EVALUATION OF HYDROCARBON DEGRADATION BY INDIGINOUS BACTERIA DEGRADE RESIDUAL HYDROCARBONS OF MATURE FINE TAILINGS UNDER ANAEROBIC CONDITIONS

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ABSTRACT
Tailing ponds are facilities to store tailings which are byproducts of bitumen extraction from the oil sands industry. Tailings consists of residual hydrocarbons, water, sand, clay and metals, which take many years to settle. Deeper layers of tailings, called Mature Fine Tailings (MFT), naturally take almost 10 years to settle. Therefore, the separation and treatment of residual hydrocarbons in MFT layers is one of the challenging problems. In this study, bacteria have been isolated from MFT layers under anaerobic conditions and then cultured on agar plates that contain crude oil as the source of carbon. The bacteria which showed oil degradation were added to the anaerobic bioreactor. The tailing ponds environment was simulated in the bioreactor, and the hydrocarbon degradation was monitored by gas chromatography. The results indicated that even in an anaerobic environment, the isolated bacteria from the polluted soil could degrade residual hydrocarbons within the first week of addition to the bioreactor.

RÈSUMÈ
Les bassins de résidus sont des installations pour stocker les résidus qui sont des sous-produits de l'extraction du bitume de l'industrie des sables bitumineux. Les résidus sont constitués d'hydrocarbures résiduels, d'eau, de sable, d'argile et de métaux, qui mettent de nombreuses années à se déposer. Les couches plus profondes de résidus, appelées résidus fins matures (MFT), mettent naturellement près de 10 ans à se déposer. Par conséquent, la séparation et le traitement des hydrocarbures résiduels dans les couches MFT est l'un des problèmes difficiles. Dans cette étude, des bactéries ont été isolées à partir de couches MFT dans des conditions anaérobies, puis cultivées sur des plaques de gélose contenant du pétrole brut comme source de carbone. Les bactéries qui ont montré une dégradation de l'huile ont été ajoutées au bioréacteur anaérobie. L'environnement des bassins de décantation a été simulé dans le bioréacteur et la dégradation des hydrocarbures a été suivie par chromatographie en phase gazeuse. Les résultats ont indiqué que même dans un environnement anaérobie, les bactéries isolées du sol pollué pouvaient dégrader les hydrocarbures résiduels au cours de la première semaine d'ajout au bioréacteur.

1. Introduction

In the oil sands industry, tailings are the combination of water, sand, clay and remaining bitumen. They are the by-products of the separation process of the “oil” from sand and clay in oil sands mining operations. These tailings are kept in a dyke called a tailing pond for storage. The water used in bitumen extraction process is separated and goes for the recycling process. Then the recovered water is sent back to the oil sand processing plant, and this is done continuously until up to 87% of water is used (capp.ca). The remaining bitumen and the structure of the tailings compounds can build significant problems in terms of fluid fine settlement. Illite which is considered as a non-expanding clay and complex layers of clay prevents the settlement of particles. A major portion of deposited oil sand tailings consists of these clay particles (Javan Roshtkhari, 2016).

At the bottom of the ponds, coarse sand grains (particles with diameters more than 44 microns) settle rapidly and are called “coarse tailings” (Foght et al. 2017). Tailings that contain 10-30% fines (particles with diameters less than 44 microns) are called Fine Fluid Tailings (FFT). They consist of 8 wt% fines, 5% un-extracted bitumen, <1% unrecovered diluent and 85% processed water and take 2-3 years to settle. Dewatering of FFT can reach 20% solids after 3-9 months. If the FFT reaches more than 30 wt%, they are called Mature Fine Tailings (MFT). They take almost 10 years to settle. It is difficult to separate the water from these fines. It has been estimated that if MFT is not treated, it can take 150 years to completely dewater and settle (Siddique et al. 2018).

The top layer water of oil sands tailings ponds (OSTP) is under oxic conditions while the deeper layers become anoxic with increasing depth (Ramos-Padrón et al. 2011). FFT and MFT sections contain a lack of oxygen because of increasing temperature and limited oxygen transfer with increasing depth and microbial metabolism (Siddique et al. 2011).

The crucial issues associated with OSTP are remaining hydrocarbons, naphthenic acid, polycyclic aromatic hydrocarbons (PAHs), greenhouse gas (GHG) emissions such as methane and carbon dioxide, and heavy metals from the large volumes that exceed 1.18 trillion liters that are continually growing. Moreover, these contaminants can threaten the health and safety of local people and wildlife and have enormous environmental impacts such as ground water contamination by chemical seepage and GHG

emissions (Foght et al. 2017). The density of the tailings and cost-effective recovery of the water bring challenges to this industry.

Both FFT and MFT sections of oil sand tailing ponds have anoxic conditions. The strict and facultative anaerobic in the FFT and MFT which are considered as indigenous bacteria can use hydrocarbons as a carbon source (Siddique et al. 2011). These microorganisms can be methanogens, nitrate reducers, iron reducers and sulfate reducers. The type of microorganisms depends on the age of the OSTP, type of diluent, use of flocculants and CO_2 injection (Burkus et al. 2014). It is noticeable that without human interference FFT and MFT degradation takes a long time. Researchers have used various ways to enhance the biodegradation of tailing ponds.

In recent decades, biological treatment of contaminant soil and water improved and solved many environmental troubles. In the oil sands industry, biological treatment was added as a remediation technique to save more money and energy and also use less chemicals. In this way, biosurfactants produced by microorganisms which are biological surfactants can reduce the surface tension, foaming capacity, emulsification and solubility. Biosurfactants have been the green revolution of bioremediation. Low toxicity and biodegradability are the two main factors of the biosurfactant that can play a key role in a range of industries such as petroleum, food and health products (Mulligan et al. 2014).

The first research of biosurfactants was conducted in 1960, and since then many research studies and experiments have been done to determine the function of the biosurfactant. In soil contamination there are well-known bacteria such as *Pseudomonas sp*. and *Bacillus sp*. which can produce biosurfactants like rhamnolipids and surfactin to enhance biodegradation of the hydrocarbons. Because of the nature of bacteria, many factors can affect their biosurfactant production such as pH, temperature, source of carbon, oxygen, etc. which must be considered by scientists when testing different ways of biosurfactant production. In most of the research, the environmental factors for biosurfactant production were examined especially the carbon and nitrogen source from the raw materials, wastes and natural sources such as vegetables oils, molasses, etc. However, the oxygen and anaerobic conditions did attract much attention. This factor is important especially for mature fine tailings (MFT) in the tailing ponds where there are low amounts of oxygen at very deep location.

Brigmon et al. (2016) used BioTiger™ (a twelve-bacteria consortium isolated from a Polish oil refinery's waste lagoon in which the bacteria can biodegrade naphthenic acids and hydrocarbons with their collaboration) to improve the separation of organic carbons from water in MFT. Also, they indicated that three strains of bacteria produce biosurfactants. They demonstrated that this bacteria consortium can increase the settling and improve the water quality. Javan Roshtkhari (2016) accelerated sedimentation of FFT by adding rhamnolipid biosurfactant and mixing it with indigenous bacteria (isolated from weathered oil).

In most research, using exogenous microorganisms is more common for the tailings bioremediation. The reason behind this could be due to the benefit of using identified and recognized microorganisms which are easier to control the growth during bioremediation. However, indigenous microorganisms can play an important role in the hydrocarbon degradation of the tailings. They are from the tailings and would be returned to perform the bioremediation.

This study objectives are to provide information regarding the role of indigenous oil-degrading bacteria in the MFT layer of oil sands tailings under anaerobic conditions, evaluate their ability to produce biosurfactant and evaluate the MFT layer condition by simulating tailing ponds samples from Alberta in the stirred tank reactor (STR) under an anaerobic environment with the isolated bacteria.

2. Material and Methods

2.1. Samples from MFT Layers of a Tailing Pond in Alberta

A mature fine tailing ponds sample was obtained from Edmonton, Alberta in a 20 L container. The sample was taken from the MFT layer of tailing ponds. The sample contained 30-50% solids (clay, metals, quartz silica, kaolinite, aluminum, calcium, iron and potassium), 5-10% bitumen, <1% naphtha. The color of sample was grey to brown and the droplets of residual bitumen were obvious on the surface.

2.2. Isolation of Bacteria

Anaerobic bacteria were isolated from the tailings ponds samples from Alberta. Minimal salt medium (MSM) enrichment culture techniques were used for isolating the bacteria under anaerobic conditions.

The medium was adjusted to the pH 7.0 and autoclaved at 121°C for 20 min to sterilize it. Crude oil was autoclaved at 121°C for 20 min as the source of carbon. Five grams of the MFT sample and sterilized crude oil (2 v/v) were added to the media bottle and sealed under a stream of argon to replace the oxygen and make the environment anaerobic; then the sample was placed on a rotary shaker at 200 rpm for 7 days at 28°C. These cycles were continued five times to grow the bacterial colonies.

2.3. Isolation of Bacterial Colonies

In order to isolate the colonies, three different types of agar plates were prepared: Luria Bertani (LB), Tryptic Soy Broth (TSB) BD Difco™ and Bushnell Haas (BH) contain $MgSO_4$ (0.2 g/l), $CaCl_2$ (0.02 g/l), KH_2PO_4 (1.0 g/l), K_2HPO_4 (1.0 g/l), NH_4NO_3 (1.0 g/l), $FeCl_3$ (0.05 g/l) and agar powder (15 g/l) Fisher BioReagents™. The reason for these 3 different media was to find out the possibility of different types of bacteria existing in the sample. Cultured agar plates were incubated at 35°C until the bacteria were grown. To create an anaerobic condition, the jar and gas pack technique was used (Thermo Scientific™ AnaeroPack™). Various bacteria were grown on the agar plates and isolating the colonies took many cycles of plating.

In addition, pure colonies were examined by microscope (OMAX 1600) and image processing software (Scopeimage 9.0) to identify the colony morphology such as form, margin and size of colonies.

2.4. Biosurfactant Production and Screening Method

In order to find out the ability of isolated and pure bacterial colonies to produce biosurfactant, the Cetyl Trimethyl Ammonium Bromide (CTAB) agar test, hemolysis test, oil displacement test, and emulsification capacity test were conducted.

The bacterial colonies with positive results to the biosurfactant screening methods, were chosen for the biosurfactant extraction. To extract the biosurfactant, isolated and pure bacteria were grown in MSM media for 7 days. The applied MSM enrichment culture contained (g/L): $NaNO_3$ (2.0), $CaCl_2$ (0.025), KCl (0.5), KH_2PO_4 (1.0), $Na_2HPO_4H_2O$ (1.0), $FeSO_4.7H_2O$ (0.001) and $MgSO_4$ (0.1) with 2ml/L trace elements which was containing: (mg/L) of $FeCl_3.6H_2O$ (60), $Na_2MoO.4H_2O$ (15) H_3BO_3 (150), $CoCl_2.6H2O$ (60), $CuSO_4.5H_2O$ (590), $ZnSO_4.7H_2O$ (600), $MnSO_4.H_2O$ (200). Cell-free supernatant of each bacteria was prepared by centrifuging the medium 10,000 rpm at 4°C for 20 min. The pH adjusted to 2.0 by 1N HCl and acidified biosurfactant was left overnight at 4°C to precipitate. Then the precipitated samples were centrifuged 10,000 rpm at 4°C for 10 min. The chloroform-methanol (2:1 v/v) was added to the cell pellet. A rotary evaporator was used to separate the organic solvent and biosurfactant (Adebajo et al., 2020; Chandankere et al., 2013).

2.5. Bacterial Colony Identification

Isolated bacterial colonies tested for the biosurfactant production were chosen for the rest of the experiment and needed to be identified. There are different identification methods such as colony color, shape of colony, gram positive/negative. In addition, there is a FTM test, which shows the type of bacteria. The FTM test is one of the methods to find out if the isolated bacteria are aerobic, anaerobic, and facultative or microaerophilic. The catalase test was used to help bacteria identification and also find the aerobic and obligate anaerobic bacteria.

2.6. Surface Tension

Surface tension was measured by Du-Nouy Ring method. In this method forced used to detach the ring from the surface of the liquid (Kim et al., 2000). In order to measure the changes in the surface tension of the saturated water in the MFT, first, the MFT sample was centrifuged at 7000 rpm for 20 min. Then the water phase was transferred to the tube and passed through the nylon syringe filter (0.4 um pore size). The filtered water was poured into the dish and the surface tension determined.

2.7. STR and Anaerobic Bottle Experiment

After isolating and purifying the desirable bacteria and finding their ability to produce biosurfactant under an anaerobic environment, the next step was the examination of the isolated bacteria on MFT aqueous layers in the stirred tank reactor (STR). The anaerobic condition was the important factor, which was provided by adding argon gas to the setup and sealing the head completely. The amount of oxygen was monitored by a DO meter. 2 L of MFT waste were added to the system, the temperature was set at 22 °C and pH was at 7.0. After a 2 week stabilization, the isolated bacteria were added to the STR and the agitation was set at 200 rpm. Sampling was done every 7 days for 8 weeks (Grishchenkov et al., 2000). At the same time, the same condition was conducted in a 1 liter bottle and the only difference was non-agitation in order to compare the effect of agitation on the bacteria function in biodegradation.

2.8. Monitoring Bacterial Population

After adding bacteria to the STR, the population and variation of bacteria were monitored. Every week 1 ml of MFT was taken from the STR and a serial dilution technique was used. One ml of sample was added to the 9 ml of distilled water at 25°C, then, cultured on BH, LB and TSB agar plates by aseptic spreading of 50 µl of each diluted sample. Plates were sealed by parafilm and incubated at 35°C. The number of observed colonies named as colony-forming units (CFU) per ml of sample was monitored.

2.9. Hydrocarbon Degradation

Every week, 5 grams of sample were taken from the STR, then 5ml n-hexane and 5 ml of acetone were added to the sample and mixed well by a vortex mixer for 2 min. After adding 25 ml of distilled water, it was mixed again for 2 min. The mixed sample was centrifuged at 1600 rpm for 20 min. 2 different phases appeared, in which the hydrocarbon phase was the upper one, and 2ml of the part was transferred to the tube containing 0.15 g silica gel. Then it was shaken well, left to settle for 10 min, and transferred to the syringe to pass through the PTFE filter (0.4 µm pore size) (Walkley & Black, 2003).

Analyses of total hydrocarbon biodegradation were conducted by a gas chromatograph (GC-FID model Varian CP-3800). The system column was a DB-6 with a length of 30 m × 0.25 mm Di and stationary phase of 0.25 µm. The helium was the carrier gas and target flow rate was 2 ml/min. The volume of the sample injected each time was 5 µl and the detector temperature was set at 300°C.

The hydrocarbon degradation was calculated by a decrease of the area compared to the sample before treatment. For this calculation, the total area of the peaks was determined and compared to the untreated MFT as the highest amount of hydrocarbons. Then the total hydrocarbon removal was calculated by percent difference, with the initial MFT.

3. Results and Discussion

3.1. Isolation and purification of bacterial colonies

Thirteen different colonies were isolated from the MFT samples. Different types of bacteria were grown on the 3 types of agar plates (LB, BH and TSB) at 35°C. Seven isolates on LB agar, 4 isolates on BH and 2 isolates on TSB agar plates were grown. The isolates were classified based on their morphology such as color, form, margin, and size. As shown in Table 1, the form is the basic shape of colony, and size is the diameter of colony that was measured by microscope and Scopeimage software. The L1, L2, L3, L4, L5, L6, L7 were isolated on LB agar plates. B1, B2, B3 and B4 were isolated on BH agar plates and 2 isolates of T1 and T2 appeared on TSB plates.

Table 1. Isolate morphology

Strain ID	Color	Form	Margin	Size
L1	White-creamy	Round	Entire	Moderate
L2	White	Round	Entire	Large
L3	White	Round	Entire	Moderate
L4	White	Round	Entire	Large
L5	Yellow-white	Round	Entire	Moderate
L6	Yellow	Round	Entire	Moderate
L7	White	Round	Entire	Large
B1	No-color	Irregular	Entire	Moderate
B2	No-color	Irregular	Entire	Moderate
B3	White	Round	Entire	Moderate
B4	Green	Punctiform	Entire	Punctiform
T1	White	Round	Entire	Moderate
T2	White-milky	Irregular	Scalloped	Large

3.2. Type of bacteria

The type of isolated bacteria based on the oxygen requirements was examined. Two different tests were conducted to identify the bacteria type: the FTM test and the Catalase test.

Based on the FTM and Catalase tests, the type of bacteria was identified (Table 2). Strains L1, L5, L6, B4 and T1 had positive reactions to the catalase test and their growth was observed throughout the FTM tube and thus they could be a facultative anaerobe or aerotolerant anaerobe. This can be considered for the strains L3, L7 and T2 as well. Although they have negative catalase results, they could be facultative (doing fermentation instead of respiration) or aerotolerant anaerobes. Isolates L2, L4, B1, B2 and B3 were grown only at the top of the FTM tube and had a positive reaction to the catalase test, and thus they were considered as obligate aerobe bacteria. Isolates had various reactions to the catalase test. L1, L2, L4, L5, L6, B1, B2, B3, B4 and T1 had positive reactions to the catalase test while L3, L7 and T2 were catalase negative.

3.3. Identification of isolated biosurfactant producing bacteria

The isolates which grew in the anaerobic condition, were tested for the biosurfactant production. CTAB, and blood agar tests were examined in both aerobic and anaerobic conditions to determine how oxygen can affect biosurfactant production. EI and oil displacement test were done under aerobic conditions.

Table 2. Types of isolated bacteria based on FTM and catalase test

Strain ID	FTM test	Catalase test	Type of Bacteria
L1	All over the tube	Positive	Facultative/aerotolerant anaerobe
L2	Top of the tube only	Positive	Obligate aerobe
L3	All over the tube	Negative	Facultative/aerotolerant anaerobe
L4	Top of the tube only	Positive	Obligate aerobe
L5	All over the tube	Positive	Facultative/aerotolerant anaerobe
L6	All over the tube	Positive	Facultative/aerotolerant anaerobe
L7	All over the tube	Negative	Facultative/aerotolerant anaerobe
B1	Top of the tube only	Positive	Obligate aerobe
B2	Top of the tube only	Positive	Obligate aerobe
B3	Top of the tube only	Positive	Obligate aerobe
B4	All over the tube	Positive	Facultative/aerotolerant anaerobe
T1	All over the tube	Positive	Facultative/aerotolerant anaerobe
T2	All over the tube	Negative	Facultative/aerotolerant anaerobe

Isolates L1, L6 and T2 showed dark blue on CTAB agar plates in both aerobic and anaerobic environments (Figure 1) which can indicate that these isolates are facultative/aerotolerant anaerobic and can produce biosurfactant in the presence or lack of oxygen. Isolate L5 had a positive reaction only in the anaerobic environment. It is interesting that these bacteria can produce biosurfactant only in the anaerobic condition. Obligate aerobe isolates L2, L4, B1, B3 only had positive reactions in the presence of oxygen which was expected. Isolates L3, L7, B2 and T1 had a negative result in both aerobic and anaerobic environments which was evaluated with another test.

The isolate L1 demonstrated a weak hemolysis in both aerobic and anaerobic situation. Isolates L3, B4 and T1 had positive reactions on blood agar in both situations. L3 and T1 didn't have a reaction in the CTAB test but they showed positive hemolysis which indicate they possible produce a biosurfactant. Isolates L2, L4, B1, B2, and B4 showed hemolysis only in the aerobic condition, which was expected as they are obligate aerobes. L5 and L6 had weak hemolysis reactions only in the anaerobic environment. L7 didn't have a hemolysis reaction in either aerobic or anaerobic environments which was the same as in the CTAB test, indicating that this isolate cannot produce any biosurfactant (Figure 1).

Figure 1. Colony growth on right, CTAB agar plates; left, hemolysis test

The emulsification activity test was conducted with 3 repetitions and the average and the standard deviation was calculated. Isolate emulsification activities measured after 24 hours was observed for all except for L7. The highest EI24 was observed in isolates L1, L5, B2 and B3. Isolates L3 and L4 had the lowest activity that may be because of the aerobic situation, pH, temperature and other factors that may affect this test. Bacteria L7 did not show any emulsification activity, which also had a negative result in CTAB and blood agar tests.

The oil displacement test was conducted to detect the biosurfactant production. The 10 µl droplet of cell free culture supernatant on the crude oil surface in the petri dish was observed and the diameter of distribution of crude oil appeared on the oil surface was measured after 30 seconds in comparison with the negative control.

The most effective biosurfactant production from aerobic media were from isolates L2, L4, B2, B3, B4 but in the anaerobic environment, obligate aerobes didn't make any clear zones which was indicated as zero. The isolate T1 had a better reaction (21.3 mm) in comparison to the other facultative/aerotolerant anaerobes.

3.4. Biosurfactant Production

Table 3 shows the biosurfactant production of each isolate in both anaerobic and anaerobic environments. Isolates L1 to L7 in the LB media, isolates B1 to B4 in the BH media and isolates T1 and T2 in the TSB media were examined. As expected, isolates L2, L4, B1, B2, and B3 didn't produce any biosurfactant under the anaerobic situation but they have produced in the aerobic environment because they were identified as the obligate aerobes. Isolates L1, L3, L5, L6, B4, T1 and T2 had biosurfactant production under both situations, which shows they are facultative or aerotolerant anaerobes. As shown in Table 3, they have lower biosurfactant production in the aerobic condition. However, isolate T2 had good biosurfactant production even in an anaerobic condition. The highest amount of biosurfactant production were isolates under aerobic conditions L5 and L6 and the lowest was isolate B3. Also, as expected, the isolate L7 did not produce any biosurfactant.

Table 3. Biosurfactant production by isolates in aerobic and anaerobic situation

Strain ID	Biosurfactant in aerobic environment (g/l)	Biosurfactant in anaerobic environment (g/l)
L1	0.86 ± 0.02	0.38 ± 0.046
L2	1.15 ± 0.07	0
L3	1.12 ± 0.06	0.53 ± 0.041
L4	0.97 ± 0.01	0
L5	1.61 ± 0.07	0.31 ± 0.026
L6	1.66 ± 0.08	0.61 ± 0.022
L7	0	0
B1	0.95 ± 0.02	0
B2	0.83 ±0.04	0
B3	0.75 ± 0.03	0
B4	1.04 ± 0.03	0.83 ± 0.077
T1	1.07 ± 0.03	0.95 ± 0.071
T2	1.37 ± 0.08	1.2 ± 0.066

Most research has indicated that the amount of biosurfactant production is related to the condition of the bacterial growth such as the type of carbon source, pH, temperature, the type of solvent (used in the extraction method) and oxygen condition. Isolated bacteria *P. aeruginosa* respond better to biosurfactant production when it used glucose as the carbon source compared to other carbon sources such as glycerol, fructose, and starch (Tomar et al., 2016). Adebajo et al. (2020) examined different types of solvents and the highest yield of biosurfactant was demonstrated by using chloroform/methanol (2:1) v/v which was 3.15 g/l in comparison with acid precipitation (1.25 g/l), acetone (2.89 g/l), dichloromethane (2.26 g/l) and ethyl acetate (1.13 g/l).

3.5. Hydrocarbon degradation

The isolated bacteria, which showed the anaerobic growth, were selected and added to the STR and the anaerobic bottle to monitor the biodegradation of residual hydrocarbon in the anaerobic environment for 8 weeks. Each week, 3 samples were taken and analyzed by gas chromatography.

In the first week, in both the STR and anaerobic bottle, the degradation started and residual hydrocarbon existing in the MFT degraded 20% and 18% respectively. The highest degradation was recorded at week 8 for STR (58.7%) and week 7 for the anaerobic bottle (55.1%) which the bottle samples didn't show any more degradation in the week 8.

In the STR, between weeks one to week 3 the degradation was fast (38.7%) and then it increased gradually to week 5. Between weeks 5 and 6, the degradation increased and reached 54.6% and then slowed at week 8 (Figure 2).

In the anaerobic bottle, between weeks one to week 5 there was fast degradation (52.4%). After week 5, the degradation slowed down to around 55% and didn't change dramatically (Figure 2).

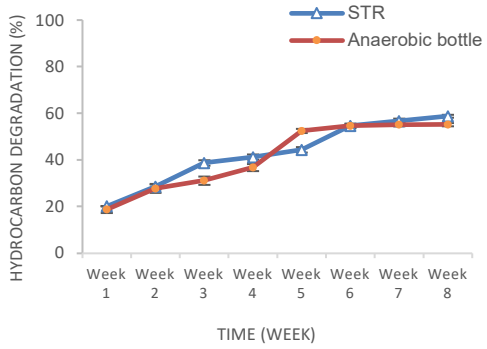

Figure 2. Hydrocarbon degradation of residual oil in MFT sample under anaerobic condition for 8 weeks in STR and anaerobic bottle.

As shown in Figures 3 and 4, the range of hydrocarbon n-alkanes degraded is between C9 to C37. In the STR, the short chain hydrocarbons, C9 and C10 peak degradation was slow at first but the second week revealed more degradation. Week 5 to week 6 demonstrated more degradation of longer chain n-alkanes which could be that some isolates started the fermentation later than the other

isolates. Weeks 7 and 8 are almost the same, with just a slight change between C15 and C22.

In the anaerobic bottle, at the first week, degradation was observed between C17 and C34 n-alkanes. Then week 2 to week 4 demonstrated more degradation between C12 to C37. During week 5 to week 8 there wasn't a large change in degradation but slight biodegradation was observed in the longer chains such as C35, C36 and C37.

As shown in results, the STR with the agitation had a bit better biodegradation than in the anaerobic bottle with was a 3% difference which is insignificant. In reality, there is no agitation in the tailing ponds and thus agitation is not required.

Siddique et al. (2006) examined the biodegradation of short chain alkanes in MFT samples under methanogenic condition. Their experiment indicated that the short chain n-alkanes can be biodegraded to methane by bacteria. An experiment revealed that in Syncrude MFT samples, the dominant bacteria are *Firmicutes* (*Peptococcaceae*) and *Proteobacteria* (*Syntrophus/Smithella*) which have the ability to degrade the short chain n-alkanes and monoaromatics (Siddique et al. 2012).

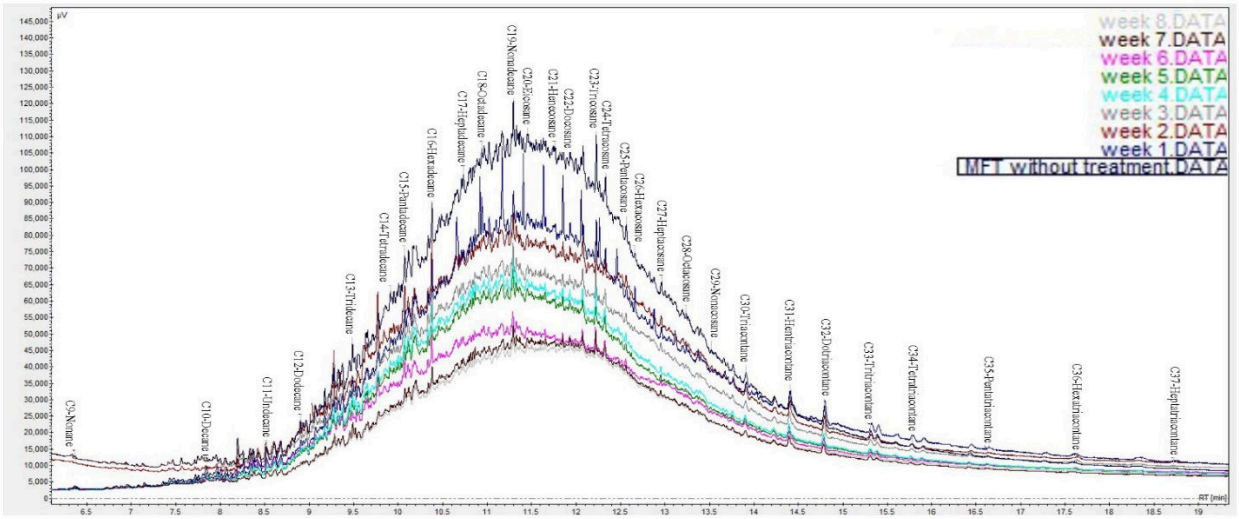

Figure 3. Gas chromatography analysis of STR. The hydrocarbons present in the MFT each week are shown.

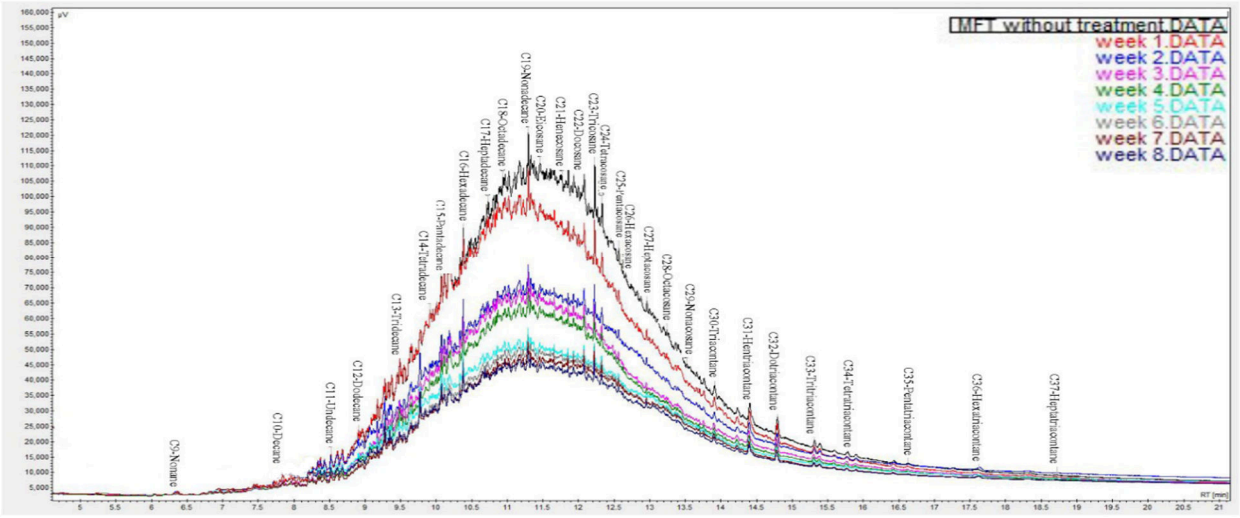

Figure 4. Gas chromatography analysis of anaerobic bottle. The hydrocarbons present in the MFT each week are shown.

3.6. Population of bacteria in the STR test

After adding bacteria to the bioreactor, the population of bacteria was monitored to be sure that growth and biodegradation was occurring. Over the first 3 weeks, the population of the bacteria was increasing but at week 4 it decreased and again, an increase was occurred and reached to the highest population at week 6 of 7.3 x 10^5 CFU/ml. The decrease of bacteria colonies was observed during the last 2 weeks of incubation (Fig. 5).

The first and second increase of bacteria could be related to the different types of bacteria in which the first growth shows the bacteria with faster growth such as T2 and L7, and the second group could be the other bacteria which start their slower growth under anaerobic conditions.

3.7. Surface tension of saturated water in MFT before and after treatment

Saturated water of MTF was extracted by centrifugation and then passed through a nylon syringe filter (0.4 µm pore size). The surface tension of the MFT sample before treatment was 68 mN/m. and was reduced to 51 mN/m and 47 mN/m in the STR and anaerobic bottle, respectively. As expected, the bacteria activities and biosurfactant production reduced the surface tension of the extracted water.

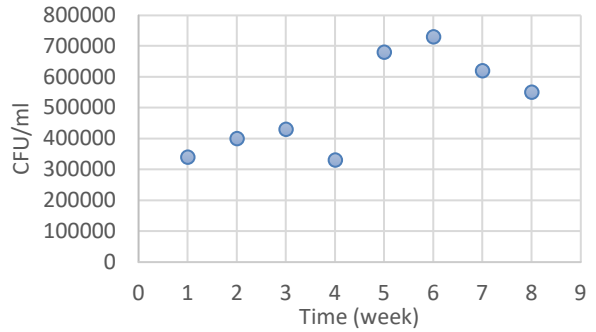

Figure 5. Bacterial population (CFU/ml) during the STR experiment

4. Conclusions

In this study, 13 indigenous bacteria were isolated and identified. Eight of the isolated bacteria were facultative or anaerobe tolerant. Their biosurfactant production and their effect of surface tension was monitored in both aerobic and anaerobic conditions. Then the potential isolates were added to the STR (with agitation) and anaerobic bottle (no agitation) to simulate the MFT condition.

The bioremediation of residual hydrocarbons was examined for 8 weeks after bacterial addition. Both STR and anaerobic bottle showed hydrocarbon degradation 58.7% and 55.1% respectively which shows agitation won't be necessary in the degradation. Surface tension of the MFT extracted water decreased from 68 to 51 and 47 mN/m after 8 weeks of bacterial growth. The number of bacteria in the STR was monitored to be sure that they can survive the anaerobic condition which shows 2 patterns. Some of the bacteria can grow faster in the anaerobic environment but some of them need more time to grow.

Conclusively, 8 bacteria were isolated from MFT of which 7 of them showed the biosurfactant production. These bacteria can be returned (bioaugmentation) to the MFT for the anaerobic bioremediation of residual oil in the MFT. Also, they can be used in the MEOR as the tertiary recovery method specifically as they can tolerate oxygen depletion.

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