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Bioprocessing-based approach for bitumen/water/fines separation and hydrocarbon recovery from oil sands tailings

Robin L. Brigmon[1*], Christopher J. Berry[1], Arielle Wade[2] and Waltena Simpson[2],

[1] Savannah River National Laboratory, Aiken, SC 29808, [2] South Carolina State University, Orangeburg, SC 29117.

*Corresponding Author

Robin L. Brigmon

Phone (803)-819-8405

Fax (803-819-8432

r03.brigmon@srnl.doe.gov

ABSTRACT

Oil sands are a major source of oil but their industrial processing generates tailings ponds that are an environmental hazard. The main concerns are mature fine tailings (MFT) composed of residual hydrocarbons, water and fine clay. Tailings ponds include toxic contaminants such as heavy metals, and toxic organics including naphthenics. Naphthenic acids and poly aromatic hydrocarbons (PAHs) degrade very slowly and pose a long-term threat to surface and groundwater as they can be transported in the MFT (Holowenko et al, 2001). Research into improved technologies that would enable densification and settling of the suspended particles is ongoing. In batch tests, BioTiger™, a microbial consortium that can metabolize PAHs, demonstrated improved oil sands tailings settling from a Canadian tailings pond. Resulting also showed, depending on the timing of the measurements, lower suspended solids and turbidity. Elevated total organic carbon was observed in the first 48 hours in the BioTiger™ treated columns and then decreased in overlying water. Oil sands tailings mixed with BioTiger™ showed a two-fold reduction in suspended solids within 24 hours as compared to abiotic controls. The tailings treated with BioTiger™ increased in microbial densities three orders of magnitude from 8.5 X 105 CFU/mL to 1.2 X 108 CFU/mL without any other carbon or energy source added indicating metabolism of hydrocarbons and other available nutrients. Results demonstrated bioaugmentation of BioTiger™ increased separation of organic carbon from particles in oil sands and enhanced settling with tailings with improved water quality.

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1. Introduction

Recovering oil from the Athabasca Oil Sands has been a major technological challenge (Masliyah et al., 2004). While many of the mining methods have been successful, the fundamental mechanisms of hydrocarbon extraction from oil sands are cumbersome and both the mining efforts and environmental impact have been costly (Thorley, 2012). The heterogeneity of the oil sands including fines content, bitumen composition, clay mineralogy, and other factors can influence the extraction process (Wallace et al., 2004). Current methods employ multi-step systems of heating, aqueous phase separation, mechanical mixing, aeration, and chemical additions to extract hydrocarbons from the oil sands. These past and recent efforts have generated large tailings ponds that still contain varying amounts of heavy molecular weight, highly viscous hydrocarbons, known as bitumen, indicating the poor efficiency in that process (Siddique et al, 2007). Recently, environmental concerns and regulations have grown for the processing water, energy cost to operate the systems, runoff from the tailings ponds, wastewater from the facilities, as well as chemical residues (e.g. paraffins) in the water effluent from the extraction process (Alberta Energy Regulator, 2009).

In the oil sands industry, the final product from bitumen extraction process is simply the primary separation froth (Xu et al., 2012). Various flotation technologies can improve froth and water recovery by generating higher-grade froth with less fine sand, thus minimizing maintenance. More efficient processing may also increase water reclamation by recycling within the system or as cleaner effluent to tailings ponds. The hot water flotation process for oil sands is a separation process where the objective is to separate

bitumen from particulate matter by exploiting the differences in their physical properties. The slurry conditioning process includes, ablation, mixing, mass and heat transfer, and chemical reactions leading to separation of bitumen from sand and mineral particles (Zhao et al., 2006). The phase separation is enhanced by the effects of mechanical mixing and factors reducing silica-bitumen adhesion. However the process leaves 3-5 % of bitumen resulting in contaminated tailings (Masliyah et al., 2004). The large scale of the operations, estimates of water utilization by the oil sands industry are over 11 million liters per day, have resulted in tailings ponds covering more than 130 square kilometers in northern Alberta (Tenenbaum, 2009). Many of these operations and tailings ponds are over 50 years old are expected to increase due to increasing oil production (Wells, 2011). Characterization of microbial activity within the Athabasca oil sands formation has indicated significant seasonal changes (Wyndham and Costerton, 1991). These changes may be expected with the associated extreme climate changes of northern Canada. However, hydrocarbon biodegradation of the oil sand-adapted mixed sediment population has not correlated well with the concentrations of bituminous hydrocarbons in specific site sediments. These results suggest that a general capability for hydrocarbon oxidation exists in the Athabasca River system and that this capability is enhanced within the natural bounds of the Athabasca oil sands. Select anaerobic bacteria within these oil sands and tailings ponds can metabolize available hydrocarbons for growth and produce methane (Siddique et al., 2011). The oil sands naturally contain high concentrations of polyaromatic hydrocarbons (PAHs) and naphthenic acids that are a complex family of cyclic and acyclic carboxylic acids present in oil sands that are a health concern (Tenenbaum, 2009). These PAHs are of concern as they are acutely toxic to aquatic

organisms, some are carcinogenic, and the run-off and effluent from oil sands processing sites is impacting aquatic systems (Kelly et al., 2009). Downstream wetland sediments exposed to oil sands process water have distinct bacterial communities based on naphthenic acids concentrations indicating adaptation and selection (Holowenko et al., 2001).

A variety of microorganisms including sulfate-reducing, nitrate-reducing, methanogenic bacteria and heterotrophic facultative anaerobic bacteria have been detected in tailings pond water (TPW) (Holowenko et al., 2001; Bordenave et al., 2010). Microorganisms in tailings ponds participate in biodegradation of the naphthenic acids, biosorption of heavy metals, and sulfate reduction (Herman et al., 1993; Wolfaardy et al., 2008). In recent years activity of the methanogenic and nitrate-reducing microbial communities have been linked to increased densification/dewatering of tailings slurry (Bordenave et al., 2010).

This research project tested bioaugmentation, the addition of natural microorganisms, as a means of improving oils sands processing including separation of hydrocarbons from particles as well as improved settling of tailings. Bioremediation has proven successful in numerous applications to petroleum contaminated soils and water both in active or engineered applications (Brigmon et al., 2002). The toxicity of hydrocarbon contaminated soil and water due to certain metal and PAH concentrations are a concern in remediation efforts (Berry et al., 2006). Decreases in petroleum hydrocarbons, e.g. PAHs, through microbiological or physical/chemical treatments, can result in production of certain metabolites that remain toxic (Story et al., 2000). Under controlled conditions the bioremediation of hydrocarbons can reduce toxicity as well as meet reclamation goals

(Płaza, et al., 2005). In this work, we tested BioTiger™, a patented consortium of natural bacteria tested and isolated from a remediation project at a Polish Oil Refinery, with Canadian oil sands and tailings (Brigmon et al., 2009; Brigmon and Berry, 2009). That the BioTiger™ strains were isolated from Northern Europe with long winters and cooler climate like Alberta, Canada, made this culture a choice for this application. Results show that addition of BioTiger™ to oil sands and tailings respectively increased separation of hydrocarbons from particles and enhanced tailings settling with improved effluent process water quality.

2. Materials and Methods

2.1 Microorganisms

BioTiger™ is a patented consortium of 12 natural aerobic bacteria (Table 1) isolated from an acidic oil refinery sludge composed of asphaltics highly contaminated with PAHs (Brigmon et al., 2009). The refinery source of the consortium originated was located near Katowice, Poland, so the microorganisms were adapted to cold winter temperatures similar to those found in Alberta, Canada. BioTiger™ components are grown separately on R2A liquid medium (Difco) and then combined when reaching 10^{7-8} cells/ml. Prior to application, the combined BioTiger™ was grown on 1% oil sands (20% Bitumen, Athabasca Oil Sand, Alberta Research Council) in Bushnell Haas Broth (Sigma–Aldrich, St. Louis, MO) to a density of 10^{7-8} cells/ml at room temperature. Control bacteria used in this project including *Sphingomonas mobilis* EPA 505 (Story et al., 2000), and *Eschericia coli K12* (American Type Culture Collection # 29425) were prepared in the same manner on R2A broth and cultured to similar densities.

2.2 Phenanthrene Degradation by BioTiger™

Liquid cultures of *E. coli* k-12 and each the components in BioTiger™ were grown in R2A broth. Each liquid culture was incubated at 30°C at 150 rpm for 24-48 hours. The components were mixed together and washed 1X in Bushnell-Haas medium (Difco). Appropriate controls were run in parallel to all experiments.

10% inoculations of both washed live and washed dead (autoclaved) cells into Bushnell-Haas saturated with phenanthrene (Sigma) (1.6ppm), and were sampled at 0 and 24 hours. The samples were extracted in ethyl acetate (Fisher); 5ml of culture to 1ml of solvent.

Phenanthrene was identified by an Agilent 6890 GC-MS in SIM mode (152, 176-179) equipped with a Restek 13323 capillary column (30m x 250µm x 0.25 µm). The following oven program was used: initial temperature at 35°C hold for 30 min, initial temperature gradient at 5°C/min from 35-200°C, final temperature gradient at 10°C/min from 200-250°C, final temperature hold at 250°C for 1 min and 5ul was injected by autosampler.

2.3 Settling Experiment

The BioTiger™ consortium and controls were tested with oilsands tailings (3% bitumen) from Ft. McMurray, Canada, kindly supplied by Shell Canada. All columns treatments were prepared in duplicate. Control columns included oil sands tailings with no bacteria added, *E. coli* (ATCC#29425), and *S. mobilis* (EPA 505). The settling experiment was performed at room temperature (25 °C) as environmental conditions at the oil sands

processing sited can vary from freezing to up to 65°C. Oil sands process water has increased temperatures to decrease the bitumen viscosity, increase surface charge of sand grains and bitumen, and enhance bitumen separation and settling of sand particles (Zhao et al., 2006). For the testing, 25 ml v/v of mixed tailings was added to 225 ml of sterile-filtered phosphate buffered saline (PBS) in 250 ml autoclaved glass columns with air stones at the bottom. Eight columns were run simultaneously with treatments in duplicate including BioTiger™, *E. coli* k-12, EPA 505, and an abiotic treatment (tailings only). All microorganisms were added to an initial concentration of $10^{7\text{-}8}$ CFU/ml. Air stones were operated for 1 hour and then all columns were allowed to settle. Measurements were taken from a 10 cm depth at times 0, 24 h, 42 h, 48 h, 72 h, 144 h, and 288 h of settling rates (cm), dissolved oxygen (D.O. %), turbidity, temperature, pH, and conductivity. Air flow, 12 mL/min. per column, was turned off @ 24 h. Total organic carbon (TOC) was measured with HACH kits (Cat# 28159-45 and 27604), settling rates was measured in mLs, turbidity was determined with a Hach kit (Cat # 2660153) in nephelometric turbidity units (NTU), chloride (Cl^-), pH, temperature (°C) and conductivity (Cond. mS/cm) were measured with an Orion 5-Star (Thermo Scientific). Heterotrophic colony forming units (CFU's) or aerobic microbial densities were determined using plates made with R2A agar medium (Sigma Aldrich, St. Louis, MO. Microbial densities, settling, and turbidity measurements were ceased after 24 hr. as removal of any overlaying water disturbed the column settling process.

2.4 Laser Confocal Microscopy

Duplicate one gram samples of Athabasca oil sands were mixed with eight mLs of filter sterilized PBS each in two separate 15 ml polypropylene tubes and vortexed for 2 min. One ml BioTiger™ was added to one vial (treated) with a concentration of 10^{7-8} CFU/ml. Then one ml of PBS was added to the second (untreated oil sands) vial. Both vials were mixed another two min and then placed in a shaking incubator for 30 min @ 25° C. After 30 min the tubes were removed from the shaker, and 10 µl aliquots aseptically placed on microscope slides. Slides were air dried in a sterile hood, overlaid with 20 µl sterile filtered 2% gelatin in PBS, heat fixed, stained with fluorescein isothiocyanate (FITC) (Sigma Chemical Co.) for two minutes, and then washed three times with filter sterilized (2 µm) deionized water. Samples were examined using a Zeiss Model 510 Laser Scanning Confocal Microscope (LSCM) excited at 488 nm with an argon laser. Photographs were taken of the treated and untreated oil sands sample with a 1000 x 1.3 oil immersion objective.

3. Results.

3.1 Single Carbon Source Experiment

Over 24 hours (Fig 1) the live consortium of BioTiger™ decreased the phenanthrene concentration below detection limits (4% relative to 100% saturated medium), and live and dead *E. coli* k-12 decreased the phenanthrene concentration less than 12%, while the autoclaved BioTiger™ was still modestly effective reducing the phenanthrene concentration by 30%. Cell-free medium had no detectable change in phenanthrene concentration.

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194 *3.2 Settling Experiment*

195 Figure 2 shows the experimental set up of the eight columns for the settling experiment at
196 Time 0 and 24 h. Total organic carbon provides an indication of the amount of organic
197 matter and dissolved fraction of organic carbon found in the oil sands tailings, in which
198 PAHs can make up 40–60% of the organic carbon content (Small et al,. 2012). TOC
199 measurements in overlay water of sedimentation cylinders out to 144 hours are shown in
200 Fig. 3. Results at 24, 42, and 48 h indicate higher TOC concentrations in the BioTiger™
201 overlay samples as compared to controls with no bioaugmentation (indigenous
202 microorganisms only) or *E. coli* k-12. However, at 144 h the BioTiger™ overlay samples
203 TOC concentrations were lower than the other treatments, indicating biodegradation (Fig.
204 3).

205 The greatest rate of sedimentation was observed in the first 48 h by all treatments
206 measured (Fig. 4). All figures from the column results represent the mean of two
207 columns for each reading with the bar being standard deviation. The columns with *S.*
208 *mobilis* EPA 505 developed floating flocs that made accurate determination of TOC,
209 settling rates, turbidity, pH, dissolved oxygen, overlay water conductivity, and chlorine
210 impossible. Oil sands tailings in the six columns demonstrated levels of sedimentation
211 ranging from 54-84 mL (v/v) in 24 h (Fig. 4). Results demonstrated that BioTiger™ had
212 more increase in tailings settling as compared to controls with no bioaugmentation
213 (indigenous microorganisms only) or *E. coli* k-12. Initial (time 0). BioTiger™ treated
214 tailings solids settled slower initially, possibly due to observed small floc formation

observed in the these two columns. The settling was most likely due to released hydrocarbons from oil sands tailings particulates. All figures from the column work represent the mean of two columns for each reading with the bar being standard deviation.

Turbidity measurements (NTU) could not be taken until 4h after enough settling had occurred of the overlay water (Fig. 5). Turbidity was highest at the initial 4 h and 6 h sampling points for the uninoculated or abiotic columns and the lowest for BioTiger™ treatments. However the abiotic columns had the lower turbidity at 24h although all the treatment means were similar at that time (Fig. 5). We did not attempt further turbidity measurements after 24 hr. as sample removal caused disturbance of settled material as well as a visible hydrocarbon layer on surface.

Dissolved oxygen (DO) was measured out to 288 h in the overlay water of the settling experiment (Fig. 6). The DO was observed to be variable by decreasing rapidly in all treatments for the first few days and then rose most likely due to some stratification observed, oxygen release from particles, and increase settling occurring in the water columns. However by 288 h variability was very low and BioTiger™ had the lowest DO measured (Fig. 6).

The overlay water conductivity (mS/cm) was highly variable and increased the first 24 h (Fig. 7). It then generally increased out to 288 h in the settling experiment for the abiotic and BioTiger™ treatments that did not seem to be significantly different. The *E. coli* k-12 treated columns decreased slightly between the 148 h and 288 h measurements (Fig.

7). Results represent the mean of two columns for each reading. Bars are standard deviation of two measurements.

The addition of the microorganisms as well as the sterile PBS for the abiotic control to the columns appeared to cause an initial 0.10-0.55 decrease in pH (Fig. 8). Over lay water pH measured out to 288 h demonstrated an overall decrease pH during the settling experiment in the 7.2-7.3 range, ideal for microbial activity.

3.2 Effect on microbial activity

Indigenous microorganisms were observed in the untreated tailings as observed in Fig. 9. There was as few as one bacterium observed per microscopic field. The active heterotrophic microorganisms in the tailings were found to be 1.5 X 10^3 CFU/ gram (wet weight). Table 2 lists the microbial concentrations in the column treatments at time 0 after immediate BioTiger™ or control addition and mixing and after 24 h. All values are volume/volume (v/v) for the tailings/PBS slurry. The abiotic control is the untreated tailings column with indigenous bacteria only. The abiotic control population varied less, starting at 3.0 X 10^4 CFU/mL v/v, and then increasing slightly to 5.1 X 10^4 CFU/mL v/v at 24 h. It was observed the indigenous culture plates had mostly small uniform white colonies. Similarly the *E. coli* K-12 also varied from 5.6 X 10^7 CFU/mL to 8.6 X 10^7 CFU/mL. The bacteria in the BioTiger™ increased significantly three orders of magnitude from 8.5 X 10^5 CFU/mL to 1.2 X 10^8 CFU/mL. A few fungi colonies were noted on the plates as well. After BioTiger™ was added to tailings, cells were observed attached to particulate material in the tailings within minutes of incubation (Fig. 9). Oil

sands tailing with no BioTiger™ added had some cells observed, as few as one per microscopic field (Figure 9).

The microbial ecology of indigenous bacteria in oilsands and tailings, both aerobic and anaerobic populations, has been well documented (Wyndham and Costerton, 1991; Hadwin et al., 2006; Siddique et al., 2011). All though indigenous microorganisms, both fungi and bacteria, were detected both microscopically and in culture here, the technique used here to determine viable aerobic heterotrophic microbial populations (CFU) does not allow identification of species.

4. Discussion

In this project we have demonstrated BioTiger™ could be potentially used in a bioprocess to increase settling of oilsands tailings. BioTiger™ has a selective advantage in this environment over certain indigenous bacteria from Athabasca watershed sediments that have been shown to be inhibited by bituminous compounds (Yergeau et al., 2013). Previous investigations have demonstrated the potential of anaerobic bioprocesses for densification of oil sands tailings (Siddique et al., 2014; Bordenave et al., 2010). Bioremediation of oil contamination, including biostimulation combined with bioaugmentation, has proven effective for a wide range of conditions including Antarctic soils, (Vázquez et al. 2009). Bioaugmentation of oil sands tailings with bacteriophages has also been tested to enhance particle aggregation and sedimentation with some success (Curtis et al., 2013). Hydrocarbons released from oilsands tailings could decrease nutrient loading of sediments and associated methane and other greenhouse gas formation. The released hydrocarbons could be recovered for processing.

Figure 1 demonstrates biodegradation of phenanthrene, a PAH, by BioTiger as a sole carbon and energy source, in 24 h. Some slight decrease by the bacteria controls was observed most likely due to sorption by the cells. The ability to degrade PAHs gives BioTiger™ an ecological advantage in this environment. Improved water quality (e.g. lower turbidity) could be managed in the bioprocess with BioTiger™. TOC in overlay water was greater in the BioTiger™ treated columns that the others up to 48 hours indicating initial hydrocarbon release from tailings (Fig. 3). It would be possible to use this action to recover hydrocarbons, e.g. through skimming, at this time if economically feasible. However, at 144 hours the TOC concentrations were lowest in the BioTiger™ columns, indication potential remediation but crucial timing needed for any future bioprocessing effort. It was observed that the BioTiger™ treatments had a lower turbidity when sampled at 6 hours (Fig. 5). However there was no difference between the treatments at 24 hours. Again timing could be a factor in developing a BioTiger™-based bioprocess that would take this timing into design considerations. Tailing separation and densification is attributed to aggregation of bacterial cells with clay particles and hydrocarbon release. It could have been after six hours materials were being released from the sediments causing turbidity to go up in the water column of the BioTiger™ treatments. The BioTiger™ columns had the lowest DO measured by day 12 (Fig. 6). This increase in oxygen consumption would be expected since the BioTiger™ treatments were also found to have significantly higher microbial activity (Table 2). Increased DO consumption from the microbial activity could also result in increased CO_2 production from respiration. The associated CO_2 released from the sediments would influence settling and turbidity. In columns chloride was observed to increase slightly in

the overlay water where measurements were taken in all treatments (data not shown). The fact that the bacteria densities in the BioTiger™ treated columns increased three orders of magnitude could indicate synergistic activity with the indigenous tailings microbial flora (Table 2). Similarly in Suncor's Northern Alberta oil sands tailings pond chloride levels have been observed to be significantly lower in the MFT pore water compared with those found in the overlaying water (Wells, 2011).

The described methodology could be employed on a larger scale to decrease the transport of hazardous hydrocarbons from theses oils sands processing sites. Parameters including mixing, temperature, and culture metabolism could be optimized for maximum hydrocarbon recovery, settling efficiency, and improved effluent quality. One of the key issues in densification and separation of hydrocarbons from particles and the MFTs is the clay minerals (Hooshiar et al., 2012). The tailings can vary greatly in settling behavior based on the initial oil sands ore quality (e.g. bitumen (4-20%), types of clay present, as well as extraction efficiency (Hooshiar et al., 2012; Xu et al, 2012). Development of a pilot-scale system would prove more flexible for industrial scale deployment than batch treatments, and future work to improve MFT settling efficiency should include continuous flow systems simulating pond conditions.

Organic carbons, including NAs have been identified as the largest component of MFTs in the tailings waters from oils sands extraction processes (Small et al., 2012). They are the major contributor to the acute toxicity of the fine tailings ponds and effluent wastewater at the oil sands extraction plants in northeastern Alberta, Canada. In this work, columns of oil sands tailings treated with microbial augmentation and abiotic

controls were studied, including BioTiger™, those organisms isolated from oil sludge lagoon-affected waters, and the influence on overlay water chemistry and settling was examined. Reducing the organic matter in tailings ponds with BioTiger™ would minimize methanogenic activity and release of other greenhouse gases that can affect these settling basins and impact environmental restoration.

Process water treatment has become of increasing concern for Canada's oil sands industry. Increased water demand and ongoing recycling of tailings pond water has contributed to a decline in water quality that has consequences for bitumen recovery, water consumption, and reclamation efforts. From 1980 to 2001, the salinity of TPW increased at a rate of 75 mg/L per year and while some biodegradation has been documented naphthenic acids continues to be the primary biohazard of concern (Allen, 2008). Current increases in process effluent water hardness, sulfate, chloride, and ammonia have raised concerns over scaling and corrosion. Naphthenic acids released during bitumen extraction are the primary source of toxicity in TPW. While biodegradation of naphthenic acids has been demonstrated in pond experiments, remaining recalcitrant hydrocarbons may contribute to slow release or chronic toxicity in reclaimed environments.

Active methanotrophic bacteria have been consistently detected in tailings pond surface indicating constant methane production (Saidi-Mehrabad et al., 2012). Molecular analysis demonstrated that the TPW methylotrophic community possessed capability for formaldehyde oxidation, carbon fixation and detoxification of nitrogenous compounds as well as methane monooxygenase. The fact that the TPW methane production is

increasing indicates the methanogenic bacterial activity in the sediments is responding to the increase organic matter loading in the sediments (Holowenko et al., 2001).

Previous applications with bacteria from the BioTiger™ consortium in a bioreactor resulted in degradation of over 20,000 mg/kg of hydrocarbon contaminated soils (Berry et al., 2006). That project demonstrated that soils co-contaminated with radionuclides and hydrocarbons could be successfully treated through bioventing and bioaugmentation while protecting workers and the environment from radiological contamination.

From the results of this study, it can be concluded that BioTiger™ can be used to treat oil sands tailings resulting in improved overlay water quality and increased settling of sediments. This fact is critical as even microorganisms cultured from Athabasca sediment have demonstrated inhibition by bituminous compounds (Yergeau et al., 2013). The ability of the BioTiger™, a natural microbial consortium, to work with this hazardous material with minimal handling demonstrates potential for pilot scale testing in larger reactors with varying types of oil sands tailings. The fact that the BioTiger™ bacteria increased three orders of magnitude in the oil sands tailings indicates this bioprocess could be a sustainable and cost effective effort with limited added energy beyond mixing and aeration. BioTiger™, a natural microbial consortium, worked effectively and synergistically with indigenous microbial populations proving that it can be applied to bioremediate hydrocarbons from oils sands processing tailings ponds. The target tailings ponds and bioprocess described here has the potential to minimize the environmental impact of oil sands processing while recovering bitumen and improving overlaying water quality.

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Tables

Table 1. Components of the BioTiger Consortium and the American Type Culutre Collection (ATCC) Accesssion number

Isolate	Identification	ATCC Accession
CZOR-L1B	*Alcaligenes-Piechaudii SRS*	PTA-5580
BP-20 (KN-2)	*Ralstonia Pickettii SRS.*	PTA-5579
CZOR-L1Bsm	*Pseudomonas-Putida* BIOTYPE B *SRS*	PTA-5581
BPB	*Flexibacter CF. SANCTI SRS*	PTA-5570
BPC	*Pseudomonas Fredriksbergensis SRS*	PTA-5571
BPE	*Staphylococcus warneri. LMG 19417 SRS*	PTA-5572
BPF	*Sphingomonas SRS*	PTA-5573
BPH	*Sphingomonas SP. S37 SRS*	PTA-5574
BPI	*Phylobacterium SRS* (α Proteobacterium TA-A1)	PTA-5575
BPJ	*Serratia ficaria SRS* (α PROTEOBACTERIUM TA12-21)	PTA-5576
BPK	*Agrobacterium Tumefaciens SRS*	PTA-5577
BPL	*Rhizobium SP. SDW045 SRS*	PTA-5578

508

509 **Table 2.** Mean* CFU/mL (v/v) from columns (two per treatment) taken at time 0 (after 5
510 min mixing) and 24 hr.

511

Treatment	**CFU/mL @ Time 0**	**CFU/mL @ 24 h**
BioTiger™	8.5E+05	1.2E+08
E. coli K-12	5.6E+07	8.6E+07
Abiotic Control	3.0E+04	5.1E+04
S. mobilis EPA 505	1.9E+05	5.4E+04

512 *Mean from duplicate plates

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515

Figures

Figure 1.

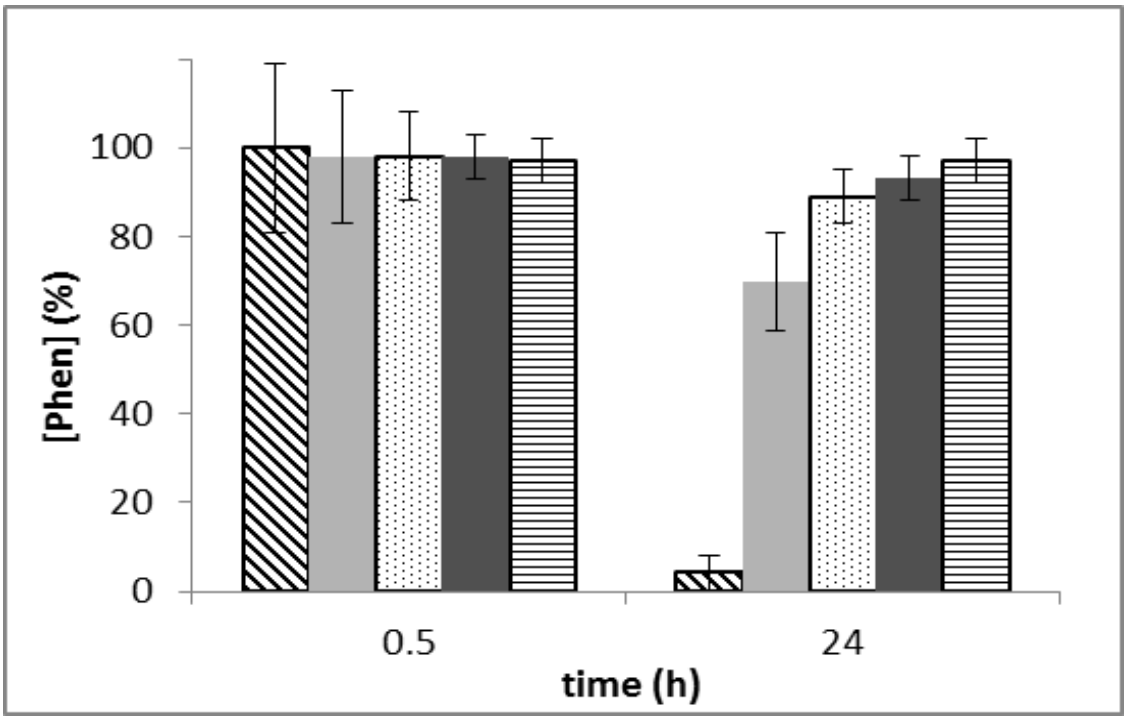

Figure 1. Single carbon source experiment. Cells inoculated in saturated phenanthrene Bushnell-Haas medium. Dotted bar is live *E. coli* (K12), Light gray bar is dead BioTiger™, Diagonal lined bar is live BioTiger™, Dark gray bar is killed *E. coli,* and horizontal lined bar is no cells added.

525

526 **Figure 2.**

527

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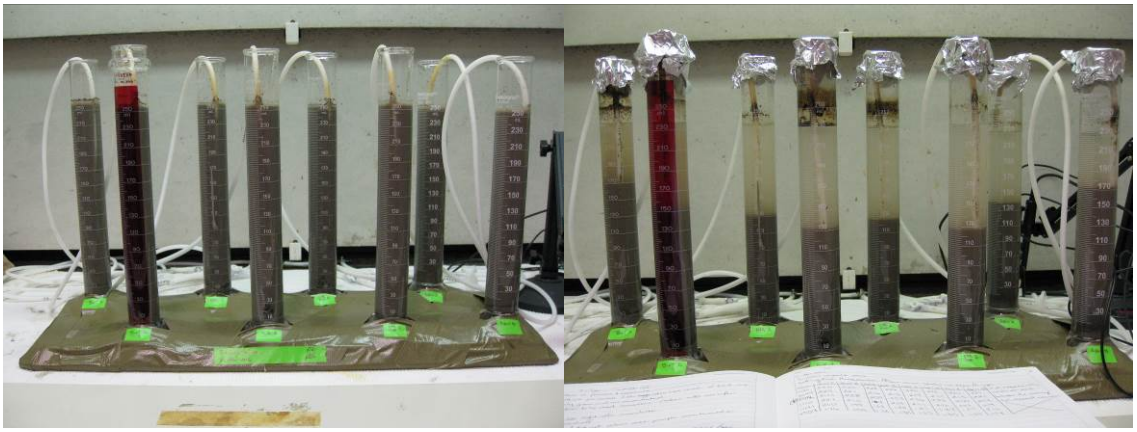


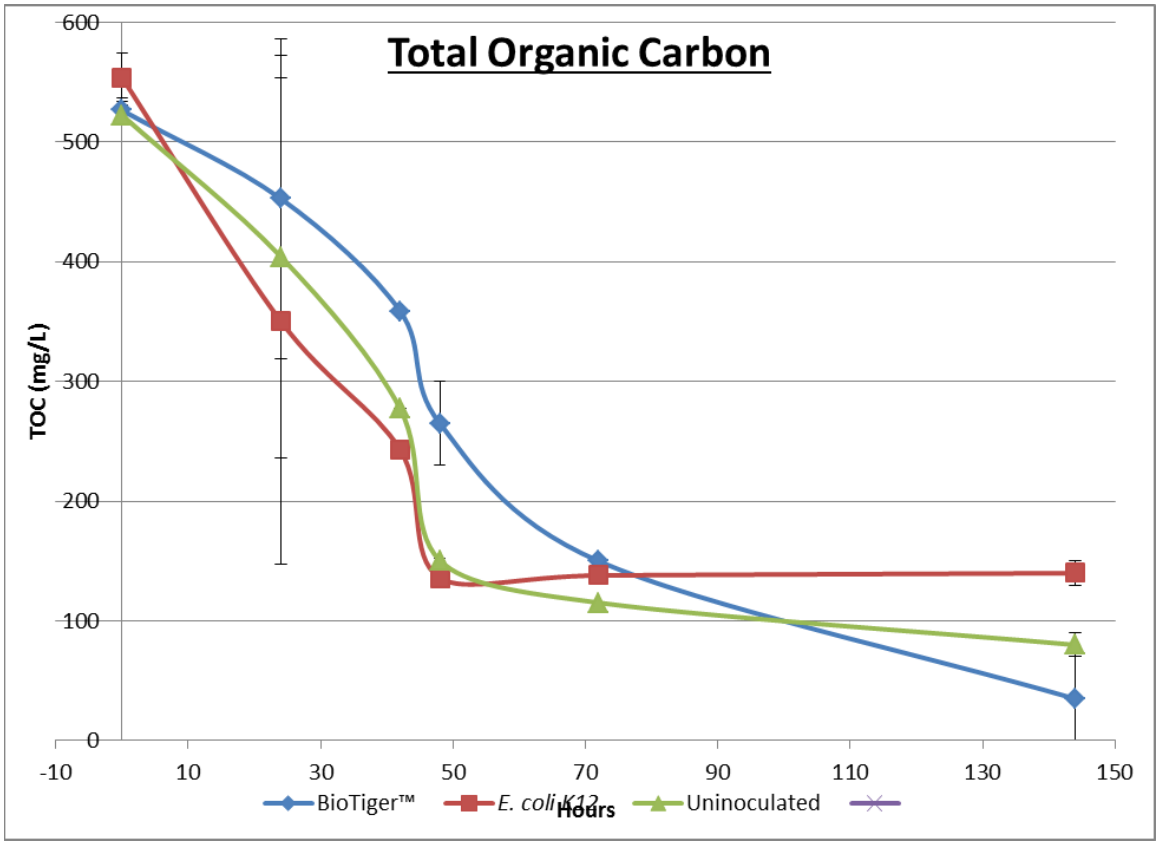
529

530 **Figure 2. Columns with oil sands tailings and treatments at time 0 (L) and after 24**

531 **hr (R)**

532

533 **Figure 3.**



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536 **Figure 3. Total Organic Carbon (TOC) concentrations in overlay water of**
537 **sedimentation cylinders over 144 hours. Results represent the mean of two columns**
538 **for each reading. Bars are standard deviation of two measurements.**

Figure 4.

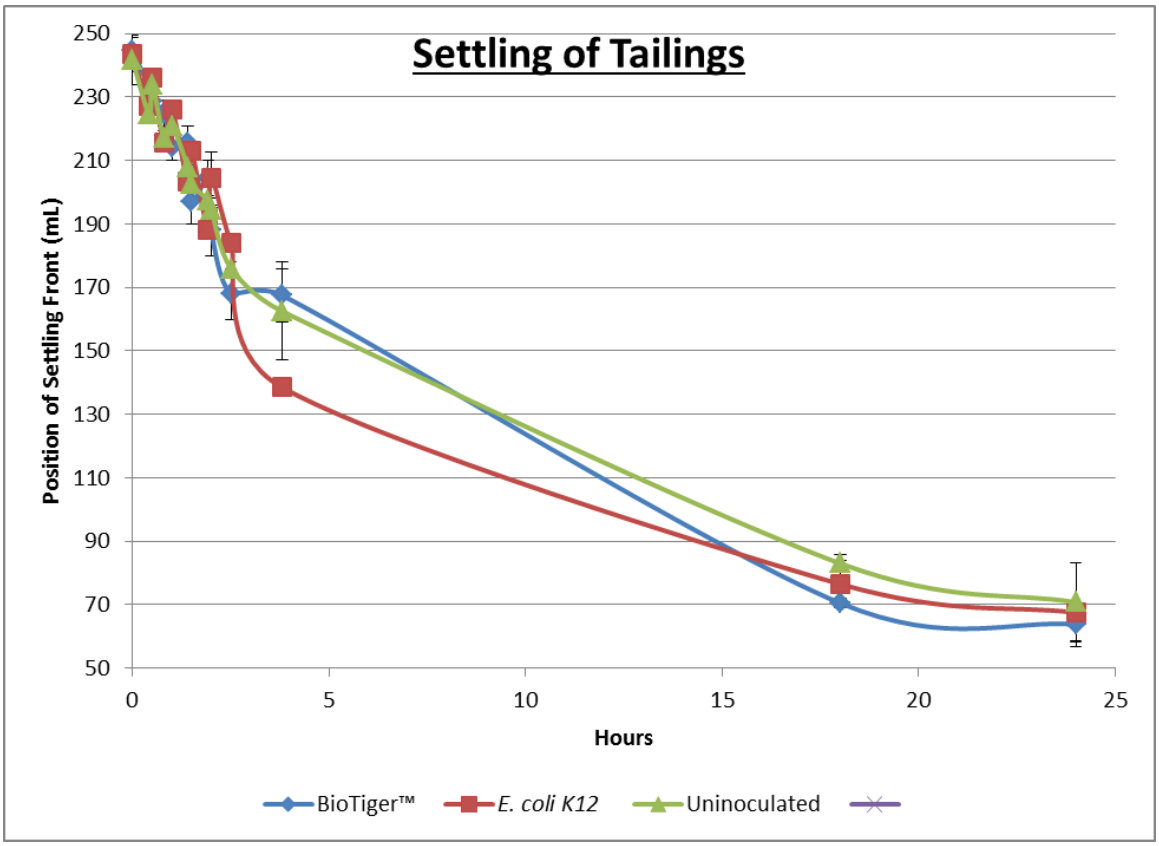
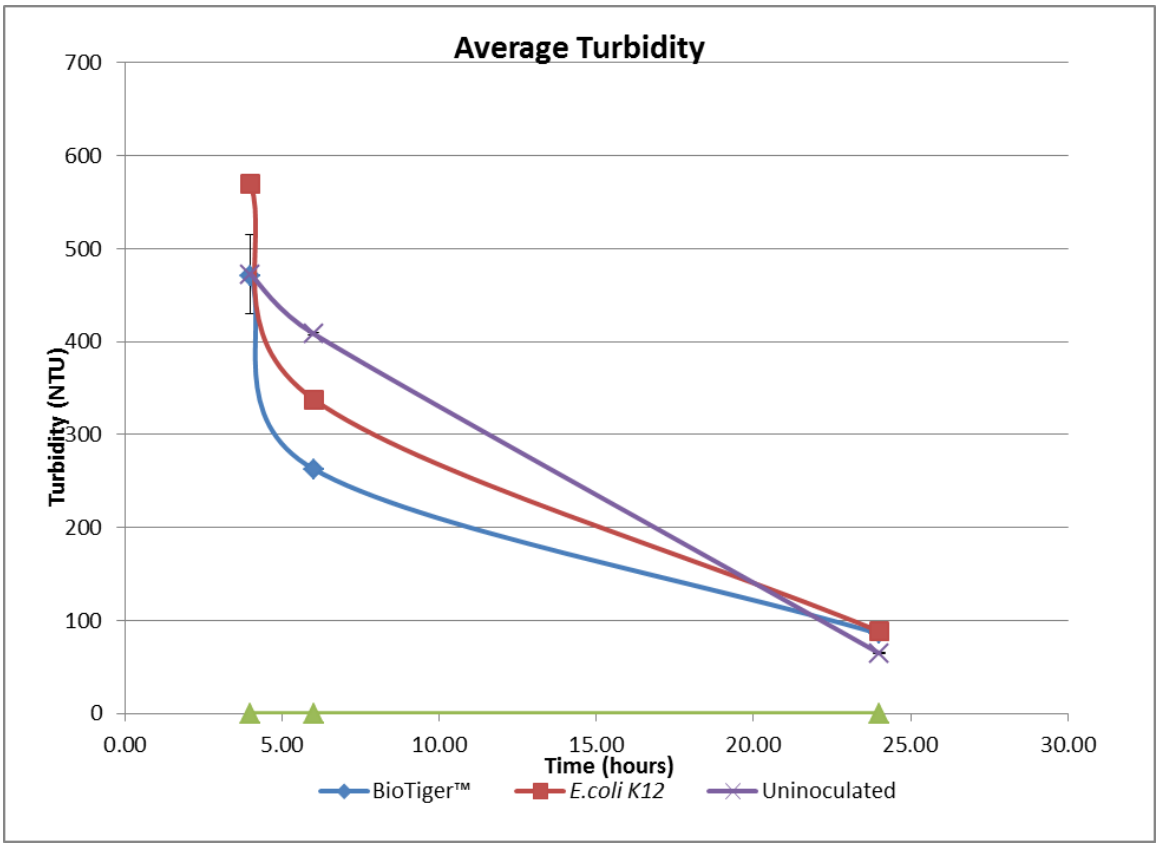


Figure 4. Oil sands tailings levels of sedimentation (v/v) per ml @ 24 hr. Results represent the mean of two columns for each reading. Bars are standard deviation of two measurements.

546 **Figure 5.**



547

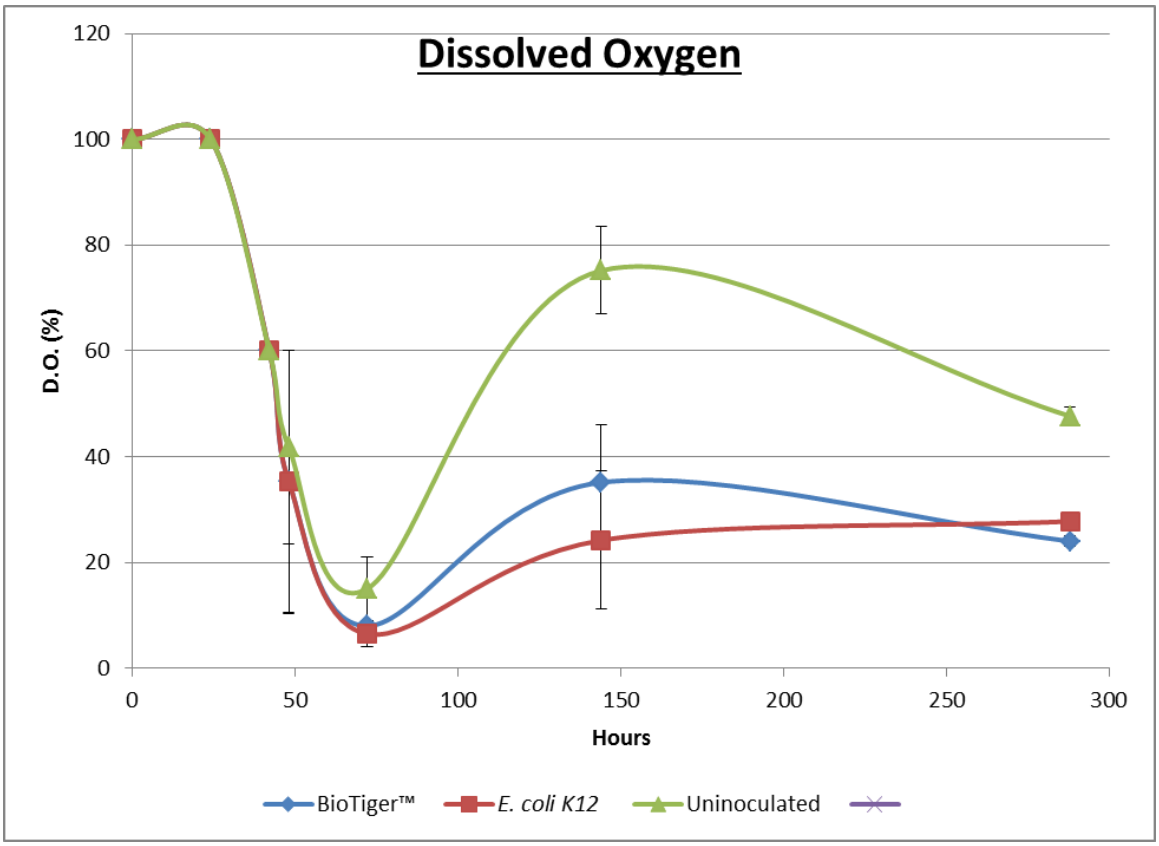
548 **Figure 5. Turdbity (NTU) of oilsands tailings in water overlay measurement for 24**

549 **h. Results represent the mean of two columns for each reading. Bars are standard**

550 **deviation of two measurements.**

551

552 **Figure 6.**



553

554 **Figure 6. Dissolved oxygen (DO) measured out to 288 h in the settling experiment.**

555 **Results represent the mean of two columns for each treatment. Bars are standard**

556 **deviation of two measurements.**

557

Figure 7.

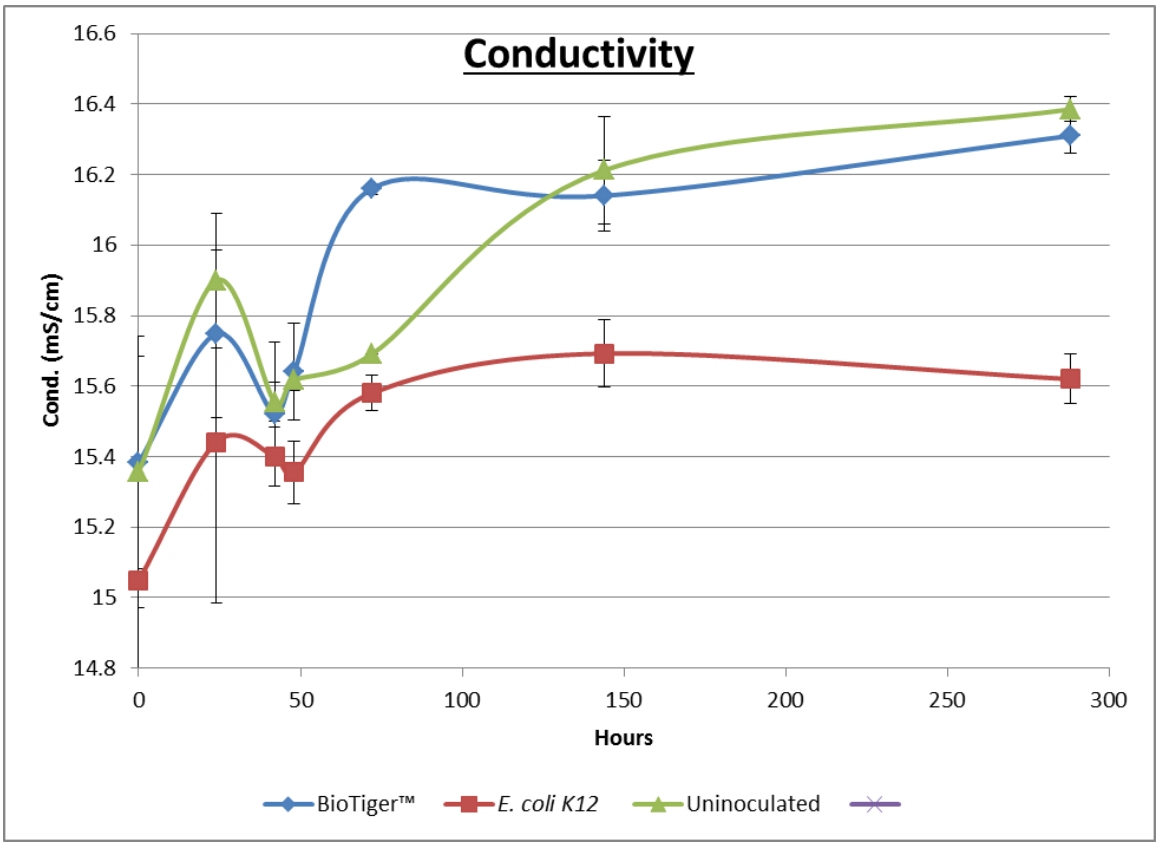
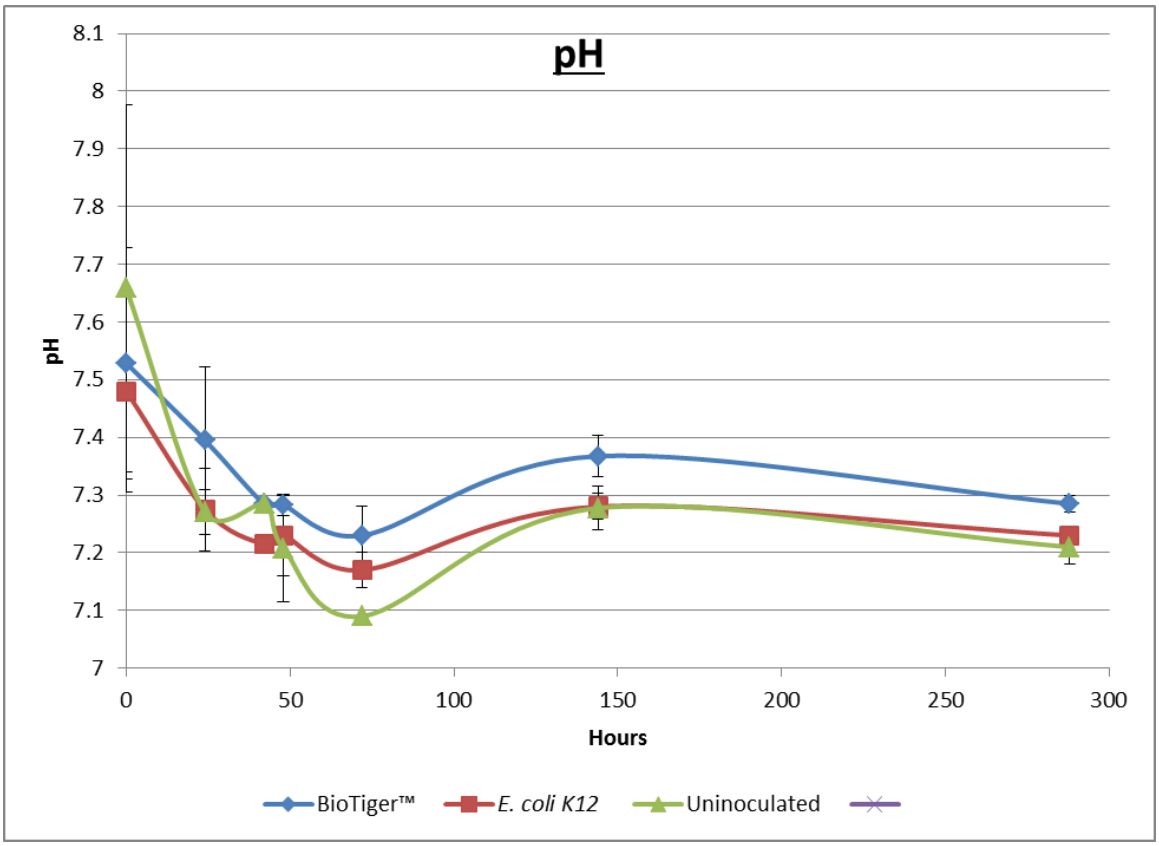


Figure 7. Conductivity (mS/cm) measured out to 288 h in the settling experiment. Results represent the mean of two columns for each reading. Bars are standard deviation of two measurements.

564 **Figure 8.**



565

566 **Figure 8. Over lay water pH measured out to 288 h in the settling experiment.**

567 **Results represent the mean of two columns for each treatment. Bars are standard**

568 **deviation of two measurements.**

569

Figure 9.

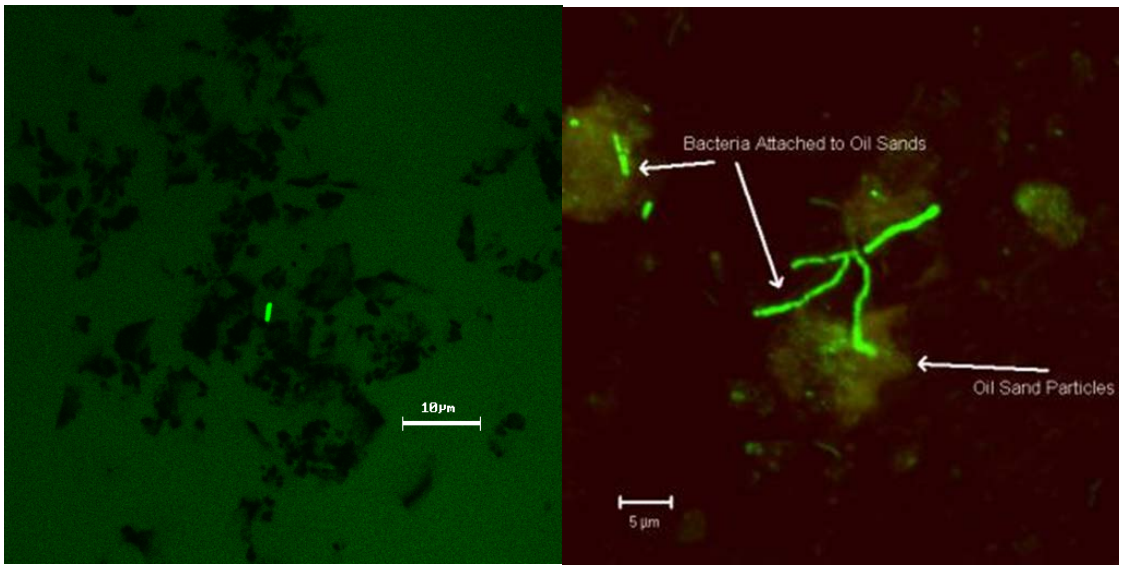


Figure 9. Bacteria observed in untreated oil sands were as few as 1 per field labeled with FITC (L). After adding BioTiger™ bacteria were observed binding to oilsands particles within 30 min (R).