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Research paper

Structural changes in smectite due to interaction with a biosurfactant-producing bacterium *Pseudoxanthomonas kaohsiungensis*

Bhabananda Biswas [a], Argha Chakraborty [a,b,c], Binoy Sarkar [a,*], Ravi Naidu [c,d,**]

[a] Environmental Science and Engineering Strand, Future Industries Institute (FII), Building X, University of South Australia, Mawson Lakes, SA 5095, Australia
[b] VIT University, Vellore 632014, Tamil Nadu, India
[c] Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE), ACT Building, University of Newcastle, Callaghan, NSW 2308, Australia
[d] Global Centre for Environmental Remediation (GCER), Faculty of Science and Information Technology, University of Newcastle, Callaghan, NSW 2308, Australia

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ABSTRACT

Clays including bentonite hold a great potential in improving the efficacy of organic contaminants degradation by bacteria. However, the mechanisms of interactions involving both biotic (microorganisms) and abiotic (clays) components during bioremediation are largely unknown. Here, we report the interaction of a biosurfactant producing bacterium, *Pseudoxanthomonas kaohsiungensis*, with bentonite clay. Using instrumental analyses including X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, inductively coupled plasma mass spectroscopy (ICP-MS), thermogravimetric analysis (TGA) and scanning electron microscopy (SEM) techniques we investigate the microstructural changes of smectite following introduction of the bacterium. The interaction resulted in a spherical clay-bacterial micro-agglomerate formation which deciphered an enhanced growth of the bacterium in a minimal medium supplemented with traces of olive oil. The bacterium brought about a significant dissolution of silicon (Si) preferentially from the tetrahedral silica edges of smectites. The deposition of bacterial biosurfactants and exopolysaccharides (EPS) slightly expanded the smectite interlayers and modified the clay's interaction with water molecules. This study has direct implication in the clay-mediated bioremediation of hydrophobic organic contaminants in the environment.

1. Introduction

Microorganisms, which are abundant in clay-rich soils and sediments, can utilise the clay minerals as their growth supplements (Lawrence et al., 2000; Kim et al., 2004; Dong, 2012). Additionally, the clay minerals can provide a protective habitat to the microorganisms for shielding them from harsh environmental conditions (Stotzky and Rem, 1966; Stotzky, 1985). Such a natural co-existence of the biotic (microorganisms) and abiotic (clay minerals) soil components has encouraged the scientific community to explore a potential clay-modulated microbial remediation strategy for cleaning up environmental contaminants (Sarkar et al., 2012; Ugochukwu et al., 2014; Biswas et al., 2015a; Biswas et al., 2015b). In this approach, while the clay mineral adsorbents pre-concentrate the contaminant molecules on the surface, the microorganisms thriving on the same particles carry out the degradation in a subsequent step (Sarkar et al., 2012). However, efficiency of this remediation approach largely depends on the type of clay-microbial interactions under specific conditions which may involve variable contaminants, clay minerals and microorganisms (Biswas et al., 2015b). Information about such interactions is very limited in the literature.

Since bacteria can play a key role in the biodegradation of organic contaminants (Mao et al., 2012), a one-to-one interaction between a particular bacterial strain and a clay mineral warrants detail investigation for understanding the clay-modulated bacterial degradation process. It is particularly important to understand whether and how the bacterium induces any change in the structure and properties of the clay mineral during an interaction.

It was suggested that hydrocarbon degrading bacterial proliferation in heavy oil polluted marine environment could be enhanced by clay minerals (Chaerun and Tazaki, 2005). The growth of bacteria for degrading contaminants was characterised by the formation of a clay-bacterial cluster which ultimately favoured biofilm formation (Lünsdorf et al., 2000). Such a biofilm could mitigate the poor bioavailability of organic contaminants in soils and sediments (Semple et al., 2003; McAllister and Semple, 2010). A co-existence of biosurfactant-producing bacteria in the clay-bacterial cluster might enhance the contaminant bioavailability even better (Johnsen and Karlson, 2004; Nayak et al., 2009) because these surface active compounds having an amphipathic structure (both non-polar and polar functional ends) could increase the solubility of hydrophobic compounds through reduction of

* Correspondence to: B. Sarkar, University of South Australia, Australia.
** Correspondence to: R. Naidu, University of Newcastle, Australia.
E-mail addresses: binoy.sarkar@unisa.edu.au (B. Sarkar), ravi.naidu@newcastle.edu.au (R. Naidu).

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surface tension (Desai and Banat, 1997; Ron and Rosenberg, 2002). While clay-modulated biofilm formation holds a great potential in contaminants bioremediation (Huang et al., 2013; Biswas et al., in press-b), the interaction of biosurfactant-producing bacteria with clay minerals is not fully understood (Rong et al., 2008; Vasiliadou et al., 2011; Hong et al., 2012; Hong et al., 2013).

Studies relating to the compatibility of biosurfactant-producing bacteria with clay minerals and possible changes in the clay mineral structure due to such interaction are scarce in the literature. Bacteria could cause elemental dissolution from crystalline clay minerals through releasing extracellular metabolites which also include the biosurfactants (Vorhies and Gaines, 2009). The release of major elements (Si and Al in clay minerals) would depend largely on the concerned bacterial species (its pH lowering and metabolite producing capacity) as well as the defects of the clay structure involved (Perdrial et al., 2009). The released interlayer cations (e.g., Na^+, Ca^{2+}, etc.) might assist in bacterial cell attachment on mineral surfaces through creation of cation-bridges (Perdrial et al., 2009; Warr et al., 2009; Biswas et al., in press-a). The dissolution would also result in the formation of water containing, biofilm related amorphous substances surrounding the clay minerals (Chaerun and Tazaki, 2005; Perdrial et al., 2009).

In this work, therefore, we aim to investigate the interaction of *Pseudoxanthomonas kaohsiungensis*, a biosurfactant-producing bacterium, with an Australian bentonite in nutrient-supplemented aqueous media, and explore the structural and physico-chemical changes in the clay due to this interaction.

2. Materials and methods

2.1. Reagents and starting clay

All the chemicals used in this study were of analytical grade, purchased from Sigma-Aldrich, Australia, and used without further purification.

A Watheroo bentonite (Bent) (purchased from Bentonite Products, Western Australia) was obtained from a desiccated stock (60 °C hot air dried material; grinded and sieved to collect ≤75 μm sized fraction). The bentonite sample possessed a cation exchange capacity (CEC) value of 85.8 cmol(p^+) kg^{-1}) and a specific surface area (SSA) of 15.4 m^2 g^{-1}. The major chemical composition of the bentonite was 47.06% SiO_2, 25.28% Al_2O_3, 6.51% TiO_2, 2.34% Na_2O, 2.81% Fe_2O_3, 0.67% CaO and 0.23% K_2O based on an energy dispersive X-ray (EDX) spectroscopy analysis. As also obtained through X-ray diffraction (XRD) analysis, the bentonite sample contained montmorillonite (84%) as the principle smectitic clay mineral along with a significant impurity of quartz (see Sections 2.4.4 and 3.4). The ζ-potential of the bentonite suspension (0.05% *w*/*v* in Milli-Q water (18.2 MΩ·cm at 25 °C), measured by Nicomp 380 ZLS, USA) was −18.6 at pH 2 and −37.5 at pH 10 with a gradual increase of negative ζ-potential as the pH values increased.

2.2. Preparation of culture inoculum

The bacterium used in this study (*Pseudoxanthomonas kaohsiungensis* J36^T) was isolated from a petroleum oil contaminated wastewater (Chang et al., 2005). Freeze-dried *P. kaohsiungensis* (PK) cells were collected from the German Collection of Microorganisms and Cell Cultures (DSMZ) and revived in Tryptic Soy Broth (TSB). The cells were then subcultured in a minimal salt media (12.8% $Na_2HPO_4 \cdot 7H_2O$, 3% KH_2PO_4, 0.5% NaCl, 1% NH_4Cl, 0.2% 1 M $MgSO_4$, 0.01% 1 M $CaCl_2$ and 0.5% glucose). At the late log phase of growth (growth curve and electron microscopic image are provided in Supplementary Information; SI Figs. 1 & 2), the cells were harvested by centrifugation at 10,000 × *g* for 10 min. The inoculum in fresh minimal media was prepared at desired cell concentration (OD_{600} = 0.4) after washing twice with 50 mM sterile phosphate buffer saline (PBS). For all the experiments, the bacterium was handled aseptically in a laminar flow assembly and only sterilised lab ware were used.

2.3. Cultivation of bacterium with bentonite

Minimal salt supplemented with 0.5% glucose (see Section 2.2) was used as the dispersion medium of the bentonite and bacterial cells. In a total 300 mL medium volume, 3 mL of the bacterial suspension was inoculated with a clay loading rate of 0.5% (*w*/*v* basis). This batch culture was grown for 7 days at 25 °C either in the presence or absence of olive oil supplements (0.1% (*v*/*v*) of the final volume). Olive oil is known to induce the biosurfactant producing ability of the bacterium (Makkar and Cameotra, 1999; Mulligan, 2009). A sample without the inoculum served as the biotic control. Another control without the clay was also maintained. All experiments were conducted in duplicate.

2.4. Characterisation of clay-bacterial interaction

2.4.1. Growth pattern of P. kaohsiungensis

An initial growth curve study was carried out with the minimal salt media, which showed that the *P. kaohsiungensis* completed its active exponential phase in 1–2 days followed by its stationary and potential decline phase within 7 days (Supplementary Information, SI Fig. 1). Based on that preliminary growth curve analysis, the actual experiment was monitored for 7 days to assess the growth pattern in the different treatments. At each sampling point, the culture treatments were mildly agitated before withdrawing 1 mL dispersion for serial dilution (10^6–10^9) in 0.1% NaCl solution. The growth of the bacterium was determined by counting the colony forming units (CFU) on TSA plates following 3–5 days of incubation at 25 °C.

2.4.2. Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analyses

The interaction of *P. kaohsiungensis* with the smectite surfaces was investigated at a microscopic level using a FEI Quanta 450 FEG scanning electron microscope (FEI, USA). The clay-bacterial agglomerate sample was prepared by diluting the original dispersion 10 times in double filtered Milli-Q (18.2 MΩ·cm) water. A dry and well-polished graphite disc was mounted on a double-sided carbon tape sitting on an aluminium stub. A small drop of the diluted sample was taken on the graphite disc and vacuum dried. A 20 kV accelerating voltage was applied to take the images in high vacuum mode using an Everhart-Thornley Detector (ETD) combined with a solid state Back Scattered Electron (BSE) detector. A cold stage was used to mount the hydrated samples, and was examined using Environmental SEM mode at a pressure of 814 Pa at 4 °C. The samples were further analysed by EDX in selected areas for 100 s at a point using an Apollo EDX detector (EDAX®, USA). A semi-quantitative analysis of the major elements was performed by eZAF using TEAM™ EDS software suite.

2.4.3. Elemental dissolution from bentonite

To monitor the potential dissolution of elements (e.g., Si and Al) from the bentonite into the culture media, a cell-free supernatant was collected through centrifugation of the culture at 13,000 × *g* for 20 min, which was further passed through a 0.45 μm multi-cellulose filter. The selected elements in the filtrate were analysed by inductively coupled plasma mass spectrometry (ICP-MS) (Model: 7500c, Agilent Technologies, USA). A subtraction of the concentration of elements in the control (without bacterium) from that in the clay-bacteria treatment was counted as the potential dissolution of elements by the action of bacteria.

2.4.4. X-ray diffraction (XRD)

Pellets from the batch culture experiments were obtained by centrifugation at 3400 × *g* for 20 min at 4 °C, and further lyophilised on a freeze-drier (Thermo Fisher ModulyoD Freeze-Dryer, USA) at a constant

temperature (−45 °C) and pressure (3.3 mbar) for 24 h. The powdered pellets were pressed in a stainless steel sample holder for collecting the XRD patterns using CuKα radiation (λ = 1.5418 Å) on a PANalytical, Empyrean X-ray diffractometer operating at 40 kV and 40 mA between 1° and 90° (2θ) at a step size of 0.016° (100 s scan step time). A fixed 0.5° divergence slit and 1° *anti*-scatter slit was used. The mineral phases were identified by the X'Pert HighScore Plus software using the International Centre for Diffraction Data (ICDD) database.

2.4.5. Fourier transform infrared (FTIR) spectroscopy

The powdered pellets (about 0.1% *w*/w) were grinded, mixed homogenously with dehydrated KBr and pressed into discs for FTIR analysis. Infrared (IR) spectra were obtained using an Agilent Cary 600 series FTIR spectrometer over the 4000 cm^{-1}–400 cm^{-1} range by the co-addition of 64 scans with a resolution of 4 cm^{-1}.

2.4.6. Thermogravimetric analysis (TGA)

Thermogravimetric Analysis (TGA) and Differential Thermogravimetric Analysis (DTGA) of the dried pellets were conducted on a Mettler-Toledo DSC-1 thermogravimetric analyser (Mettler-Toledo International Inc., USA). Approximately 25 mg of finely grinded and homogenised sample was heated in an open platinum crucible. The temperature for TGA was raised at a rate of 10 °C min^{-1} ranging from 25 °C to 1000 °C with a resolution of 6 °C under continuous N_2 flow (50 mL min^{-1}).

3. Results and discussion

3.1. Proliferation of P. kaohsiungensis in bentonite suspension

Bentonite enhanced the growth of *P. kaohsiungensis* (PK) depending on the growth phase of this bacterium (Fig. 1). In the control (no clay), PK completed its log phase at day 2 by reaching a maximum of 81×10^8 CFU mL^{-1}; this growth pattern was similar to the previous description of this bacterium (Chang et al., 2005). The growth then encountered a quick decline and remained steady for 3–7 days with an estimation of 18–41 $\times 10^8$ CFU mL^{-1} (Fig. 1A). However, the presence of bentonite in the culture media influenced the early log phase significantly where PK proliferated up to 66×10^8 CFU mL^{-1} at its log phase from the initial value of 15×10^8 CFU mL^{-1} (Fig. 1B). Bentonite also promoted the recovery of growth decline at day 3 and onwards (Fig. 1B). This extended stationary growth of PK reiterated that bentonite could provide a microbial friendly microenvironment with the supportive surface and elemental properties (Chaerun and Tazaki, 2005; Warr et al., 2009; Chaerun et al., 2013; Biswas et al., 2015b). A supplement with olive oil (0.1%) in both the control and bentonite-amended culture further shifted the growth of PK (Fig. 1C and D). Since PK is a biosurfactant-producing bacterium (Chang et al., 2005), the olive oil supplement could induce the production of biosurfactant by the microorganism by increasing the availability of organic compounds (e.g., additional carbon source in the culture media) and thus enhance the growth pattern accordingly. Interestingly, a combination of bentonite and olive oil considerably shortened the growth decline period of PK after the first log phase (see day 2, Fig. 1D). The recovery of this initial growth-fall was remarkable and reached afterwards up to 167×10^8 CFU mL^{-1} at day 6 (Fig. 1D). No treatment other than the combined bentonite plus olive oil medium could impart such a positive effect to the bacterial growth. It could be assumed that the clay layers might have acted as a slow release sink for supplying the growth supplement (olive oil) (Choy et al., 2007), which would have supported the bacterial proliferation over a longer period of incubation.

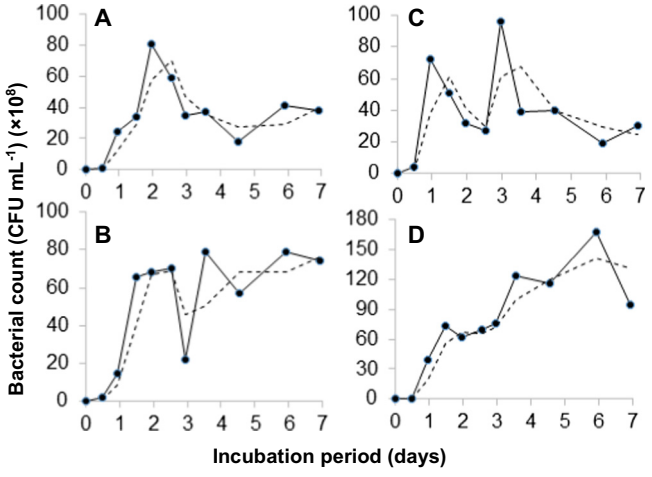

Fig. 1. Growth patterns of PK in (A) control media (minimal salt with 0.5% glucose), (B) supplemented with bentonite, (C) supplemented with 0.1% olive oil, and (D) supplemented with 0.1% olive oil plus bentonite. The solid lines with circles represent the original curve and the dotted lines represent the average projection of two replicates).

3.2. Visualisation of clay-bacterial interaction and elemental profiling

Clay minerals can form hutch-like structure when interacting with bacteria (Lünsdorf et al., 2000; Biswas et al., in press-a). Such a hutch could form in the culture media where the clay particles and bacterial cells mutually attach each other in a cluster, which might provide a microhabitat to the bacteria. In the current study, spherical and well-structured agglomerates were observed in the interaction of the bentonite and the biosurfactant-producing bacterium (*P. kaohsiungensis*) (Fig. 2B and C). The control (without the bacterium) did not show any such structure (Fig. 2A). This could be attributed to the production of biosurfactants and other extracellular polymeric substances (EPS) in the bentonite-supplemented PK culture, which could bind the abiotic (clay) and biotic (PK) components together to make a distinguished structure (Fig. 2B and C). The EPS might contain protein, carbohydrate, lipid and nucleic acids, which could form different moieties of those major components (e.g., EPS-nitrogen moiety from protein-rich EPS) (Mangwani et al., 2014). Such organic moieties could influence the attraction of bacterial cells onto the solid surfaces (Biswas et al., 2015b). The organic nature of these clay-bacterial agglomerates was confirmed by EDX microanalysis (see corresponding spectra in Fig. 2). Comparatively higher amount of carbon (48.47 ± 9.74%) and oxygen (26.64 ± 11.8%) species were detected in these agglomerates due to the possible contribution of the EPS moieties (Table 1). The presence of olive oil contributed even more organic substances (61 ± 7.92% C and 14.49 ± 13.41% O, Table 1) in a smear type clay-based bacterial biofilm (Fig. 2C). The presence of bentonite was also confirmed in the agglomerates by the appearance of Si and Al (Fig. 2B and C, Table 1). As expected, the intensity of the clay signature (Si and Al) was less in the PK containing agglomerates (Fig. 2B & C) than the bentonite alone treatment (Fig. 2A) likely because a less proportion of clay per unit mass of the material was present in the clay-bacterial agglomerates (Table 1). These results also indicated a possible dissolution of Si and Al from the clay structure as a result of the bacterial interaction, which has been discussed in Section 3.3.

3.3. Elemental dissolution from bentonite

The bacterial activity increased the dissolution of major elements (Si and Al) from the smectite structure into the salt media (Fig. 3). At the end of the incubation period, Si (4536 ± 98.9 μg g^{-1} clay) and Al (119.1 ± 1.0 μg g^{-1} clay) were detected in the cell-free clear supernatant, which was ca. 0.45% and 0.012% loss of Si and Al, respectively, from the original composition (Section 2.1). This elemental release occurred due to the intervention of PK, which was significantly higher ($p < 0.05$) than the dissolution rate in the biotic control suspension (no PK) (Si = 244.4 ± 11.0 and Al = 0.12 ± 0.1 μg g^{-1} clay) (Fig. 3). Similar trend of Si and Al dissolution was previously reported from a

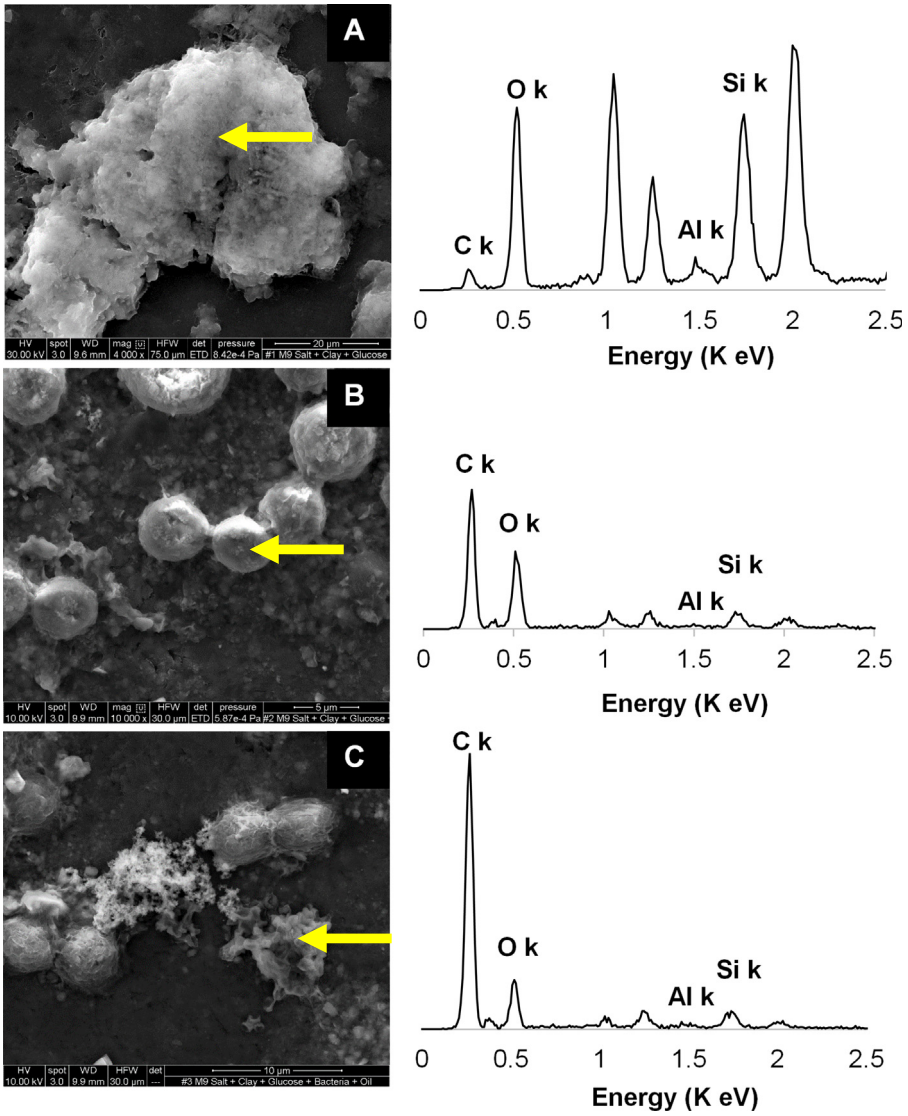

Fig. 2. SEM micrographs of clay-bacterial interactions and the corresponding EDX spectra from the arrow-indicated selected spots: (A) only bentonite, (B) bentonite-based PK agglomerates, (C) bentonite-oil-PK agglomerates and films.

Chinese bentonite by the action of a *Bacillus* sp. (Zhu et al., 2011). The dislodgement of major elements was also positively correlated with the increase of incubation time of the bacterial culture in liquid media (Zhu et al., 2011). In the present study, the 7 day-incubation allowed the viable PK cells to disintegrate the structural elements from the mineral structure. A higher amount of Si released into the solution than Al (Fig. 3), which indicated a preferential dissolution sites at the tetrahedral silica edges (Bickmore Barry et al., 2001). It could be assumed that the metabolites produced by the bacterium and thus the acidic pH might have accelerated the elemental dissolution (Zhu et al., 2011). This was confirmed by the lowering of pH value of the bacterial growth medium by 1.2 ± 0.2 unit at the end of 7 days of incubation. The ζ-potential profile of the bentonite with the change of pH also showed that the lower pH induced a comparatively positive surface charge (as indicated by the less negative ζ-potential value) (Supplementary Information, SI Fig. 3). This further indicated that the dislodgement of Si (Fig. 3) during the bacterial activity might also be due to the influence of pH (Au and Leong, 2013). At a comparatively lower pH value, the clay surface was more positively charged, which would

Table 1
Elemental composition of clay and clay-bacterial agglomerates.

EDS spot	Interaction	C (%wt ± error)	O (%wt ± error)	Si (%wt ± error)
Fig. 2A	Bentonite	7.67 ± 21.59	36.16 ± 11.19	4.75 ± 8.75
Fig. 2B	Bentonite-PK	48.47 ± 9.74	26.64 ± 11.8	3.48 ± 11.65
Fig. 2C	Bentonite-Oil-PK	61.17 ± 7.92	14.49 ± 13.41	2.9 ± 11.09

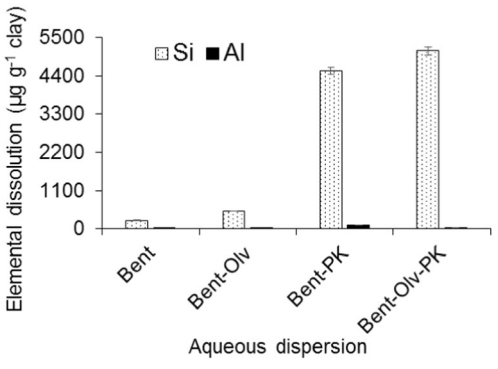

Fig. 3. Si and Al dissolution from bentonite into the culture media.

adhere more number of negatively charged bacterial cells through electrostatic attraction and impart a greater bacterial activity on the clay structure. The utilisation of the dislodged Si and Al by the bacterium is still hypothetical, which warrants further studies. While investigating the interaction of smectite with *Shewanella* sp., Perdrial et al. (2009) reported a possible uptake of Si by the bacterium. Contrarily, an excess of Al could be toxic to bacterial growth (Chaerun and Tazaki, 2005). Also, attachment of these elements on the bacterial cells might impart a bridging effect with the mineral surfaces and the food sources (Perdrial et al., 2009; Chaerun et al., 2013). The surface bound Si or Al could be released back to the solution when cells underwent death phase (Perdrial et al., 2009). In the current study, the elemental dissolution was observed at the end of 7 days, which coincided with a decline in cell growth (Fig. 1). However, a further study on such elemental dissolution at every growth phase of PK in the presence of bentonite would provide a confirmation of this growth rate-dissolution relation.

With the addition of olive oil in the growth media, the bacterial action caused slightly higher dissolution of Si (5114 ± 121.6 μg g^{-1} clay) and Al (36.6 ± 2.1 μg g^{-1} clay). Evidently the olive oil supplement enhanced the bacterial activity (Fig. 1D), which further induced the production of polysaccharides and other EPS, and thus a biofilm (Fig. 2). As will be described in the following section (Fig. 4, Section 3.4), these substances could expand the basal space by entering into the clay interlayers, which also might have accelerated the gradual dissolution of the major elements (Si and Al) (Chaerun et al., 2013).

3.4. Interlayer properties of montmorillonite

The characteristic XRD reflection (*001*) of montmorillonite appeared at 2θ = 6.81° (1.24 nm) (Fig. 4). Quartz impurity (2θ = 26.7°, 3.34 nm) was present in the raw bentonite and was not affected by the bacterial cultivation (Fig. 4). The addition of olive oil did not also make any noticeable change in the interlayer properties of the montmorillonite (Fig. 4B). This indicated that the small proportion of olive oil (0.1% in the 0.5% clay suspension) only adsorbed on the bentonite surfaces during the incubation period (7 days). On the other hand, due to the insertion of other organic molecules (EPS and other macromolecules produced by the bacterial activity), the basal space of montmorillonite increased slightly from 1.24 nm up to 1.30 nm (Fig. 4C and D). The slight increase of basal space indicated that the expansion of the interlayer of montmorillonite was a potential influence of the bacterial products (e.g., biosurfactant/EPS), and a higher production of these compounds might have a significant impact in the interlayer structure. While the functional relationship between the expansion rate and the loading of naturally produced bacterial substances would seek a further research, the current study indicated that the smectite structure was impacted by *P. kaohsiungensis*. As can also be seen in the SEM image (Fig. 2), this impact was from the active growth of bacterium on the bentonite by inducing a clay-based biofilm formation (Fig. 2) (Alimova et al., 2009; Chaerun et al., 2013).

3.5. Grafting of functional groups on smectite surfaces

The FTIR characteristic bands of montmorillonite at 1010 (Si—O stretching), 663 (Al—O + Si—O out of plane vibration), 462 (Si—O—Si deformation) and 3440–3625 cm^{-1} (broad OH stretching) (Madejová, 2003) remained unchanged in the bentonite sample in all the treatments (PK inoculated and "no bacterial" control) (Fig. 5). The olive oil in the culture media adsorbed on the montmorillonite surface, which was visible by the IR bands at 1720 cm^{-1} due to the ester carbonyl functional group of olive oil (Rohman and Man, 2010) (Fig. 5B). Additional bands at 2854 cm^{-1} and 2931 cm^{-1} were due to the stretching of CH_2 group which likely originated from the oil (Elkhalifah et al., 2012). However, the interaction of bacterium (PK) slightly shifted the bands of the ester carbonyl groups to 1735 cm^{-1}. This could be a direct evidence of the interaction between bacterial cellular substances and the oil ester compounds adsorbed on the clay surface. The IR band at 2360 cm^{-1} represented CO_2 from the atmosphere. However, in PK interacted montmorillonite, the peak intensity at that region (2360 cm^{-1}) could be due to the *anti*-symmetric stretching of –N = C = O group likely from EPS and other organic macromolecules (Mishra and Jha, 2009). While interacting with a clay mineral, bacteria would interfere with the water molecules present in the mineral structure (Alimova et al., 2009). *P. kaohsiungensis*, in this study also showed a similar interaction with bentonite as the water molecules (band at 1635 cm^{-1} of OH deformation of water) was interfered and the band shifted to 1666 cm^{-1} (Fig. 5C and D). This band shift (modified vibration pattern) also indicated the adsorption of EPS and biosurfactant on the clay surfaces (Cao et al., 2011). A further structural change in the smectite was observed by the IR band at 1535 cm^{-1} (NH_2 bending and C═O, C═N stretching), which was due to the presence of cellular proteins of PK (Filip and Hermann, 2001; Sheng et al., 2006) (Fig. 5C and D). A probable nitrogen impurity (band at 1465 cm^{-1} in montmorillonite, Fig. 5A and B) was also largely interfered by the bacterial interaction unless other physico-chemical factors were involved. As seen in Fig. 5 (C and D), band at 1465 cm^{-1} disappeared and two new bands (1403 cm^{-1} and 1450 cm^{-1}) appeared, which represented the fatty acid regions of the bacteria-produced organic molecules (Helm et al., 1991).

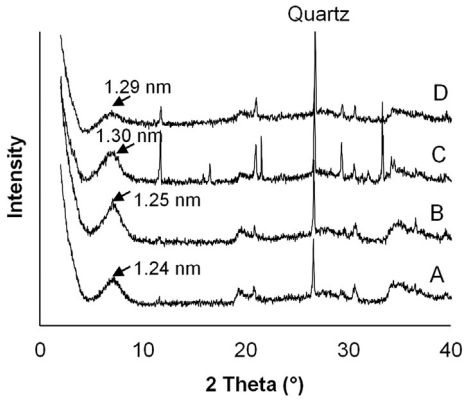

Fig. 4. XRD patterns of (A) bentonite, (B) bentonite–oil, (C) bentonite–PK, and (D) bentonite–oil–PK.

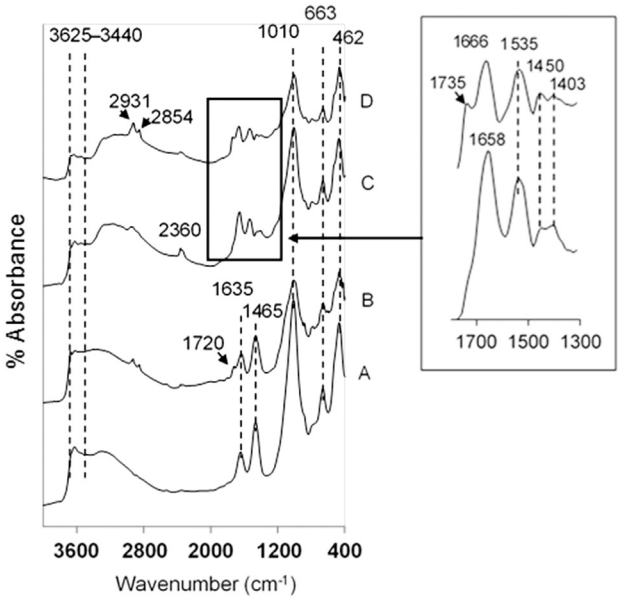

Fig. 5. FTIR spectra of (A) bentonite, (B) bentonite-oil, (C) bentonite-PK, and (D) bentonite-oil-PK.

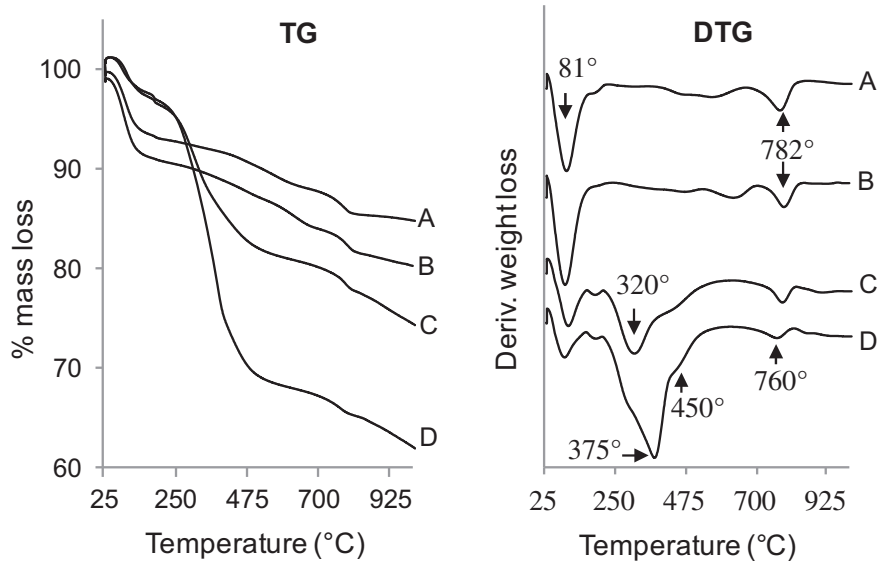

Fig. 6. Thermogravimetric and differential thermogravimetric patterns of (A) bentonite, (B) bentonite-oil, (C) bentonite-PK, and (D) bentonite-oil–PK.

3.6. Thermal properties of bentonite-bacterial products

The TG (thermogravimetric) and DTG (differential TG) patterns of the bentonite-PK composites showed significant differences than that of the raw clay (Fig. 6). The bentonite sample showed its first mass loss at 81 °C due to desorption of surface water, and second at 780 °C due to the smectite dehydroxylation of the aluminosilicate layers (Yue et al., 2007). The mass loss was further accelerated due to the presence of olive oil, which had lowered the thermal stability of the oil-adsorbed bentonite. A subsequent steep mass loss at 175–450 °C (~15.30% mass loss of bentonite-PK) (Fig. 6C) indicated the load of organic substances originated due to the bacterial activity (Li et al., 2014). These organic molecules were deposited on the surfaces and in the interlayer space of the montmorillonite (Fig. 4 and Fig. 5). The two consequent derivative peaks at 175–450 °C region indicated two phases of the potential surfactant/EPS products which originated from the bacterium. The first peak at 320 °C corresponded to the decomposition of the adsorbed surfactant/EPS on the outer surface of the clay and the second peak at 450 °C indicated the elimination of the clay intercalated bacterial products (Xi et al., 2007; Sarkar et al., 2010; Biswas et al., 2016). The addition of olive oil further increased the production of surfactant/EPS materials by the bacterium as indicated by a higher mass loss of bentonite-oil-PK (~29.08%) at 175–450 °C regions (Fig. 6D). The relatively higher intensity peak at 450 °C and slightly early dehydroxylation (760 °C) of bentonite-oil-PK could also be an effect of the higher load of bacterial products in the interlayer of montmorillonite (Xi et al., 2005).

4. Conclusions

In this study, we explored the interaction mechanism of the biosurfactant producing bacterium *Pseudoxanthomonas kaohsiungensis* with bentonite clay and observed an enhancement of growth of the bacterium by the presence of the clay in the culture media. This growth enhancement was achieved by the microenvironment which formed as a clay-bacterial cluster. The smectite surface also acted as a slow-release sink for olive oil which could supply carbon source to the bacterium over a longer period of time and maintained an enhanced bacterial proliferation. The bacterial action brought about significant changes in the clay structure including dissolution of Si and Al, and grafting of bacteria-produced organic moieties. The biosurfactants produced by *P. kaohsiungensis* along with other EPS resulted in the formation of a well-defined clay-bacterial structure. Hence, this study highlighted some of the most important characteristics of an interaction of bentonite with a biosurfactant producing bacteria, which would have direct implication in the remediation of hydrophobic organic contaminants in the environment.

Contributions

B.B. and A.C. contributed equally in this work and should be considered as the joint first authors. This study was conducted as a part of A.C.'s master by research degree.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.clay.2016.11.008.

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