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Pseudomonas putida Biofilm Facilitates Fine Solids, Water and Oil Separation from Oil Sands Tailings

Victoria Kostenko[1*], Robert John Martinuzzi[2] and Geir Hareland[1]

[1]Chemical and Petroleum Engineering Department, 2500 University Drive NW, Calgary, T2N 1N4, Canada.
[2]Mechanical and Manufacturing Engineering Department, 2500 University Drive NW, Calgary, T2N 1N4, Canada.

Authors' contributions

The work is a fruition of multi-disciplinary collaboration. Author VK contributed to the microbiological expertise and conducted experiments, author RJM contributed expertise in transport, mass transfer and dispersion. Author GH contributed expertise in hydrocarbon chemistry. All authors have read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The generation of the tailings, poor settling slurry contaminated with emulsified bitumen, significantly increases the negative impact of oil sands operations on the environment and human health (contamination of surface and ground water with hydrocarbons and naphthenic acids, methane emission), as well as operation cost. Poor effectiveness of conventional tailings settling and clean-up technologies contributes to the daily increase of the quantity of tailings deposited in ponds covering now more than 130 km^2. There is an urgent need for development of novel tailings settling technologies. The aim of the present study is a comparative analysis of the impact of *Pseudomonas putida* planktonic and biofilm populations on oil, solids and water separation in tailings, and the investigation of the mechanisms involved in bioseparation.
Methodology: Mature fine tailings (MFT) were exposed to *Pseudomonas putida* planktonic populations and biofilms at agitation followed by static conditions for settling. Oil-solids-water separation was determined by water and oil release from MFT in comparison with untreated tailings. Interaction of tailings with microbial populations was

**Corresponding author: E-mail: v.kostenko@ucalgary.ca;*

investigated with scanning electron microscopy (SEM), confocal scanning laser microscopy (CSLM) and energy-dispersive X-ray (EDAX) spectroscopy.
Results: The exposure of mature fine tailings to microbial cultures, and especially to biofilms, significantly increase tailings densification, dewatering and bitumen release. The separation efficiency is associated with fine clay aggregation due to the interaction with the microbial cells, biofilm colonies and extracellular polymeric substances (EPS).
Conclusion: The mechanism driving the observed biodensification is the aggregation of fine solids via flocculation by biofilm-produced EPS and bacterial cells. Microorganisms were also observed to destabilize emulsions and enhanced residual bitumen release from tailings.

Keywords: Oil sands tailings; oil-solids-water separation; biodensification; microbial biofilm; fine clay aggregation; flocculation.

ABBREVIATIONS

***MFT** – mature fine tailings; **CT** – composite/consolidated tailings; **TRO** – tailings reduction operations; **UT** – untreated tailings; **PL** – planktonic cultures; **BFG** – biofilm granules; **SBF** – surface biofilm (developed on silicon coupons); **CFU** – colony forming units; **EPS** – extracellular polymeric substances; **SEM** – scanning electron microscopy; **EDAX** – energy-dispersive X-ray analysis; **CSLM** – confocal scanning laser microscopy; **AO** – actidine orange.*

1. INTRODUCTION

The safe disposal of oily waste slurry generated during oil and gas drilling, petroleum production and refining, and bitumen extraction from oil sands is a major problem faced by the oil industry. These slurries contain water, oil and significant concentrations of fine solids. The presence of fine solids in the processes involving the contact of oil and water often results in the formation of solid-stabilized emulsions. Generally, the particles accumulate on the oil-water interface and serve as mechanical emulsifiers stabilizing the emulsion [1]. Another possible mechanism of oily slurry stabilization is fine clay gelation in water or oil continuous phases [2]. These thixotropic gels entrap coarser solids and droplets that prevent the separation of oil, water and solids [3,4]. The build-up of fine solids in emulsions is detrimental to either bitumen recovery or drilling operation as a result of dramatically increased viscosity [5]. Moreover, oil and water trapped in the slurry are consequently lost, while oily slurry becomes a big danger for the environment and human health. The petroleum-contaminated slurries are sources of polycyclic aromatic hydrocarbons (PAH), naphthenic acids, heavy metals and other toxic chemicals [6]. As a result of the high toxicity of the petroleum slurries, environmental regulations (e.g., the Alberta Environmental Protection and Enhancement Act) prohibit the release of potentially toxic petroleum slurry into aqueous and terrestrial environment. The only possible way to manage petroleum slurries is the separation of oil and water from solids and recycling them in the drilling and petroleum production. However, in the processes involving the formation of solids-stabilized emulsions and dispersions, the slurries are proved to be so stable that they do not coagulate in spite of the use of the traditional demulsification and solid control technologies [7]. Mostly, these oily slurries end up being stored in tanks or tailing ponds or discharged into landfills thereby increasing the cost of the oil production and the environmental risk.

Over the last decades, many strategies have been attempted to dewater petroleum slurries such as mature fine tailings (MFT), a waste product of the bitumen extraction from oil sands. The most popular technologies for MFT consolidation are composite/consolidated tailings (CT) and tailings reduction operation (TRO) based on chemical coagulation and polymers-induced flocculation of fine solids, which results in non-segregating sediments with release of entrapped water [8,9]. While these technologies have been in use for more than 10 years, several challenges remain associated with the process in term of the very long remediation and reclamation time required. To generate CT, MFT are mixed with coarser solids and coagulant-gypsum (calcium sulfate) and then pumped to the tailings ponds where, over time it will become denser and water will be released. The consolidation of the CT-treated tailings pond may take up to 30 years before the trafficable surface can be reclaimed. Moreover, CT must be regularly deposited into the CT layer within the tailings pond. Otherwise, the material will segregate in the pond and once again form MFT [10]. It has also been observed that calcium, used in tailing densification, impacts the total ionic strength of released water and can inhibit the bitumen floatation process required for bitumen extraction from oil sands when the released water is recycled [11,12].

TRO is the process of mixing small portions of MFT with a polymer flocculant, then depositing it in thin layers with shallow slopes. In a matter of weeks, the material dries resulting in a product that can be reclaimed in-situ or moved for final reclamation [10]. However, since only a small portion of MFT can be mixed, the treatment time for an entire pond is comparable to that for CT technology. The alternative, bulk flocculation, requires high concentrations of polymer and its availability to clay particles in order to interact, which is difficult to achieve *in situ*. Resultant flocs are usually loosely packed and trap large amounts of water [13], which may be released only with additional treatment with dewatering agents and/or vacuum filtration [14,15]. Because of these challenges, oil sands operators continue to research other tailings management technologies.

A promising way for tailings management is biodensification. Microorganisms indigenous to tailings ponds are reported to have the capacity to degrade residual hydrocarbons and naphthenic acids, while simultaneously improving tailings consolidation [16-19]. While microorganisms inhabit tailings ponds, no additional treatment is required and the biodensification process is sustainable. The microbially-induced tailing densification can be reinforced by biofilms, well-organized communities of microbial cells attached to the surface or each other and embedded in polymeric matrix, that give them advantages over the planktonic (free-swimming) microbial cells. Biofilms accumulate high numbers of bacterial cells and extracellular polymers, which can facilitate fine clay flocculation, and protect community members from toxic compounds and environmental stresses that provides the opportunity for microorganisms within biofilms to be active even under very unfavorable conditions such as oil sands tailings. We hypothesize that biofilm-based tailings densification/dewatering is attributed to complex mechanism, including flocculation of fine solids by bacterial cells and extracellular polymeric substances produced by biofilms and absorption of the clay particles by bacterial cells within biofilm matrix. Additionally, the ability of bacterial cells to grow on oil-solids and oil-water interfaces would facilitate oil displacement and release from emulsions. Thus, the proposed biological treatment aims to release residual bitumen and water from MFT without impairing water quality. Water can be recycled for the bitumen extraction process or discharged without additional treatment. The residual solid deposit will be appropriate for reclamation or reuse.

The goal of the present research is a comparative study of the impact of *Pseudomonas putida* planktonic populations and biofilms on oil, solids and water separation in MFT, and

the investigation of the mechanisms involved in bioseparation. To explain the mechanism of enhanced MFT dewatering and residual oil release via microbial activity, the size distribution and composition of the aggregates in tailing samples were characterized using a Scanning Electron Microscope (SEM) and an energy-dispersive X-ray (EDAX) spectroscopy.

2. MATERIALS AND METHODS

2.1 Tailings and Media

The mature fine tailings (MFT) samples were obtained from Albian Sands and Syncrude tailings ponds. The M9 minimal media with glucose (M9-G) consists of 12.8 g/l Na_2HPO_4•$7H_2O$; 3 g/l KH_2PO_4; 0.5 g/l NaCl; 1 g/l NH_4Cl; 10 g/l glucose; 1 mM $MgSO_4$; 100 µM $CaCl_2$; 3×10^{-8}M $CoCl_2$•$6H_2O$; 1×10^{-8}M $CuSO_4$•$5H_2O$; 8×10^{-8}M $MnCl_2$•$4H_2O$; 1×10^{-8}M $ZnSO_4$•$7H_2O$; 1×10^{-6}M $FeSO_4$•$7H_2O$.

2.2 Microorganism and Biofilm Formation

The strain *Pseudomonas putida*ST6 isolated from oil sands tailings was used in this study as a model organism to investigate impact of the bacterial planktonic and biofilm populations on the oil sands tailings dewatering and residual bitumen release. Planktonic cultures (PL) were grown in M9-G to the concentration of 10^8 colony forming units (CFU) per ml. Then, microbial cells were separated from incubation media by centrifugation at 4000 rpm for 20 min (here and below, centrifugation speed and time were optimized for separation efficiency, data not shown). Settled PL cells were resuspended in the tailings samples to final concentration of 10^6 CFU/ml.

P. putida biofilm granules (BFG) were developed on bentonite particles suspended in viscous (with 0.1 % agar) M9-G with agitation at 120 rpm over 72 hours (here and below, agitation speed and time were optimized for microbial growth and interaction with clay, data not shown). BFG were separated from suspended microbial cells and incubation media by centrifugation at 4000 rpm for 10 min. Settled BFG were resuspended in the tailing samples to final microbial cell concentration of 10^6 CFU/ml.

The surface biofilms (SBF) were developed on silicon coupons immersed in wells of 6-well micro dishes with M9-G. Biofilms were grown with an agitation at 120 rpm to reach final concentrations of 10^8 CFU per cm^2. Coupons with biofilms were rinsed to wash out planktonic cells and incubation media, and submerged in the tailings samples.

CFU values were verified by plate count as previously described [20]. All incubations were performed at 25ºC.

2.3 Tailings Dewatering

The tailing samples were supplemented with *P. putida* planktonic cultures, biofilm granules or surface biofilms as described above. The concentration of microorganisms injected was 10^6 CFU per ml of tailings. The tailings samples without addition of the microbial cultures were used as controls (mentioned in the paper as untreated sample, UT). The samples were incubated at 25ºC with agitation at 120 rpm for 24 hours and then under static conditions for an additional 24 hours. The dewatering rate (D) were determined as the percentage of the

clear water volumes released after settling (*W*) compared to the initial total volume of tailings samples (T) according to formula (1):

$$D = \frac{W}{T} \times 100\% \quad (1)$$

The contamination of the released water with solids was determined by optical density (OD) at 650 nm.

2.4 Oil Release from Slurry

The tailings samples were supplemented with *P. putida* planktonic cultures, biofilm granules or surface biofilms. The concentration of microorganisms injected was 10^6 CFU per ml of tailings. The tailings samples without addition of microbial cultures were used as controls. After 48 hour incubation as above, oil released from slurry was skimmed from the top of the samples and centrifuged at 4000 rpm for 20 min. The volume of oil fraction was measured in the centrifuge tube after separation from water. The volume of oil released was calculated as the percentage relative to the initial total volume of tailings samples.

2.5 Scanning Electron Microscopy and Elemental Analysis

Scanning electron microscopy (SEM) was performed in order to investigate morphology of the tailing samples with respect to particle sizes, and interaction of fine solids with bacterial cells and biofilms. The samples from settled tailings: untreated and treated with PL, BFG and SBF were fixed with 5 % glutaraldehyde for 24 hours at 4ºC on the membrane of vacuum filtration system. The biofilms on silicon coupons exposed to tailings were fixed with 5 % glutaraldehyde for 24 hours at 4^{o}C on the original carrier. The samples were dehydrated with serial ethanol dilutions, air-dried, sputter coated with gold, and viewed with a FEI ESME XL30 SEM (Microscopy and Imaging Facility, University of Calgary). Particle size distributions were analyzed with ImageJ software. Content of Al, Si, C and P in tailings samples and biofilms were determined with an energy-dispersive X-ray (EDAX) spectroscopy as atomic percentage. Al and Si were used to indicate the presence of clay; C and P indicate presence of biomass (bacterial cells and EPS). EPS were determined by increased C to P ratio. C without P indicates bitumen [19].

2.6 Confocal Scanning Laser Microscopy

The Confocal Scanning Laser Microscope (CSLM) observations and recombinant strain *P. aeruginosa*PA01 containing the fluorescence reporter *mCherry* [21] were used to study the dynamics of the biofilm-fine solids interaction. *P. aeruginosa*PA01 and *P. putida*ST6 were observed to form biofilms with similar morphological and chemical characteristics (data not shown). *P. aeruginosa*PA01 was used in this experiment because of the capacity to produce protein with red fluorescence encoded by gene *mCherry, w*hich allows visualizing microbial biofilm under CSLM. *P. aeruginosa* surface biofilms were developed on glass cover slips incubated in M9-G media under agitation of 120 rpm at 37ºC to a final concentration of 10^8 CFU per cm^2. Coupons with biofilms were rinsed to wash out planktonic cells and incubation media, and submerged in the tailings samples for 48 hours as above. After incubation, cover slips with biofilms and absorbed fine solids were rinsed to wash out loosely attached microbial cells and solid particles and stained with 10 µg/ml acridine orange (AO) for 15 min.

AO was used to visualize clay particles (green spots). Bacterial cells were determined by *mCherry* expression (red spots). Stained cover slips were examined for bacterial cells and clay particles percentage in aggregates with an Olympus FV1000 CSLM and analyzed by the Fluorview image analysis software.

2.7 Statistical Analysis

At least three independent experiments were performed. A one-way analysis of variance (ANOVA) was used to analyze the difference between experimental groups. The probability values of $p<0.05$ were considered to indicate statistical difference between experimental groups with 95% of confidence.

3. RESULTS

3.1 Tailings Dewatering via Biosedimentation

The inherent dewatering of the oil sands mature fine tailings (MFT) is a very slow process. In the present study, MFT samples obtained from Albian sands and Syncrude tailings ponds have been exposed to mechanical forces (agitation at 120 rpm) for 24 hours followed by the sedimentation under the static conditions for extra 24 hours. As a result, sediment volumes were reduced by 5% for Albian sands samples and by 7% for Syncrude samples, thus, providing the measure of the natural dewatering rate for these (untreated) samples (Fig.1). In general, treatment of MFT with microbial cultures, either planktonic or biofilms, resulted in enhanced MFT sedimentation and release of the entrapped water. 16 – 24 % of entrapped water from samples exposed to planktonic culture and significantly more (38 – 53 %) of water from samples treated with biofilms was released from tailing samples as a result of biosedimentation. The biosedimentation and dewatering of Syncrude tailings induced by planktonic cultures and granular biofilms were higher than those of tailings from Albian sands pond ($p<0.05$); while, the treatment with SBF did not let to any difference between biosedimentation of both samples.

Water released from untreated tailings contains a significant amount of dispersed solids as determined by optical density (Fig. 2). The treatment with planktonic *P. putida* reduced turbidity of the released water 2 – 3 folds, while treatment with granular biofilms provided nearly clean water (OD = 0.02). The treatment with the coupon biofilms also markedly improved release water purity compared to untreated MFT (4 – 8 fold better), but turbidity of released water after treatment with coupon biofilms was 2 – 4 times higher than those treated with granular biofilms. Untreated Albian sands tailings showed nearly twice better water purification capacity during sedimentation than Syncrude tailings. However, levels of the improvement of the water quality after treatment with planktonic cultures and granular biofilms were very close for Albian sands and Syncrude tailings. In contrast, SBF had much better impact on water quality release from Syncrude samples ($p<0.05$).

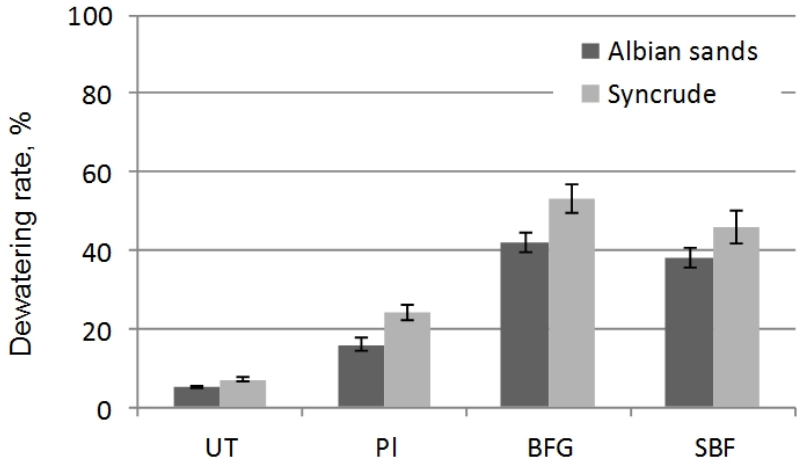

Fig. 1. Dewatering in tailings samples: untreated (UT) and exposed to either planktonic culture (PL), biofilm granules (BFG) or surface biofilms (SBF) on silicon coupons

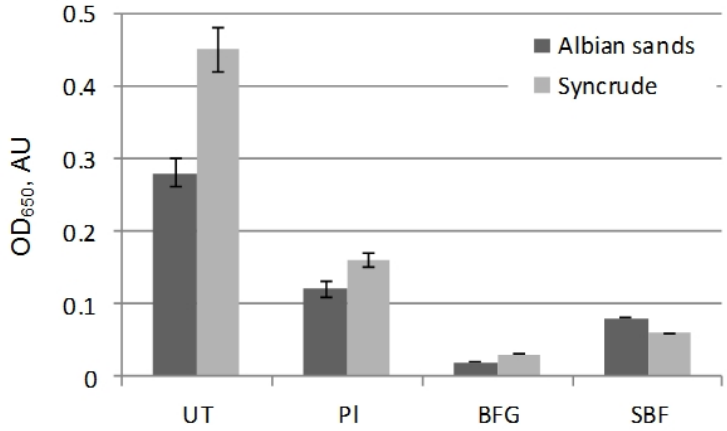

Fig. 2. Optical density of the water released from tailings samples: untreated (UT) and exposed to either planktonic culture (PL), biofilm granules (BFG) or surface biofilms (SBF) on silicon coupons

3.2 Microbially Induced Oil Release from Tailings

The agitation of MFT samples over 24 hours at 120 rpm resulted in residual oil release up to 1% of total tailings volume (Fig. 3). However, exposure of MFT to planktonic *P. putida* enhanced oil release to 4.5 – 6.0 %. The treatment with biofilms resulted in the release of 3.5 – 4.5 % of oil. No difference was observed between amounts of oil released from Albian sands and Syncrude samples under all tested conditions (p>0.05).

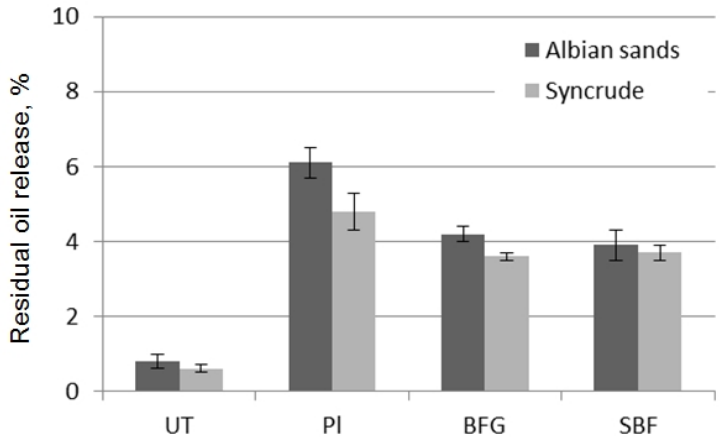

Fig. 3. Residual oil release rates from tailings samples: untreated (UT) and exposed to either planktonic culture (PL), biofilm granules (BFG) or surface biofilms (SBF) on silicon coupons

3.3 Additional Dewatering and Oil Release via Centrifugation

Centrifugation of the tailings samples after biosedimentation led to release of additional portion of entrapped water and oil. An extra 8 – 12 % of water was released from settled tailings slurry after treatment with planktonic *P. putida* and 12 – 16 % from biofilm-treated tailings compared to 2 – 3 % of water released from untreated samples (Fig. 4). Higher centrifugation-induced water release was observed for Albian sands tailings for both untreated and treated with planktonic cultures ($p<0.05$), while water release in response to treatment with biofilms (both GBF and SBF) did not showed any differences between tailings from different ponds ($p>0.05$). Centrifugation also allowed releasing an extra 1.5-1.7 % of oil from MFT; while biological pre-treatment enhanced oil release due to centrifugation 2 – 4 times (Fig.5).No difference between oil release from Albian sands and Syncrude tailings was observed ($p>0.05$).

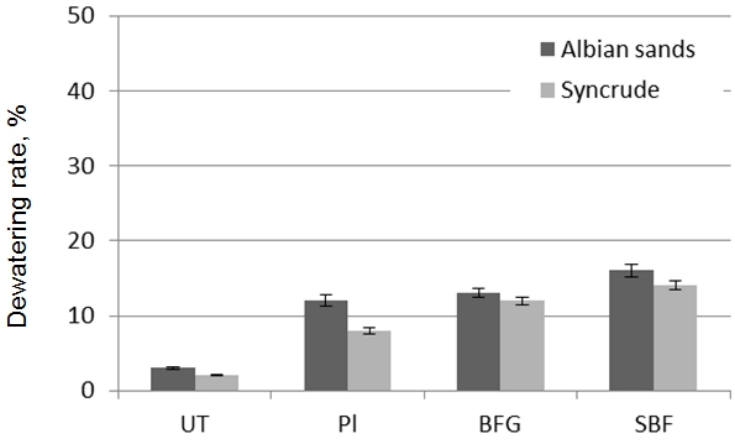

Fig. 4.Centrifugation-induced dewatering in the tailings samples: untreated (UT) and exposed to either planktonic culture (PL), biofilm granules (BFG) or surface biofilms (SBF) on silicon coupons

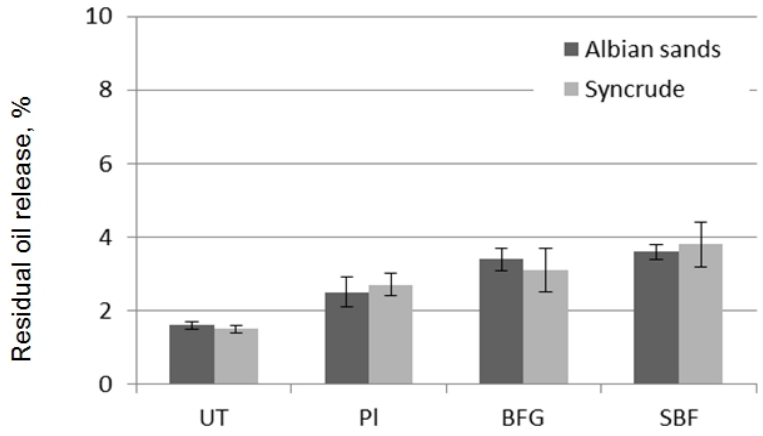

Fig. 5. Centrifugation-induced residual oil release rates from tailings samples: untreated (UT) and exposed to either planktonic culture (PL), biofilm granules (BFG) or surface biofilms (SBF) on silicon coupons

3.4 Size Distribution and Composition of the Aggregates in Tailings

To explain the mechanism of enhanced MFT dewatering and residual oil release via microbial activity, the size distribution and composition of the aggregates in tailing samples were investigated with Scanning Electron Microscope (SEM) and an energy-dispersive X-ray (EDAX) spectroscopy. SEM observations demonstrated that untreated tailings samples mostly contain plate-like fine and ultra-fine particles: 98 % of the particle pool in Albian Sand samples and 92% in Syncrude samples (Fig. 6). Exposure of tailings samples to *P. putida* planktonic populations facilitated aggregation of fines and ultra-fine particles: 45 – 49 % of the particles observed in PL-treated tailings exceeded 50 μm, and half of them in Syncrude samples and one eighth in Albian Sand samples were marginally bigger than 100 μm.

Initially tailings supplemented with BFG contained approximately 70% tailings (fine and ultra-fine particles) and 30 % biofilm granules of 100 – 300 μm in diameter (data not shown). After 48 hour incubation, the amount of fine and ultra-fine solids was reduced to 20 – 22 % of total particles pool; the rest fine particles either aggregated to form larger particles (16 -18 % of particles of 50 – 100 μm) or were absorbed by BFG (28 – 32 % of particles of > 300 μm). The tailings supplemented with SBF contained 92- 98% fines and ultra-fines and biofilms inserted on coupons. After 48 hours incubation, amount of fines reduced to 18 – 20 %; aggregates of 50 – 100 μm represented 24 – 26 % of particle pool, aggregates of 100 – 300 μm – 12 – 18 %, and aggregates of >300 μm – 36 - 38 %. In the untreated state and after treatment with planktonic cultures, Syncrude tailings contained higher relative numbers of clay aggregates than Albian sands samples; while the size distributions of solid particles in both tailings samples after treatment with biofilms were equivalent.

Table 1. Elemental analysis for particles of different size from tailings samples after biosedimentation

Samples	Elements, atomic %	Albian sands				Syncrude			
		< 50 μm	50–100μm	100–300μm	>300 μm	< 50 μm	50–100μm	100–300μm	>300 μm
UT	Al	21.9±1.2	18.4±0.8	*NA**	*NA*	22.4±1.2	16.2±0.4	*NA*	*NA*
	Si	59.1±2.3	51.6±1.2	*NA*	*NA*	62.7±4.1	43.7±1.8	*NA*	*NA*
	C	9.3±0.5	19.2±2.4	*NA*	*NA*	9.6±0.8	34.1±0.9	*NA*	*NA*
	P	0.05**	0.7±0.1	*NA*	*NA*	0.06	0.9±0.0	*NA*	*NA*
PL	Al	23.9±1.8	11.2±0.9	10.1±0.6	*NA*	23.8±0.2	12.4±0.9	10.4±0.1	*NA*
	Si	59.7±3.6	27.6±2.4	28.3±0.4	*NA*	64.2±0.8	32.2±2.6	28.1±0.6	*NA*
	C	11.4±0.4	51.4±1.1	52.6±2.6	*NA*	7.0±0.0	48.2±2.8	53.7±1.2	*NA*
	P	0.03	2.7±0.2	2.0±0.0	*NA*	0.02	2.4±0.6	2.1±0.1	*NA*
BFG	Al	22.1±0.8	6.8±0.2	3.2±0.1	7.2±0.4	22.9±0.6	7.1±0.4	4.3±0.0	8.1±0.2
	Si	61.8±2.8	18.4±0.4	8.4±0.2	19.4±1.2	61.2±3.8	19.2±1.2	12.1±0.3	21.1±1.1
	C	10.1±0.9	66.5±2.2	79.4±2.4	65.9±1.8	9.6±0.7	67.6±4.8	75.5±1.6	62.9±2.4
	P	0.05	1.2±0.2	3.8±0.1	2.8±0.1	0.03	1.1±0.2	3.1±0.2	2.9±0.4
SBF	Al	23.2±1.2	7.2±0.8	6.1±0.3	6.8±0.4	22.9±1.1	7.8±0.6	5.6±0.3	7.8±0.1
	Si	62.4±2.4	20.1±1.8	16.7±1.2	17.3±1.2	64.1±2.6	21.1±1.2	14.5±0.8	20.7±0.6
	C	9.4±1.1	64.9±2.4	68.2±3.6	66.8±2.8	8.2±0.4	64.5±3.6	71.2±2.6	62.4±1.8
	P	0.06	1.3±0.1	2.9±0.4	2.5±0.1	0.02	1.6±0.5	3.1±0.1	2.9±0.1

** NA – non-applicable.*

***SD is less than 0.01.*

The elemental (EDAX) analysis (Table 1) and SEM images (Fig. 7) of the particle of different sizes demonstrated the strong correlation between the particle size and the interaction of bacterial cells with fine clay. According to elemental analysis, fine and ultra-fine particles in all samples (UT, PL, BFG and SBF) were mostly clay as indicated by high relative contents of Al and Si (21 – 23 % and 59 – 64 %, respectively) and low relative contents of C and P (7 – 11 % and 0.02 – 0.06 %, respectively). C indicated the presence of organic matter, but very low P-to-C ratios indicated the absence of microbial cells in fine particles [19]. Thus, C may be attributed to the presence of residual bitumen attached to clay particles. The composition of lager particles depended on the sample type. The particles of more than 50 µm in PL-treated tailings contained both clay and bacterial cells as indicated by approximately 38 % Al+Si and 54 % C+P. The presence of residual bitumen in the aggregates is expected. However, high (0.04 – 0.05 %) P/C ratios are associated with the presence of bacterial cells in the aggregates [19], which is confirmed by SME images of the corresponding aggregates (Fig. 7). The particles of 50 – 100 µm in biofilm-treated MFT along with clay contained a significant amount of EPS as indicated by high content of carbon (64 – 68%) with low P/C ratio (0.02). The particles of 100 – 300 µm were observed only in microbially-treated tailings. These aggregates contained clay, bacterial cells and EPS as indicated by elemental analysis (3 – 10 % Al, 8 – 28 % Si, 50 – 70 % C and 2 – 3 % P) and SEM images. The relative amount of clay was higher in PL-treated MFT. The amount of bacterial cells was higher in BFG-treated samples. The particles of >300 µm were observed in biofilm-treated samples. These particles contained higher amount of clay compared to 100 – 300 µm particles in biofilm-treated MFT.

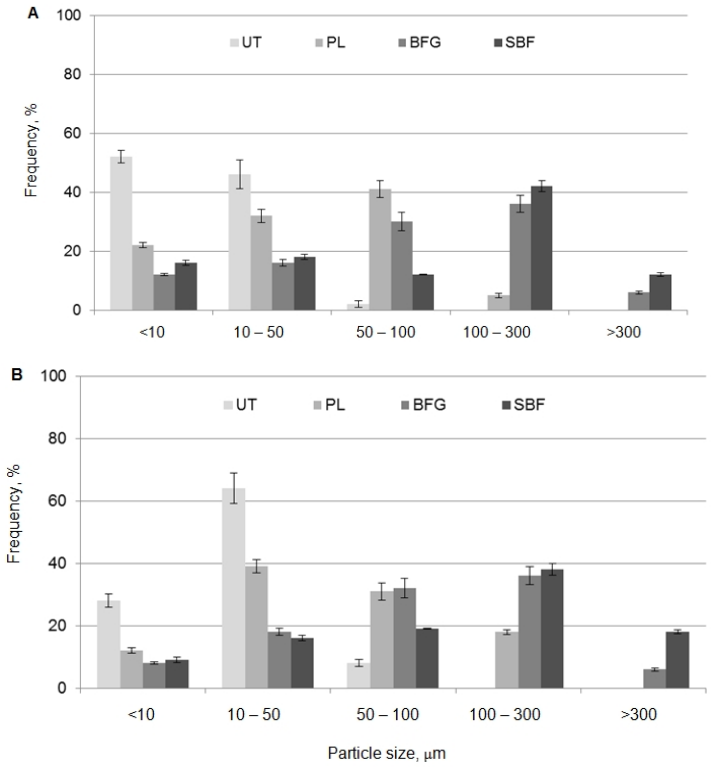

Fig. 6. Particle size distribution in Albian Sands (A) and Syncrude (B) tailing untreated (UT) and exposed to either planktonic culture (PL), biofilm granules (BFG) or surface biofilms (SBF) on silicon coupons

Significant amounts of clay were accumulated within *P. putida* coupon biofilms after two days exposure to MFT (Fig. 8). The biofilm colonies, initially containing tightly packed bacterial cells embedded in EPS, absorbed fine particles, which underwent aggregation and incorporated into the biofilms. EDAX analysis confirmed presence of bacterial cells, EPS and minerals within the biofilm colonies.

Thus, even untreated tailings contain organic matter, which might be residual bitumen or microorganisms. While bitumen deposition was equal in tailings samples, the relative amount of indigenous microbial population in Syncrude samples was higher than in Albian sands tailings. Biological treatment significantly increased the load of microbial cells in the tailings regardless the tailings source.

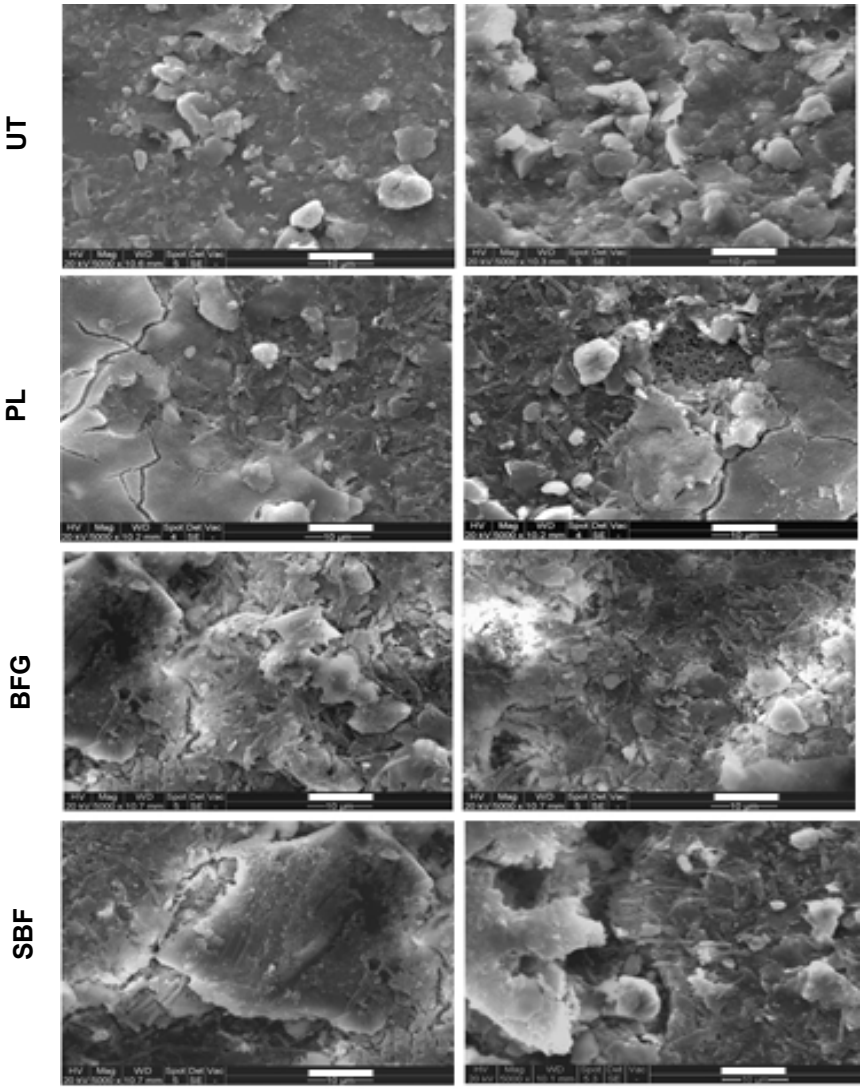

Fig. 7. SEM images of Albian Sands (left column) and Syncrude (right column) tailings samples: untreated (UT) and exposed to either planktonic culture (PL), biofilm granules (BFG) or surface biofilms (SBF) on silicon coupons. Bar is 10 μm

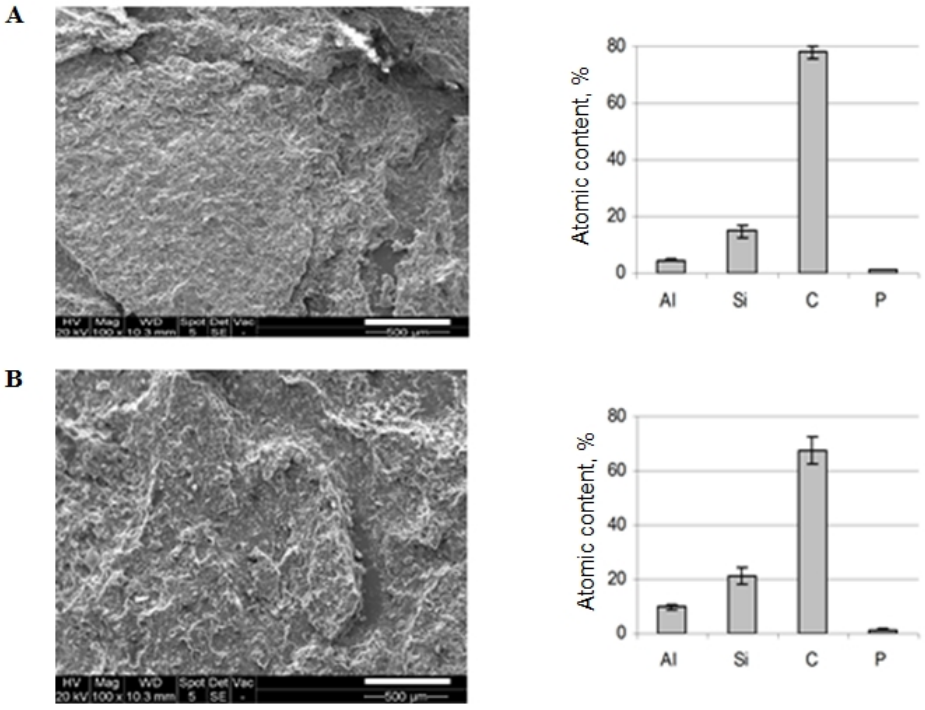

Fig. 8. Left column: SEM images of the *P. putida* biofilms developed on silicon coupons and then exposed to the Albian Sands (A) and Syncrude (B) tailings samples for two days. Bar is 500 µm. Right column: Relative concentrations of Al, Si, C and P in the corresponding samples as determined by EDAX analysis

3.5 Dynamics of Biofilm-Tailings Interaction

P. aeruginosa biofilms developed on glass cover slip consist of large colonies of bacterial cells embedded in EPS (Fig. 9). No sign of clay particles on SEM and CLSM images was supported by EDAX analysis indicating presence of biomass (around 100 % of C+P) and the absence of clay (no Al+Si). Exposure of the biofilm colonies to tailings resulted in the absorption of clay (green) within biofilm (red). After three day exposure, 54 % of biofilm was covered with clay. Further incubation results in nearly complete coverage of biofilms with clay particles. CLSM observation is supported by the observation of plate-like fine particles entrapped in biofilm colonies and increased contents of Al and Si.

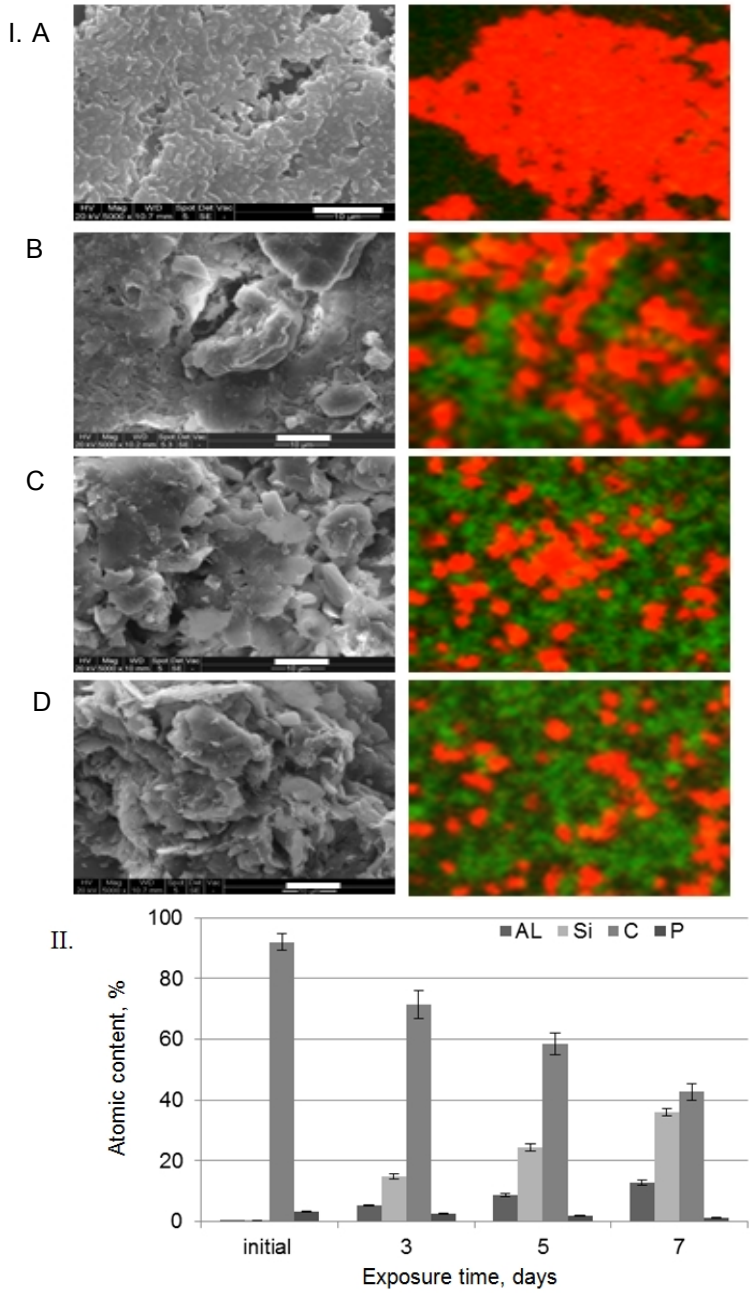

Fig. 9. Dynamics of the surface biofilm-fine solids aggregates formation. I: SEM images (left column) show morphology of aggregates, CSLM images (right column) demonstrate clay (green) accumulation on biofilm colony (red) before (A) and after 3 days (B), 5 days (C) and 7 days (D) exposure to MFT. Bar is 10 μm. II: Relative concentrations of Al, Si, C and P in corresponding samples as determined by EDAX analysis

4. DISCUSSION

This paper addresses the challenge in oil sands operation associated with low settling rate and water release from tailings, a waste product of bitumen extraction from oil sands. We demonstrated that, in general, the exposure of MFT to microbial cultures, and especially biofilms, significantly increases tailings densification and dewatering. Moreover, biotreatment allows residual bitumen recovery from tailings. The observed oil, water and fine solids separation is attributed to aggregation of fine solids via flocculation by bacterial cells and EPS. The effectiveness of biodensification/dewatering depends on the composition of the tailings samples: planktonic cells and GBF were more effective in Syncrude tailings, while SBF had equal effects on both Albian sands and Syncrude samples. This observation correlates with higher amounts of indigenous microbial cells and clay aggregates in Syncrude tailings, which may serve as nucleation centers for aggregation of both clay and injected microorganisms. The biofilms developed on coupon surface (SBF) absorb clay from tailings without interaction with the indigenous population. However, purification of released water had an opposite trend: SBF better improved water quality in Syncrude tailings, while planktonic cultures and GBF had equal effects on both tailings samples. This observation indicates that SBF better absorb clay particles from water. In contrast, planktonic cells and GBF aggregate clay particles in suspension, but to the level which not necessary sufficient to allow sedimentation. Notwithstanding, clay-microbes interaction led to the destabilization of emulsions and release of the entrapped bitumen which was equal for both tailings samples under corresponding conditions. Thus, destabilization and separation of tailings is based on the aggregation of clay by bacterial cell and EPS with subsequent densification of tailings.

Previously, biodensification was linked to gas production via degradation of organic matter claimed by methanogenic and sulfate-reducing bacteria in tailing pounds [17]. Released gases form pockets in viscous media such as MFT. As the number of gas pockets increases, they coalesce to form vertical drainage channels which facilitate the settling capacity of the fine solids in tailing ponds and floating up of the oil droplets. However, *P. putida*, used in this study, does not produce significant amounts of gas. Thus, enhanced sedimentation must be explained by different mechanisms.

Challenges in tailing densification and dewatering result from poor solids/water separation in stable gel-like sludge, which is a network of fine and ultra-fine solid particles, typically clay, dispersed via the alkaline hot water process used for bitumen extraction from oil sands [3, 4]. Deprotonation of edge AlOH and SiOH at alkaline pH can cause edge charge reversal from positive to negative [22,23]. The charge reversal increases the repulsive double layer and prevents edge-to-face heterocoagulation [24]. Electrostatic repulsion along with mechanical forces facilitates clay dispersion to fine and ultra-fine levels. At a critical gelation concentration (the limiting concentration under which no gel can be formed), dispersed fines form a gel-like colloidal suspension [23]. Emulsification is another mechanism contributing to the stability of the tailings gel. Organic matter (asphaltenes and humic acids) absorbed on fine clay produces biwettable particles, known to be surface active, which redistribute themselves on the oil-water interface to form colloidal systems that are extremely difficult to separate [25-27]. Therefore, MFT are a highly hydrated gel-like network of fine solids with entrapped solid-stabilized bitumen colloidal droplets [28]. The gel and stable emulsion formation retards water efflux whether through applied dewatering forces (e.g., centrifugation) or by sedimentation under gravity [29]. Thus, the dewatering of the MFT requires simultaneous destabilization of the gel-like clay dispersion and the solid-stabilized emulsion, together with fluocculation/aggregation of the dispersed fine particles with subsequent settling by gravity [30]. Microbial biofilms can contribute in both processes. The

metabolic activity of microbial biofilms results in the production of organic acids, solvents, surfactants, EPS and microbial cells. A reduction of pH due to acid production causes protonation of the clay edges that inverts edge negative charge to positive and leads to spontaneous clay platelets aggregation due to the attraction between oppositely charged base and edge sites [31]. Attraction forces destabilize the MFT gel and lead to clay self-aggregation and subsequent sedimentation. The presence of microbial cells and EPS facilitate this process via flocculation.

P. putida forms biofilm granules in suspension and large biofilm colonies on the coupon surface, which produce large amounts of EPS. The EPS are polyelectrolyte networks, which can chelate clay particles [32,33]. This mechanism of the flocculation issimilar to those observed for polyacrylamide and other flocculant used in tailings management [34]. Nevertheless, EPS produced by microbial biofilms possess a wide variety of properties that are advantageous in flocculation. Biofilm EPS contains a variety of monosaccharides, uronic acid, alginate, lactones, proteins, DNA, products of hydrolysis and cell lysis [35] that provide an affinity to a variety of substances with different charges, charge densities, hydrophobicity, *etc*. The composition of EPS is flexible and may be changed in response to environment conditions. Thus, biofilm EPS are effective collectors of dispersed particles under different tailings and water chemistry as it was demonstrated in our study for samples from different ponds. In contrast to conventional polymeric flocculants, biofilm EPS is viscoelactic polymeric network, which flocculates clay via bridging increasing the tensile strength of the aggregates [36].

One more advantage of biofilm-based bioflocculation is the accumulation of a high number of microbial cells within the biofilm matrix that enhances the flocculation ability of the biofilms. Bacteria play an important role in determining the properties and behavior of clay minerals in natural environments and such interactions have a great potential for creating stable biofilms in soil. In soil, bacteria colonize clay aggregates to form biofilm colonies. Clay minerals have a high cationic exchange capacity and high absorbance potential for organics, so they may function as nutrient shuttles for dissolved nutrients [37, 38], as well as solid surface for bacterial attachment [39]. Upon aggregation with biofilms, clay expands and undergoes restructuring being filled with bacterial metabolites [40]. Bacteria were also reported to accelerate clay formation (neoformed fine-grained aluminosilicate deposits) by concentrating relevant chemical species for mineral formation in localized microenvironments [41]. The organization of the microorganisms within biofilm provides high accumulation of microbial cells, which perform as surface for clay adsorption and significantly increase fine solids aggregation and subsequent settling.

Biofilm-produced cell aggregates and EPS can behave as suspended flocculates causing the destabilization of a colloidal dispersion through the agglomeration of the fine particles. The flocs thus obtained settle under gravity. Additionally, clay is adsorbed and accumulated within surface-attached biofilms that may be removed from the tailings and used for soil maturation. In both cases, the biofilms behave as nucleation sites for fine solids aggregation. The maturation of the biofilm colonies causes layer-by-layer absorption of clay to microbial cells and EPS and leads to formation of stable microbes-clay aggregates (in contrast to loose aggregates generated by conventional flocculation), which may be easily removed or settled from tailings providing effective tailings densification and water release.

Microbial cells are also able to grow on oil-solid-water interfaces, which can result in the destabilization of the oil-water emulsions and release of entrapped bitumen and water [42-44]. This process is enhanced by surfactants and solvents produced by microbial biofilms.

Bitumen recovery from MFT has a huge economic impact. The amount of residual bitumen lost in MFT represents up to 7% of tailings volume [45]. Given a tailing production of approximately 10^5 m^3/day, daily bitumen loss in tailings ponds can reach 7000 m^3. Even a partial recovery of residual bitumen from tailings would lead to significant economic benefit. Bitumen recovery would also improve tailings dewatering and reduce tailings toxicity associated with high bitumen content. Released water can be recycled thereby significantly reducing the demand for fresh water during bitumen extraction. Densified tailings with low hydrocarbon content can be effectively reclaimed.

In summary, the present paper represents perspective and environmentally friendly approach for oil sands tailings management leading to recovery of the lost bitumen, recycling of the produced water and tailings pond reclamation. In contrast to previously reported gas-channels –associated drainage of oil and solids from slurry [17], this study revealed that oil-water-solids separation in tailings is attributed to the microbial biofilms activity leading to destabilization of MFT gel and emulsion with simultaneous fine clay flocculation and settling. This study tested three ways of the biofilm injection into the tailings: (i) planktonic cells which developed biofilm colonies *in situ*; (ii) biofilm granules prior developed on clay particle in viscous incubation media, and (iii) large biofilm colonies developed on solid carrier. All these methods were effective in MFT dewatering and bitumen release; although, effectiveness of biofilms developed prior the injection was higher in fine solids aggregation and settling with subsequent water release and purification; while, planktonic cultures provided higher release of bitumen. It may be explained by high concentrations of EPS and microbial cells in developed biofilms which are easily available for fine solids flocculation and adsorption. In this case, granular biofilms had an advantage over the biofilm on coupons to be spread and flocculate clay over the tailings volume. Higher oil release in PL-treated tailings is attributed to the ability of microbial cells to grow and build up biofilm on oil-solid-water interface which resulted in displacement and floatation of entrapped bitumen. These observations put a question on development of complex biological treatment approach based on application of planktonic and biofilm populations.

5. CONCLUSION

Microbial biofilms significantly improved oil sands tailings densification, dewatering and residual bitumen release. This oil-solids-water separation is attributed to the microbial activity: flocculation of fine clay by microbial cells and EPS; absorption of clay by biofilm colonies; and demulsification by biosurfactants. Biofilms can be introduced into tailings in different ways: development in the tailing by injecting planktonic cell or injection of the developed biofilms. The latter approach showed greater effectiveness.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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