass could be efficient for rapid radionuclide removal from dilute solutions (Figure 4). The efficiency is noteworthy since the soluble uranium remaining after biosorption is considerably low (<1 mg L$^{-1}$ U). Apparently, aerobic granular biomass could be useful for removing uranium from low-level liquid wastes (say, 1 to 15 mg L$^{-1}$ U), where physico-chemical methods may not work or are expensive.

The experimental time-course data fitted well with the second-order equation. Reaction order of biosorption is dependent upon the characteristics of the heavy metals as well as the nature of the sorption sites available on the biosorbent. Recently, van Hullebusch et al. described that experimental data on biosorption of Ni$^{2+}$ and Co$^{2+}$ on anaerobic granular sludge fitted well with pseudo second-order reaction. However, biosorption of Cd$^{2+}$ on aerobic granular sludge followed a first-order reaction. The relationship between the equilibrium metal uptake capacity and residual metal ion concentration was fitted using Langmuir, Freundlich and Redlich–Peterson isotherm equations. The experimental data fitted well with the Redlich–Peterson isotherm ($r^2 = 0.98$). From the graph, the maximum uranium biosorption capacity was estimated to be 218 ± 2 mg g$^{-1}$ dry biomass. Sar et al. reported a maximum uranium uptake of 541 mg g$^{-1}$ Pseudomonas dry biomass. However, uranium loading capacity of the strain was significantly reduced when the cells were immobilized in a polymerized acrylamide matrix. Maximum uranium loading observed in the present study is comparable to that reported in the earlier works on Cd$^{2+}$ or Zn$^{2+}$ biosorption by aerobic granular biomass. Metal-loading capacity of greater than 15% of dry biomass is economically viable for practical application as biosorbents when compared to alternative methods. Therefore, uranium removal capacity of granular biomass observed in the present study (about 22%) surpasses the economic threshold, from the viewpoint of its practical application.

Recently, Pollman et al. have emphasized that pH stability, ability to be employed in repeated cycles and ease of immobilization are of special importance for successful application of biomaterials as biosorbents. Microbial granules can be successfully developed in sequencing batch reactors by employing appropriate selection pressure (high shear coupled with aerobic starvation and short setting times). Biogranulation under aerobic conditions is relatively rapid and mature granules could be obtained within a few weeks of reactor operation. Analysis of bulk solution revealed that metal ions such as Na$^+$, K$^+$, Ca$^{2+}$ and Mg$^{2+}$ were released during uranium uptake (Figure 6). It is clear from these results that ion exchange mechanism is involved in the uptake of uranium by granular biomass. Release of light metal ions such as K$^+$, Mg$^{2+}$ and Ca$^{2+}$ during nickel (Ni$^{2+}$) uptake by granular biomass was reported earlier. Immobilization of heavy metal ions or radionuclides can result from (1) sorption to cell wall components, (2) sorption to the EPS matrix, (3) intracellular sequestration as insoluble organic or inorganic compounds, or (4) complexation with minerals present in the granular sludge. Presently, research on biofilm–metal interactions is focused on understanding the mechanisms underlying metal immobilization by biofilms for developing viable technological solutions. Extraction techniques yield limited information on the nature and localization of the interactions and, therefore, other in situ techniques are needed to get insight into metal–biofilm interactions.

Recent developments in analytical tools have allowed understanding the biofilm structure and interactions in situ. Application of CLSM has led to better understanding of the architecture of biofilms and their temporal development. CLSM can be used for determining the spatial organization of bacteria within fully hydrated microbial granules. CLSM analysis of granules revealed that bacterial cells were organized into clusters and enmeshed in an extensive EPS matrix. Several large void spaces/channels seen within the granules may facilitate the transfer of water/nutrients in and out of the granules. Earlier workers have used CLSM for studying the distribution of metals within biofilms. For example, Wurz et al. have used the fluorescent dye Newport Green with CLSM to study the penetration of nickel, zinc and cobalt into biofilms. In another study, the spatial distribution of zinc in biofilms was determined using a zinc-binding fluorophore (8-hydroxy-5-dimethylamino-quinoline) and two-photon laser scanning microscopy. It was reported that zinc was distributed evenly in thin (12 μm) biofilms but was located only at the surface of thick biofilms, penetrating less than 20 μm after 1 h incubation time. In comparison, the granular biofilm used in the present study was very thick (mean diameter 1.1 mm) and therefore, surface localization of metals appears likely. Results of XPS analysis, which is basically a surface analysis tool, showed loading of uranium on the granular biomass, indicating that uranium was probably concentrated on the granule surface. Porosity of granular biofilms would determine the diffusivity of metal ions into the biofilms. Using a combination of scanning electron microscopy and energy dispersive X-ray detection, it has been shown that cobalt was strongly interacting with iron minerals, in addition to binding with EPS and carbonates in anaerobic granular sludge. Further experimentation is needed to understand the mechanisms underlying uranium immobilization onto aerobic granular biomass.

The use of aerobically grown granular biomass as novel biomaterials for removing soluble uranium has been demonstrated. XPS analysis showed that after the biosorption process, the biomass was extensively loaded with ura-
nium. Light metal ions such as Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+} and Mg\textsuperscript{2+} were simultaneously expelled from the granules, indicating involvement of an ion-exchange-driven uranium uptake process. Uranium removal from very low strength solutions suggests that granular biomass could find application in treatment of low-level liquid wastes, where other physicochemical methods may not be suitable or are expensive. In conclusion, low cost, ideal physical characteristics, fast kinetics and high metal uptake capacity make aerobically cultured granules ideal biomaterials for metal/radiouclide immobilization from dilute effluents.


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