

Liberación de agua intersticial y concentración (Bio-densificación), mediada por microorganismos, de partículas finas de las arenas de alquitrán, suplementadas con residuos agro-industriales
Microbially-mediated de-watering and consolidation (“Biodensification”) of oil sands mature fine tailings, amended with agri-business by-products

Marcela Cárdenas-Manríquez^{1,6}

Rozlyn F. Young²

Kathleen M. Semple²

Carmen Li²

Debora Coy¹

Eleisha Underwood³

Tariq Siddique⁴

Selma Guigard³

David C. Bressler¹

Rajender Gupta⁵

Julia M. Foght²

¹ University of Alberta, Edmonton, Department of Agricultural, Food and Nutritional Science, AB, T6G 2P5

² University of Alberta, Edmonton, Department of Biological Sciences, AB, T6G 2E9

³ University of Alberta School of Mining & Petroleum Engineering, Edmonton, Department of Civil & Environmental Engineering, AB, T6G 2W2

⁴ University of Alberta, Edmonton, Department of Renewable Resources, AB, T6G 2E3

⁵ University of Alberta, Edmonton, Department of Chemical and Materials Engineering, AB, T6G 2V4

⁶ Tecnológico Nacional de México, Campus Celaya (IT Celaya), Department of Environmental Engineering.

Autora para correspondencia: Marcela Cárdenas-Manríquez, E-mail: marcar@itcelaya.edu.mx

Resumen

Introducción: La extracción de hidrocarburos en el Noreste de Alberta, en Canadá, genera una gran cantidad de residuos conocidos como “arenas de alquitrán”. Las arenas de alquitrán son una suspensión acuosa de arcillas finas, arena, betún de petróleo e hidrocarburos. Estos residuos son depositados y retenidos en estanques de sedimentación, donde los sólidos coloidales precipitan y se concentran muy lentamente por efecto de la gravedad y el agua intersticial que se libera se colecta para reúso. La concentración, también llamada densificación, de las arenas de alquitrán mediada por los microorganismos nativos que producen metano o dióxido de carbono, es un fenómeno observado *in situ* e *in vitro*, a través del cual, los residuos se precipitan, y se libera agua intersticial más rápidamente que la sedimentación por gravedad.

Método: En este trabajo, se aplicaron enmiendas orgánicas para estimular la actividad de microorganismos nativos de los estanques de sedimentación, con el objetivo de acelerar la densificación y liberación de agua intersticial. Se utilizaron muestras de arenas de alquitrán de tres distintas compañías petroleras (Syn crude Canada Ltd.; Suncor Energy Inc.; y Shell Albian Sands), y se enmendaron en condiciones anaeróbicas con seis materias primas de la industria agrícola: (residuos de la extracción de aceite de canola; harina de huesos y harina de sangre de res hidrolizados; suero de leche hidrolizado; glicerol y granos secos de maíz usados para la producción de cerveza y bioetanol).

Se utilizó acetato de sodio como control positivo de metanogénesis y 2-bromoetanosulfonato (BES) 10 mM como inhibidor específico de metanogénesis. Los experimentos de densificación se realizaron en botellas serológicas de 100 ml en condiciones anaeróbicas, y en columnas de sedimentación de vidrio de 1.5 L; y se siguió la producción de metano, recuperación de agua intersticial y densificación. Se tomaron muestras durante los experimentos para la cuantificación de bacterias por número más probable, y para el seguimiento de los cambios en la dinámica de comunidades microbianas por pirosecuenciación de los genes ribosomales 16S. Al término de los experimentos de densificación, se realizaron algunas determinaciones de características físicas y químicas de las arenas densificadas y del agua intersticial liberada.

Resultados: Varias enmiendas incrementaron la producción de metano y aceleraron la densificación, en comparación con los controles negativo y sin enmienda. Residuos de canola, hidrolizados de harina de hueso, y de sangre y glicerol, aceleraron la densificación, estimularon la producción de metano e incrementaron el crecimiento de microorganismos metanogénicos y fermentadores. Las enmiendas alteraron algunos parámetros químicos de las arenas de alquitrán sedimentadas; generalmente disminuyeron el pH, incrementaron la conductividad eléctrica e incrementaron las concentraciones de magnesio, potasio, sodio y bicarbonato.

Discusión o Conclusión: La Densificación mediada por microorganismos representa una tecnología potencial para acelerar los procesos de recuperación de agua intersticial y densificación de las arenas de alquitrán almacenadas en los estanques de precipitación de las industrias petroleras en Canadá.

Palabras clave: biodensificación; arenas de alquitrán; biodegradación anaeróbica; metanógenos

Abstract

Oil sands surface mining operations in northeastern, Alberta, Canada produce enormous volumes of fluid fine tailings, an aqueous suspension of fine clays, sand, unrecovered bitumen, and diluent hydrocarbons. The tailings are deposited and retained on-site in large settling basins where the colloidal solids sediment and consolidate very slowly by gravity and pore water collects at the surface for re-use. Tailings “biodensification”, mediated by indigenous microbes that produce methane and/or carbon dioxide, is a phenomenon observed *in situ* and *in vitro* whereby tailings with active anaerobic microbial communities consolidate and de-water faster than predicted by gravitational (self-weighted) consolidation alone. To exploit this phenomenon, we used organic amendments to stimulate endogenous anaerobic tailings microorganisms. Tailings from three different operators were amended with agri-business by-products, placed in 100-mL microcosms and 1.5-L settling columns, and monitored for methanogenesis, pore water recovery, and solids densification. Several amendments increased methane production and accelerated biodensification compared to unamended and negative controls. Hydrolyzed canola, blood meal, bone meal and glycerol generally accelerated biodensification, stimulated methane production and supported growth of methanogens and fermentative microbes. Amendment altered the chemistry of the tailings, generally decreasing pH, increasing conductivity and magnesium,

potassium, sodium, and bicarbonate concentrations. Biodensification is a potential engineered technology for accelerating water recovery and reducing the volume of stored oil sands tailings.

Keywords: biodensification; oil sands tailings; anaerobic biodegradation; methanogens

Recibido en: 01-11-2019

Aceptado en: 07-11-2019

Introduction

The Athabasca oil sands deposits (colloquially called “tar sands”) in northeastern Alberta, Canada comprise a vast reserve of biodegraded petroleum, known as bitumen, that is surface-mined by several companies (reviewed by Foght *et al.*, 2017). Bitumen extraction requires huge volumes of water, including recycled and makeup water from the Athabasca River to separate bitumen from the sand, clay and minerals of the ore. A light hydrocarbon diluent, the composition of which differs between mining companies, is used in one processing step to reduce bitumen viscosity and enhance separation of bitumen from fine solids. Following extraction, fluid fine tailings comprising suspended sand, solids, clays, unrecovered bitumen, and residual diluent are stored in large settling basins (tailings ponds) holding hundreds of millions of cubic meters of tailings. The solids separate from water relatively quickly initially, allowing the uppermost layer of process-affected water to be re-used in ore-processing activities. Thereafter, the solids (predominantly fine clay particles) suspended beneath the surface water layer form a semi-solid colloid that consolidates very slowly while expressing pore water; gravitational densification of fines to a “trafficable” physical state has been estimated to take 125-150 years (Eckert *et al.*, 1996). Several chemical and physical methods have been developed to accelerate consolidation (*e.g.*, BGC Engineering, 2010) but they can be cost-intensive and degrade the quality of recycle water. Thus, new technologies to enhance and accelerate separation of pore water from colloidal solids (“de-watering”) accompanied by faster aggregation and decreased volume of clay particles (“consolidation”) might augment existing tailings management practices.

Oil sands tailings ponds are biologically active and contain complex, predominantly anaerobic, communities of microorganisms (An *et al.*, 2013; Ramos-Padrón *et al.*, 2011; Holowenko *et al.*, 2000; Penner and Foght, 2010). Each tailings pond appears to have a unique microbiota reflecting the ore source, bitumen extraction process and tailings operations (Wilson *et al.*, 2016). The diverse bacterial communities in tailings ponds include nitrate-, iron-, sulfate-reducing, and hydrocarbon-degrading genera, and the Archaea are almost exclusively methanogens (reviewed by Foght *et al.*, 2017). Analysis of metagenomic data has also been used to correlate community composition with microbial activity in tailings ponds (An *et al.*, 2013; Wilson *et al.*, 2016). It is presumed that certain hydrocarbons, predominantly from fugitive processing diluent, are the major microbial substrate in the tailings ponds, being biodegraded anaerobically to carbon dioxide and methane *in situ*. Syncrude's Mildred Lake Settling Basin began to release bubbles of methane and carbon dioxide at the surface in the early 1990s and has been estimated to produce >40 million L methane/day (Holowenko *et al.*, 2000; Siddique *et al.*, 2008). Other tailings ponds receiving diluent-treated tailings also produce biogenic gases (Burkus *et al.*, 2014; Kong *et al.*, 2019).

Fedorak *et al.*, (2003) observed in laboratory trials that the consolidation rate of methanogenic tailings was greater than that of non-methanogenic tailings, indicating that microbial activity enhances densification. Arkell *et al.*, (2015) confirmed this observation by amending tailings with acetate, suggesting the potential for an on-site engineered process. Although using a pure substrate such as acetate is economically impractical for implementation at field scale, many agricultural industries produce large amounts of low- or negative-value by-products that are susceptible to anaerobic biodegradation. Siddique *et al.*, (2014a, b) amended oil sands tailings with hydrolyzed canola meal and observed enhanced biodensification compared to an unamended control; a biogeochemical model involving various biodensification processes was developed to explain the phenomenon. Thus, engineered biodensification has potential environmental benefits by increasing the volume of pore water for reuse in the bitumen extraction process (thereby decreasing the fresh water demand) and reducing the legacy tailings inventory (thus reducing the land surface area required for storing tailings). An additional benefit could be production and capture of biogenic methane on-site as a clean-burning fuel, as commonly achieved in municipal biosolids waste treatment plants. However, exploiting this phenomenon on

the vast scale of the oil sands tailings ponds would require a cost-effective carbon source and studies using tailings from different operators.

In the current study several agri-business by-products including canola meal, glycerol, blood meal, bone meal, whey powder and corn-based dried distillers' grains were tested for their potential to stimulate tailings biodensification. These experiments are part of a larger study to identify the optimal conditions for biodensification of fine tailings and to address the physical and chemical factors that contribute to increased consolidation in microbially active tailings.

Methods

Sources of oil sands tailings and organic amendments

Tailings ponds operated by three different oil sands companies (Syncrude Canada Ltd., Suncor Energy Inc., and Shell Albian Sands) were sampled for mature fine tailings below the water:sediment interface ("mud line") and process-affected water at the surface, using sterile containers filled to overflowing to eliminate aerobic headspace, per Foght *et al.*, (2014). Prior to sub-sampling, the bulk tailings samples were gently stirred by hand under a stream of anaerobic N₂ gas to avoid introducing oxygen. Gravimetric Dean-Stark analysis (as described by Foght *et al.*, 2014) was performed to determine the water, solids and bitumen content of the tailings. Microbiological enumeration of four different metabolic classes of bacteria in the bulk tailings was performed as described below.

Six agribusiness feedstocks and one model substrate were tested as amendments in biodensification experiments. The agricultural by-products from commercial crops or agri-products (provided by Sanimax Canada) included: canola meal (post-oil extraction); hydrolyzed blood-meat meal and bone meal (slaughterhouse wastes, rendered safe to Canadian Food Inspection Agency standards); whey powder (dairy waste); corn-based dried distillers grains with

solubles (corn-DDGS; brewing or bioethanol waste); and glycerol (representing biodiesel waste). Sodium acetate, a methanogenesis substrate, was used as a positive control amendment. The negative control treatment consisted of adding 10 mM 2-bromoethanesulfonate (BES) to specifically inhibit methanogens (Smith and Mah, 1978); BES was added in combination with acetate to distinguish it from a control comprising unamended (endogenous carbon) tailings. Unless otherwise indicated, pure chemicals were purchased from Fisher Scientific, Ontario, Canada.

Canola meal, blood meal and bone meal were hydrolyzed as previously described (Siddique *et al.*, 2014a) to render them water soluble and so avoid adding bulk to the tailings. Elemental analysis (C, H, N, and S) was performed on the organic amendments using a Carlo Erba EA 1108 elemental analyzer in the Analytical and Instrumentation Laboratory at the University of Alberta (Department of Chemistry) so that amendments could be added to tailings on a carbon-equivalent basis. The agricultural amendments (which contained 33.7 to 45.9% C; Supplementary Table S1) and pure glycerol were added individually at 400 mg C/L tailings; due to a calculation error, acetate was added at 72 mg C/L tailings.

Biodensification (de-watering and consolidation) columns

Amendments were dissolved individually in 75 mL of anoxic tailings pond water and gently mixed by hand into 1.5 L tailings under N₂ gas to maintain anaerobic conditions. The amended tailings were transferred to sterile 2-L glass cylinders and capped with aluminum foil or several layers of latex secured with tape, to reduce water evaporation from the surface. An unamended (endogenous substrate) control that received tailings pond water but no organic substrate addition was prepared in parallel. Columns were incubated stationary in the dark at room temperature (~20°C), which is similar to the tailings temperature in situ (*e.g.*, 12–21°C; Penner and Foght 2010). Initially the tailings were homogeneous with no pore water observed at the surface, but during incubation a water layer representing expressed pore water appeared above the mud line.

The volumes of these two phases were measured at intervals during incubation for 56–188 d until de-watering and consolidation measurements reached a plateau.

At the end of incubation (168 d) of the Syncrude columns, the composition of randomly selected biogenic gas bubbles trapped in the tailings layer was measured by using a cannula to withdraw a portion of the bubble, followed by gas chromatography (GC) (Holowenko *et al.*, 2000). During decommissioning of these Syncrude columns the overlying expressed pore water as well as sub-samples of the uppermost few cm and lowermost few cm of solid phase from each column were collected for standard chemical analyses by Syncrude Canada Ltd.

Measurement of cumulative methane production by tailings in microcosms

We could not directly measure methane production from the consolidation columns because they were not gas-tight and furthermore some gas remained trapped as gas voids within the solids. Therefore, we determined the methane-producing potential of amended tailings in sealed anaerobic microcosms. For these experiments, tailings samples were mixed with cognate anoxic tailings pond water at 3:1 by volume, then replicate 100-mL subsamples were added to sterilized 158-mL serum bottles having a headspace of 30% CO₂/balance N₂ (Holowenko *et al.*, 2000). Organic amendments were added to the bottles based on the same carbon-equivalent basis as for the biodensification columns (400 mg C/L tailings) and acetate at 72 mg C/L. Microcosms were incubated stationary in the dark at room temperature (ca. 20°C). At intervals the bottle contents were mixed gently by inversion and 0.1 mL of headspace was removed using sterile needle and syringe for methane analysis using GC (Holowenko *et al.*, 2000).

Microbial enumeration and identification (pyrosequencing of 16S rRNA genes)

Viable methanogens, sulfate-reducing prokaryotes, nitrate-reducing Bacteria and fermentative prokaryotes were enumerated in the solids fraction of initial bulk tailings samples and also after biotransformation by using the 3-tube most probable number (MPN) method and previously described media (Hulecki *et al.*, 2010; Penner and Foght, 2010). MPN tubes were incubated in the dark at room temperature (ca. 20°C) for 60 d before scoring; results were reported on a volumetric basis. Statistical differences between MPN values were determined using Cochran's method (Cochran, 1950).

During decommissioning of consolidation columns, total genomic DNA was extracted from the solids phase in each column using a bead-beating method (Foght *et al.*, 2004). Thereafter, pyrotag sequencing of 16S rRNA genes in total genomic DNA was performed as described by Siddique *et al.*, (2014b). Briefly, triplicate aliquots (300 µl each) of every sample were extracted twice (total six extractions per sample), pooled, then PCR-amplified in triplicate using "universal" primers (An *et al.*, 2013) and a touchdown method (Siddique *et al.*, 2014b). Pyrosequencing of pooled amplicons was performed at Genome Quebec Innovation Centre, Montreal QC using a GX FLX Titanium Series XLR70 kit (Roche Diagnostic Corporation). Negative control extraction blanks and amplification reactions were included for each set of samples to ensure quality control. Sequence analysis was performed using the Phoenix 2 pipeline (Soh *et al.*, 2013), which filters sequences through a series of stringent conditions, removes chimeras, and determines consensus sequences for each operational taxonomic unit using MOTHUR and the SILVA database. To compare communities in samples, non-metric multidimensional scaling analysis was implemented in Phoenix 2. Pyrotag sequences were submitted to the NCBI Short Read Archive under accession numbers SRR617546-SRR617557 (Albian); SRR617558-SRR617568 (Suncor) and SRR617569-SRR617579 (Syncrude).

Results

Chemical and microbial analyses of initial bulk tailings samples

The three oil sands tailings samples from Syncrude, Suncor and Albion contained similar proportions of solids upon receipt, ranging from 21.9–24.0 wt%, whereas the oil content (predominantly bitumen) varied by source, from 0.9–4.2 wt% (Table 1).

Table 1. Composition of mature fine tailings and most probable number (MPN) of four anaerobic metabolic types in bulk samples received from different oil sands companies.

Tailings source	Extraction Solvent ^b	Dean-Stark Gravimetric Analysis of Tailings (wt%) ^a				MPN/mL x 10 ⁻³ (95% confidence limits)			
		Oil	Water	Solids	Total	Methano-gens	Sulfate-reducers	Nitrate-reducers	Fermenters
Syncrude	Naphtha	1.6	72.5	23.6	97.7	2,300 (36-12,000)	930 (150-3,800)	280 (90-850)	93 (15-380)
Suncor	Naphtha	4.2	71.3	21.9	97.4	43 (7-210)	24,000 (3,600-130,000)	49 (17-130)	430 (70-2,100)
Albian	C ₅ and C ₆ paraffins	0.9	73.6	24.0	98.4	8 (1-23)	43 (7-210)	220 (57-700)	93 (15-380)

a, performed per Foght *et al.*, (2014); **b**, for additional detail see Burkus *et al.*, (2014)

The initial bulk tailings samples harboured substantial numbers of methanogens, with Syncrude tailings having 2×10^6 MPN/mL and Suncor tailings having 4×10^4 MPN/mL; Albion tailings (a younger tailings pond; Foght *et al.*, 2017) had the fewest methanogens at 8×10^3 MPN/mL (Table 1). Sulfate-reducing prokaryotes were prevalent in all samples, with Syncrude and Suncor tailings having 9×10^5 and 2×10^7 MPN/mL, respectively, and Albion tailings containing 4×10^4 MPN/mL. Nitrate-reducing prokaryotes and fermenters were also detected in all samples at 10^4 - 10^5 MPN/mL (Table 1). Thus, all tailings samples harboured a viable endogenous anaerobic microbiota, comparable in magnitude to previous surveys of tailings ponds (summarized by Foght *et al.*, 2017). Based on the anaerobic types tested, Syncrude tailings were dominated by methanogens, Suncor tailings by sulfate-reducing prokaryotes, and Albion tailings were somewhat enriched in nitrate-reducers with a smaller microbiota overall.

Methane production from tailings incubated in microcosms

Because previous studies found a correlation between methanogenesis and biodensification (Fedorak *et al.*, 2003; Siddique *et al.*, 2014a, b), we measured the potential methanogenic activity of tailings samples amended with different agricultural by-products compared to three control treatments: unamended tailings as a control for endogenous substrates; acetate amendment as a positive control for methane production; and acetate plus BES to inhibit methanogens. For technical reasons methane production was measured in sealed anaerobic microcosms constructed in parallel with the consolidation columns rather than directly in the columns. However, production of methane in the columns is inferred from the occurrence of gas voids (particularly in the Syncrude tailings columns; see below).

All three tailings samples produced methane in amended microcosms (Supplemental Figs. S1, S2, S3). Unamended (endogenous control) microcosms produced a small amount of background methane above that produced by BES-amended (negative control) cultures. More specifically, the Suncor and Syncrude tailings samples supplemented with glycerol, bone meal, blood meal, corn DDGS, whey or canola produced >1 mM methane during ~170 d incubation with a lag period of ~25 d before significant methane began to be produced (Supplemental Figs. S2, S3). Less methane was generated in the acetate-amended positive control microcosms because these cultures inadvertently received approximately one-sixth the amount of substrate (72 mg C/L) as the other amendments (400 mg C/L); arithmetically, the mass of methane produced in the acetate controls is proportional to that observed in the test microcosms. In contrast to Syncrude and Suncor tailings, the Albian tailings microcosms were slower to begin producing methane, having lag phases of 40-60 d, and exhibited substrate preference in the order canola>bone meal>corn DGGs>whey>blood meal, with glycerol being a poor methanogenic substrate (Supplemental Fig. S1). The observed methane yield compared with the theoretical maximum methane was calculated for the Syncrude microcosms using Buswell's equation as implemented by Roberts (2002). After correcting for endogenous methane production in the unamended control microcosms, the agricultural amendments produced 56-129% of the maximum theoretical methane yield (Supplemental Table S2). Thus, the agricultural substrates supported similar methanogenesis by the Syncrude and Suncor microbiota but the Albian microbiota were more selective and slower to produce methane.

De-watering and consolidation of methanogenic tailings in column studies

The classic self-weighted (gravitational) densification behaviour of oil sands mature fine tailings is that expressed pore water accumulates above the mud line (*i.e.*, cumulative values >0) and the underlying solids volume decreases (*i.e.*, values <0) as fine clay particles pack more closely with expression of pore water. This pattern was observed in the Albian and Suncor tailings consolidation columns (Figs. 1A, B and 2A, B). As observed for methane production, the Albian tailings exhibited substrate preference, with acetate yielding the greatest volume of pore water (500 mL from an initial 1.5 L of amended tailings placed in the column) and the negative control (acetate + BES) producing the least pore water (~ 50 mL) during 100 d incubation (Fig. 1A). Correspondingly, acetate amendment resulted in the greatest consolidation of solids, decreasing by ~ 500 mL, whereas the BES control changed little (Fig. 1B). The unamended (endogenous control) Albian tailings showed slightly more biodensification than the negative control, but less than the other amended treatments.

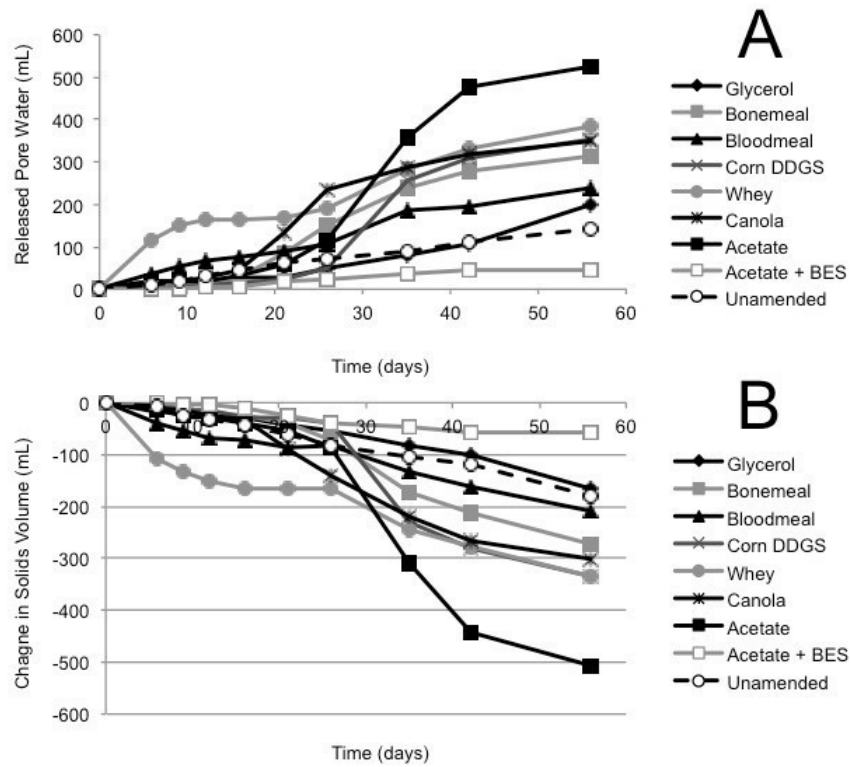


Fig. 1 (A, B). Biodensification of 1.5 L Albian tailings, amended with substrate or unamended, and incubated in columns for 56 d. **(A)** Cumulative volume of expressed pore water measured above the mud line (*i.e.*, de-watering); **(B)** Volume of solids layer below the mud line (*i.e.*, consolidation). **(C)** MPN estimations of four metabolic types in tailings solids at the end of incubation in columns; error bars are 95% confidence intervals and asterisks (*) indicate MPN values that are statistically different from the unamended control value.

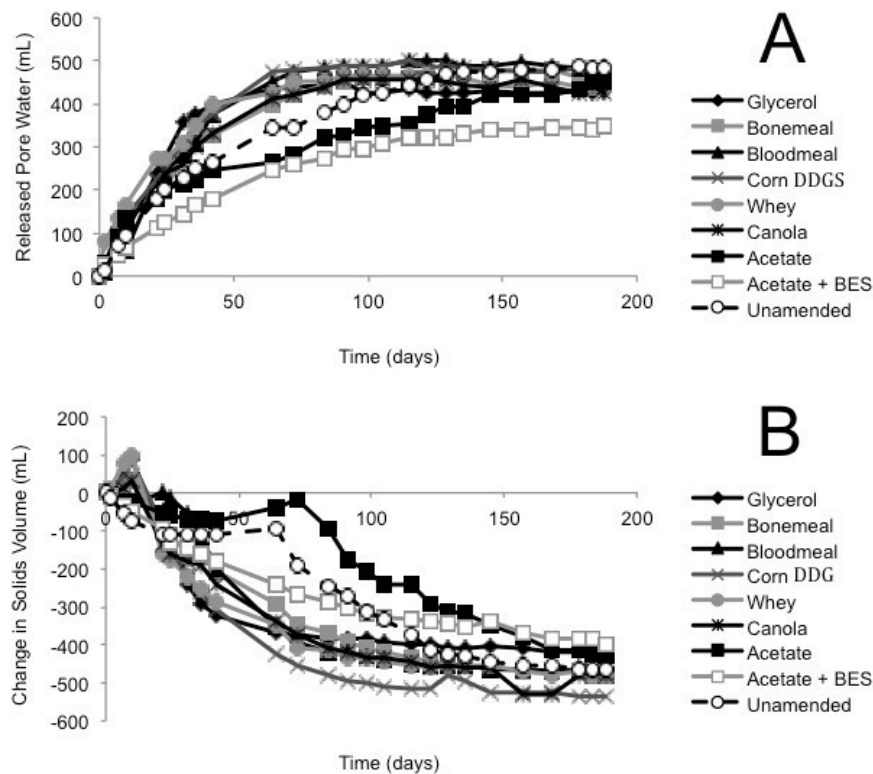


Fig. 2 (A, B). Biodesulfurization of 1.5 L Suncor tailings, amended with substrate or unamended, and incubated in columns for 188 d. **(A)** Cumulative volume of expressed pore water measured above the mud line (*i.e.*, de-watering); **(B)** Volume of solids layer below the mud line (*i.e.*, consolidation). **(C)** MPN estimations of four metabolic types in tailings solids at the end of incubation in columns; error bars are 95% confidence intervals and asterisks (*) indicate MPN values that are statistically different from the unamended control value.

In contrast, the Suncor tailings showed little substrate preference and even the BES negative control exhibited substantial biodesulfurization (Fig. 2A, B). This outcome suggests that methanogenesis is a less important factor in Suncor tailings where sulfate-reducing prokaryotes predominate the endogenous microbiota by several orders of magnitude (Table 1). Instead, sulfate reduction and/or fermentation by facultative sulfate-reducing prokaryotes may be the dominant biodesulfurization mechanism.

A different pattern of biodesulfurization was observed in the Syncrude tailings columns. As expected, little pore water (<50 mL) accumulated in the BES negative control (Fig. 3A) but a larger proportion of pore water (~200 mL) was expressed from the unamended control column, suggesting that endogenous metabolism in Syncrude tailings is robust. Moreover, during the early

stages of incubation, biogenic gases accumulated as gas voids visibly trapped in the solids layer in all amended Syncrude tailings except the BES negative control. This gas production resulted initially in increased rather than decreased solids volumes (Fig. 3B). The bloating effect diminished over time, presumably due to coalescence and ebullition of water-insoluble methane near the mud line and to CO₂ dissolution into pore water (Siddique *et al.*, 2014b), reducing the volume of gases trapped below the mud line. The composition of gases in these voids was tested during decommissioning of the Syncrude columns by sampling individual gas voids using a cannula and analyzing the gases by GC. The trapped bubbles comprised methane and CO₂, with CH₄:CO₂ molar ratios ranging from 4 for corn DDGS to 13 for acetate (data not shown). Detection of methane in the gas voids confirmed that methanogenic conditions prevailed in the solid fraction of the columns even though they were not gas-tight.

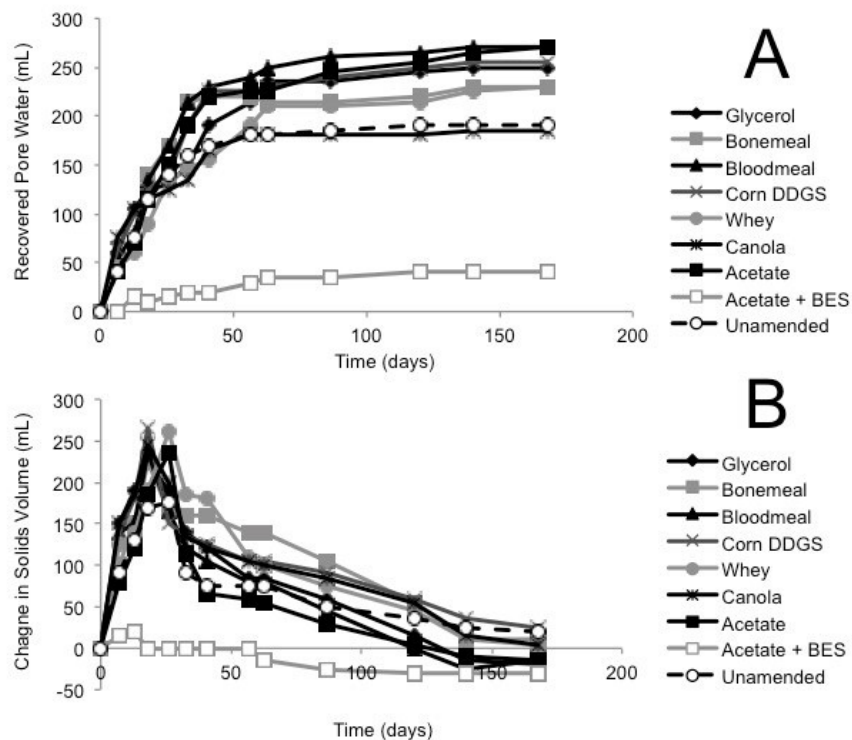


Fig. 3 (A, B). Biodensification of 1.5 L Syncrude tailings, amended with substrate or unamended, and incubated in columns for 168 d.

(A) Cumulative volume of expressed pore water measured above the mud line (*i.e.*, de-watering); (B) Volume of solids layer below the mud line (*i.e.*, consolidation). (C) MPN estimations of four metabolic types in tailings solids at the end of incubation in

columns; error bars are 95% confidence intervals and asterisks (*) indicate MPN values that are statistically different from the unamended control value.

Effect of amendment on chemistry of pore water and solids in Syncrude biodensification columns

During decommissioning of the Syncrude biodensification columns after 168 d incubation, the overlying expressed pore water was collected for analysis of pH, conductivity, major soluble cations, anions, and metals (Supplemental Table S3). pH values were not substantially altered by amendment, but conductivity, magnesium, potassium, sodium, and bicarbonate concentrations were greater for all amended versus unamended tailings. There were also increased concentrations of ammonium in corn DDGS- and blood meal-amended tailings water and increased calcium in tailings amended with corn DDGS, whey, and canola meal; the addition of the methanogen inhibitor BES increased conductivity, sodium (the BES counter-ion), calcium and, of course, sulfur concentrations.

During decommissioning, two subsamples of the tailings solids were also collected: one near the mud line to determine whether an oxidation gradient existed in the columns or whether ebullition events affected the solids chemistry, and one near the bottom of the column to determine whether biogenic CO₂ dissolution altered the chemistry of pore water in the tailings layer. The same suite of chemical analyses were performed as for the pore water sample by measuring bulk pH of the solids, and concentrations of major soluble anions, cations and metals in the pore water still entrained in the solids fractions, as well as gravimetric Dean-Stark analysis. Amended solids samples collected from both positions in the column showed minor differences compared with the corresponding unamended control tailings, including slightly lower pH, increased conductivity and increased cations and bicarbonate concentrations (Supplemental Tables S4, S5). Comparing Dean-Stark results from the BES-treated tailings to amended tailings shows the magnitude of methanogen-influenced biodensification.

Microbial enumeration and pyrotag sequencing of 16S rRNA genes

Microbial enumeration of tailings was performed during column decommissioning. MPN analysis revealed a general increase in fermentative bacteria and methanogens in tailings amended with complex agricultural by-products compared with the corresponding unamended control tailings and the BES negative control (Figs. 1C, 2C, 3C).

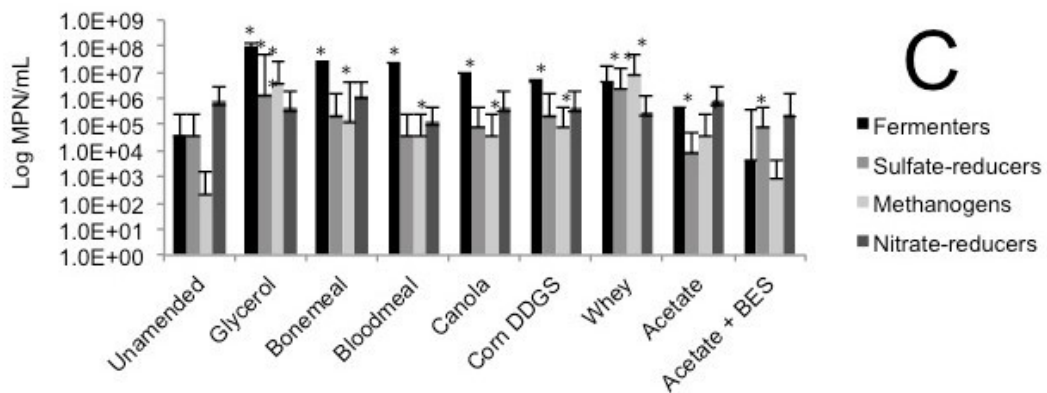


Fig. 1 (C). Biodensification of 1.5 L Albian tailings, amended with substrate or unamended, and incubated in columns for 56 d.

(A) Cumulative volume of expressed pore water measured above the mud line (*i.e.*, de-watering); (B) Volume of solids layer below the mud line (*i.e.*, consolidation). (C) MPN estimations of four metabolic types in tailings solids at the end of incubation in columns; error bars are 95% confidence intervals and asterisks (*) indicate MPN values that are statistically different from the unamended control value.

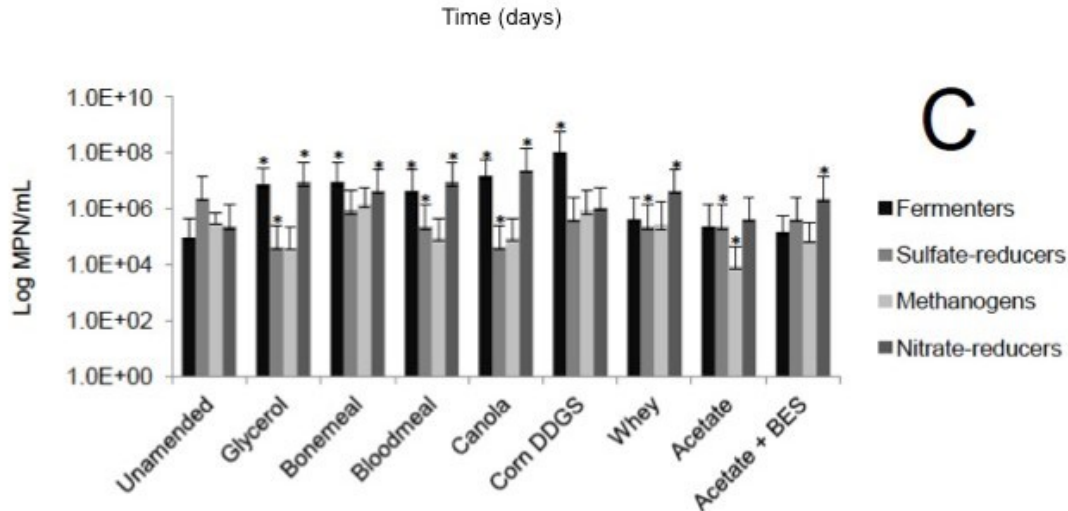


Fig. 2 (C). Biodensification of 1.5 L Suncor tailings, amended with substrate or unamended, and incubated in columns for 188 d. **(A)** Cumulative volume of expressed pore water measured above the mud line (*i.e.*, de-watering); **(B)** Volume of solids layer below the mud line (*i.e.*, consolidation). **(C)** MPN estimations of four metabolic types in tailings solids at the end of incubation in columns; error bars are 95% confidence intervals and asterisks (*) indicate MPN values that are statistically different from the unamended control value.

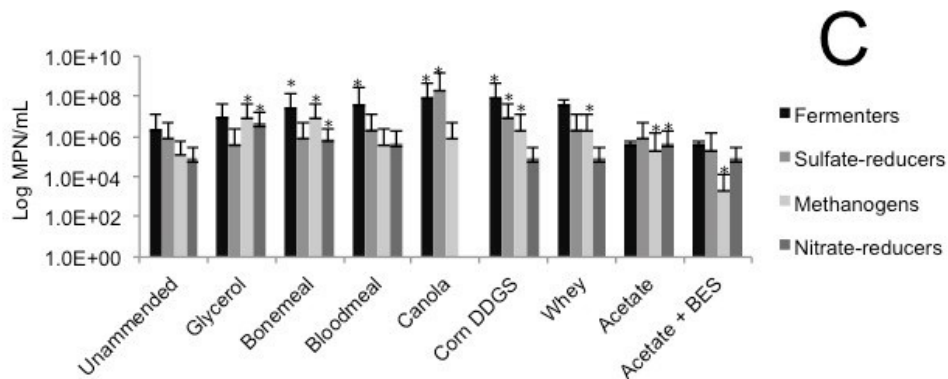


Fig. 3 (C). Biodensification of 1.5 L Syncrude tailings, amended with substrate or unamended, and incubated in columns for 168 d. **(A)** Cumulative volume of expressed pore water measured above the mud line (*i.e.*, de-watering); **(B)** Volume of solids layer below the mud line (*i.e.*, consolidation). **(C)** MPN estimations of four metabolic types in tailings solids at the end of incubation in columns; error bars are 95% confidence intervals and asterisks (*) indicate MPN values that are statistically different from the unamended control value.

In addition to enumeration, samples of tailings solids were collected for pyrotag sequencing of 16S rRNA genes. Non-metric multidimensional scaling was applied to compare the microbial

communities in Syncrude, Suncor and Albian tailings at a global level. This revealed that the three sources of tailings harboured distinct microbiota, and that all amendments shifted the microbial community compositions away from the unamended controls (Fig. 4). The BES negative control community shifted the greatest distance from the unamended community in both Albian and Syncrude tailings, whereas BES had less effect on the Suncor community that is dominated by sulfate-reducers (Table 1).

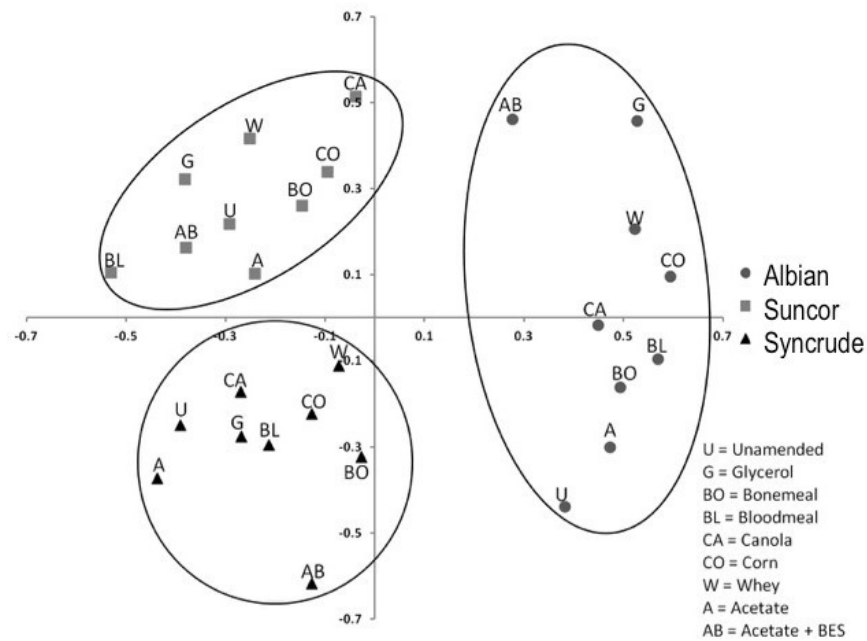


Fig. 4. Non-metric multidimensional scaling plot of 16S rRNA gene pyrosequences representing microbial communities in Syncrude, Suncor and Albian tailings solids amended with substrate or unamended, after incubation in columns.

Taxonomic analysis of the column tailings samples, identified to the family level, revealed that the unamended samples of all three tailings share a wide range of dominant taxa including those affiliated with known methanogens, fermenters, and syntrophs (Fig. 5). Syncrude and Suncor tailings communities had high proportions of reads affiliated with methanogens, particularly Methanosaetaceae. These proportions correlate with the MPN values for amended Syncrude tailings (Fig. 3C) but not for Suncor (Fig. 2C). The unamended Albian tailings had fewer methanogen-affiliated reads than Suncor and Syncrude tailings but higher proportions of

Comamonadaceae (including Acidovorax) than either Syncrude or Suncor tailings. Unamended Syncrude tailings were enriched in reads affiliated with Desulfuromonadaceae versus the other two unamended tailings types. After incubation with amendments, Syncrude and Suncor tailings showed either decreased or unchanged proportions of methanogens but Albian tailings amended with bone meal, blood meal, canola, corn DGGS, or whey were enriched in methanogen sequences, particularly Methanoregula and Methanosaetaceae. All of the amendments resulted in a decrease in the proportion of Comamonadaceae reads in Albian tailings: however, many of the amendments stimulated an increase in Comamonadaceae in Syncrude tailings, with the largest increase observed in bone meal-amended samples (Fig. 5). All amendments also resulted in a decrease in Desulfuromonadaceae reads in Syncrude tailings and decreased Syntrophaceae reads in Suncor tailings.

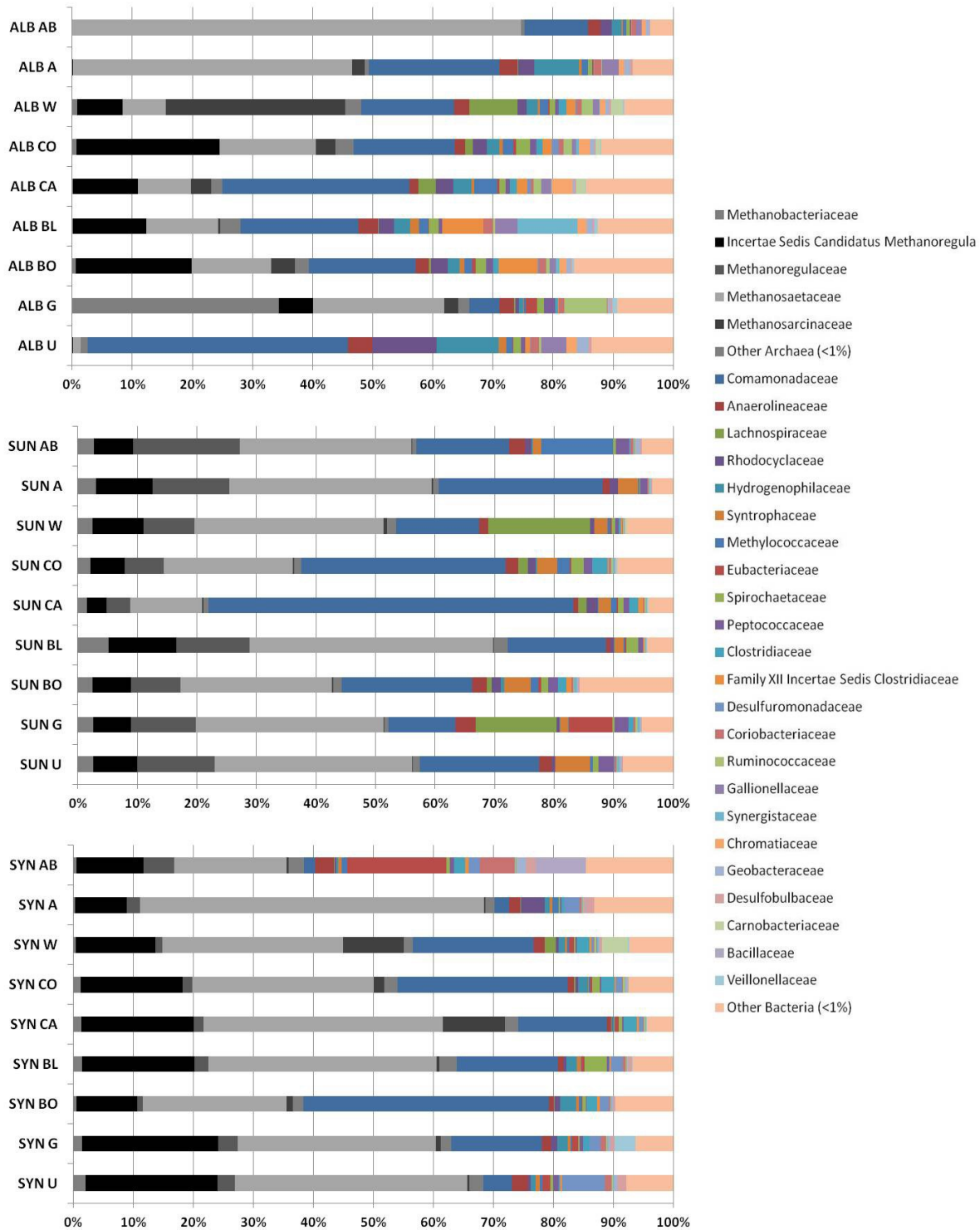


Fig. 5. Archaeal and bacterial 16S rRNA gene pyrosequences identified to family level in tailings samples unamended (U) or amended with glycerol (G); bone meal (BO); blood meal (BL); canola (CA); corn DDGS (CO); whey (W); acetate (A); or acetate plus BES (AB). ALB, Albian; SUN, Suncor; SYN, Syncrude. “Other Archaea” and “Other Bacteria” are the sum of all taxa present at <1% of total reads, as well as reads that could not be identified to family level.

Discussion

Previous studies of biodensification of oil sands tailings ponds have been based on experiments using a limited number of tailings ponds and organic substrates (*e.g.*, Fedorak *et al.*, 2003; Siddique *et al.*, 2014a, b; Arkell *et al.*, 2015). The current study expanded the range of substrates and tested different tailings types that initially had similar physical characteristics (Table 1, Dean-Stark analysis) but were from three companies using unique combinations of ore extraction and post-extraction tailings management methods. Syncrude and Suncor use naphtha (of different compositions) as the extraction diluent, whereas Albian uses a light aliphatic diluent (Table 1). Suncor adds gypsum (calcium sulfate) to fluid tailings as they are deposited in the tailings pond, whereas Albian amends fresh tailings with citrate (reviewed by Foght *et al.*, 2017). At time of sampling the Syncrude pond was ~40 years old whereas the Suncor and Albian tailings ponds were ~15 years old. Such processing and age differences contribute to the distinct endogenous microbiota in the ponds, both taxonomically (Fig. 4 and Wilson *et al.*, 2016) and numerically (Table 1). In fact, the shorter lag phase before onset of methanogenesis and the maximum rate of methane production in Syncrude and Suncor microcosms (Supplementary Figs. S1, S2, S3) correlated with the greater MPN estimates of methanogens in these tailings (Table 1). Thus, we expected – and observed – different responses of the tailings samples to amendment with different organic substrates based on tailings sources and their microbiota.

The agribusiness amendments were selected based on their regional availability and low (or negative) value in their respective industries. Acetate was chosen as the positive control substrate because it was previously demonstrated to be effective in small-scale biodensification trials with Syncrude tailings (Arkell *et al.*, 2015). BES was selected for the negative control treatment under the assumption that it would suppress biodensification as it did in previous experiments with Syncrude tailings (Arkell *et al.*, 2015). In both the Albian and Syncrude tailings columns, little or no methane production (Supplementary Figs. S1 and S3) and slow biodensification (Figs. 1 and 3) were observed in the BES-inhibited controls, with no evidence of

gas void formation in the BES-containing columns, suggesting that methanogenesis was the main source of biogenic gas in these substrate-amended tailings. Because BES specifically affects methanogens (Smith and Mah, 1978), whose inhibition can have upstream effects on the activity of synergistic anaerobes, any settling observed in the BES-inhibited tailings presumably represents primarily gravitational (self-weighted) consolidation and de-watering, without a significant contribution by the methanogenic microbial community. Interestingly however, in the current study, BES was far less effective at stalling biodensification of Suncor tailings (Fig. 2) that are dominated by sulfate-reducing prokaryotes and fermenters (Table 1). This indicates that methanogenesis *per se* is not essential for biodensification and suggests that anaerobic metabolism generating CO₂ and/or organic acid wastes may contribute to or substitute for methanogenic activity during biodensification, given an appropriate microbial community composition.

The column method used to quantify biodensification at small scale could be criticized for the fact that the headspace and pore water are not incubated under strictly anaerobic conditions, since the columns are sealed (initially under a N₂ headspace) using only a foil or thick latex cap to prevent evaporative losses from the water surface. However, although the water and upper few mm of solids may have been suboxic rather than anoxic, it was obvious that the underlying tailings remained sufficiently anaerobic (due to minimal gas penetration into the dense tailings) to permit production of gas bubbles comprising methane and CO₂ to form throughout the solids layer.

A small degree of de-watering and consolidation was observed in all unamended control tailings columns with all three tailings sources. This is interpreted to be due to metabolism of endogenous hydrocarbons (*e.g.*, residual diluent; Siddique *et al.*, 2006, 2007, 2011) supporting microbial activity. In contrast, each tailings type responded differently to amendment. Albian tailings exhibited substrate specificity for methanogenesis, with very little methane being produced in unamended and BES-treated microcosms (Supplemental Fig. S1). Notably, acetate was the most effective amendment for Albian tailings biodensification (Fig. 1) even though it was inadvertently amended at approximately one-sixth the concentration (by C content) of the other amendments. This preference may result from Albian tailings having been exposed *in situ* to citrate as a water conditioning agent (Li, 2010), which may have enriched for microbes capable of fermenting acetate. Correspondingly, MPN enumeration of Albian tailings from the columns

showed significant enrichment of methanogens and fermenters for all amendments (Fig. 1C). Suncor tailings exhibited a slight preference for blood meal for methane production (Supplementary Fig. S2) but a slight preference for canola for biodensification (Fig. 2A, B). MPN enumeration of Suncor columns showed significant increases in fermenters and nitrate-reducers for most amendments (Fig. 2C) but little change in proportions of methanogens and an overall trend to decreased proportions of sulfate-reducers. Bearing in mind that both nitrate- and sulfate-reduction can be facultative properties, and that some taxa may be enumerated by more than one type of MPN medium, the shift towards more fermentation capability is likely the stronger observation. Syncrude tailings showed no strong substrate preferences for either methane production (Supplementary Fig. S3) or biodensification (Fig. 3). In fact, even the unamended control tailings exhibited biodensification similar to the amended columns. No consistent patterns of enrichment were noted in Syncrude MPN enumerations (Fig. 3C).

Non-metric multidimensional scaling analysis of 16S rRNA pyrosequences from the tailings after incubation in columns confirmed the unique compositions of the initial endogenous microbiota (*i.e.*, unamended control communities) and showed that amendments caused the community compositions to shift (Fig. 4). Interestingly, this analysis also indicates that amendments did not simply select for the same taxa regardless of tailings source: the communities remained unique even after amendment with the same substrates or inhibitor. Examining the taxa at the family level (Fig. 5) revealed the complexity of the prokaryotic communities. In general, substrate amendment increased the proportion of archaeal to bacterial sequence reads but did not perturb the bacterial community in any consistent patterns. For example, Albion and Suncor tailings amended with canola were enriched in Comamonadaceae, but Syncrude tailings were not; Syncrude amended with bone meal was enriched in Acidovorax but Albion and Suncor were not. Because these communities are represented by a single time point at the end of incubation, no strong conclusions can be drawn from the molecular analysis. Additionally, the full metabolic potential of many of the anaerobic taxa detected is incompletely known, so correlating their presence with the observed behaviour of the tailings is tenuous. Future studies must include more comprehensive replication, temporal sampling and analysis of the microbial communities.

Chemical analyses of the changes in pore water and solid phase chemistry of biodensified oil sands tailings previously revealed that microbial activity also altered the clay chemistry of

tailings (Siddique *et al.*, 2014 a, b) where dissolution of biogenic CO₂ lowered the pH of tailings, dissolving carbonate minerals and releasing calcium, magnesium and bicarbonate ions, which reduced the surface charge potential (repulsive forces) of the clay particles. Transformation of Fe^{III} minerals to amorphous Fe^{II} minerals, and the formation of FeS, also reduced the surface charge potential of clay particles to aid the aggregation of clays in tailings and could be related to the formation of de-watering channels in microbially active tailings. The mechanism underlying biodensification of oil sands tailings appears to be a complex combination of geobiological processes activated by indigenous microorganisms. The detailed chemical analyses of the pore water retained in tailings from the upper and lower column reported here (Supplementary Tables S4 and S5) showed similar increases in cations and bicarbonate as well as greater conductivity and lower pH in amended tailings compared with unamended control tailings (which also showed endogenous biodensification activity). Future studies should examine the geochemistry of the solids phase to determine whether the factors for different tailings sources and amendments are the same as reported for Syncrude with canola amendment (Siddique *et al.*, 2014b).

Regardless of recent developments of physico-chemical processes for de-watering tailings, oil sands surface mining companies continue to generate and deposit fresh tailings, adding to the inventory of microbially active tailings ponds. Amendment of oil sands fine tailings with agri-business by-products has been shown here to enhance the production of methane, and in turn the consolidation and de-watering (biodensification) of tailings. Accelerated de-watering of tailings would increase the water available for reuse in oil sands extraction processes thus decreasing the demand for make-up water from the Athabasca River, and reduce the volume of existing tailings. The success of organic amendment suggests that a flow-through biodensification system analogous to sewage treatment facilities could be possible for oil sands tailings treatment. The generation of methane as a clean-burning on-site fuel could be an additional benefit to exploiting biodensification of methanogenic tailings. However, to implement an oil sands tailings treatment technology that accelerates consolidation and water recovery from amended tailings, a better understanding of the physical, chemical and microbiological processes of biodensification is required. It is important to recognize that each tailings pond is unique in terms of endogenous microbiota and metabolic potential, requiring optimization of amendment type and concentration for each pond. In addition, the consequences of altered pore water and tailings solids chemistry for water re-use and ultimate solids reclamation must be considered.

Acknowledgments

The authors thank Alberta Innovates–Technology Futures for funding and Syncrude Canada Ltd., Suncor Energy Inc., and Shell Albian Canada for providing samples and chemical analyses (Syncrude). MCM thanks Tecnológico Nacional de México for the fellowship for Academic leave in Canada.

Supplemental information

Four Tables (S1-S4), and three Figures (S1-S3) are provided as supplemental information.

References

- An, D., Caffrey, S. M., Soh, J., Agrawal, A., Brown, D., Budwill, K., Dong, X., Dunfield, P. F., Foght, J., Gieg, L. M., Hallam, S. J., Hanson, N. W., He, Z., Jack, T.R., Klassen, J., Konwar, K. M., Kuatsjah, E., Li, C., Larter, S., Leopatra, V., Nesbø, C. L., Oldenburg, T., Pagé, A. P., Ramos- Padrón, E., Rochman, F. F., Saidi-Mehrabad, A., Sensen, C. W., Sipahimalani, P., Song, Y. C., Wilson, S., Wolbring, G., Wong, M., and Voordouw, G. (2013). Metagenomics of hydrocarbon resource environments indicates aerobic taxa and genes to be unexpectedly common. *Environmental Science and Technology*, 47(18):10708-10717. DOI: 10.1021/es4020184.
- Arkell, N., Kuznetsov, P., Kuznetsova, A., Foght, J. M., Siddique, T. (2015). Microbial metabolism alters porewater chemistry and increases consolidation of oil sands tailings. *Journal of Environmental Quality*. 44(1): 145-153. DOI:10.2134/jeq2014.04.0164.
- BGC Engineering. (2010). *Oil Sands Tailings Technology Review*. OSRIN Report No. TR-1. 136 pp. <http://hdl.handle.net/10402/era.17555> (Accessed 30 October 2019).
- Burkus, Z., Wheler, J., Pletcher, S. (2014). GHG emissions from oil sands tailings ponds: Overview and modelling based on fermentable substrates. *Alberta Environment Sustainable Resources Development*. DOI: doi.org/10.7939/R3F188 (Accessed 22 Oct 2019).
- Cochran, W. (1950). Estimation of bacterial densities by means of the Most Probable Number. *Biometrics* 6(2): 105-116.

- Eckert, W. F., Masliyah, J. H., Gray, M. R., Fedorak, P. M. (1996). Prediction of sedimentation and consolidation of fine tails. *AIChE J* 42(4): 960-972. doi.org/10.1002/aic.690420409.
- Fedorak, P. M., Coy, D. C., Dudas, M. J., Simpson, M. J., Rennenberg, A. J., MacKinnon, M. D. (2003). Microbially-mediated fugitive gas production from oil sands tailings and increased tailings densification rates. *Journal of Environmental Engineering and Science*. 2(3):199-211. doi.org/10.1139/s03-022.
- Foght, J., Aislabie, J., Turner, S., Brown, C. E., Ryburn, J., Saul, D. J., Lawson, W. (2004). Culturable bacteria in subglacial sediments and ice from two Southern Hemisphere glaciers. *Microb Ecol* 47(4): 329–340. DOI: doi.org/10.1007/s00248-003-1036-5.
- Foght J. M., Gieg L. M., Siddique T. (2017). The microbiology of oil sands tailings: past, present, future. *FEMS Microbiology Ecology*. 93(5): DOI: doi:10.1093/femsec/fix034.
- Foght, J. M., Siddique, T. and Gieg, L. M. (2014). Protocols for Handling, Storing, and Cultivating Oil Sands Tailings Ponds Materials for Microbial and Molecular Biological Study. In: T.J. McGenity *et al.* (eds.), *Hydrocarbon and Lipid Microbiology Protocols, Springer Protocols Handbooks*, DOI: 10.1007/8623_2014_26, Springer-Verlag Berlin Heidelberg.
- Holowenko, F. M., MacKinnon, M. D., and Fedorak, P. M. (2000). Methanogens and sulfate-reducing bacteria in oil sands fine tailings waste. *Canadian Journal of Microbiology*. 46(10):927-937.
- Hulecki J. C., Foght J. M., and Fedorak P. M. (2010). Storage of oil field-produced waters alters their chemical and microbiological characteristics. *Journal of Industrial Microbiology*. 37(5):471–481. DOI: doi.org/10.1007/s10295-010-0693-x.
- Kong, J. D., Wang, H., Siddique, T., Foght, J., Semple, S., Burkus, Z., and Lewis, M. A. (2019). Second-generation stoichiometric mathematical model to predict methane emissions from oil sands tailings. *Science Total Environment*. 694:133645
DOI: doi.org/10.1016/j.scitotenv.2019.133645.
- Li, C. (2010). *Methanogenesis in oil sands tailings: An analysis of the microbial community involved and its effects on tailings densification*. University of Alberta MSc thesis, 177 pp.

- Penner, T. J., and Foght, J. M. (2010). Mature fine tailings from oil sands processing harbour diverse methanogenic communities. *Canadian Journal of Microbiology*. 56(6): 459-470. doi.org/10.1139/w10-029.
- Ramos-Padrón, E, Bordenave S., Lin S., Bhaskar I. M., Dong X., Sensen C. W., Fournier J., Voordouw G., and Gieg L. M. (2011). Carbon and sulfur cycling by microbial communities in a gypsum-treated oil sands tailings pond. *Environmental Science and Technology*. 45(2): 439-446. DOI: 10.1021/es1028487.
- Roberts, D. J. (2002). *Methods for Assessing Anaerobic Biodegradation Potential*. In: *Manual of Environmental Microbiology*. Hurst, C.J. R.L. Crawford, G.R. Knudson, M.J. McInerney and L.D. Stetzenbach (eds.) ASM Press, Washington CD, pp. 1008-1017.
- Siddique, T., Fedorak, P. M., and Foght, J. M. (2006). Biodegradation of short-chain *n*-alkanes in oil sands tailings under methanogenic conditions. *Environmental Science and Technology*. 40(17): 5459-5464. doi.org/10.1021/es060993m.
- Siddique, T., Fedorak, P. M., MacKinnon, M. D., and Foght, J. M. (2007). Metabolism of BTEX and naphtha compounds to methane in oil sands tailings. *Environmental Science and Technology*. 41(7): 2350-2356. DOI: doi.org/10.1021/es062852q.
- Siddique, T., Gupta, R., Fedorak, P. M., MacKinnon, M. D., and Foght, J. M. (2008). A first approximation model to predict methane generation from an oil sands tailings settling basin. *Chemosphere*, 72:1573-1580.
- Siddique, T., Penner, T., Semple, K., and Foght, J. M. (2011). Anaerobic biodegradation of longer-chain *n*-alkanes coupled to methane production in oil sands tailings. *Environmental Science and Technology* 45(13): 5892-5899. DOI: doi.org/10.1021/es200649t.
- Siddique, T., Kuznetsov, P., Kuznetsova, A., Arkell, N., Young, R., Li, C., Guigard, S., and Foght, J. M. (2014a). Microbially-accelerated consolidation of oil sands tailings. Pathway I: changes in porewater chemistry. *Frontiers in Microbiology*. 5: 106. DOI: 10.3389/fmicb.2014.00106.
- Siddique, T., Kuznetsov, P., Kuznetsova, A., Arkell, N., Young, R., Li, C., Guigard, S., and Foght, J. M. (2014b). Microbially-accelerated consolidation of oil sands tailings. Pathway II: solid phase biogeochemistry. *Frontiers in Microbiology*. 5: 107. DOI: 10.3389/fmicb.2014.00107.

- Smith, M. R., and Mah, R. A. (1978). Growth and methanogenesis by *Methanosarcina* Strain 227 on acetate and methanol. *Applied and Environmental Microbiology* 36(6): 870-879.
- Soh, J., Dong, X., Caffrey, S. M., Voordouw, G., and Sensen, C. W. (2013). Phoenix 2: a locally installable large-scale 16S rRNA gene sequence analysis pipeline with Web interface. *Journal of Biotechnology*. 167(4): 393-403. DOI:10.1016/j.jbiotec.2013.07.004.
- Wilson, S. L., Li, C., Ramos-Padrón, E., Nesbø, C., Soh, J., Sensen, C. W., Voordouw, G., Foght, J., and Gieg, L. M. (2016). Oil sands tailings ponds harbour a small core prokaryotic microbiome and diverse accessory communities. *Journal of Biotechnology*, 235: 187–196. DOI:10.1016/j.jbiotec.2016.06.030.

Supplementary information

Supplementary Table S1. Elemental analysis of agricultural by-products.

Feedstock	Elemental Analysis (%) ^a			
	C	H	N	S
Blood meal	33.8 ± 0.2	4.8 ± 0.1	9.2 ± 0.0	0.4 ± 0.0
Bone meal	39.6 ± 4.8	5.7 ± 0.7	8.7 ± 1.1	0.4 ± 0.2
Canola	42.6 ± 0.3	6.0 ± 0.0	5.7 ± 0.2	0.5 ± 0.1
Corn DDGS	45.9 ± 0.1	6.9 ± 0.1	4.3 ± 0.1	0.2 ± 0.0
Whey	33.7 ± 0.1	5.9 ± 0.0	1.3 ± 0.0	1.0 ± 0.2

^a Results are the mean of five analyses for hydrolyzed canola and blood meal, and three analyses for bone meal, whey and corn DDGS.

Supplementary Table S2. Calculated maximum theoretical methane production, measured methane produced in microcosms containing Syncrude tailings, and actual yield of net methane in proportion to theoretical production.

Substrate	Theoretical Molecular Formula ^b	Weight added (mg/100 ml tailings)	% Carbon ^b	Carbon added (mg/L) ^c	Expected yields of gases ^a			Theoretical maximum CH ₄ (μmol)	Measured CH ₄ (μmol) ^d	Net Measured CH ₄ (μmol) ^e	Yield (%) ^f
					Moles CO ₂	Moles CH ₄	Ratio CH ₄ /mmolC added				
Bone meal	C ₆ H ₁₀ O ₅ N ₁	103.3 ± 1.2	39.6	409	2.4	2.6	0.43	1464	1285 ± 44	1,107	76
Blood meal	C ₆ H ₁₀ O ₇ N ₁	119.7 ± 1.5	33.8	404	2.9	2.1	0.33	1192	1715 ± 24	1,537	129
Corn DDGS	C ₆ H ₁₁ O ₄ N ₁	89 ± 1	45.9	409	2.0	3.0	0.52	1769	1382 ± 13	1,204	68
Whey	C ₆ H ₁₃ O ₈ N ₀	121.7 ± 0.6	33.7	410	3.4	2.6	0.42	1454	1411 ± 31	1,233	85
Canola	C ₆ H ₁₀ O ₅ N ₁	96 ± 1	42.6	409	2.4	2.6	0.47	1596	1230 ± 9	1,052	66
Glycerol	C ₃ H ₈ O ₃	103	39.1	400	1.25	1.75	0.58	1965	1288 ± 101	1,110	56
Acetate	C ₂ H ₄ O ₂	40.6	24.5	72	1	1	0.5	300	468 ± 4	290	97

^a Calculated using Buswell's Equation, as implemented by Roberts (2002).

^b From elemental analysis (Online resource 8).

^c Target amount was 400 mg Carbon/L tailings, however acetate was added as 72 mg Carbon/L tailings due to calculation error.

^d Measured at 120 d incubation of microcosms; value is the mean of triplicates \pm standard deviation.

^e Mean measured methane value minus unamended (endogenous) methane value of $178 \pm 23 \mu\text{mol}$; *i.e.*, methane production from amendment, corrected for endogenous methanogenesis.

^f The ratio of net measured methane to maximum theoretical methane yield.

Supplementary Table S3. Analysis of pore water expressed from Syncrude tailings after 168 d incubation in biodensification columns. Analyses were performed by Syncrude Canada Ltd.

Water Chemistry (units)	Unamended	Glycerol	Bone meal	Blood meal	Corn DDGS	Whey	Canola	Acetate	Acetate + BES
pH	8.9	9.0	9.0	9.0	8.7	9.0	9.0	9.1	8.0
Conductivity (μS)	3790	5020	4960	4930	4080	4500	4630	4970	9070
Anions (ppm)									
CO ₃ ²⁻	0.2	NA ^a	NA	NA	NA	NA	NA	NA	NA
HCO ₃ ⁻	1200	NA	NA	NA	NA	NA	NA	NA	NA
Cations (ppm)									
Na ⁺	1020	1200	1170	1130	957	1110	1100	1250	2270
K ⁺	13.4	18.4	18.8	22.9	19.6	26.2	23.6	14.3	37.0
Mg ²⁺	9.4	18.1	14.0	18.0	16.0	20.5	20.4	10.6	99.4
Ca ²⁺	7.3	6.2	6.4	6.4	10.5	11.1	9.4	4.0	400
NH ₄ ⁺	7.3	4.0	BDL ^b	26.8	18.1	BDL	BDL	7.6	17.9
Metals (ppm) ^c									
Aluminum (Al)	4.5	3.1	0.5	2.3	0.9	BDL	BDL	4.3	0.1
Boron (B)	3.7	4.8	4.5	4.3	3.9	4.1	3.9	3.3	4.9
Barium (Ba)	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	4.3
Iron (Fe)	1.0	0.7	0.0	0.4	0.1	BDL	BDL	0.8	BDL
Manganese (Mn)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	2.1
Phosphorus (P)	BDL	BDL	0.5	0.5	1.2	2.0	1.0	BDL	BDL
Sulfur (S)	18.2	28.9	33.6	16.8	10.8	15.0	27.8	16.7	518.0
Silicon (Si)	10.1	7.9	3.0	7.5	6.3	3.9	2.6	9.2	4.3
Strontium (Sr)	0.4	0.5	0.4	0.5	0.6	0.6	0.5	0.4	7.0

^a NA not analyzed.

^b BDL below detection limits.

^c Concentrations of cadmium, cobalt, chromium, copper, lithium, molybdenum, nickel, lead, antimony, selenium, titanium, vanadium, zinc and zirconium were analyzed but found to be below detection limits for all samples.

Supplementary Table S4. Analysis of Syncrude tailings solids near the mud line after 168 d incubation in biodensification columns. Analyses were performed by Syncrude Canada Ltd.

Analysis (units)	Unamended	Glycerol	Bone meal	Blood meal	Corn DDGS	Whey	Canola	Acetate	Acetate + BES
------------------	-----------	----------	-----------	------------	-----------	------	--------	---------	---------------

pH	8.1	7.9	7.9	7.9	7.9	7.9	8.0	8.4	6.9
Conductivity (\square S)	3790	4190	4370	4460	4050	4480	4390	4670	7650
Bitumen (% by wt.) ^a	1.8	1.7	1.8	1.7	2.1	2.0	2.0	2.0	1.5
% Water ^a	63.0	63.9	60.6	60.5	62.4	61.3	61.1	62.3	65.9
% Solids ^a	35.4	34.9	36.2	36.2	35.6	35.8	36.2	36.0	31.7
Anions (ppm)									
CO ₃ ²⁻	138	42.3	0.0	33.0	83.4	25.2	30.9	127	0.0
HCO ₃ ⁻	1150	1580	1570	2040	1530	1910	1780	1980	1850
Cations (ppm)									
Na ⁺	893	901	905	960	931	1000	999	1240	1970
K ⁺	14.2	15.6	18.1	22.6	20.6	27.1	23.3	15.7	34.8
Mg ²⁺	12.1	15.7	16.8	23.6	17.9	22.9	23.5	11.8	95.2
Ca ²⁺	21.8	19.6	27.5	47.4	36.7	57.9	78.2	16.1	341.0
NH ₄ ⁺	10.8	9.3	27.5	65.2	23.3	17.3	21.2	10.7	23.8
Metals (ppm) ^b									
Aluminum (Al)	0.3	0.1	BDL ^c	BDL	BDL	BDL	BDL	0.6	BDL
Boron (B)	3.1	2.9	2.8	2.9	3.2	3.1	2.9	2.7	4.0
Barium (Ba)	0.4	0.4	0.6	0.9	0.6	0.9	1.1	0.4	3.7
Iron (Fe)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.1	BDL
Lithium (Li)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Manganese (Mn)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	3.6
Phosphorus (P)	BDL	BDL	BDL	0.3	0.8	1.7	1.0	BDL	BDL
Sulfur (S)	23.0	26.2	23.4	13.0	19.3	7.0	30.1	34.1	414
Silicon (Si)	4.6	3.8	4.1	4.6	5.1	5.2	4.4	4.2	4.4
Strontium (Sr)	0.8	0.8	1.0	1.5	1.1	1.5	1.7	0.8	5.6

^a Dean-Stark analysis as implemented by Syncrude Canada Ltd. (Syncrude, 1979).

^b Concentrations of cadmium, cobalt, chromium, copper, lithium, molybdenum, nickel, lead, antimony, selenium, titanium, vanadium, zinc and zirconium were analyzed but found to be below detectable limits for all samples.

^c BDL below detection limits.

Online Resource 8. Analysis of Syncrude solids fraction near the bottom of the column after 168 d incubation in biodensification columns. Analyses were performed by Syncrude Canada Ltd.

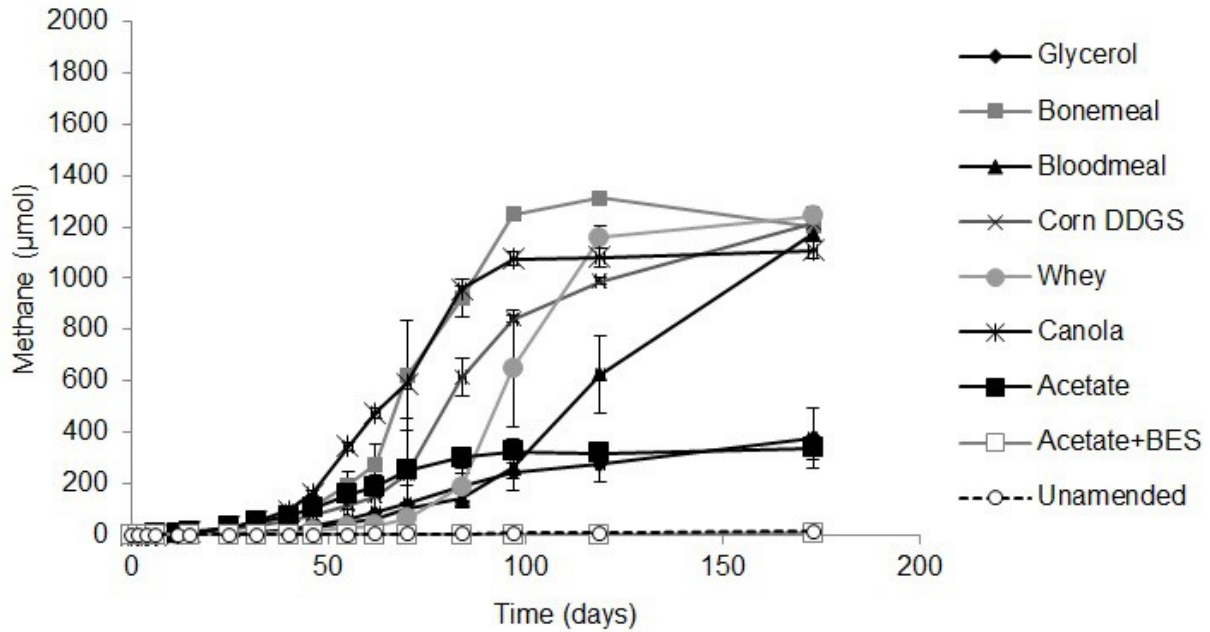
Analysis	Corn								
	Unamended	Glycerol	Bonemeal	Bloodmeal	DDGS	Whey	Canola	Acetate	Acetate + BES
pH	8.1	7.8	7.9	7.9	7.7	7.8	7.8	8.2	7.0
Conductivity (μ S)	3690	4140	4170	4580	4150	4550	4500	4570	7320
Bitumen (% by wt.) ^a	1.9	1.8	1.7	1.4	2.1	1.9	1.9	2.0	1.5
% Water ^a	61.8	60.0	58.4	58.1	60.0	64.0	62.2	59.9	64.4
% Solids ^a	36.6	38.0	37.9	38.8	37.4	36.0	35.6	37.8	33.7
Anions (ppm)									
CO ₃ ²⁻	28.5	27.0	20.1	26.4	39.0	0.0	16.5	262	0.0
HCO ₃ ⁻	1340	1740	1750	2050	1690	1990	1810	1610	1740
Cations (ppm)									
Na ⁺	873	927	951	961	960	1000	1010	1190	1720

K ⁺	14.1	17.3	20.3	24.9	22.3	29.5	25.3	17.0	33.7
Mg ²⁺	12.7	21.1	19.5	26.2	21.1	28.6	27.7	13.9	83.2
Ca ²⁺	21.5	31.9	46.1	55.1	42.4	75.6	73.4	17.0	304
NH ₄ ⁺	11.3	13.0	33.7	65.2	23.8	20.6	26.1	12.1	23.9
Metals (ppm)^b									
Aluminum (Al)	0.3	BDL ^c	BDL	BDL	BDL	BDL	BDL	0.5	BDL
Boron (B)	2.9	3.0	3.2	2.8	3.7	3.2	3.1	2.8	4.0
Barium (Ba)	0.4	0.7	0.8	1.2	0.8	1.1	1.1	0.4	3.5
Iron (Fe)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.1	0.0
Manganese (Mn)	BDL	BDL	0.0	BDL	0.0	0.0	BDL	BDL	2.8
Phosphorus (P)	BDL	BDL	0.3	BDL	0.4	0.8	0.7	BDL	BDL
Sulfur (S)	22.9	11.5	16.8	14.3	21.3	16.1	24.3	31.5	359
Silicon (Si)	4.6	4.8	5.4	4.8	6.1	5.6	5.1	4.7	5.1
Strontium (Sr)	0.8	1.3	1.3	1.8	1.4	1.9	1.9	0.9	5.3

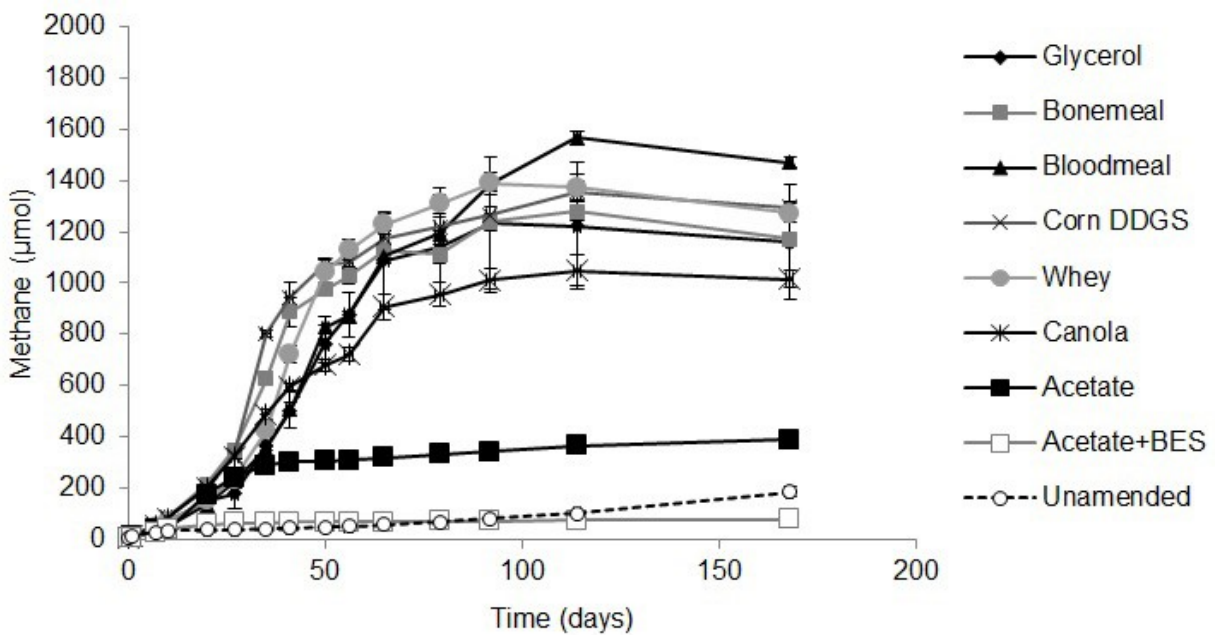
^a Dean-Stark analysis (Dean and Stark, 1920), as implemented by Syncrude Canada Ltd. (Syncrude, 1979).

^b Concentrations of cadmium, cobalt, chromium, copper, lithium, molybdenum, nickel, lead, antimony, selenium, titanium, vanadium, zinc and zirconium were analyzed but found to be below detectable limits for all samples.

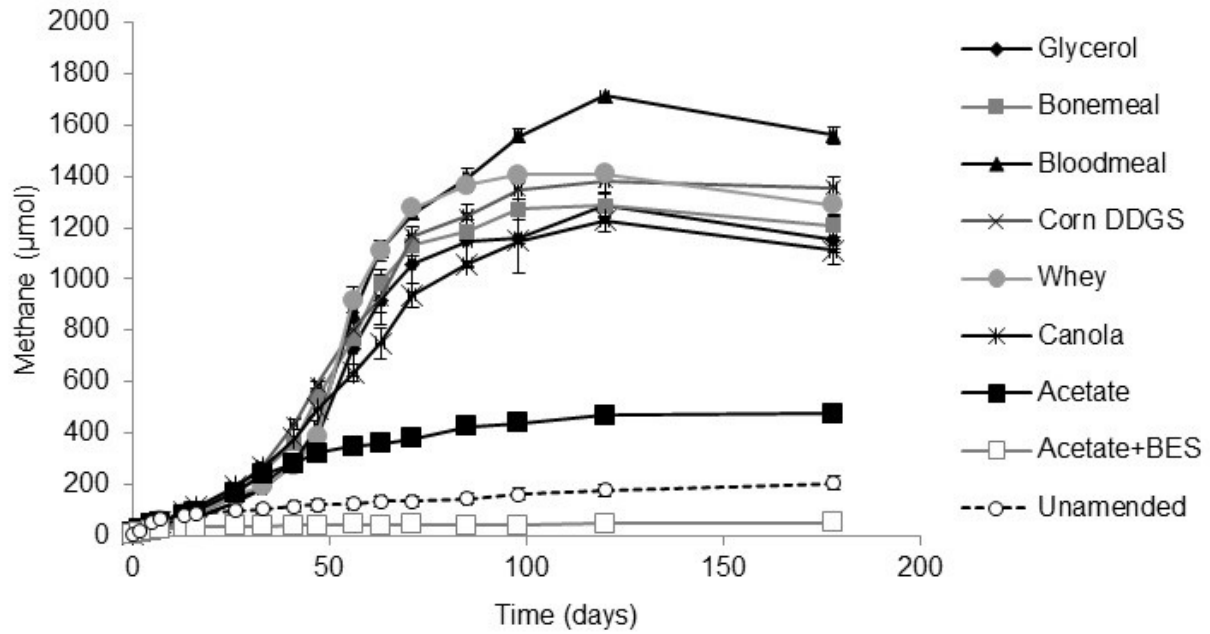
^c BDL below detection limits.



Supplementary Fig. S1. Cumulative methane production by unamended and amended Albian tailings incubated in anaerobic microcosms. Agri-business amendments were added at 400 mg C/L tailings and acetate was added at 72 mg C/L tailings.



Supplementary Fig. S2. Cumulative methane production by unamended and amended Suncor tailings incubated in anaerobic microcosms. Agri-business amendments were added at 400 mg C/L tailings and acetate was added at 72 mg C/L tailings.



Supplementary Fig. S3. Cumulative methane production by unamended and amended Syncrude tailings incubated in anaerobic microcosms. Agri-business amendments were added at 400 mg C/L tailings and acetate was added at 72 mg C/L tailings.