Biodegradation of Polyacrylamide in Oil Sands Tailings by Indigenous Microorganisms

by

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ABSTRACT

Hydrolyzed polyacrylamide (HPAM) is a common flocculant used in oil sands tailings dewatering technologies. Although HPAM is regularly used in tailings treatment and will eventually be incorporated into the reclaimed landscape of the oil sands, there are knowledge gaps surrounding the environment fate of HPAM, specifically its susceptibility to microbial degradation. Microcosm studies were established to evaluate the biodegradation of HPAM by indigenous oil sands tailings microorganisms under oxic, sulfate-reducing, and methanogenic conditions. Under each redox condition, the ability of microorganisms to use HPAM as a carbon and/or nitrogen source, or degrade HPAM through co-metabolic processes was investigated. Biodegradation was assessed using Size-Exclusion Chromatography, while the formation of hydrolytic ammonium indicated the use of HPAM as a nitrogen source.

After 110 days, HPAM experienced the greatest biodegradation when provided as a co-substrate (99%) and nitrogen source (99%) under oxic conditions, with lower molecular weight oligomers (< 6000 g/mol) as biodegradation products. For anoxic conditions, HPAM biodegradation was observed when provided as a co-substrate (77% after 420 days) for sulfate-reducing conditions, and as a co-substrate (60% after 374 days) and nitrogen source (60% after 374 days) for methanogenic conditions. Under anoxic conditions, biodegradation produced oligomers with a molecular weight around 2-4 x 10^6 g/mol. An increase in ammonium was observed under all redox conditions. When HPAM was provided as a nitrogen source, this decreased the lag time of sulfate-reduction and methane production. HPAM biodegradation was not found to significantly increase acute toxicity towards *Aliivibrio fischeri*.

Microbial communities identified in HPAM degrading cultures included *Pseudomonadaceae, Flavobacteriaceae*, *Lentimicrobiaceae*, and *Acholeplasmataceae* for oxic conditions,

Methanosarcinaceae, *Methanosaetaceae*, Desulfuromonadia, *Acholeplasmataceae*, and *Hydrogenophilaceae* for sulfate-reducing conditions, and *Methanosarcinaceae*, *Flavobacteriaceae*, and *Pseudomonadaceae* for methanogenic conditions. This study demonstrated the conditions in which HPAM biodegradation could potentially occur in oil sands tailings systems or reclamation landscapes, and the bacteria and archaea involved.

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INTRODUCTION

1.1 Alberta Oil Sands Overview

The Alberta Oil Sands is the fourth largest oil reserve in the world, after Venezuela, Saudi Arabia, and Iran, consisting of about 160.1 billion barrels (Government of Alberta, 2023a). These reserves are situated beneath approximately 142,200 km^2 of land distributed across the Peace River, Athabasca, and Cold Lake regions as presented in Figure 1-1 (Government of Alberta, 2023a). Oil sands deposits are considered unconventional oil, since they cannot be extracted using conventional methods such as drilling and pumping. Oil sands consist of sand, silt, clay, water, and bitumen, a heavy oil incapable of flowing or being pumped under natural environmental conditions due to its viscous nature. Surface mining is therefore used to extract bitumen reserves up to 75 meters below ground level, while deeper reserves are extracted using in-situ technologies (Chalaturnyk et al., 2002). The Athabasca region is the only deposit in Alberta that can be recovered using surface mining. In 2021, bitumen production was about 3.3 million barrels per day (Government of Alberta, 2023a). Of the 4,800 km^2 area capable of surface mining, the total active footprint of the oil sands was 1,055 km^2 in 2020 (Alberta Environment and Parks, 2022). In addition to land disturbance, surface mining has resulted in the accumulation of over 1.3 billion m^3 of tailings (Alberta Energy Regulator, 2022a).

1.2 Oil Sands Tailings

Tailings are a waste by-product of surface mining, consisting of sand, silt, clay, process-affected water, inorganics, organics, and residual bitumen. Surface-mined bitumen is extracted from the oil sands ore using the Clark Hot Water Process, where caustic soda (sodium hydroxide) and hot water are mixed with crushed oil sands ore (Clark and Pasternack, 1932). The Clark Hot Water Process produces a bitumen froth that floats to the surface of a separation vessel, while the remaining tailings slurry is transferred and temporarily stored in above ground facilities or mine pits referred to as tailings ponds (Clark and Pasternack, 1932). In the bitumen froth, residual water and solids can be further removed using naphtha or paraffinic solvents (Chalaturnyk et al., 2002). After three to five years in the tailings pond, the minerals settle to a solids content of 30-40 wt% producing tailings commonly referred to as mature fine tailings (MFT) (Kasperski and Mikula, 2011). The high clay content (fines <2 μm) of the MFT results in low flocculation and slow consolidation characteristics, decreasing the tailings settling rate and requiring decades for tailings to reach a

solids content suitable for reclamation (Chalaturnyk et al., 2002). In addition, poor settling prevents process water from being recycled back to the extraction facility, placing dependance on water withdrawals from the Athabasca River.

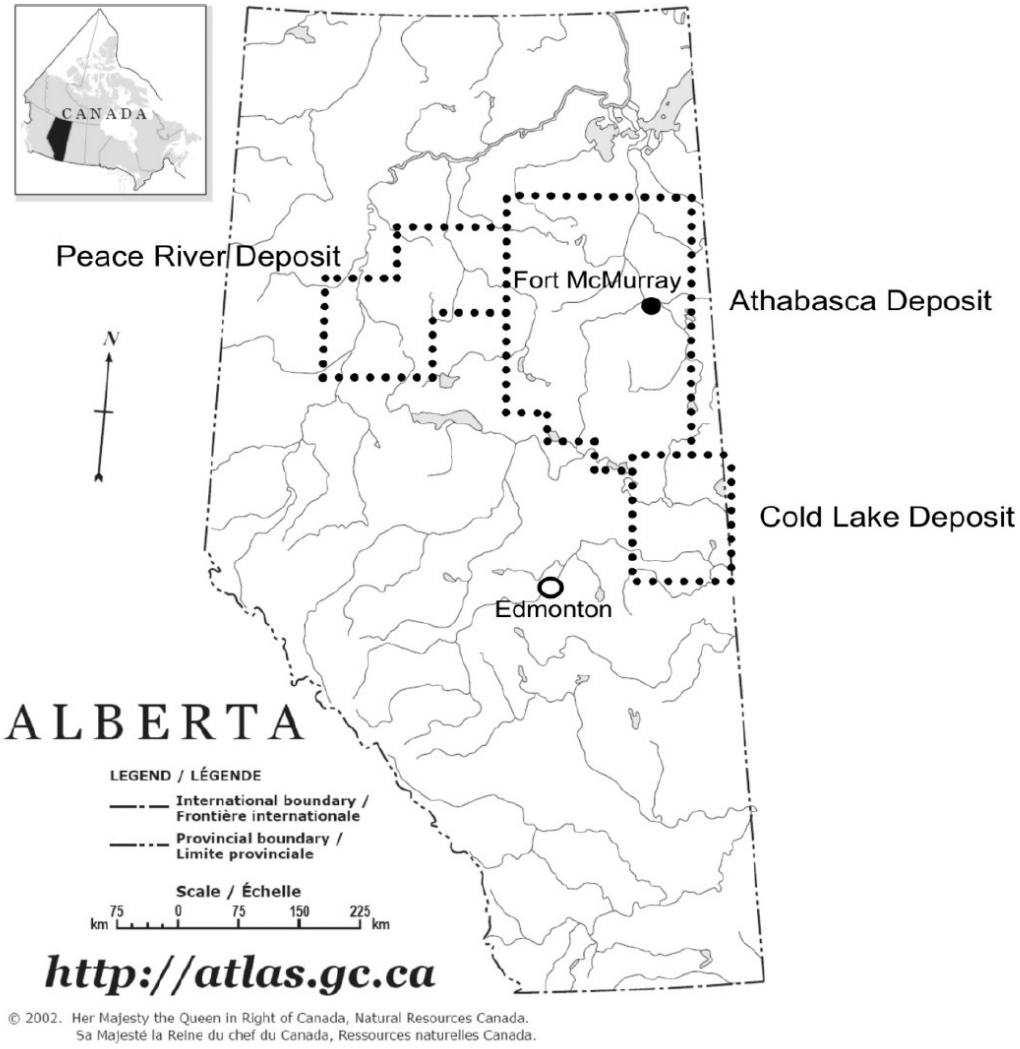


Figure 1-1 Major oil sands areas in Alberta, Canada (Allen 2008).

The large accumulation and land use of tailings presents a considerable environmental liability and has prompted concerns over their permanence and if reclamation is possible. In 2020, Alberta oil sands tailings facilities occupied a surface area of 270 km^2 (Alberta Environment and Parks, 2020). Other potential issues associated with oil sands tailings facilities include toxicity, the exposure to

surface and groundwater systems, and greenhouse gas emissions, particularly from microbial activity (Holden et al., 2011; Small et al., 2015; Li et al., 2017).

1.3 Tailings Treatments

To address the environmental risks and land occupancy associated with oil sands tailings, operators have relied upon physical and/or chemical amendments to increase tailings settling and dewatering. Treatments include the use of mechanical thickeners, centrifuges, coagulants, and flocculants, as well as mixing with coarse tailings sand. Currently, majority of tailings dewatering technologies rely heavily on the use of polymer flocculants, specifically those based on polyacrylamide (PAM) (Vedoy and Soares, 2015). Initially designed for municipal wastewater, metal mining, and paper-making industries, PAM is also now used for soil conditioning (erosion control), enhanced oil recovery, biomedical applications, and oil sands tailings dewatering (Caulfield et al., 2002; Vedoy and Soares, 2015).

The dependance on PAM as a flocculant is largely due to its ability to be customized to meet various industry specifications. The non-ionic form of PAM, which consists of acrylamide monomers, can be modified using comonomers to produce anionic or cationic PAM (Vedoy and Soares, 2015). In the case of oil sands tailings, anionic PAM with a high molecular weight (MW) and medium charge density (20-30%) has been most successful with solids flocculation (Xu and Cymerman, 1999; Long et al., 2006; Vedoy and Soares, 2015). The most common form of anionic PAM used in oil sands tailings is hydrolyzed PAM (HPAM), where acrylamide and acrylic acid make-up the polymer chain. The addition of HPAM to oil sands tailings increases the settling rate; producing tailings with a solids content typically between 35 and 50 wt% (Hyndman et al. 2018). Figure 1-2 provides a visual comparison of untreated tailings (~26 wt%) and HPAM treated tailings (~60 wt%).

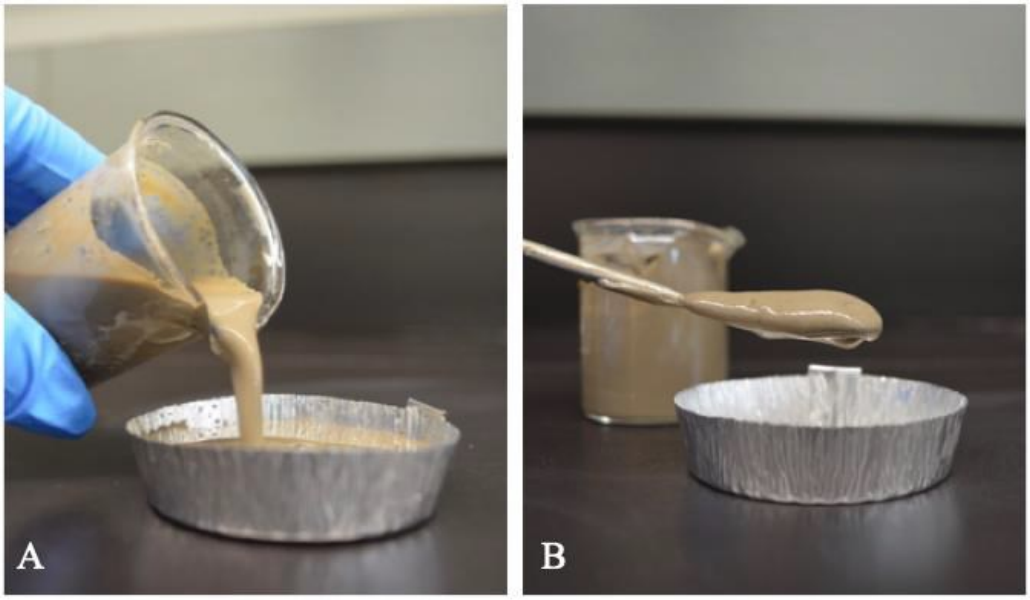


Figure 1-2 Untreated tailings (A) and HPAM treated tailings (B) (Cossey et al., 2021).

1.4 Polyacrylamide Biodegradation

Although PAM is the most common flocculant use for oil sands tailings dewatering, little is known about its interactions with tailings microbial communities and susceptibility to biodegradation in tailings systems. The biodegradation of PAM and HPAM has been investigated under oxic and anoxic conditions. Under oxic conditions, PAM is thought to serve as a nitrogen source through the hydrolysis of the amide group. To support this mechanism, Kay-Shoemake et al. (1998b) observed that aerobic microorganisms produce extracellular amidase in the presence of PAM, releasing ammonium which can serve as a nitrogen source. Due to PAMs high molecular weight and a lack of enzymes capable of depolymerizing the polymer chain it is thought that PAM cannot serve as a carbon source for aerobic microorganisms (Kay-Shoemake et al., 1998a). However, studies have observed a decrease in the polymer molecular weight when subject to aerobic activity, suggesting cleavage of the carbon chain through monooxygenase (Bao et al. 2010; Liu et al., 2012; Yu et al., 2015; Li et al. 2023).

Under anoxic conditions, the presence of PAM has been found to stimulate methanogens and sulfate-reducing bacteria (Grula et al., 1994; Haveroen et al., 2005; Hu et al., 2018). Haveroen et al. (2005) found that methanogenic activity was higher in nitrogen deficient cultures in the

presence of HPAM compared to cultures without HPAM. Similar to aerobic processes, it is thought that anaerobic microorganisms can hydrolyze the amide group through amidase (Grula et al., 1994; Hu et al., 2018). PAM and HPAM may also serve as a carbon source for anaerobic microorganisms, with studies reporting a change in polymer molecular weight, biogenic gas production, and a change in water chemistry that aligns with cleavage of the carbon chain (Dai et al., 2014; Dai et al., 2015; Song et al., 2017; Hu et al., 2018). It is proposed that once PAM has been hydrolyzed to polyacrylic acid through amidase, further biodegradation occurs through alcohol dehydrogenase (Dai et al., 2015). However, the enzymes involved in anaerobic carbon chain cleavage of PAM and polyacrylic acid have not been thoroughly investigated. Various biodegradation products have been proposed and a select few identified, but the primary metabolite of concern is acrylamide. The acrylamide monomer is a neurotoxin and carcinogen (United States Environmental Protection Agency, 2010), prompting concerns over its release into the environment following degradation. There is limited research confirming the biodegradation of PAM to acrylamide, with studies observing that any residual acrylamide in the initial polymer solution are quickly degraded by aerobic microorganisms (Li et al., 2023).

To date, only three studies have investigated HPAM biodegradation or its used as a nutrient source for tailings microbial communities. Under oxic conditions, Li et al. (2023) found oil sands tailings isolates and indigenous microbial communities were capable of HPAM biodegradation, with a greater decrease in biodegradation by tailings isolates. For anoxic conditions however, contradicting evidence for HPAM biodegradation was obtained from Haveroen et al. (2005) and Collins et al. (2016). As previously stated, Haveroen et al. (2005) found that HPAM stimulated methanogenesis under nitrogen deficient conditions in comparison to cultures without HPAM. However, Collins et al. (2016) found that methanogenesis was not a result of HPAM, but instead nitrogen fixation. It was also determined that HPAM could not be used as a carbon source (Collins et al., 2016). The contradicting results for the use of HPAM as a nitrogen source to support methanogenesis, as well as limited investigation into the use of HPAM as a carbon source for anaerobic activity, highlight the importance in further investigating the role of HPAM in oil sands tailings. Currently no oil sands studies have investigated the biodegradation of HPAM or PAM under sulfate-, nitrate-, or iron-reducing conditions.

Though acrylamide is a concern when it comes to toxicity, PAM based polymers are considered relatively non-toxic (Buczek et al., 2017). Currently there is limited research on the toxicity of PAM biodegradation metabolites. Li et al. (2023) found a slight increase (<10%) in the inhibition effect of pure polymers solutions following biodegradation of HPAM by tailings isolates, suggesting HPAM metabolites may influence the potential toxicity of HPAM treated tailings deposits. Limited research exists on the toxicity of oil sands tailings following aerobic HPAM biodegradation, with no studies performed on anoxic conditions. Therefore, the toxicity in oil sands tailings and the surrounding environment following PAM biodegradation is not well understood.

1.5 Research Objectives

Majority of PAM and HPAM biodegradation studies have been reported in municipal wastewater systems, treated soils, and enhanced oil recovery, while only a few peer-reviewed studies on oil sands tailings exists. For anaerobic studies, Haveroen et al. (2005) and Collins et al. (2016) reported contradicting results. They also did not measure HPAM directly, but instead based findings from biogenic gas production and changes in water chemistry due to microbial activity. For aerobic studies, Li et al. (2023) determined HPAM biodegradation by measuring pore water concentration using Size Exclusion Chromatography. There is currently a lack of studies monitoring a shift in HPAM molecular weight due to indigenous microorganisms throughout the incubation period. Indigenous tailings microorganisms capable of PAM biodegradation were also not identified in studies performed by Haveroen et al. (2005) and Li et al. (2023). To address these knowledge gaps, the two research objectives were developed for this thesis dissertation.

1. ***Develop a method for measuring hydrolyzed polyacrylamide using Size-Exclusion Chromatography***

Research question: Can Size-Exclusion Chromatography elucidate HPAM biodegradation in an oil sands tailings context?

Hypothesis: We hypothesized that Size-Exclusion Chromatography (SEC) will be able to elucidate a change in HPAM molecular weight and concentration over the course of the experiment and make comparisons among redox conditions. SEC has been used to compare the initial and final molecular weight of PAM in activated sludge, polymer flooded produced water, and oil sands

tailings (Li et al. 2023; Song et al., 2017; Liu et al. 2012; Hu et al. 2018), as well as measure the concentration of HPAM overtime in oil sands tailings studies (Li et al 2023).

Due to the strong adsorption of PAM to soil and clay particles (Guezennec et al., 2015), we hypothesized that the high clay content of oil sands tailings may influence the detection limit and create interferences when measuring HPAM in the pore water. SEC has achieved detection limits of 0.1 mg/L for PAM, and has been applied to pure polymer solutions, activated sludge, enhanced oil recovery, and oil sands tailings (Xihua et al., 2007; Song et al., 2017; Hu et al., 2018; Li et al., 2023). Therefore, this method holds promise for our research.

2. ***Investigate the biodegradation of hydrolyzed polyacrylamide under methanogenic, sulfate-reducing, and oxic conditions.***

Research questions: Are indigenous oil sands tailings microorganisms capable of HPAM biodegradation under methanogenic, sulfate-reducing, and oxic conditions? Are these microorganisms capable of using HPAM as a carbon and/or nitrogen source, or degrade HPAM through co-metabolic processes? How do microbial communities in fresh tailings respond to the introduction of PAM and shift over time under different redox conditions?

Hypothesis: We hypothesized that HPAM would undergo biodegradation in each redox condition, with the most significant biodegradation observed when HPAM is provided as a co-substrate, followed by as a sole nitrogen source, and then sole carbon source. Due to the presence of other organics in tailings, it is possible that biodegradation could occur through co-metabolic pathways. Wen et al. (2010) observed increased PAM degradation in the presence of a carbon source (glucose), suggesting the use of PAM as a co-substrate. When comparing the use of PAM as a nutrient source, Bao et al. (2010) observed the most biodegradation when PAM was the sole nitrogen source, compared to the sole carbon source.

Based on current literature, there is evidence that HPAM serves as a nitrogen source for aerobic and anaerobic activity (Grula et al., 1994; Kay-Shoemake et al., 1998a; Haveroen et al., 2005; Bao et al., 2010; Hu et al., 2018; Berdugo-Clavijo et al., 2019). Using various analytical techniques, the partial biodegradation of HPAM has been recorded in previous studies, suggesting the potential use of HPAM as a carbon source under oxic and methanogenic conditions (Bao et al., 2010; Dai et al., 2014; Hu et al., 2018; Li et al., 2023). There is limited research confirming the use of HPAM

and PAM as a carbon source for sulfate-reducing bacteria. We therefore hypothesized that HPAM will undergo biodegradation either through co-metabolism or as a nitrogen source under sulfate-reducing conditions, but likely no or limited biodegradation when provided as the sole carbon source. For methanogenic and oxic conditions, HPAM will undergo biodegradation when provided as a co-substrate, or sole nitrogen and/or sole carbon source.

In oil sands tailings deposits, redox conditions transition from oxic to anoxic at the surface water and tailings interface, with reducing conditions increasing with depth (Reid and Warren, 2016). Deep oil sands cores in the Athabasca River Region also revealed networks of aerobic, facultative, and anaerobic bacteria, including methanogens and sulfate-reducing species (An et al., 2013). We therefore hypothesized that when provided with suitable terminal electron acceptors for oxic, sulfate-reducing, or methanogenic conditions, initial tailings microbial communities will transition to the dominant redox condition. We also hypothesized that HPAM amendments will increase microorganisms capable of degrading HPAM and the associated metabolites in oil sands tailings. Li et al. (2023) isolated *Pseudomonas* sp*., Bosea* sp*., and Sphingopyxis* sp. capable of aerobic HPAM biodegradation, while *Clostridia* have been associated with the presence of HPAM but not necessarily capable of HPAM biodegradation (Kuzntesov et al., 2015; Collins et al., 2016). Other microorganisms associated with HPAM biodegradation that have also been identified in oil sands tailings include but are not limited to, *Gammaproteobacteria*, *Betaproteobacteria*, *Flavobacterium* sp., *Brevundumonas* sp*., Desulfovibrio* sp*., Methanomicrobiales,* and *Methanosarcinales* (including M*ethanosaeta sp.)* (Penner and Foght et al., 2010; Foght et al., 2017; Warren et al., 2016; An et al., 2023). It is likely that microorganisms capable of producing amidase will also be identified, including *Brevundimonas* sp. and *Pseudomonas* sp. (Kay-shoemake et al., 1998b).

Oil sands tailings (some PAM treated) will ultimately be deposited into closure landscapes and developed into either end pit lakes, wetland systems, or upland forests. This highlights the importance of understanding the interactions of tailings microbial communities with PAM treated tailings, and the potential for biodegradation in these systems. In doing so, this research will provide insight into PAM biodegradation and environmental fate in tailings, and therefore the conditions in which biodegradation may occur in treated tailings materials as they progress to reclamation.

1.6 Thesis Outline

This thesis consists of five chapters. A literature review on the current knowledge of methodological techniques for measuring PAM and HPAM biodegradation and studies that have observed biodegradation is presented in Chapter 2. The methodology for measuring HPAM and elucidating HPAM biodegradation is discussed in Chapter 3. The microcosms biodegradation experiment is discussed in Chapter 4, with subsections describing (I) microbial activity in the presence of HPAM and biodegradation, (II) acute toxicity, and (III) tailings microbial diversity. Chapter 5 will present the final conclusions and recommendations for future research. The Appendices include supplementary information for Chapter 3 and 4.

2 LITERATURE REVIEW

2.1 Oil Sands Tailings

Bitumen recovery occurs through open pit mining or in-situ processes (reserves deeper than 75 meters below ground surface) (Chalaturnyk et al., 2002). The most prevalent in-situ methods are steam assisted gravity drainage (SAGD) and Cyclic Steam Stimulation (CSS) (Oil Sands Magazine, 2023). Both methods apply steam to reduce bitumen viscosity and enhance recovery of otherwise immobile reserves (Butler et al., 1981; Alberta Energy Regulator, 2023). SAGD uses two separate horizontal wells, where steam is injected into the reservoir through the upper well (Butler et al., 1981). The higher temperatures reduce the viscosity of the bitumen, mobilizing the reserves which flow towards the lower production well (Butler et al., 1981). During CSS, steam is injected using a single horizontal or vertical well (Alberta Energy Regulator, 2023). After the bitumen is allowed to soak for several days or week, the bitumen and water emulsion is pumped to the surface through the same well (Oil Sands Magazine, 2023). 80% of bitumen reserves in Alberta are extractable through in-situ techniques, which would result in the disturbance of approximately 136 000 km^2 of land (97% of the oil sands area) (Jordaan, 2012; Alberta Energy Regulator, 2023).

Alternatively, 20% of bitumen reserves can be recovered through surface mining. Surface mining involves the removal of forest and overburden, typically muskeg and topsoil, to expose the oil sands deposits (Oil Sands Discovery Center, 2016; Government of Alberta, 2023b). The oil sands are transported to a crusher and then to the extraction plant to separate the sands from the bitumen (Oil Sands Discovery Centre, 2016). The land use for surface mining typically comprises of the mine site, overburden storage, tailings ponds, and reclamation sites (Jordaan, 2012). Both extraction techniques have impacts on the landscape. Surface mining has a large, localized impact from the conversion of land, while in-situ techniques create landscape fragmentation. Each technique will have different impacts on the landscape, water requirements, land management, and reclamation. In this review, surface mining will be the focus.

Although a smaller percentage of bitumen reserves are accessible through surface mining, this continues to be the predominant process for bitumen extraction due to greater recovery (Kasperski and Mikula, 2011). The rapid growth in surface mining will present challenges for water and land management and reclamation, especially when it comes to the production of oil sands tailings

(Kasperski and Mikula, 2011; Jordaan, 2012). Surface mining has resulted in the disturbance of 895 km^2 of land and the accumulation of over 1.3 billion m^3 of tailings (Alberta Energy Regulator, 2022a; Government of Alberta, 2023a).

2.1.1 Bitumen Extraction

Bitumen recovered through surface mining is extracted from the oil sands ores using the Clark Hot Water Process (Chalaturnyk et al., 2002). The bitumen extraction process is provided in Figure 2-1. During extraction, the crushed oil sands ore is mixed with caustic soda (sodium hydroxide) and high temperature water ranging from 35 to 93°C (Small et al. 2014; Foght et al. 2017). With the addition of sodium hydroxide (NaOH), organic acids (mainly aromatics possessing oxygen functionalities such as phenolic, carboxylic, and sulphonic groups) in the bitumen are ionized, producing a surfactant that aids in the liberation of bitumen from the oil sands ore (Sanford, 1983; Chalaturnyk et al., 2002; Masliyah et al., 2008). Increasing the pH using NaOH reduces the surface and interfacial tension in the mixture, disintegrating the bonds holding the ore components (bitumen, sand, water, clay) together (Chalaturnyk et al., 2002). The slurry produced during the caustic, hot water treatment is then pumped to a primary separation vessel where entrained or introduced air produces a froth, promoting bitumen flotation (Chalaturnyk et al., 2002; Masliyah et al., 2008). The bitumen froth consists of approximately 60 wt% bitumen, 30 wt% water, and 10 wt% solids, where the froth is collected with skimming rakes and pumped to froth treatment (Chalaturnyk et al., 2002; Masliyah et al., 2008). While the bitumen froth floats to the surface of the separation vessel, the remaining slurry is separated into a coarse mineral matter that settles to the bottom, and a middle layer known as middlings (consists of sand, clay, water, and bitumen) (Chalaturnyk et al., 2002; Oil Sands Discovery Centre, 2016). A rake at the bottom of the primary separation vessel collects the coarse mineral matter, where this mixture is then transferred to settling basins referred to as tailings ponds (Clark and Pasternack, 1932; Oil Sands Discovery Centre, 2016). The middling stream moves onto secondary separation, where air is injected into the flotation tank for further separation and recovery of residual bitumen (Oil Sands Discovery Centre, 2016). This removes an additional 2-4% bitumen, which is then recycled back to the primary separation vessel (Oil Sands Discovery Centre, 2016).

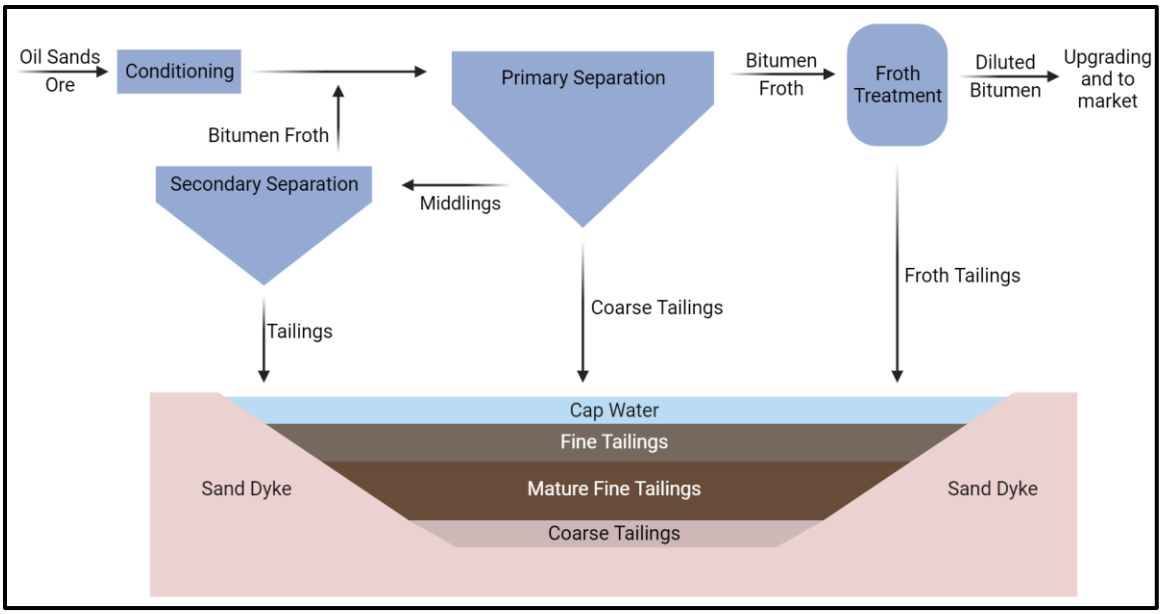


Figure 2-1 General pathway for bitumen extraction and tailings generation following surface mining. Bitumen extraction and tailings pathways may vary among operator depending on tailings dewatering methods used.

During froth treatment, the bitumen froth is treated with a diluent to reduce water-in-bitumen emulsion stability, decrease bitumen viscosity, and encourage separation of bitumen from sand and fine particles (Chalaturnyk et al., 2002; Kasperski and Mikula, 2011). Naphtha is the more commonly used, comprised of aliphatic hydrocarbons(~C5-C16) and monoaromatics including BTEX (benzene, toluene, ethylbenzene, and xylene) (Burkus et al., 2014; Foght et al., 2017). Light paraffinic diluent comprised of C5-C6 alkanes are also used by some operators (Foght et al., 2017). After diluting with a diluent, water and residual solids are further removed from the bitumen using inclined plate settlers and centrifuges (Oil Sands Discovery Centre, 2016). The diluted bitumen is then ready to be upgraded into synthetic crude oil (Oil Sands Discovery Centre, 2016).

Though the hot water extraction process can achieve over 90% bitumen recovery (Chalaturnyk et al., 2002), significant environmental impacts originate from this process, with the predominant issue being tailings generation. For every 1 m^3 of bitumen produced, about 3 to 5 m^3 of tailings are produced (Holowenko et al., 2000; Kasperski and Mikula, 2011).

2.1.2 Oil Sands Tailings

After bitumen is extracted during the primary and secondary separation, and froth treatment, the remaining slurry is comprised of sand, silt, clay, oil sands process-affected water (OSPW), dissolved salts, organics, minerals, and residual bitumen (Allen, 2008). The tailings composition

will vary depending on ore quality and source, extraction processes, and tailings age (Allen, 2008). Bitumen extraction results in three tailings streams: coarse tailings, fine tailings, and froth treated tailings (Figure 2-1). Coarse tailings are collected from the bottom of the separation vessels and possess a solids content of 55 wt%, of which 82 wt% is mineral particles greater than 44 μm, 17% are fines less than 44 μm, and 1% is residual bitumen (Chalaturnyk et al., 2002; Kasperski and Mikula, 2011). Fine tailings possess a solids content of 6-10 wt% and consist predominantly of fines (Chalaturnyk et al., 2002; Kasperski and Mikula, 2011). Silt and clay make up the fines fraction of the fine tailings, with minerals being predominantly quartz, kaolinite, and illite (Kasperski and Mikula, 2011). Froth treatment tailings will have a combination of water, sand, fines, organics and inorganics, and residual bitumen, along with diluent added during froth treatment (Kasperski and Mikula, 2011).

When it comes to tailings handling and discharge, combining tailings steams will depend on the operator (Kasperski and Mikula, 2011). Upon being discharged into tailings ponds, the coarse sand particles settle quickly forming a beach that can be used to build containment dykes, while the fines remain suspended in the pond (Chalaturnyk et al., 2002; Kasperski and Mikula, 2011). The fine tailings quickly settle to 20 wt%, and after three to five years the fines settle to 30-40 wt% producing tailings commonly referred to as mature fine tailings (MFT) (Chalaturnyk et al., 2002; Kasperski and Mikula, 2011). MFT possesses low flocculation and consolidations characteristics, producing a stable slurry that will persist for decades (Chalaturnyk et al., 2002). MFT stability is thought to be a result of residual organics, the presence of clays, and water chemistry. Water-soluble asphaltic acids that are derived from bitumen are thought to reduce the surface and interfacial tension of water, resulting in clay dispersal (Chalaturnyk et al., 2002). Low flocculation and consolidation may also be due to ultrafine clay particles less than 0.2 μm, where the particles create a gel-like structure with high water holding capacity (Ripmeester et al., 1993; Chalaturnyk et al., 2002). In addition, pH is thought to influence the interactions of organic compounds with clay surfaces, assisting with the clay structure formation within the tailings slurry (Chalaturnyk et al., 2002). As the pH increases above 10 or decreases below 6, the clay structure collapses (Chalaturnyk et al., 2002).

Oil sands producers operate under a zero-discharge water policy, requiring all tailings and OSPW to be stored on site in tailings ponds (Allen, 2008). Release restrictions and MFT stability has

resulted in the large accumulation of tailings and prolonged presence of tailings ponds (Allen, 2008). Bitumen extraction and tailings ponds present significant environmental challenges including greenhouse gas emissions particularly from microbial activity and impacts to surface and groundwater systems from potential OSPW seepage (Holden et al., 2011; Small et al., 2015). Fines dominated tailings prevents water recycled back to the extraction facility, delaying reclamation and prolonging environmental liabilities and disturbance to the surrounding ecosystems (Allen, 2008; Jordaan, 2012).

2.1.3 Tailings Treatment and Reclamation

To address the challenges faced by the slow consolidation and dewatering rate of oil sands tailings, operators have investigated various tailings management and reclamation strategies. Tailings treatment includes various physical and/or chemical amendments to improve consolidation and dewatering and prepare tailings for reclamation. Treated tailings may be deposited into a tailings pond or dedicated disposal area, where they then undergo further dewatering (Cossey et al., 2021). Eventually these tailings and disposal areas will be incorporated or converted into wet or dry reclamation landscapes. Anticipated reclamation landscapes for oil sands tailings include end pit lakes (EPLs), wetlands, and upland forests. Treated tailings, including chemical additives such as polyacrylamide (PAM), will ultimately be incorporated into the reclaimed landscape of the oil sands.

2.1.3.1 Tailings Dewatering and Management

Tailings dewatering methods currently in commercial operation include consolidated tailings (CT), non-segregating tailings (NST), centrifugation, thickened tailings (TT), and flocculated tailings (Hyndman et al., 2018). Composite tailings are a collective term to describes tailings management that produces fines-enriched sand tailings, which include CT and NST. Following deposition, composite tailings typically possess a solids content of 75-84 wt% and have the potential to be capped with water, floating coke, or sand (Hyndman et al., 2018). CT involves combining fine and coarse tailings with a coagulant, typically gypsum ($CaSO_4.H_2O_2$) (Hyndman et al., 2018). This process produces tailings with an average fines content of 20 wt% and approximately 60 wt% solids (BGC Engineering Inc., 2010). This non-segregating slurry is then discharged into a tailings pond, where it rapidly consolidates forming a deposit that can be capped and incorporated into reclamation landscapes (BGC Engineering Inc., 2010). Composite tailings may also be discharged to a beach and the liquid portion further removed (Hyndman et al., 2018).

NST is similar to CT, in that it uses coarse sand tailings and a coagulant, except instead of fine tailings, TT are used. Issues associated with composite tailings include the potential for material mobility if saturated, low fines density, and poor trafficability for reclamation equipment (Hyndman et al., 2018).

Centrifuge tailings combine the use of a centrifuge with coagulant and/or flocculant treatment. Tailings dosed with a coagulant (typically $CaSO_4.H_2O_2$) or flocculant (typically PAM), are fed into a centrifuge and separated into an outlet solid stream and liquid stream yielding water, bitumen, and residual fines (BGC Engineering Inc., 2010; Hyndman et al., 2018). The solid stream, typically referred to as "cake" possess a solids content of 45-60 wt% and can be deposited in deep in-pit deposits or shallow cells constructed of overburden or sand tailings (BGC Engineering Inc., 2010; Hyndman et al., 2018). The deposits can later be capped with water or floating coke (Hyndman et al., 2018). Issues associated with centrifuge tailings include material mobility if saturated, poor trafficability for reclamation equipment when initially deposited, difficulty with capping topography, and post-reclamation settlement (Hyndman et al., 2018).

TT are produced through the use of a mechanical thickener and flocculant additions, while flocculated tailings are produced through an in-line flocculation process. In-line flocculation typically uses PAM as a flocculant and injects the flocculant solution either in a pipe or a mixing system during fine tailings discharge (Hyndman et al., 2018). In-line thickening has been found to produce tailings that undergo rapid sedimentation from a solids content of 4 wt% to 30 wt% in days (BGC Engineering Inc., 2010). Overall, PAM treated tailings typically possess a solids content of 35-50 wt% and may be deposited in thin lifts or deep deposits, and can later be capped with water, floating coke, or sand (Hyndman et al., 2018). TT and in-line flocculation present similar issues as those described for centrifuge tailings (Hyndman et al., 2018). Overall, PAM is widely used in tailings dewatering, and can be found in NST, centrifuge tailings, TT, and flocculated tailings. The use of tailings treatments, such as PAM, allows for released water to be recycled back to the extraction facility.

2.1.3.2 End Pit Lakes

Oil sands EPLs are engineered bodies of water consisting of freshwater or a mixture of freshwater and OSPW, in decommissioned mining pits or tailings ponds, and may or may not contain oil sands by-product materials (Westcott and Watson, 2007; Canada's Oil Sands Innovation Alliance,

2021). Oil sands by-product materials that could potentially be stored in EPLs include fine tailings, coarse tailings, treated tailings, overburden, petroleum coke, and OSPW (BGC Engineering Inc., 2010). Of the 23 EPLs planned for northern Alberta, 10 of these currently are incorporated with untreated or treated tailings (Canada's Oil Sands Innovation Alliance, 2021). EPLs with tailings storage will typically have a tailings deposit 10-80 m thick, capped with 3-10 m of freshwater (Cossey et al., 2021). Due to particle resuspension in the water cap, capping untreated tailings may presents more challenges than capping treated tailings (Hyndman et al., 2018).

Syncrude Base Mine Lake (BML) was the first full scale EPL demonstration in the oil sands. Established in 2013, BML consists of approximately 186 Mm^3 of untreated fine tailings, with a fine tailings layer of 45 m and a water cap of 12 m (Dompierre et al., 2016; Canada's Oil Sands Innovation Alliance, 2021). When it comes to treated tailings, specifically tailings treated with PAM, Suncor is currently researching a commercial scale permanent aquatic storage structure (PASS). PASS technology involves the in-line treatment of tailings with a coagulant (alum) and flocculant (PAM). PASS will be implemented at Suncor's Upper Pit Lake, Lake Miwasin, and Fort Hills (Canada's Oil Sands Innovation Alliance, 2021, Suncor Energy Operating Inc., 2022). The overall goal of EPLs is to store oil sands by-product materials until they have been naturally attenuated, eventually becoming a self-sustaining ecosystem with suitable water quality and ecological and societal function (BGC Engineering Inc., 2010).

2.1.3.3 Wetlands

Wetlands are valuable features of the boreal forest, with peat-forming wetlands such as bogs and fens covering 43% of the pre-disturbed landscape (BGC Engineering Inc., 2010) Wetlands play a crucial role in carbon storage, groundwater recharge, and storm water run-off, and provide wildlife habitat for an abundance of fauna (Foote, 2012). Muskegs of the boreal forest hold significant value to Cree, Dene, and Métis communities in Fort McKay (Garibaldi, 2009) Based on these ecosystem services and values and their presence through the landscape prior to oil sands operations, wetlands have been identified as important features for meeting the regulatory requirements for oil sands reclamation (Alberta Environment, 2008). Due to their remedial properties, wetlands are a viable option for toxic organics such as hydrocarbon and naphthenic acids and reclaiming treated tailings and OSPW (Foght et al., 2017).

Operators are targeting terrestrial landscapes with a wetland for TT, NST, CT, and centrifuge tailings (Alberta Energy Regulator, 2022a). For tailings to be considered ready to reclaim, CT, centrifuge cake, and flocculant tailings (TT and NST) must achieve a solids content of 65, 50, and 50 wt%, respectively, within one year of deposit (Alberta Energy Regulator, 2022a). Once tailings are considered ready to reclaim, they are removed from the fluid tailings inventory (Alberta Energy Regulator, 2022b). Treated tailings have a higher strength than fluid tailings, and therefore can be capped using solid materials such as overburden, coarse sand tailings, or coke (Hyndman et al., 2018). Once deposited and capped, tailings may exhibit residual subsidence (OSTC and COSIA, 2012). Wetlands could potentially be designed to accommodate for this subsidence, however, settlement greater than 2 meters could result in the generation of a lake, altering the intended landscape (OSTC and COSIA, 2012; Hyndman et al., 2018).

Ongoing research for wetlands constructed on reclaimed tailings deposits include Suncor's Nikanotee Fen and Sandhill Fen. Nikanotee Fen is constructed of coarse sand tailings capped with fine grained soil (Sutton and Price, 2020), while Sandhill Fen consists of CT capped with sand (Biagi et al., 2019). Though these pilot wetland systems do not demonstrate the presence of PAM in wetland systems, meso-scale column studies have investigated plant survival and microbial community structure for TT capped with peat mineral mix and established with boreal and wetland plant species (Samad et al., 2023; Degenhardt et al., 2023). Wetlands will eventually be developed on landforms containing PAM treated tailings. .

2.1.3.4 Upland Forests

Tailings deposits suitable for terrestrial reclamation must undergo sufficient water removal so that residual subsidence in the closure landform is limited (OSTC and COSIA, 2012). Deposits must also be able to support surface access, vehicles, and reclamation activities (OSTC and COSIA, 2012). Dry stack tailings deposits, in which coarse and/or fine tailings form an unsaturated, dense, and stable tailings deposit, requires tailings with a solids content of 70 wt% (BGC Engineering Inc., 2010). This can be achieved with filtered tailings or treated tailings that achieve a suitable solids content (BGC Engineering Inc., 2010). TT can be deposited in thin-lifts, where tailings are spread on a shallow slope allowing for drainage and natural evaporation (Kasperski and Mikula, 2011; Hyndman et al., 2018). PAM could be potentially incorporated into terrestrial landscapes through the addition of TT, NST, or centrifuge tailings with sufficient solids content.

Oil sands tailings reclamation predominantly focus on settling and dewatering issues, leading to the incorporation of PAM throughout the reclaimed oil sands landscape. More recently, there has been greater focus on environmental performance and the biogeochemistry of tailings containing landforms. Microbial communities play a significant role in tailings systems, through biogeochemical cycling, tailings consolidation and bio densification, bioremediation of toxic organic compounds (hydrocarbons and naphthenic acids), and greenhouse gas emissions (Herman et al., 1994; Siddique et al., 2014a; 2014b; Small et al., 2015; Foght et al., 2017). Microbial activity is therefore expected to influence reclamation in oil sands landforms (Foght et al., 2017). The dependence on PAM for various tailings treatment methods suggests its abundance throughout the reclaimed landscape of the oil sands, and furthermore its exposure and potential interactions with indigenous oil sands tailings microbial communities.

2.2 Properties and Environmental Fate of Polyacrylamide

2.2.1 Polyacrylamide Overview

Currently, the majority of tailings dewatering technologies rely heavily on the use of polymer flocculants, specifically PAM (Vedoy and Soares, 2015). The ability to customize PAM to meet various specifications makes it ideal for industry applications (Vedoy and Soares, 2015). Initially designed for municipal wastewater, metal mining, and paper-making industries, PAM is also now used for soil conditioning (erosion control), enhanced oil recovery, biomedical applications, and oil sands tailings dewatering (Vedoy and Soares, 2015; Caulfield et al., 2002).

2.2.1.1 Polyacrylamide Chemistry and Adsorption Mechanisms

The non-ionic form of PAM, which consists of acrylamide monomers, can be modified using comonomers to produce anionic or cationic PAM (Vedoy and Soares, 2015). Along with varying charges, PAM can also have a range of charge densities, chemical functionalities, molecular weights (10^3 – 10^6 g/mol), and molecular weight distributions (MWD) (Lipp and Kozakiewicz, 2000). Anionic polymers with high charge densities exhibit less adsorption to negatively charged clay particles, therefore polymers with low or medium charge densities are typically relied upon (Bolto and Gregory, 2007). In the case of oil sands tailings, anionic PAM with a high molecular weight and medium charge density (20-30%) has been most successful with solids flocculation (Xu and Cymerman, 1999; Long et al., 2006; Vedoy and Soares, 2015). The most common form of anionic PAM used in oil sands tailings is hydrolyzed PAM (HPAM), where acrylamide and

acrylic acid make-up the polymer chain. This can be achieved through the partial hydrolysis of acrylamide monomers, or co-polymerization of acrylamide with acrylic acid (Moody, 2007).

Polymer flocculation mechanisms include adsorption, bridging, and charge neutralization (Bolto and Gregory, 2007). Polymer adsorption can take place through hydrogen bonding, ion binding/salt linkages, and electrostatic attraction (Bolto and Gregory, 2007; Moody, 2007). Regarding the interactions of HPAM with oil sands tailings, the amide groups of PAM can form hydrogen bonds with silica oxides and aluminol groups of kaolinite and illite, while ions such as Ca^{2+} and Mg^{2+} can act as bridges between anionic groups on the polymer and negatively charged sites on kaolinite, illite, and quartz (Figure 2-2) (Laird, 1997; Bolto and Gregory, 2007; Guezennec et al., 2015). HPAM may also interact with negatively charged mineral particles through electrostatic attraction to cationic patches (Vedoy and Soares, 2015).

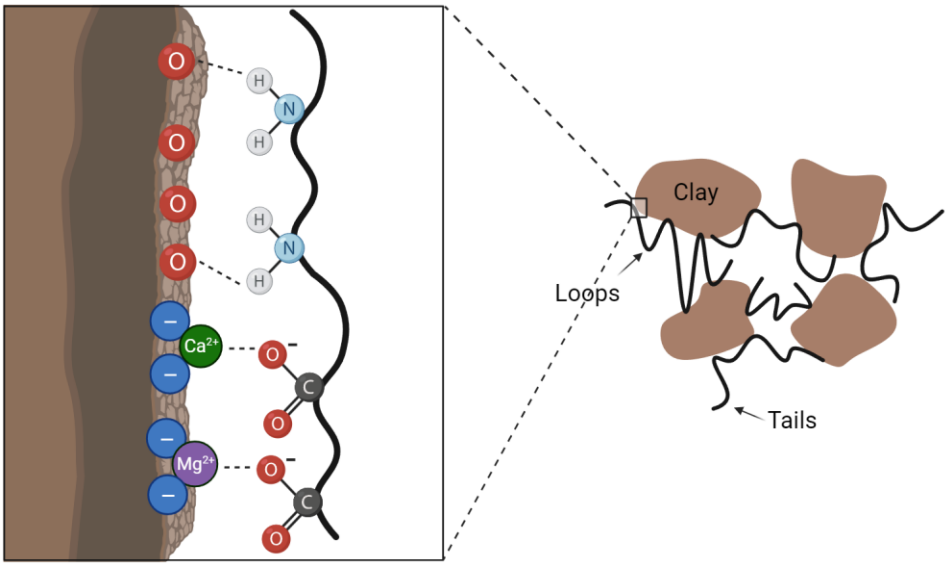


Figure 2-2 Bridging of clay particle and adsorption of hydrolyzed polyacrylamide to anionic clay particles through hydrogen bonding and salt linkages.

When polymers are added to a suspension full adsorption does not occur immediately, resulting in polymer "loops" and "tails" extending into the solution (Figure 2-2) (Bolto and Gregory, 2007). This allows for attachment to other particles, often referred to as "particle bridging". Flocs formed through particle bridging are considered much stronger than those formed through charge neutralization (Bolto and Gregory, 2007). Particles are typically negatively charged, as is with negatively charged clay particles in oil sands tailings. Therefore, it is unlikely that charged neutralization is occurring with the addition HPAM. Polymer adsorption and bridging through

hydrogen bonding, salt linkages, and electrostatic attraction are likely the dominant mechanisms for tailings flocculation using HPAM.

2.2.1.2 Environmental Fate

The adsorption of PAM to soil and clay particles is typically considered irreversible due to its strong adsorption properties and low mobility (Nadler et al., 1992; Lu and Wu, 2003b; Deng et al. 2006; Guezennec et al., 2015). Deng et al. (2006) found that less than 3% of adsorbed anionic PAM was removed from smectite, illite, and kaolinite after four consecutive washes. In soil systems, PAM was predominantly retained in the top 2 cm of the soil matrix with limited movement deeper than 20 cm (Lu and Wu, 2003b). Upon soil drying, Nadler et al. (1992) found that PAM became irreversibly bonded to the soil, suggesting limited mobility in terrestrial landscapes. In general, PAM is considered incapable of penetrating a soil system more than a few centimeters (Lu and Wu, 2003b; Guezennec et al., 2015).

These observations obtained from soil studies suggest that HPAM mobility in oil sands tailings systems is unlikely once adsorbed to the mineral component. However, the PAM monomer, acrylamide, as well as acrylic acid and lower molecular weight polymers have higher mobility in soil systems as indicated by their low adsorption ($\log K_{oc} < 1$) and high solubility in water ($\log K_{ow} < 10$; $H < 0.3$) (Table 2-1) (Sojka et al., 2007; Guezennec et al., 2015). The biodegradation of PAM could therefore lead to increased mobility in oil sands tailings systems, resulting in the potential transfer of acrylamide, PAM oligomer, and other degradation products in the surrounding environment. Polymer oligomers and other biodegradation products of PAM may possess different chemical functionalities from their parent polymer, potentially influencing their adsorption to tailings minerals and mobility in OSPW. The slightly alkaline nature of OSPW could also result in the deprotonation of PAM biodegradation products, further influencing mobility and interactions.

Table 2-1 Environmental constants for proposed hydrolyzed polyacrylamide degradation products (Yaws, 2003).

Compound	Adsorption ($\log K_{oc}$)	Solubility ($\log K_{ow}$)	Henrys Law (H)
Acrylamide	0.95	-0.67	1.35×10^{-8}
Acrylic Acid	1.56	0.35	4.73×10^{-4}

2.2.1.3 Treatment Concentrations

The concentration of HPAM depends on the application, with variability due to oil sands tailings operator, treatment method, and types of tailings. To obtain optimal flocculation and water release, flocculant studies have used 5 to 50 mg HPAM/L tailings slurry (Xu and Cymerman 1999; Cymerman et al., 1999; Li et al., 2005; Li et al., 2009; Wang et al., 2010). More recent oil sands tailings studies determined doses of 1000 to 2000 g HPAM/tonnes solids to be suitable (Li et al., 2021; Zhang et al., 2021). Concentrations used in biodegradation studies are provided in Table 2-2, with activated sludge and enhanced oil recovery studies using 500 and 1000 mg PAM or HPAM/L. For oil sands biodegradation studies, Collins et al. (2016) used 100 mg HPAM/kg, Li et al. (2023) used 1000 g HPAM/tonne solids, and Haveroen et al. (2005) used 3000 mg HPAM/L medium. As PAM concentration varies among studies and applications, doses are also not consistent among oil sands operators and tailings treatments. PAM doses can range from 70 to 600 g HPAM/tonne solids depending on the tailings deposit (Canadian Natural Resources Limited, 2021; Imperial Oil Resources Ltd., 2022). Other operators use doses of 900 to 1000 g HPAM/tonne dry fines (Jordan Hamilton, personal communication, May 2023).

PAM and other polymers used for industrial applications are susceptible to complete or partial degradation through biological, mechanical, photolytic, thermal, and chemical mechanisms (Caulfield et al. 2002). Once introduced to soil systems, PAM readily adsorbs to soil and clay minerals with limited desorption. Excess polymer in hydrolytic systems will remain in the pore water until encountering unoccupied mineral adsorption sites. Dosage requirements for effective PAM adsorption and flocculation, the potential degradation of PAM in soil and water systems, and the unknown environmental fate of PAM place importance on monitoring parent PAM polymers and their degradation products.

2.2.2 Analytical Techniques for Determining Polyacrylamide Biodegradation

This literature review will focus on biodegradation studies and the methods used to detect PAM and corresponding degradation products. PAM degradation products can vary depending on the mechanism (biological, mechanical, etc.), and since biodegradation was the focus of this study, the methods reviewed and selected for monitoring PAM were based on biological processes. Various analytical techniques have been established to determine the use of PAM as a nitrogen or carbon source, determine the degree of biodegradation, identify biodegradation products, and monitor

microbial response. Analytical techniques used to determine HPAM biodegradation and elucidate biodegradation products are provided in Figure 2-3 and 2-4.

2.2.2.1 Nitrogen Source

PAM or HPAM hydrolysis is one of the more well studied mechanisms for PAM biodegradation and has typically been determined using the starch-cadmium iodide colorimetric method (SCI). When PAM or HPAM are hydrolyzed, the amino group (NH_2) is converted to ammonia/ammonium, which can further be used as a nitrogen source. The SCI method measures the concentration of amino groups in acrylamide-based polymers, indicating hydrolysis with removal of NH_2. Since this method is based solely on the presence of amide, reported biodegradation percentages can appear misleading and are often higher than other measurements such as viscosity and total organic carbon (TOC) (Nyyssölä and Ahlgren, 2019; Sang et al., 2015; Berdugo-Clavijo et al., 2019). SCI assesses biodegradation of PAM and HPAM as a nitrogen source, but not cleavage of the carbon chain. It is therefore important to clearly state how PAM biodegradation was evaluated and be cautious when comparing the extent of biodegradation among studies.

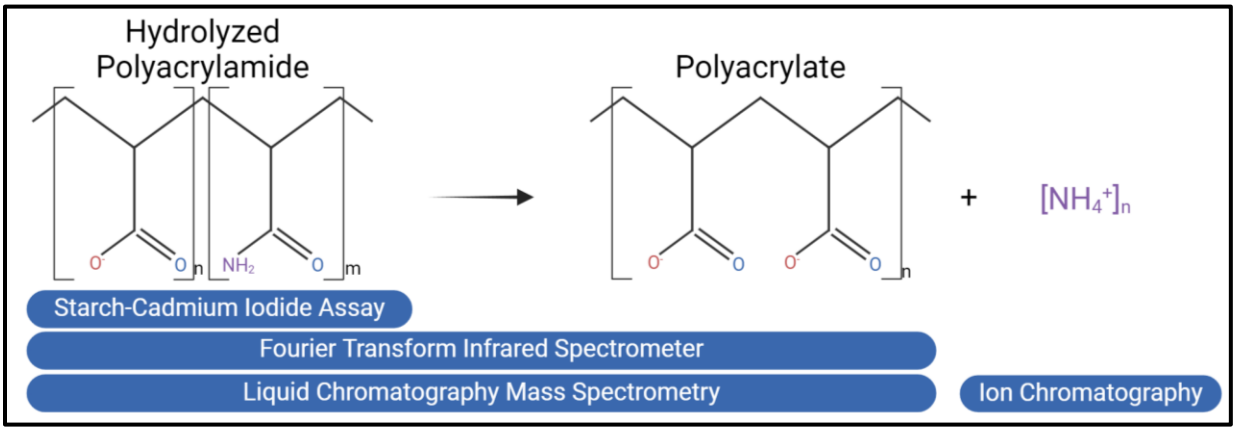


Figure 2-3 Analytical techniques for monitoring hydrolyzed polyacrylamide as a nitrogen source at each. The same techniques can be applied to non-ionic polyacrylamide biodegradation. The placement of each analytical technique corresponds with which stage in the biodegradation process it can measure.

The role of PAM as a nitrogen source and the conversion of the amide group to carboxylic has also been measured using liquid chromatography-mass spectrometry (LC-MS), Fourier Transform Infrared Spectroscopy (FTIR), and ion chromatography (IC). Mass spectrometry (MS) can be used

to characterize polymer microstructure, by analyzing a compound's molecular weight and chemical structure. MS can therefore be used to identify unknown degradation products or support information obtained from other methods. LC-MS has been able to detect acrylic acid oligomers following biodegradation, suggesting the use of PAM as a nitrogen source (Liu et al., 2012). FTIR is used for identifying functional groups and covalent bonds in the polymers before and after biodegradation. PAM and their biodegradation products can either be quantitatively or qualitatively measured, however, most biodegradation studies interpret FTIR results qualitatively. FTIR spectrums for parent PAM polymers will have peaks representing C=O, C-N, and N-H bonds. Following biodegradation, FTIR spectrums typically exhibit a decrease in peaks associated with C-N and N-H bonds, and an increase in peaks associated with O-H and C=O bonds, signifying conversion of the amide group to carboxylic acid (Bao et al., 2010; Dai et al., 2014; Sang et al., 2015; Yu et al., 2015; Hu et al., 2018; Ma et al., 2008; Li et al., 2023). Based on FTIR spectrums, polyacrylic acid has been proposed as a biodegradation product (Bao et al., 2010). IC has been used for measuring nitrogen species such as ammonium, nitrite, and nitrate (Hu et al., 2018; Li et al., 2023)

2.2.2.2 Carbon Source

PAM and HPAM biodegradation through their use as a carbon source is typically assessed by measuring changes in the polymers physical properties or detecting biodegradation products. Viscosity, TOC, and molecular weight can be used as indicators of carbon chain degradation to smaller polymer fragments. Size-Exclusion Chromatography (SEC) is used to measure the molecular weight, MWD, and concentration of polymers in a solution. Hydrolysis does not decrease the polymers molecular weight, therefore change in molecular weight or viscosity can be attributed to cleavage of the carbon chain (Caulfield et al., 2002).

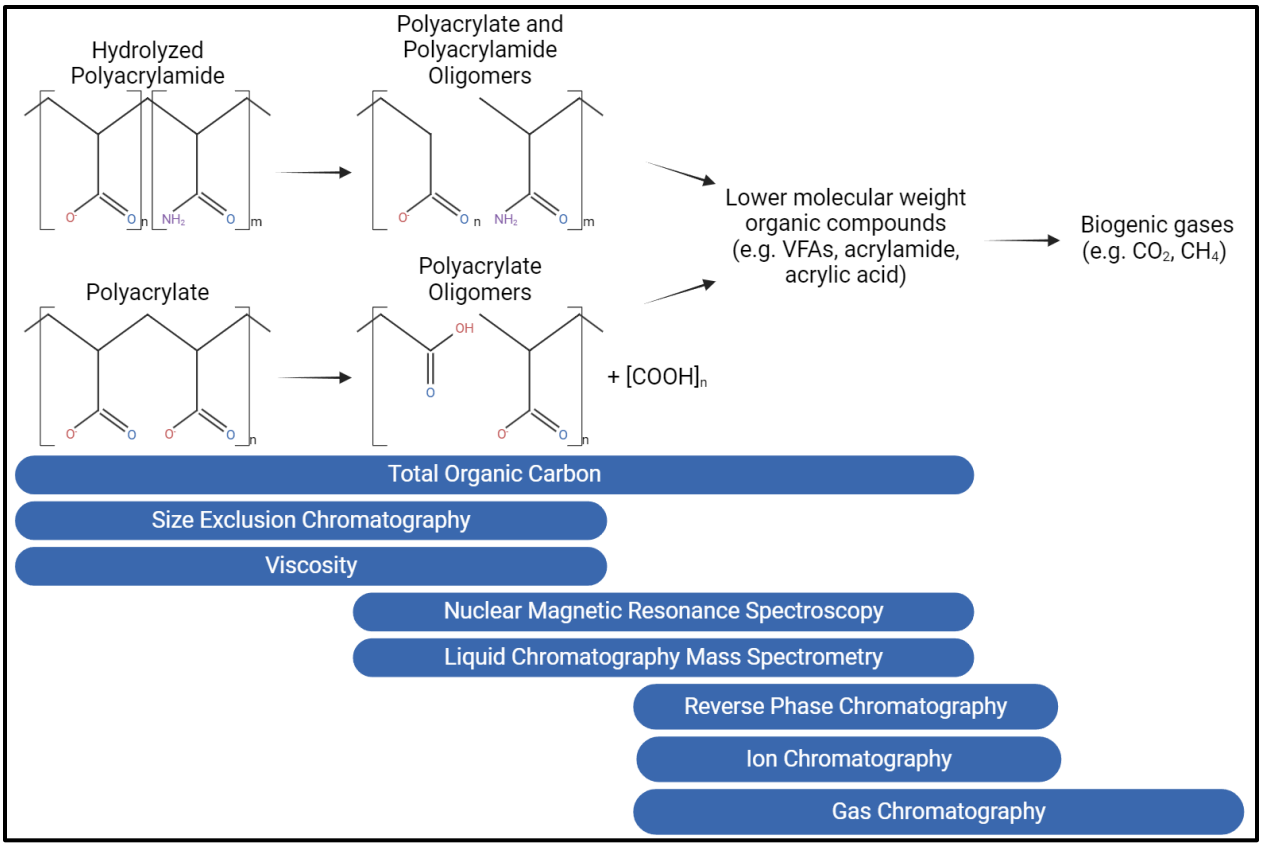


Figure 2-4 Analytical techniques for monitoring hydrolyzed polyacrylamide as a carbon source. The same techniques can be applied to non-ionic polyacrylamide biodegradation. The placement of each analytical technique corresponds with which stage in the biodegradation process it can measure.

MS and Nuclear Magnetic Resonance Spectroscopy (NMR) are techniques capable of identifying unknown organic compounds and characterizing polymer microstructure when PAM and HPAM are used as a carbon source. The identification of polyacrylic acid oligomers using LC-MS would also suggest the use of PAM as a carbon source (Liu et al., 2012). GC-MS has been used to detect and measure volatile fatty acids (VFAs) (Ma et al. 2008; Berdugo-Clavijo et al. 2019). Biodegradation along the carbon chain backbone, and the formation of acrylamide oligomers was determined using NMR (Nakamiya and Kinoshita 1995; Nakamiya et al., 1997).

When proposing biodegradation products, IC and reverse-phase chromatography (RP-HPLC) can be used to determine their formation. Using standards, IC has been used for measuring VFAs, while RP-HPLC is frequently used to detect and measure acrylamide (Bao et al. 2010; Dai et al.

2014; Sang et al. 2015; Li et al. 2015; Yu et al. 2015; Hu et al. 2018; Li et al. 2023). RP-HPLC can be used to detect the presence of new peaks following biodegradation, however, without standards identification is limited. IC and RP-HPLC can be used to identify other organics or ions present in a system, while SEC indicates changes to the parent polymer.

2.2.2.3 Indirect Methods

To assess the use of PAM as a nitrogen and/or carbon source, microbial biomass, biogenic gas production, and consumption of terminal electron acceptors and additional nutrients have been used as indicators. Optical density (OD) and most probable number (MPN) were used to determine microbial biomass (Grula et a., 1994; Nakamiya and Kinoshita, 1995; Matsuoka et al., 2002; Bao et al., 2010), while the formation of CO_2 and CH_4 were indicators of microbial activity and mineralization (Haveroen et al., 2005; Dai et al., 2014; Hu et al., 2018; Berdugo-Clavijo et al., 2019). Sulfate-reduction was observed when HPAM was provided as the sole nitrogen source, while nitrate-reduction was observed when HPAM was provided as the sole carbon source, indicating the potential presence of sulfate- and nitrate-reducing bacteria (Hu et al., 2018). When HPAM was provided as the sole nitrogen source, the consumption of acetate and benzoate as the carbon source were compared between HPAM amended and unamended controls (Haveroen et al., 2005). An increase in microbial activity in the presence of PAM compared to unamended and abiotic controls can indicate if microorganisms are potentially using PAM as a carbon or nitrogen source. This method does not elucidate the mechanisms in which PAM is used, however, it can guide further analysis and suggest how PAM supports microorganisms.

When it comes to the use of PAM as a nitrogen source, techniques such as SCI, FTIR, LC-MS, IC, microbial growth, and biogenic gas production are suitable. PAM as a carbon source can be measured using TOC, SEC, viscosity, NMR, LC-MS, RP-HPLC, IC, microbial growth, and biogenic gas production. A summary of analytical techniques used to determine PAM and HPAM biodegradation are provided in Table 2-2. Overall, the use of multiple techniques is necessary to determine the presence of PAM degradation, propose and elucidate degradation mechanisms, and make comparisons among previous studies.

Table 2-2 Summary of analytical techniques used to determine the biodegradation of polyacrylamide and hydrolyzed polyacrylamide.

Compound	Substrate/source	Polymer Concentration	Analytical Method	% degradation	Degradation Product(s)	Biodegradation pathway	Reference
HPAM 9-10 x 10^6 g/mol 25-35% hydrolyzed	Soil		Biomass (OD), GC, Viscosity	6.4-30.2% (Viscosity)		Nitrogen Source	Grula et al., 1994
PAM 2 x 10^6 g/mol	Activated Sludge and Soil	10,000 mg/L	TOC, Biomass (OD), SEC, H-NMR	15-20% (TOC) 75% (SEC)	Degradation of main carbon chain	Carbon and nitrogen source	Nakamiya and Kinoshita 1995
PAM 2 x 10^6 g/mol	Purified Enzyme Solution	1000 mg/L	SEC, H-NMR, FTIR	99% (SEC)	1-dodecene-2, 4, 6, 8, l0-pentacarboxyamide and l-hexadecane-2, 4, 6, 8, 10, 12, 14-heptacarboxyamide (Oligomer with 5 and 7 acrylamide units and a terminal methyl group and terminal double bond)	Carbon source	Nakamiya et al., 1997

Table 2-2 (Continued)

Compound	Substrate/source	Polymer Concentration	Analytical Method	% degradation	Degradation Product(s)	Biodegradation pathway	Reference
HPAM 1-2 x 10^7 g/mol 18% anionic	Soil	0.05% w/w	Plate count, enzyme activity, IC	Not reported	Ammonium	Nitrogen Source	Kay-Shoemake et al., 1998a
PAM 2.3 x 10^6 g/mol	Soil	1% PAM in media (units not specified)	Biomass (OD), TOC, SEC	16-19% (TOC) 78% (SEC)	Lower MW compounds	Carbon** and nitrogen source	Matsuoka et al., 2002
HPAM 29 % hydrolyzed	Oil Sands Tailings (TT and MFT)	3000 mg/L	GC	Not specified	CH4, ammonium*, polyacrylate*	Nitrogen source	Haveroen et al., 2005
HPAM	Oil Field System		FTIR, GC-MS, viscosity,	30.8% (Viscosity)	Carboxylic acid functional groups, HPAM fragments, epoxy	Carbon and nitrogen source	Ma et al., 2008
HPAM 23% hydrolyzed 2 x 10^7 g/mol	Polymer Flooded Produced Water	300 mg/L	SCI, Biomass (MPN), SEM, FTIR, RP-HPLC	13.6 - 36.3% (SCI)	Polyacrylic acid*, Smaller HPAM fragments, unidentified organics	Carbon or nitrogen source	Bao et al., 2010

Table 2-2 (Continued)

Compound	Substrate/source	Polymer Concentration	Analytical Method	% degradation	Degradation Product(s)	Biodegradation pathway	Reference
PAM 1.6 x 10^7 g/mol	Activated Sludge and Soil	50-1000 mg/L	SCI, viscosity, FTIR	74-76% (SCI)		Co-metabolism with glucose, carbon, and nitrogen source	Wen et al., 2010
PAM > 3 x 10^6 g/mol	Simulated polymeric flooding water treated with aerobic granules	0.67 mg/L	SCI, COD, SEC, RP-HPLC, LC-MS, SEM	50%	Ammonia, low MW compounds, polyacrylic acid oligomers	Carbon** and nitrogen source	Liu et al., 2012
HPAM	Dewatered sewage sludge	500 mg/L	SEC, RP-HPLC, SCI, FTIR	35.9% (SCI)	Lower MW HPAM fragments, ammonia, CH4, VFAs (acetic, propionic, butyric, and valeric), acrylamide	Carbon and nitrogen source	Dai et al., 2014
PAM	Waste Activate Sludge	200 mg COD/L	SCI, Enzyme assays	86.64% (SCI)	Polyacrylic acid*, acetyl-CoA*, pyruvic acid*, acetyl phosphate*, acetic acid*	Carbon, nitrogen, and co-metabolism through co-fermentation with sludge	Dai et al., 2015

Table 2-2 (Continued)

Compound	Substrate/source	Polymer Concentration	Analytical Method	% degradation	Degradation Product(s)	Biodegradation pathway	Reference
HPAM 32% hydrolyzed	Oil field Pipeline System	5240 mg/L	Viscosity, SCI, IC, GC	34.47% (viscosity) 43% (SCI) 26.35% (hydrolysis) 87.52% (MW)	CO2	Carbon and nitrogen source	Li et al., 2015
HPAM 10% hydrolyzed 2.2 x 10^7 g/mol	Polymer Flooded Produced Water	500 mg/L	SCI, TOC, viscosity, RP-HPLC, FTIR, SEC	89.8% (SCI) 32.9% (TOC) 72.6-78.6% (Viscosity) 97.06 – 99.68% (SEC)	Carboxylic functional group, smaller HPAM fragments	Co-metabolism with glucose and NH_4Cl	Sang et al., 2015
PAM 1.7-2.2 × 10^7 g/mol	Dewatered sludge	1000 mg/L	SEC, FTIR, RP-HPLC	45% (SEC) (Based on concentration)	Lower MW compounds, carboxylic acid functional groups	Carbon and nitrogen source	Yu et al., 2015
HPAM	Oil Sands Tailings	100 mg/kg (dry weight)	GC, ^{15}N	No degradation	None associated with PAM		Collins et al., 2016

Table 2-2 (Continued)

Compound	Substrate/source	Polymer Concentration	Analytical Method	% degradation	Degradation Product(s)	Biodegradation pathway	Reference
HPAM 1.5-3 x 10^6 g/mol 24.14% hydrolyzed	Enhanced Oil Recovery Wastewater	500 mg/L	SCI, TOC, SEM, FTIR, HPAM OD	79.4% (SCI) 80.39% (TOC)	Carboxylic acid functional groups, lower MW compounds	Carbon and nitrogen source	Liu et al., 2016
HPAM 2.2 x 10^7 g/mol 10% hydrolyzed	Activated Sludge	500 mg/L	SCI, FTIR, HPLC, TOC	54.69% (SCI) 70.14% (TOC)	Higher polarity HPAM fragment, ammonium, carboxylic acid functional groups, ether functional groups	Co-metabolism with glucose, carbon source, nitrogen source	Yan et al., 2016
HPAM 2.17 x 10^7 g/mol 24% hydrolyzed	Activated Sludge	150 mg/L	Viscosity, FTIR, COD, SEC	20.8-28.2% (Viscosity) 77.8% (SEC)	unidentified organics, lower MW compounds, ammonium	Carbon**	Song et al., 2017
HPAM 2.2 x 10^7 g/mol	Activated Sludge	500 mg/L	SEM, SEC, SCI, COD	64.36% (SCI)	Lower MW compounds, ammonia	Carbon** and nitrogen source	Song et al., 2018

Table 2-2 (Continued)

Compound	Substrate/source	Polymer Concentration	Analytical Method	% degradation	Degradation Product(s)	Biodegradation pathway	Reference
HPAM 3.0 x 10^6 g/mol 20% hydrolyzed	Polymer Flooded Produced Water	1000 mg/L	GC, IC, RP-HPLC, FTIR, SEC	Not specified	CO2, CH4, ammonium, Sulfite, sulfide, VFAs (propionic acid, formic acid, acetic acid), carboxylic acid groups, lower MW compounds	Co-metabolism with sucrose, carbon source, and nitrogen source	Hu et al., 2018
HPAM 2.2 x 10^7 g/mol 10% hydrolyzed	Enhanced Oil Recovery Wastewater	500 mg/L	SCI, FTIR, TOC, Nitrogen Species, SEM	55.93% (SCI)	Ammonium, carboxylic acid functional groups	Carbon source	Song et al., 2019
PAM and HPAM 25-30% hydrolyzed	Hydraulic Fracturing Produced Water and Activated Sludge	500 mg/L	GC, SCI, viscosity, RP-HPLC	PAM 13-20% (SCI) HPAM 22-34% (SCI) 18-21% (Viscosity)	CO_2	Nitrogen source	Berdugo-Clavijo et al., 2019

Table 2-2 (Continued)

Compound	Substrate/source	Polymer Concentration	Analytical Method	% degradation	Degradation Product(s)	Biodegradation pathway	Reference
HPAM	Activated Sludge	500 mg/L	FTIR, RP-HPLC, SEM, SCI, TOC, Nitrogen Species	40.5% (SCI) 38.9% (TOC)	Ammonia, HPAM Fragments, acetic acid	Carbon and nitrogen source	Zhao et al., 2019
PAM 5 x 10^6 g/mol	Activated Sludge	10-50 g/kg TSS	GC, RP-HPLC	76-78%	CH4, VFAs (acetic acid, n-butyric acid, iso-butyric acid), acrylamide	Carbon source	Akbar et al., 2020
PAM 3 x 10^6 g/mol	Soil	1.5 mg/g soil	SCI, water-extractable organic carbon	11-69.1% (SCI)	Ammonia, lower MW compounds	Carbon and nitrogen source	Ma et al., 2021
HPAM 2.12 x 10^6 g/mol Medium anion charge	Oil Sands Tailings	1000 mg/L	SEC (concentration), RP-HPLC, FTIR, TOC, COD, IC	41.0% (SEC concentration 6-91.46% (SEC MW) 24.3% (TOC) 32.8% (COD)	Lower MW compounds, ammonium, polyacrylic acid*	Carbon and nitrogen source	Li et al., 2023

*Proposed biodegradation products
**An alternative carbon source was provided initially to stimulate the system.

2.2.2.4 Size Exclusion Chromatography

Size Exclusion Chromatography (SEC) is a separation technique dependent on the size or hydrodynamic volume of polymers in solution, with larger molecules being eluted earlier than smaller molecules (Figure 2-5). The terms gel permeation chromatography and gel filtration chromatography are also used for separation techniques based on size, but for the purpose of this research SEC will be used. Initially developed for the separation of water-soluble polymers, SEC is commonly used for the analysis of polymers, lipids, proteins, humic substances, and other organic compounds (Trathnigg, 2000). Through column calibration, retention time can be correlated with molecular weight, providing information on a molecule's average molecular weight and MWD.

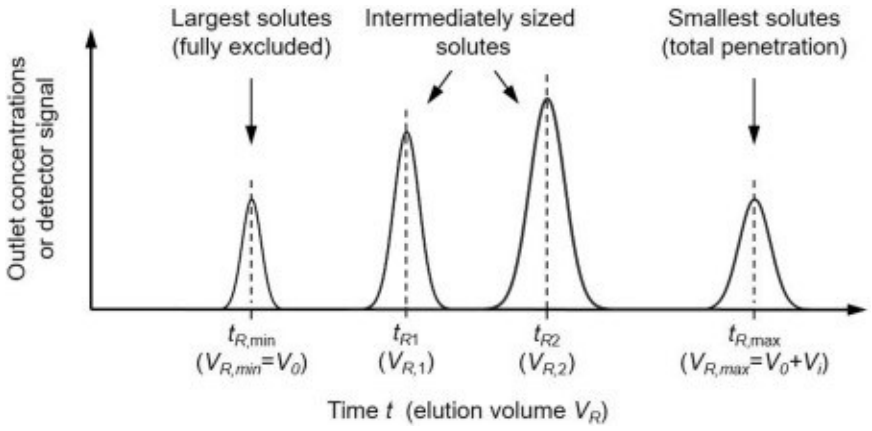


Figure 2-5 Schematic chromatogram illustrating the separation of differently sized monodisperse sample components (Peukert et al., 2022).

To achieve high performance, important components for SEC methods include the mobile phase, pump and flowrate, sample preparation, column selection, and detector. When measuring polymers, a mobile phase capable of dissolving the polymer must be selected. Buffer concentration, salt concentration, and pH are important parameters to consider during SEC method development. The buffer influences polymer dissolution and peak shape, as well as maintaining pH during analysis (Striegel, 2009). The presence of electrolytes (ex. Salt such as NaCl or $NaNO_3$) assists with electrostatic repulsion of the polymer chain. For anionic PAM there is potential for repulsion between negatively charged sites, and therefore salts can shield these electrostatic interactions and create a more compact polymer shape (Striegel, 2009). This may result in earlier elution time and improve resolution narrowing peaks. pH will impact polymer and stationary phase dissociation and influence ionic interactions with the stationary phase (Striegel, 2009). Mobile

phases must also be compatible for the column, suitable for the detector, and safe to handle, to create an acceptable method. Studies have used water (Dai et al., 2014; Hu et al., 2018), phosphate buffer (Liu e al., 2012; Song et al., 2018; Liu et al., 2023) and sodium salt (Nakamiya and Kinoshita, 1995; Yu et al., 2015) as the mobile phase.

Flowrate and pump quality will influence the elution time and peak resolution. Lower flowrates will increase resolution; however, this increases run times. Pump performance will also influence elution and resolution, therefore high-quality pumps are necessary for SEC (Moody, 2007). Flowrates for PAM analysis ranges from 0.5-1 mL/min, and were likely selected based on a balance between resolution and run times (Liu et al., 2012; Dai et al., 2014; Yu et al., 2015; Hu et al., 2018; Li et al., 2023).

One of the most important components for SEC method development is column selection. Originally designed using cross-linked, semi grid polystyrene gel packings, columns now typically consist of small rigid inorganic particles such as silica (Striegel, 2009). Parameters to consider during column selection include separation range, column particle size, particle pore size, column length and diameter. Smaller particles (5 μm) can provide better resolution, however, can lead to shear degradation when separating larger polymers (Trathnigg, 2000). Therefore, it is recommended to use larger particles sizes (10-20 μm) for higher molecular weight polymers (Trathnigg, 2000). Pore size will influence the molecular weight range in which separation can occur. Pore volumes will typically be more accessible to smaller molecules, resulting in later elution times. Smaller pores are therefore suitable for the separation of smaller compounds, while larger pore sizes are necessary for higher molecular weight compounds. Selecting a pore size or pore size distribution that allows for separation of the polymer of interest is important, however manufacturer information on pore size is typically not available (Striegel ,2009). Instead, the molecular weight separation range is provided. Column length will influence analysis time and resolution, while the column diameter will also influence analysis time, along with shear stress to polymers and flow rate (Striegel, 2009).

Using SEC, polymers of different sizes are separated and detected as a function of retention volume (V_R). Retention volume is calculated using the mobile phase flow rate (F) and retention time t_R (Equation 2-1). Though retention time can be used for calibrating polymer molecular weight, retention volume is most suitable since it minimizes the effect of flow rate change and can allow

for comparison with other studies (Striegel, 2009). As the polymer decreases in size, retention volume increases.

$$V_R = Ft_R \quad [2\text{-}1]$$

In order to obtain the molecular weight of a polymer, a calibration curve relating retention volume to molecular weight is necessary. Standards of known molecular weight and chemistry are typically used for SEC, where the peak apexes on a chromatogram are given molecular weights based on the peak average molecular weight (M_P) of the standards (Striegel, 2009). These values are provided by the manufacturer (Striegel, 2009). A calibration curve is then plotted with retention volume or time as the abscissa and log M_P as the ordinate. Polymers may appear as separate narrow peaks or broad peaks possessing a broader MWD. MWD is typically described by the polydispersity index. Polymers with broad molecular weight distributions possess higher polydispersity. It is not uncommon for calibration curves constructed from precise, linear, and narrow polydispersity standards to be used to calculate the molecular weight of polymers with various distributions and chemistries (Figure 2-6) (Striegel, 2009). In addition, polymers of similar molecular weight but different hydrodynamic volumes can exhibit different retention volumes (Striegel, 2009). Caution is therefore required when interpreting SEC results, especially when using standards with different chemistries than the analyte of interest. Stating the method conditions, column, detection method, and standards are important when it comes to reproducibility (Striegel, 2009).

The molecular weight of chromatograms with multiple peaks or peaks of narrow MWD can be calculated using Equation 2-2, where h_i is the peak height at the at the retention volume i and M_i is the molecular weight at the ith retention volume (Striegel, 2009).

$$M_W = \frac{\sum_{i=1}^{N}(h_i M_i)}{\sum_{i=1}^{N} h_i} \quad [2\text{-}2]$$

Standards with broad MWD, and therefore broad peaks, can be calibrated through linear or integral techniques. Integral calibration requires the complete MWD for a sample, while linear uses the average or peak molecular weight. Linear calibration is often more practical due it its convenience, versatility for analyzing various polymers, and the attainability of average molecular weight values for standards (Streigel, 2009).

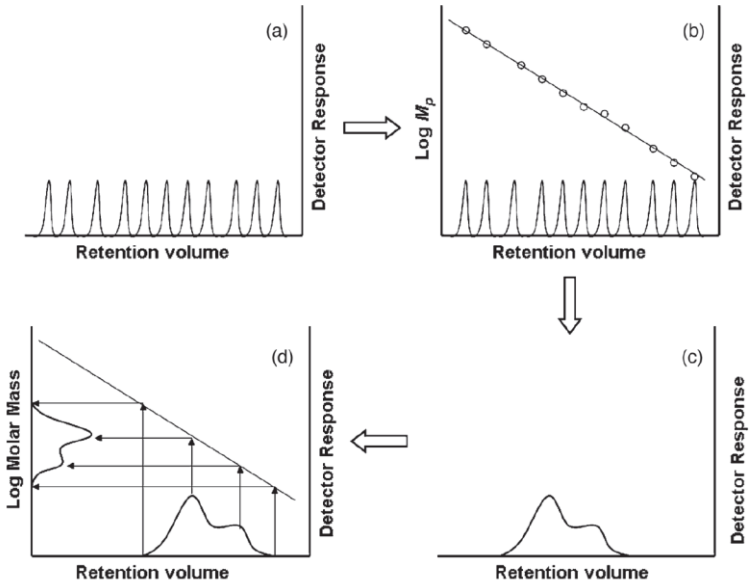


Figure 2-6 Constructing peak-position calibration curve to obtain MWD of unknown sample: (a) narrow polydispersity standards of known molecular weight are analyzed by SEC with a concentration-sensitive detector; (b) Peak apexes of chromatograms of standards are assigned molecular weight values consistent with the peak-average molecular weight, M_p. A calibration curve is constructed from the relation between retention volume and M_p of the standards. (c) Unknown sample is analyzed on the same system and experimental conditions as were the calibration standards. (d) Elution profile of the unknown is "reflected" off the calibration curve onto the molecular weight axis, to obtain the MWD of the unknown (Striegel, 2009)

Parent polymer molecular weight has been identified in various PAM biodegradation studies using SEC, along with the detection of lower molecular weight compounds following biodegradation (Table 2-3). SEC has also been used to monitor the removal efficiency based on pore water concentration (Liu et al., 2023). Due to the limited understanding of PAM biodegradation in oil sands tailings or reclamation landscapes, an analytical technique suitable for determining biodegradation is necessary. Though SEC does not provide qualitative information on the chemical composition, the ability to measure molecular weight and quantify molecular weight change can give insight to how PAM can change under various environmental conditions. The use of PAM as a nitrogen source has been demonstrated in many studies, however its use as a carbon source varies between systems. SEC is therefore a suitable method for determining its use as a carbon source. Changes in the molecular weight or chemistry of the parent polymer will be reflected through retention volume shifts, combined with monitoring of microbial activity and water chemistry, will provide a suitable method for determining PAM biodegradation in oil sands tailings.

Table 2-3 Size exclusion chromatography parameters for measuring polyacrylamide.

Compound	Column	Mobile Phase	Injection Volume and Temperature	Flow Rate	Sample Preparation	Detector	Detection Limit/Range	Reference
HPAM	Synchrompak GPC 100, 250 mm X 4.6 mm	0.1 M NaCl and 0.005 M $C_5H_{12}O_3S$	Ambient temperature	1 mL/min	Polymer dissolved in 0.1 M $NaClO_4$	190 nm	1 to 100 mg/L	Beazley, 1985
Anionic PAM 15-30% hydrolyzed		0.1 M KH_2PO_4	200 µL	2 mL/min		Diode Array Detector 200 and 205 nm	1 mg/L	Gharfeh and Maradi-Araghi, 1986
Nonionic, anionic, and cationic PAM	TSKgel G2000PW guard column TSKgel G5000PW column	0.05 M Na_2SO_4	250 µL	0.75 mL/min		UV-VIS Detector 208 nm	5 to 100 mg/L	Leung et al., 1987
PAM 2 x 10^6 Da	Toyoperal HW-65	0.2 M NaCl				210 nm		Nakamiya and Kinoshita, 1995
PAM 2 x 10^6	TSK gel G30OOSW	0.1 M KH_2PO_4 and 0.2 NaCl		1 mL/min		UV-VIS 210 nm		Nakamiya et al., 1997

Table 2-3 (Continued)

Compound	Column	Mobile Phase	Injection Volume and Temperature	Flow Rate	Sample Preparation	Detector	Detection Limit/Range	Reference
Anionic PAM 12-15 x 10^6 g/mol 21% hydrolyzed	TSK-Gel GMPWXL	0.05 M KH_2PO_4	25 °C 100 µL	1 mL/min	Centrifugation and dilution in 2 M KH_2PO_4 (3 parts sample, 1 part buffer)	195 nm	0.2 to 80 mg/L	Lu et al., 2003a
PAM 22 x 10^6 g/mol	TSKgel GMPWxl	0.1 M KH_2PO_4	50 µL	0.8 mL/min		UV-VIS detector 200 nm	0.1 to 75 mg/L	Xihua et al., 2007
PAM > 3 x 10^6 g/mol	G3000XLSW column	0.025 M NaH_2PO_4 + 0.025 M Na_2HPO_4 0.1 M NaCl		1 mL/min		210 nm		Liu et al., 2012
HPAM		Milli-Q water		0.5 mL/min				Dai et al., 2014
PAM 1.7-2.2 × 10^7 g/mol		0.1 M $NaNO_3$		0.5 mL/min	Filtered using 0.45 µm filter	RID-10A differential detector		Yu et al., 2015

Table 2-3 (Continued)

Compound	Column	Mobile Phase	Injection Volume and Temperature	Flow Rate	Sample Preparation	Detector	Detection Limit/Range	Reference
HPAM 2.17 x 10^7 g/mol 24% hydrolyzed	TSK gel GMPWxl	0.1 M CH_3COONa		0.7 mL/min				Song et al., 2017
HPAM 20% hydrolyzed 3.0 x 10^6 g/mol		Water		0.5 mL/min	100 mg of the dried sample dissolved in deionized water			Hu et al., 2018
HPAM medium anion charge 2.12 x 10^6 g/mol	TSK-Gel GMPWXL column TSK-Gel guard PWXL column	0.05 M KH_2PO_4	ambient temperature 100 µL	0.7 mL/min	Filtered using 0.45 µm filter	Diode Array Detector 205 nm		Li et al., 2023
HPAM 8-10 x 10^6 g/mol		Water	40 °C	0.5 mL/min	Filtered using 0.22 µm filter			Zhang et al., 2023

2.3 Biodegradation of Polyacrylamide

Although HPAM is the most common flocculant used for oil sands tailings dewatering, little is known about its interactions with tailings microbial communities and susceptibility to biodegradation in tailings systems. Studies have investigated the biodegradation of PAM and HPAM under oxic and anoxic conditions.

2.3.1 Biodegradation Under Oxic Conditions

2.3.1.1 Nitrogen Source

Under oxic conditions, PAM is thought to serve as a nitrogen source through the hydrolysis of the amide group. To support this mechanism, Kay-Shoemake et al. (1998b) observed that aerobic microorganisms produced amidase in the presence of PAM, which hydrolyses the amide group, producing polyacrylic acid and ammonium at low pH, and polyacrylate and ammonia at high pH under anoxic conditions. Though both intracellular and extracellular amidase was identified, the extracellular fraction was likely responsible for nitrogen utilization due to the limited ability of high molecular weight polymers such as PAM to be transported into the cell (Kay-Shoemake et al., 1998b). The use of PAM as a nitrogen source is well understood, with various studies measuring amidase activity, hydrolysis of the amide group using IC and FTIR, and production of ammonia/ammonium (Bao et al., 2010; Wen et al., 2010; Liu et al., 2012; Yu et al., 2015; Yan et al., 2016; Song et al., 2019; Ma et al., 2021; Li et al., 2023; Zhang et al., 2023). Studies have also observed higher cell biomass, biogenic gas production, and changes in polymer viscosity in cultures amended with PAM, compared to unamended controls (Berdugo-Clavijo et al., 2019). Following hydrolysis of the amide group, the produced ammonium can further be used as a nitrogen source to support microbial communities (Liu et al., 2012; Yan et al., 2016).

Other enzymes identified in PAM degrading systems capable of amide hydrolysis include aliphatic amidase and urease (Yu et al., 2015; Yan et al., 2016). Aliphatic amidase hydrolyzes short- or middle-chain aliphatic amides, converting them to organic acids (Yu et al., 2015). Many studies propose the production of polyacrylic acid or polyacrylate based on hydrolysis of the amide group and the detection of carboxylic acid using FTIR, however a study by Liu et al. (2012) is one of the few to identify polyacrylic acid with 7 acrylic acid monomers using LC-MS.

2.3.1.2 Carbon Source

Due to PAMs high molecular weight and a limited knowledge of enzymes capable of depolymerizing the polymer chain, it is thought that PAM cannot serve as a carbon source for aerobic microorganisms (Kay-shoemake et al. 1998a). However, studies have observed a decrease in the polymers molecular weight when subject to aerobic activity, suggesting cleavage of the carbon chain through oxidation (Bao et al., 2010; Wen et al., 2010; Yu et al., 2015; Li et al., 2023). A decrease in molecular weight, viscosity, TOC, or COD was typically used as an indicator for a decrease in PAM size, and therefore breakdown of the polymers carbon chain (Nakamiya and Kinoshita, 1995; Nakamiya et al. 1997; Matsuoka et al. 2002; Yu et al. 2015; Liu et al. 2016; Yan et al. 2016; Berdugo-Clavijo et al. 2019; Li et al. 2023;). While the enzymes and mechanisms responsible for PAM hydrolyzation have been widely investigated, the enzymes involved in cleavage of the carbon chain have only recently been reported. Nakamiya et al. (1997) observed the biodegradation of PAM in the presence of hydroquinone peroxidase extracted from *Azotobacter beijerinckii.* Hydroquinone peroxidase was believed to react with hydrogen peroxide to generate hydroxyl radicals, producing corresponding radicals capable of abstracting a hydrogen atom from the carbon chain (Nakamiya et al., 1997). Using H-NMR, biodegradation products were identified as 2 PAM oligomers, one with 5 acrylamide units and the other with 7, both with a terminal methyl group and terminal double bond (Table 2-2 for scientific name). Additional enzymes believed to be responsible for cleavage of the carbon chain and biotransformation of PAM in oxic conditions include monooxygenase, cytochrome, and dehydrogenase (Bao et al., 2010; Yan et al., 2016; Song et al., 2019).

The biodegradation of PAM may sometimes take place after initial stimulation of cultures with an alternative carbon source, or through co-metabolic processes from the continuous supply of additional carbon and nitrogen sources. Glucose (Liu et al., 2012) and yeast (Matsuoka et al., 2002) have been used to stimulate microbial communities, gradually decreasing carbon source concentrations until the microorganisms have acclimated to the PAM treated system. Wen et al. (2010) also observed improved PAM biodegradation with the addition of glucose at low concentrations. Additional carbon sources may also be provided through the substrate source, including residual organics or hydrocarbons found in oil produced water or oil sands tailings (Song et al., 2019). Therefore, the use of PAM as a carbon source, and the enzymes correlated with PAM biodegradation should be carefully considered based on the system (Berdugo-Clavijo et al., 2019).

Aside from the identification of the PAM oligomers by Nakamiya et al. (1997), descriptions of biodegradation products through the use of PAM as a carbon source are limited to lower molecular weight compounds identified through SEC, or unidentified organics observed using RP-HPLC (Table 2-2). Elucidating biodegradation products requires resources, various analytical techniques, and can be time consuming, therefore studies primarily focus on a metabolite of concern, acrylamide. The acrylamide monomer is a neurotoxin and carcinogen (United States Environmental Protection Agency, 2010), prompting concerns over its release into the environment following degradation. There is limited research confirming the biodegradation of PAM to acrylamide. Li et al. (2023) found that any residual acrylamide in the initial polymer solutions was quickly degraded by aerobic microorganisms. Acrylamide has also been used as a carbon source to support PAM degrading cultures in soil (Kay-Shoemake et al. 1998a).

Various aerobic biodegradation studies have been performed using activated sludge, produced water, and soil. A list of the microorganisms associated with PAM biodegradation can be found in Table 2-4. To date, only one study has investigated the interactions of aerobic tailings microbial communities with PAM. Li et al. (2023) isolated 6 HPAM degrading strains from oil sands tailings. These strains were identified as *Pseudomonas chloritidismutans, Bosea lathyri*, *Sphingopyxis witflariensis, Pseudomonas vancouverensis*, and *Pseudomonas knackmussii*. HPAM biodegradation was studied in tailings augmented with these isolates, and tailings with indigenous microorganisms. A greater shift in HPAM molecular weight and decrease in TOC and COD was observed for augmented tailings, suggesting that indigenous tailings microorganisms are not as efficient in HPAM biodegradation (Li et al. 2023). However, indigenous microorganisms were still capable of decreasing molecular weight, and tailings TOC and COD, suggesting the use of PAM as a carbon source (Li et al. 2023). In addition, ammonium production and FTIR analysis indicated hydrolysis of the amide group, suggesting the use of PAM as a nitrogen source (Li et al. 2023). Overall, oil sands tailings microorganisms were capable of aerobic PAM biodegradation, however the differences observed between augmented and indigenous tailings suggest a difference in degradation efficiency depending on the age of the tailings deposit. As microorganisms become acclimated to PAM treated tailings in tailings ponds, disposal areas, or reclamation landscapes treated with PAM, PAM biodegradation may increase. The augmented tailings may therefore represent biodegradation efficiency in older deposits or reclamation landscapes, while the indigenous tailings represent initial biodegradation efficiencies.

Table 2-4 Aerobic microorganisms and enzymes associated with PAM and HPAM biodegradation.

Microorganism or Culture	Enzymes	% Biodegradation	Experiment Duration	Reference
Enterobacter agglomerans *Azomonas macrocytogenes*		15-20% (TOC) 75% (SEC)	27 hours	Nakamiya and Kinoshita 1995
Azotobacter beijerinckii	Hydroquinone peroxidase	99% (SEC)	30 minutes	Nakamiya et al. 1997
Bacillus sphaericus *Acinetobacter* sp.		16-19% (TOC) 78% (SEC)	14 days	Matsuoka et al., 2002
filamentous and bacilli bacteria		50%	3 days	Liu et al., 2012
Bacillus cereus *Bacillus sp.*		13.6 - 36.3% (SCI)	7 days	Bao et al., 2010
Bacillus cereus *Bacillus flexu*		74-76% (SCI)	5 days	Wen et al. 2010
Acinetobacter *Sphingobacterium* *Flavobacterium* Betaproteobacteria Gammaproteobacteria Alphaproteobacteria Lentisphaerae		34.47% (viscosity) 43% (SCI) 26.35% (hydrolysis) 87.52% (MW)		Li et al., 2015
Pseudomonas putida	Aliphatic amidase	45% (SEC) (Based on concentration)	7 days	Yu et al. 2015
Pseudomonas aeruginosa UCBPP-PA14		79.4% (SCI) 80.39% (TOC)	7 days	Liu et al., 2016
Pseudomonadota Bacteroidetes Planctomycete Chloroflexi *Chlorobi* sp.	Urease Dehydrogenase	54.69% (SCI) 70.14% (TOC)	4 days	Yan et al. 2016

Table 2-4 (Continued)

Microorganism or Culture	Enzymes	% Biodegradation		Experiment Duration	Reference
Phylum Bacteroidetes Pseudomonadota Planctomycetes Chloroflexi Genus *Methylibium* sp. *Flavobacterium* sp. *Planctomyces* sp. *Flavobacterium* sp. *Luteolibacter* sp. *Caldilinea* sp.	Amidase	20.8-28.2% (Viscosity) 77.8% (SEC)		470 days	Song et al. 2017
Family *Xanthomonadaceae* *Rhodanobacteraceae* *Hydrogenophilaceae* *Paenibacillaceae* *Streptosporangiaceae* *Micromonosporaceae* *Burkholderiaceae* *Beijerinckiaceae* *Blastocatellaceae* *Anaerolineaceae* *Bacillaceae* Genus *Pseudoxanthomonas* sp. *Chelatococcus* sp. *Bellilinea* sp. *Anoxybacillus* sp.		*PAM* 13-20% (SCI)	*HPAM* 22-34% (SCI) 18-21% (Viscosity)	43 days	Berdugo-Clavijo et al., 2019
Bacillus megaterium	Urease Cytochrome	55.93% (SCI)		7 days	Song et al., 2019

Table 2-4 (Continued)

Microorganism or Culture	Enzymes	% Biodegradation	Experiment Duration	Reference
Augmented species *Klebsiella sp.* Indigenous Culture Firmicutes Actinomycetota Chloroflexi Alphaproteobacteria Deltaproteobacteria *Rhizobiaceae* *Roseiflexaceae* *Clostridiaceae* *Gemmatimonadaceae* *Nocardioides* sp. *Bacillus* sp.	Amidase	69.1% (SCI) 11% with indigenous microorganisms (SCI)	30 days	Ma et al., 2021
Pseudomonas chloritidismutans strain AW-1 *Bosea lathyri* strain R-46060 *Sphingopyxis witflariensis* strain W-50 *Pseudomonas vancouverensis* strain DhA-51 *Pseudomonas knackmussii* strain B13		41.0% (SEC concentration 6-91.46% (SEC MW) 91.46% with augmented tailings 24.3% (TOC) 32.8% (COD)	77 days	Li et al., 2023
Tistrella bauzanensis *Brucella* sp.	Amidase	63.59-75.3% (SCI) 94% (SEC)	1-1.5 days	Zhang et al., 2023

2.3.2 Biodegradation Under Anoxic Conditions

2.3.2.1 Nitrogen Source

Under anoxic conditions, the presence of PAM has been found to stimulate methanogens and sulfate-reducing bacteria (Grula et al., 1994; Ma et al., 2008; Haveroen et al., 2005; Hu et al., 2018). Iron-reducing bacteria have also been identified in anaerobic cultures from HPAM amended produced water (Hu et al., 2018). Haveroen et al. (2005) found that methanogenic activity was higher in nitrogen deficient cultures in the presence of HPAM compared to cultures without

HPAM. As has been observed in oxic studies, it is thought that anaerobic microorganisms can hydrolyze the amide group through amidase (Grula et al., 1994; Hu et al., 2018). With HPAM provided as a nitrogen source and glucose, xylose, and acetate provided as alternative carbon sources. Grula et al. (1994) observed activity in sulfate-reducing activity in *Desulfovibrio* cultures, suggesting that PAM can be used as a nitrogen source. They also observed higher microbial activity in cultures amended with 25-35% hydrolyzed PAM in comparison to 1-4% hydrolyzed PAM, suggesting that the degree of hydrolyzation may increase microbial activity (Grula et al., 1994). Hu et al. (2018) observed a decrease in sulfate, and an increase in sulfide and sulfite for anaerobic cultures, suggesting the presence of sulfate-reducing bacteria. Microbial analysis did not reveal sulfate-reducing bacteria, possibly due to low concentrations (Hu et al., 2018). Additional studies have also observed an increase in biogenic gas production (CH_4 and CO_2) when HPAM was provided as a nitrogen source, in comparison to unamended controls (Haveroen et al., 2005; Hu et al., 2018).

Similar to aerobic microorganisms, anaerobic microorganisms are capable of hydrolyzing the amide group and producing polyacrylic acid/polyacrylate and ammonium/ammonia. Amidase and urease have been identified in anoxic conditions and associated with the use of PAM as a nitrogen source (Dai et al., 2015; Song et a., 2017). An increase in VFAs such as formic acid, acetic acid, and propionic acid has also been observed when HPAM was provided as the sole nitrogen source, and sucrose as the carbon source (Hu et al., 2018). Production of VFAs suggests that the presence of PAM as a sole nitrogen source promotes the conversion and production of organic carbon species.

2.3.2.2 Carbon Source

PAM may also serve as a carbon source for anaerobic microorganisms, with studies reporting a change in polymer molecular weight, biogenic gas production, and water chemistry that aligns with cleavage of the carbon chain (Dai et al., 2014; Dai et al., 2015; Song et al., 2017; Hu et al., 2018). It is proposed that once PAM has been hydrolyzed to polyacrylic acid through amidase, further biodegradation occurs due to enzymes such as alcohol dehydrogenase, laccase, acetate, kinase, butyrate kinase, and CoA transferase (Dai et al., 2015; Song et al. 2018; Zhao et al., 2019). However, the enzymes involved in anaerobic carbon chain cleavage of PAM (or PAA) have not been thoroughly investigated. The presence of such enzymes may also be due to the presence of

alternative carbon or nitrogen sources initially added to stimulate microorganisms, or nutrient inputs from the substrate source. Song et al. (2018) relied on the addition of glucose and urea to accelerate the formation of granular sludge and biofilms. The biodegradation of HPAM was still observed following complete reduction of glucose, however biodegradation should not be completely attributed to PAM serving as the sole carbon source. Activated sludge can also be a source for carbohydrates and proteins, supporting PAM biodegradation through co-fermentation processes (Dai et al., 2015).

The formation of these enzymes due to the presence of carbon sources does not disregard the enzyme's role in PAM biodegradation. It only suggests that cleavage of the carbon chain is more likely through co-metabolic processes. Due to the presence of PAM and HPAM in surface water and groundwater systems, studies for municipal wastewater and enhanced oil recovery application are interested in the transformation of PAM and HPAM into innocuous substances (Bao et al., 2010; Sang et al., 2015). This would suggest why studies place importance on the identification of the acrylamide monomer during biodegradation, as opposed to complete elucidation of the biodegradation products. Sang et al. (2015) was able to utilize glucose and NH_4Cl to support microbial growth and improve HPAM biodegradation without the formation of acrylamide.

Although acrylamide may be the primary concern, studies have still been able to identify certain biodegradation products when PAM was provided as a carbon source. An increase in biogenic gas production (CO_2 and CH_4) and the formation of VFAs such as formic acid, acetic acid, propionic acid, and butyric acid has been observed (Hu et al., 2018; Zhao et al., 2019). TOC and SEC measurements have also indicated a decrease in the parent polymer molecular weight, suggesting the formation of PAM oligomers and other polymer fragments (Grula et al., 1994; Dai et al., 2014; Sang et al., 2015; Yan et al., 2016; Song et al., 2017; Zhao et al., 2019).

Currently only two studies have investigated the use of HPAM as a nutrient source in oil sands tailings under anoxic conditions. Haveroen et al. (2005) and Collins et al. (2016) found contradicting evidence as to whether PAM could be used as a nitrogen source to support methanogenic activity. As previously stated, Haveroen et al. (2005) found that HPAM stimulated methanogenesis under nitrogen deficient conditions in comparison to cultures without HPAM. Benzoate and acetate provided as a carbon source were not consumed in the nitrogen deficient unamended controls, confirming that microbial activity was dependent on the presence of a

nitrogen source, at least within 80 days (Haveroen et al. 2005). In contrast to the findings from Haveroen et al. (2005), Collins et al. (2016) found that methanogenesis was not a result of HPAM degradation, but instead nitrogen fixation. Methane production was similar in citrate and HPAM amended cultures and cultures without HPAM (Collins et al. 2016). Additionally, cultures amended only with HPAM yielded similar CH_4 production as controls, suggested HPAM could not be used as a carbon source (Collins et al. 2016). The discrepancy between the findings by Haveroen et al. (2005) and Collins et al. (2016) was attributed to (I) different microorganisms as each study used different tailings, (II) different carbon sources, as Haveroen et al. (2005) used benzoate and acetate and Collins et al. (2016) used citrate, (III) different HPAM concentrations (Table 2-2), and (IV) that Haveroen et al. (2005) removed endogenous nitrogen through serial dilutions (Collins et al. 2016). Though Collins et al. (2016) did not determine that HPAM could serve as a nitrogen source, they did observe an increase in *Clostridium* sp. for HPAM amended cultures, suggesting that HPAM played a role in their growth. Methanosarcinales was observed in HPAM amended cultures, however this archaeal order was also observed in cultures not amended with HPAM (Collins et al., 2016). Additional anaerobic microorganisms associated with PAM and HPAM biodegradation in other applications can be found in Table 2-5.

Table 2-5 Anaerobic microorganisms and enzymes associated with PAM and HPAM biodegradation.

Microorganism or Culture	Enzymes	% Biodegradation	Experiment Duration	Reference
Desulfovibrio sp.		6.4-30.2% (viscosity)		Grula et al., 1994
		Not specified	80 days	Haveroen et al., 2005
PAM degrading bacteria isolated from activated sludge		35.9% (SCI)	26 days	Dai et al. 2014
<u>Bacteria</u> Bacteroidetes Firmicutes Pseudomonadota Spirochaetes <u>Archaea</u> *Methanosaeta* sp.	Amidase Alcohol dehydrogenase oxidase Acetate kinase Butyrate kinase Phosphotransacetylase Phosphotransbutyrylase Oxaloacetate transcarboxylase CoA transferase	86.64% (SCI)	17 days	Dai et al., 2015
<u>Bacteria</u> Betaproteobacteria Epsilonproteobacteria Gammaproteobacteria Alphaproteobacteria Deltaproteobacteria Spirochaetes Lentisphaerae Flavobacteria <u>Archaea</u> Methanomicrobiales Methanosarcinales Methanobacteriales Thermoplasmatales		34.47% (viscosity) 43% (SCI) 26.35% (hydrolysis) 87.52% (MW)		Li et al., 2015
Bacillus cereus strain FM-4 EU794727 *Rhodococcus* sp. strain EF028124.1		89.8% (SCI) 32.9% (TOC) 72.6-78.6% (viscosity) 97.06 – 99.68% (SEC)	20 days	Sang et al., 2015

Table 2-5 (Continued)

Microorganism or Culture	Enzymes	% Biodegradation	Experiment Duration	Reference
Bacteria *Clostridium* sp.		No degradation	112 days	Collins et al., 2016
Archaea Methanosarcinales				
Phylum Chloroflexi Bacteroidetes	Amidase	20.8-28.2% (viscosity) 77.8% (SEC)	470 days (HRT 2 days)	Song et al., 2017
Genus *Bacteroides* sp. *Anaerolinea* sp. *Longilinea* sp. *Syntrophobacter* sp. *Paludibacter* sp.				
Bacteria Pseudomonadota Planctomycetes Firmicutes Deferribacteres		Not specified	328 days	Hu et al., 2018
Archaea *Methanofollis* sp.				
Bacteria *Trichococcus* sp. *Brooklawnia* sp. *Bacillus* sp. *Pseudomonas* sp.	Laccase Dehydrogenase	64.36% (SCI)	360 days	Song et al., 2018
Archaea *Methanosaeta* sp.				
Pseudomonadota Bacteroidetes Chloroflexi	Phosphatase Urease Dehydrogenase	40.5% (SCI) 38.9% (TOC)	49 days	Zhao et al., 2019
Bacteroidetes Firmicutes Pseudomonadota Actinobacteria Chloroflexi Thermotogae		76-78%	15 days	Akbar et al., 2020

In summary, there is limited research on the biodegradation of PAM and its use as a nutrient source to support oil sands tailings microorganisms. Li et al. (2023) was able to compare HPAM biodegradation between augmented and indigenous tailings, however the microorganisms in the indigenous tailings were not identified. For anoxic conditions, Haveroen et al. and Collins et al. only focused on methanogenesis. Currently there are no studies investigating interactions between PAM and oil sands tailings sulfate-reducing, nitrate-reducing, and iron-reducing bacteria. Though Haveroen et al. (2005) was able to identify PAM as a nitrogen source, neither the polymers properties nor biodegradation products were investigated. Due to the variability in tailings minerology, chemistry, sulfide content (use of gypsum in CT), diluent, microbial communities, and reclamation plans, it is unlikely that PAM biodegradation will be consistent among different studies and operators. Long-term tailings deposits are also heterogenous in nature, with redox conditions transitioning from oxic to anoxic with increasing tailings depth (Cossey et al., 2021). Therefore, it is possible for different PAM biodegradation rates within a single tailings deposit. Understanding the mechanisms behind PAM biodegradation and its role as a nutrient source for aerobic and anaerobic microorganisms can provide insight into its environmental fate, as well as what is to be expected for oil sands reclamation landscapes.

3 SIZE-EXCLUSION CHROMATOGRAPHY FOR MONITORING POLYACRYLAMIDE BIODEGRADATION

3.1 Introduction

Oil sands tailings dewatering technologies rely heavily on the use of polymer flocculants, specifically PAM based polymers (Vedoy and Soares, 2015). The increasing presence of anionic PAM, typically hydrolyzed PAM (HPAM), in treated tailings has prompted the need for quantifying HPAM in oil sands systems, specifically tailings pore water. An analytical method for measuring HPAM in oil sands tailings should have a detection limit suitable for operator doses, be able to tolerate interferences from other analytes, demonstrate precision and accuracy, and have suitable requirements based on lab or user limitations. Interferences from oil sands tailings could arise from the high clay content and presence of organics and dissolved salts. Dissolved organic matter, surfactants, and organic compounds consisting of amide or carboxylic groups have been found to interfere with analytical techniques that measure the chemistry of PAM, including the starch-cadmium iodide method (SCI) and Fourier transform infrared (FTIR) (Xihua et al., 2007; Lu and Wu, 2001). SCI is a common technique used for determining PAM biodegradation, which measures the amino (NH_2) groups of PAM (Lu and Wu, 2001; Berdugo-Clavijo et al., 2019). This method can determine the removal of NH_2 in PAM and therefore polymer hydrolyzation but does not determine cleavage of the carbon chain backbone. Since this method is based solely on the presence of amides, reported biodegradation percentages can appear misleading and are often higher than other measurements such as viscosity and total organic carbon.

Since naphthenic acids found in oil sands tailings consist of carboxylic groups, there is potential for these compounds to interfere when measuring the functional groups of HPAM using FTIR. Amide and carboxyl groups can be found in dissolved organic matter, or other organic materials, suggesting interference in oil sands tailings from compounds other than naphthenic acids (Lu et al., 2003). Dissolved salts can interfere with techniques based on the physical properties of PAM such as flocculation, viscosity, and turbidity measurements (Lu and Wu, 2003a). Viscosity and turbidity techniques may also lack sensitivity for measuring PAM concentrations less than 10 mg/L (Lu and Wu, 2001). Analytical techniques must therefore be capable of separating PAM from other organic compounds and be immune to dissolved salts.

Size Exclusion Chromatography (SEC) presents a promising analytical technique for separating HPAM from interferences in oil sands tailings and measuring its concentration and molecular weight in tailings pore water. SEC has proved effective in measuring PAM and HPAM concentrations in pure polymer solutions (Li et al., 2023), activated sludge (Song et al., 2017), enhanced oil recovery (Xihua et al., 2007; Hu et al., 2018; Gharfeh and Mradi-Araghi, 1986; Beazley, 1985), oil sands tailings (Li et al., 2023), coal process water (Leung et al., 1987), and soil supernatant and leachate (Lu et al., 2003). Lu et al. (2003) was able to measure anionic PAM in soil water containing dissolved organic matter obtaining a detection limit of 0.2 mg/L. Anionic PAM with an average molecular weight of 12-15 x 10^6 g/mol was successfully separated from dissolved organic matter and salts in various soil samples (Lu et al., 2003). Li et al. (2023) was able to measure the concentration of anionic PAM using SEC in oil sands tailings pore water when tailings were dosed at 1000 g PAM/tonne solids, although a detection limit was not specified. SEC has also been used to compare the initial and final molecular weight of PAM and HPAM (Liu et al., 2012; Song et al., 2017; Hu et al., 2018; Li et al., 2023). We therefore hypothesize that this method will be capable of elucidating HPAM biodegradation in oil sands tailings.

The adsorption of PAM to soil and clay particles is typically considered irreversible due to its strong adsorption properties and low mobility (Nadler et al., 1992; Lu and Wu, 2003b; Deng et al. 2006; Guezennec et al., 2015). We therefore also hypothesize that the high clay content of oil sands tailings may influence the detection limit and create interferences when measuring HPAM in the pore water. The results from this study will elucidate the suitability of SEC for measuring HPAM concentration and molecular weight in an oil sands context, and the ability of SEC to monitor shifts in HPAM molecular weight during biodegradation studies.

3.2 Materials and Methods

3.2.1 Chemicals and Standards

Ultrapure water from Millipore Milli-Q was used for sample and mobile phase preparation. Reagents used for the mobile phase were obtained from Fischer Scientific. Mobile phases used for SEC method development are provided in Table 3-1, with the selected mobile phase described in subsection 3.2.2.

SNF FLOPAM A3332 and FLOPAM A3338 were used as standards for quantifying HPAM concentration (mg/L) in the liquid phase (SNF Canada Ltd.). A3332 is a linear anionic PAM while

A3338 is a branched anionic PAM possessing a crosslinking agent. Both are 30% anionic with a molecular weight range of 8-10 million Daltons (Da).

Standards for determining HPAM molecular weight were obtained from American Polymer Standards Corporation (Ohio, USA). Non-ionic PAM standards were used for molecular weight calibration: PAAM9000K (9450 10^3 g/mol), PAAM7000K (7300 10^3 g/mol), PAAM6000K (5352 10^3 g/mol), PAAM1000K (1145 10^3 g/mol), PAAM500K (540 10^3 g/mol), PAAM350K (382 10^3 g/mol), PAAM80K (81 10^3 g/mol), PAAM60K (59 10^3 g/mol), PAAM20K (23 10^3 g/mol), and PAAM9K (9 10^3 g/mol).

3.2.2 Instrument and Chromatography Conditions

The HPLC system was equipped with an Agilent 1260 Infinity Quaternary Pump, Agilent 1260 Infinity Standard Autosampler, Agilent 1260 Infinity Thermostatted Column Compartment, and Agilent 1260 Infinity Diode Array Detector. The separation was performed using a PL aquagel-OH MIXED-H column (300 x 7.5 mm, 8um) along with a PL aquagel-OH guard column (50 x 7.5 mm, 8um) from Agilent. The column was eluted isocratically using 0.01 M NaH_2PO4 and 0.1 M NaCl at a pH of 7. At 20 °C and a flowrate of 0.750 mL/min, 20 uL of sample was injected and analyzed at 210 nm. Run times were 20 minutes, however samples passed through the column in less than 14 minutes. The detection limit for A3332 and A3338 was 10 mg/L.

3.2.3 Sample Preparation

Oil sands tailings samples containing HPAM were first centrifuged at 5000 rpm for 20 minutes. The supernatant was then filtered using a 0.45 µm nylon filter to remove any remaining suspended solids. Samples were transferred to a 2 mL autosampler vial and diluted using milli-Q water to obtain a desired concentration. The total volume in the autosampler vial was 1 mL. Samples were prepared a week prior to analysis to allow for complete dissolution.

3.2.4 Method Validation

3.2.4.1 Polyacrylamide Concentration Calibration Curves

The calibration curve for PAM pore water concentration was based upon A3332 and A3338. Concentrations were prepared at 10, 50, 100, 200, 300, and 400 mg/L. By the time of this experiment, A3332 and A3338 were used simultaneously in oil sands tailings treatment, with A3332 used for the thickener and A3338 for secondary injection (Imperial Oil Resource Limited,

2020). Therefore, standards were prepared with both polymers (1:1 ratio), and individually for each polymer. Linearity was determined by plotting concentration (mg/L) versus retention time (min).

3.2.4.2 Polyacrylamide Molecular Weight Calibration Curves

The molecular weight calibration curve was based on a series of non-ionic PAM standards (9450, 7300, 5352, 1145, 540, 382, 81, 59, 23, and 9 10^3 g/mol). Each standard was prepared at 100, 200, and 300 mg/L. Standards of 300 mg/L were injected in triplicate during the same day, as well as during different days. Linearity was determined by plotting the logarithm of molecular weight versus retention time (min).

3.2.4.3 Precision and Accuracy

The precision of the SEC method was assessed for concentration and molecular weight. Triplicate injections for A3332 and A3338 at 50, 100, 200, and 400 mg/L were used to determine standard deviation and percent relative standard deviation (RSD) for retention time and peak area (mAU). The molecular weight of A3332 and A3338 was also calculated and assessed for precision. A3332 and A3338 standards were injected in triplicate during the same day to determine reproducibility within a single sequence.

molecular weight standards were assessed for precision by comparing standards injected on the same day. molecular weight standards were also injected on different days to determine reproducibility among different sequences. Standard solutions of 300 mg/L were injected in triplicate and assessed for mean retention time, standard deviation, and RSD. Comparisons for retention time, standard deviation, and RSD were made between samples injected in the same day and samples injected on different days.

Determining the accuracy of the SEC method was difficult, as only a molecular weight range was provided for A3332 and A3338, and no commercial solutions were available. The molecular weight of A3332 and A3338 were calculated using the molecular weight calibration curve and compared to the molecular weight range provided by SNF Canada Ltd.

3.2.5 Adsorption of Hydrolyzed Polyacrylamide in Oil Sands Tailings

To understand the adsorption of hydrolyzed polyacrylamide in oil sands tailings, HPAM was added to 30 mL of fine tailings (n = 10) and milli-Q water (n = 2). The fine tailings possessed a solids

content of 26.5% and bulk density of 1.21 g/mL. Samples were amended to 1 g HPAM/L tailings (equal parts A3332 and A3338). Following HPAM addition, samples were left for 2 hours with 150 rpm gyratory shaking. To minimize mechanical and biological degradation, samples were then placed in a cold room for two weeks. Samples were inverted daily to ensure complete adsorption.

Pore water samples were centrifuged at 5000 rpm for 15 minutes and filtered using 0.45 µm nylon filters prior to analysis. HPAM concentration was measured using SEC (subsection 3.2.2). Using a mass balance of HPAM, the adsorption capacity (g HPAM/ tonne solids) in fine tailings was calculated (Equations 3-1 to 3-4).

$$\frac{1\ g\ HPAM}{L\ tailings} \times \frac{mL\ tailings}{1.21\ g\ tailings} \times \frac{1\ L}{1000\ mL} \times \frac{g\ tailings}{0.265\ g\ solids} \times 10^6 = \frac{3119\ g\ HPAM}{tonne\ solids} \quad [3\text{-}1]$$

$$\frac{447.07\ g\ HPAM}{g^6\ liquid} \times 26.8\ g\ liquid = 0.0120\ g\ HPAM_{(aq)} \quad [3\text{-}2]$$

$$0.0301\ g\ HPAM_{(total)} - 0.0120\ g\ HPAM_{(aq)} = 0.0181\ g\ HPAM_{(s)} \quad [3\text{-}3]$$

$$\frac{0.0181\ g\ HPAM}{9.66\ g\ solids} \times 10^6 = \frac{1874\ g\ HPAM}{tonne\ solids} \quad [3\text{-}4]$$

3.3 Results and Discussion

3.3.1 Size-Exclusion Chromatography Method Development

3.3.1.1 Stationary phase and column dependant components

In SEC, the stationary phase primarily determines polymer retention and resolution (Striegel, 2009). The stationary phase was selected based on the size of the parent polymers A3332 and A3338. The HPAM compounds have a proposed molecular weight ranging from 8-10 million Da, therefore the PL aquagel-OH MIXED-H column and PL aquagel-OH guard column with a molecular weight range of 6000 to 10 million g/mol was selected. A wide molecular weight range will allow for detecting molecular weight shifts if a parent polymer degrades into smaller polymer fragments. The molecular weight range is dictated by the pore size of the column packing material. Smaller pores are therefore suitable for the separation of smaller compounds, while larger pore

sizes are necessary for higher molecular weight compounds. Selecting a pore size or pore size distribution that allows for separation of the polymer of interest is important, however manufacturer information on pore size is typically not available (Striegel, 2009). Pore size was not provided for the PL aquagel-OH columns, only that they consisted of mixed pore sizes. A wide pore size distribution will ensure good separation of PAM compounds and smaller organic substances (Lu et al., 2003).

Columns have specific requirements and limits for pH range, mobile phase chemistry, flow rate, temperature, and injection volume (Striegel, 2009). The column can therefore influence further method optimization. A flow rate of 0.750 ml/min was selected to balance resolution with parent polymer elution time and total run time. Ambient temperature (20 °C) was selected to prevent any thermal degradation of HPAM. Recommended injection volumes for the SEC column was 20-50 uL. 20 uL was selected as the injection volume. Increasing the injection volume to 30 uL did not improve resolution or limit of detection (LOD), instead it led to peak fronting likely due to overloading the column (Wang et al., 2016). Peak fronting from a higher injection volume is presented in Appendix A, Figure A-1.

3.3.1.2 Mobile phase optimization

Mobile phase optimization is important for sample solubility and to minimize interferences from the sample or stationary phase (Striegel, 2009; Wang et al., 2016). The mobile phase must also be compatible with the stationary phase (Podzimek, 2011). Mobile phase ionic strength, pH, and concentration can potentially influence sample interactions with the stationary phase and mitigate non-size-exclusion effects (Striegel, 2009). Non-size-exclusion effects that can interfere in SEC analysis include ion-exchange effects, adsorption effects, and electrostatic repulsion (Striegel, 2009). An example of ion-exchange effects is the repulsion of anionic polyelectrolytes by anionic groups on the column packing material (Striegel, 2009). Adsorption effects can arise from hydrophobic interactions, hydrogen bonding, or ion exchange between the analyte and stationary phase. Electrostatic repulsion arises from the fixed charges on the polyelectrolyte such as anionic PAM, resulting in the expansion of the polymer chain and increase in hydrodynamic volume. Mobile phase ionic strength and pH can minimize ion-exchange and adsorption effects (Striegel, 2009; Lu et al., 2003). pH can influence ionic interaction between polymer and stationary phase through dissociation. For mobile phase concentration, too low of a concentration can result in poor

separation of PAM and other high molecular weight organics (Lu et al., 2003). High concentrations can increase background absorbance (Lu et al., 2003).

The mobile phase parameters optimized for the mobile phase are provided in Table 3-1. A phosphate buffer was selected for its ability to dissolve HPAM and maintain pH, which would ensure detection consistency if sample pH were to change. The slightly alkaline nature of OSPW (Allen, 2008), along with the presence of microbial activity or tailings treatment presents potential for pH change. Therefore, the use of a buffer would allow for comparisons among different water samples regardless of the sample pH. A sodium phosphate monobasic (NaH_2PO_4) buffer at 0.01, 0.02, and 0.03 M was tested with A3332 and A3338. Mobile phase success was assessed qualitatively based on peak resolution, shape, and reproducibility. Peak tailing was the primary issue initially encountered during mobile phase method development. Increasing NaH_2PO_4 concentration did not improve peak shape, therefore 0.01 M was selected as the buffer concentration. To limit the risk of salt precipitation and build-up in the liquid chromatography systems, the lowest phosphate buffer concentration that achieved clear peak resolution was selected.

The addition of an electrolyte increases mobile phase ionic strength, which can assist in controlling electrostatic repulsion of polyelectrolytes and influence sample and stationary phase interactions (Striegel, 2009). Peak tailing observed during the buffer tests was likely a result of electrostatic repulsion from the anionic PAM species, therefore the addition of an electrolyte was evaluated. NaCl was selected as the electrolyte, however, $NaNO_3$ is another common electrolyte used in HPLC methods (Gómez-Ordóñez et al., 2012). $NaNO_3$ is an irritant and can cause damage to organs if exposed to for prolonged periods of time without proper safety equipment (Global Safety Management Inc., 2014). To limit exposure risks, NaCl was selected. 0.01 M of NaH_2PO_4 and NaCl at 0.05, 0.1, and 0.2 M were compared. Peak tailing was improved with the addition of 0.1 M NaCl (Appendix A, Figure A-2). Peak resolution was then compared among pH 6, 7, 8, and 9. A pH outside of 2 and 8 can potentially degrade the silica packing within an SEC column (Striegel, 2009), however the column used in this study has a pH range of 2-10. Peak quality was not influenced by the different pH values, therefore a pH of 7 was selected for the mobile phase. Through mobile phase optimization, a mobile phase with 0.01 M NaH_2PO_4, 0.1 M NaCl, and a pH of 7 was selected and further used for method validation.

Table 3-1 Mobile phase optimization for polyacrylamide detection using Size Exclusion Chromatography. The bolded row indicates the mobile phase that had the best peak resolution.

Buffer Concentration (NaH_2PO_4, mol/L)	Electrolyte Concentration (NaCl, mol/L)	pH
0.01	0	7
0.02	0	7
0.03	0	7
0.01	0.05	7
0.01	**0.1**	**7**
0.01	0.2	7
0.01	0.1	6
0.01	0.1	8
0.01	0.1	9

3.3.1.3 Sample Preparation

Method development for analytical techniques is not only dependent on the analytical equipment, but also the sample preparation steps leading up to analysis. Due to the complexity of the sample matrix, sample preparation was an important parameter for analyzing HPAM in oil sands tailings. Before measuring HPAM in tailings, HPAM was first added to milli-Q water to observe peak shape. This allowed for peak assessment without any interactions from OSPW or the presence of particulates. Centrifugation removes insoluble material within tailings samples, while filtration is a common practice for water samples (Wang et al., 2016). The HPAM samples dissolved in milli-Q water were treated in the same manner as tailings samples, with centrifugation and filtering. It was determined that centrifuging and filtering with a 0.45 μm nylon filter did not impact the structure or concentration of PAM (Appendix A, Figure A-3). Peak quality did not differ between milli-Q water and OSPW dosed with PAM (Appendix, Figure A-4).

Samples for SEC analysis are commonly dissolved in the mobile phase, however exceptions can be made so long as the polymer can be dissolved in the alternative solvent (Podzimek, 2011). HPAM is water-soluble and therefore capable of dissolving in OSPW and milli-Q water (Vedoy and Soares, 2015). However, dissolving the tailings sample in the mobile phase could decrease the concentration below the detection limit. Considering these limitations, samples were dissolved in the mobile phase and milli-Q water and compared for resolution and retention. No significant differences were observed, therefore milli-Q water was used for standards and samples for biodegradation studies conducted in this thesis (Chapter 4). Dissolving a polymer often requires

several days, with dissolution rates decreasing with increasing polymer molecular weights (Podzimek, 2011). When dissolving HPAM granules, we found that complete dissolution required at least a week, with concentrations above 500 mg/L requiring almost 2 weeks. Insufficient dissolution time may lead to insoluble polymer parts within the solution (Appendix A, Figure A-7). If diluting samples, complete dissolution was typically achieved after a couple days, however samples were prepared a week prior to analysis to allow for sufficient dissolution. The chromatogram for the finalized method is provided in Appendix A, Figure A-5.

3.3.2 Size-Exclusion Chromatography Method Validation

3.3.2.1 Calibration Curve Linearity

The concentration linearity was calibrated using standards created from A3332 and A3338. A3332 and A3338 are typically used simultaneously during tailings treatment, therefore standards with combined polymers (1:1) were also calibrated. Each calibration curve presented satisfactory correlation between concentration (x, mg/L) and peak area (y, mAU), with $R^2 > 0.99$ (Figure 3-1).

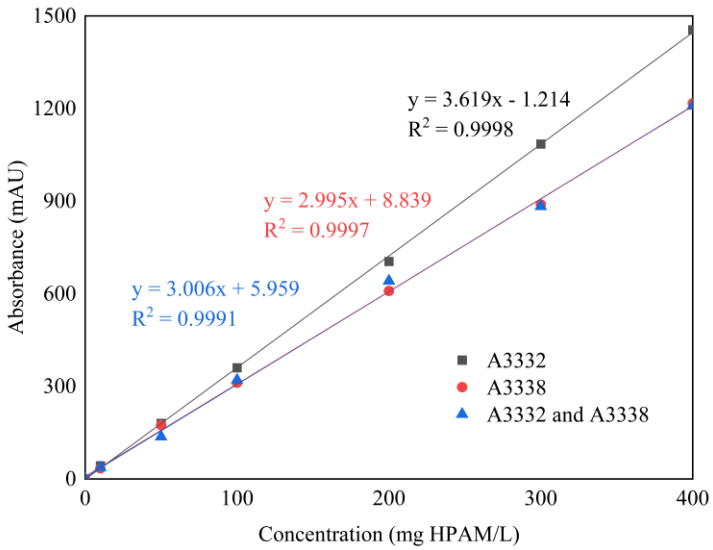


Figure 3-1 Concentration calibration curves for A3332, A3338, and combined polymer standards using a PL aquagel-OH MIXED-H column (300 x 7.5 mm, 8um) and PL aquagel-OH guard column (50 x 7.5 mm, 8um), 0.01 M NaH_2PO_4 and 0.1 M NaCl mobile phase, 0.750 mL/min flowrate, 20 uL injection volume, and ultra-violet detection at 210 nm.

The limit of detection for A3332 and A3338 standards was 10 mg/L. Similarities in slope were observed for combined polymer and A3338 standards. The calibration curve varied slightly for

A3332 (Figure 3-1). To compare calibration curves the concentration of an unknown HPAM sample was calculated. The sample possessed a peak area of 1059.8 mAU. Using the calibration curves, the concentration of the unknown sample was 292.5, 350.9, and 350.6 mg/L for A3332, A3338, and combined polymer standards, respectively. These results indicated that a lower concentration of A3332 would yield the same absorbance of A3338. Although there were slight differences in the calculated concentration for A3332, compared to A3338 and combined polymer standards (RSD of 8.29%), standards with combined polymers appeared suitable for quantifying HPAM in tailings samples.

The linearity for molecular weight was calibrated using non-ionic PAM standards (Figure 3-2). Table 3-2 presents the retention time and linearity for standards ranging from 9 to 9450 10^3 g/mol. Standards were plotted as retention time (x) vs molecular weight on a log scale (y). The PAM calibration curve presented a satisfactory correlation between retention time and logMW (R^2 = 0.9505), however, it was lower than the R^2 for concentration. This is possibly due to the broad molecular weight distribution (MWD) of certain standards. Polymers with higher polydispersity indexes will possess broader MWD, which could result in inconsistencies when detecting retention time in the chromatogram, thus influencing linearity. Polydispersity indexes for the PAM standards can be found in Appendix X, Table A-1.

Table 3-2 Retention time, linearity, and calibration curve components for non-ionic polyacrylamide molecular weight standards

Standards	Molecular Weight (10^3 g/mol)	LogMW	Retention Time (min) (n=3)
PAAM9000K	9450	6.98	9.35 ± 0.16
PAAM7000K	7300	6.86	9.77 ± 0.07
PAAM6000K	5352	6.73	10.02 ± 0.08
PAAM1000K	1145	6.06	10.10 ± 0.03
PAAM500K	560	5.73	10.63 ± 0.10
PAAM350K	382	5.58	11.20 ± 0.09
PAAM80K	81	4.91	11.84 ± 0.13
PAAM60K	59	4.77	11.93 ± 0.07
PAAM20K	23	4.35	12.80 ± 0.01
PAAM9K	9	3.95	13.46 ± 0.56

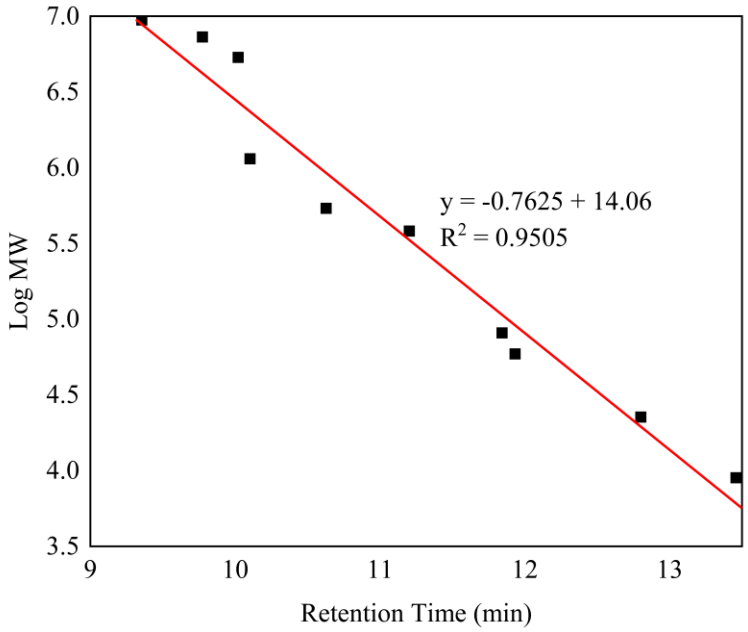


Figure 3-2 molecular weight calibration curves obtained for non-ionic PAM standards using a PL aquagel-OH MIXED-H column (300 x 7.5 mm, 8um) and PL aquagel-OH guard column (50 x 7.5 mm, 8um), 0.01 M NaH_2PO4 and 0.1 M NaCl mobile phase, 0.750 mL/min flowrate, 20 uL injection volume, and ultra-violet detection at 210 nm.

3.3.2.2 Precision and Accuracy

Precision represents the variability and reproducibility of a method. Precision for the SEC method was evaluated based on the reproducibility of retention time, peak area, and molecular weight. Retention time and peak area reproducibility were expressed as mean time ± one standard deviation. Anionic (A3332 and A3338) and non-ionic (PAAM9-9000k) PAM standards were used to calculate the precision of the SEC method. The RSD of retention time reproducibility ranged from 2.1-3.8% for A3332 and 1.0-2.9 % for A3338 (Table 3-3). In coal process water, RSD for anionic PAM was 0.38-1.03% for samples injected on the same day, and 0.87% for samples injected on separate days (Leung et al., 1987).

For peak area, RSD ranged from 2.4-6.2% for A3332 and 1.4-6.2% for A3338. Background noise has the potential to impact peak area when interpreting chromatograms, potentially leading to higher RSD for peak area. RSD for PAM and HPAM concentrations were 0.79-1.96% in soil water (Lu et al., 2003), 0.59-1.4 and 9.2% in oil field process water (Xihua et al., 2007; Gharfeh and Moradi-Araghi, 1986), and 5.8-7.73% in coal process water (Leung et al., 1987). The RSD for

peak area is within the range of concentration RSD from previous studies, suggesting that our method is suitable for determining HPAM concentration in oil sand tailings.

Table 3-3 Retention time, peak area, and molecular weight reproducibility for A3332 and A3338.

Standards	Concentration (mg/L)	Retention Time (n=3)		Peak Area (n=3)		Molecular Weight (10^3 g/mol) (n=12)	
		Mean (min)	RSD (%)	Mean (mAU)	RSD (%)	Mean (10^3 g/mol)	RSD (%)
A3332	50	10.06 ± 0.35	3.4	180.31 ± 5.41	3.0	9707 ± 1457	15.0
	100	10.13 ± 0.30	2.9	360.33 ± 10.13	2.8		
	200	9.94 ± 0.21	2.1	704.66 ± 43.86	6.2		
	400	9.90 ± 0.38	3.8	1454.89 ± 34.63	2.4		
A3338	50	10.02 ± 0.10	1.0	174.68 ± 2.45	1.4	10524 ± 1432	13.6
	100	10.05 ± 0.12	1.1	311.97 ± 19.30	6.2		
	200	9.86 ± 0.13	1.3	609.23 ± 27.36	4.5		
	400	9.87 ± 0.29	2.9	1217.01 ± 30.77	2.5		

Calibration curve used for molecular weight was y = -0.7077x + 14.06

The RSD for non-ionic PAM retention times ranged from 0.2-3.4%, indicating good reproducibility when analyzed on the same day (Table 3-4). Standards injected on the different days yielded a higher RSD of 11.2-13.6%. Gomez-Ordonez et al. (2012) measured retention time RSD of seaweed polysaccharides. Standards injected on the same day had RSD <0.7%, while RSD for standards injected on different days were 0.6-2.6%. Our results suggest that retention time precision is acceptable for samples analyzed on the same day, but reproducibility decreased for the SEC method for different days.

Throughout the duration of the method development and biodegradation experiment, HPLC pump pressure was variable, with high pressure sometimes leading to a shift in retention time by ~1 minute in the middle of a sequence. Samples were rerun if the pressure and standard retention times shifted within a sequence. The retention time of A3332 and A3338 standards also shifted from ~6.5 to ~9.9 minutes over the course of 2 years. Pump performance and column quality can influence retention and resolution (Moody, 2007). Standards were injected for each day to obtain

precise measurements. To stabilize the pump or identify issues with high pressure, the HPLC was flushed with warm water followed by the mobile phase prior to injections.

Though the retention time shift was consistent among samples and standards run at the same time, this does inhibit retention time comparisons among results for different days. Gradual shifts in retention time are common and are typically due to the column or HPLC system. Sudden increase or changes in the pump pressure and chromatograms can happen on occasion and are usually a sign that either the column or HPLC system needs maintenance. After ~40 sequences the HPLC would typically exhibit changes in retention times and an increase in pump pressure. Maintenance can include minor actions such as flushing the system with an organic solvent, or more involved actions such as disassembling the pump to clean and replace parts. Pump maintenance was an important factor when it came to reproducibility and peak quality.

Table 3-4 Retention time reproducibility within the same day, and reproducibility among different days for non-ionic polyacrylamide molecular weight standards.

Standards	Same Day (n=3)		Different Day (n=3)	
	Retention Time (min)	RSD (%)	Retention Time (min)	RSD (%)
PAAM9000K	9.24 ± 0.13	1.4	9.39 ± 1.05	11.2
PAAM7000K	9.99 ± 0.16	1.6	10.08 ± 1.21	12.0
PAAM1000K	10.20 ± 0.12	1.2	10.61 ± 1.36	12.8
PAAM350K	11.06 ± 0.06	0.5	11.96 ± 1.35	11.3
PAAM80K	11.87 ± 0.02	0.2	12.71 ± 1.54	12.1
PAAM20K	12.79 ± 0.02	0.2	12.73 ± 1.73	13.6
PAAM9K	13.35 ± 0.46	3.4	14.78 ± 1.80	12.2

Accuracy describes how close the experimental result is to the true value. The molecular weight range for A3332 and A3338 provided by the manufacturer is 8-10 million g/mol. The calculated molecular weight was 9707 10^3 g/mol for A3332 and 10524 10^3 g/mol for A3338. Though these values were within the range provided by the manufacturer, the average molecular weight was not provided and therefore molecular weight accuracy for the SEC method could not be determined. If we were to assume the average molecular weight is 9 million Da, then the relative error would be 7.86% for A3332 and 17.0% for A3338. In previous studies, molecular weight error was ≤ 9.8% in seaweed polysaccharides (Gomez-Ordonez et al., 2012), and ranged from 0.76-16% in various proteins (Folta-Stogniew and William, 1999). For PAM and HPAM concentrations, previous

studies have obtained a relative error of ≤ 9.2% in oil field process water (Xihua et al., 2007) and ≤ 2.5% in coal process water (Leung et al., 1987). Though the accuracy was not investigated for HPAM concentration in our study, relative error for molecular weight aligns with other SEC studies. This suggests the SEC method is suitable for measuring HPAM biodegradation in oil sands tailings studies.

3.3.3 Application for Oil Sands Tailings

When developing an analytical technique for a specific application, some important considerations include the application concentration and system, sample volume and preparation requirements, ease of use and required training, and detection limit. By evaluating these parameters, the use of SEC for detecting HPAM in oil sands tailings and determining biodegradation can be evaluated.

3.3.3.1 Concentration and Tailings System

Polyacrylamide doses will vary depending on the application, and within oil sands applications there is variability among industry, lab studies, and types of tailings. In-line flocculation pilot studies at Imperial's Kearl Mine found that polymer dosage was optimized at around 400 to 1200 g/tonne depending on the sand to fines ratio and clay content (Imperial Oil Resources Limited, 2021). When applied to non-segregated tailings, Canadian Natural found that polymer doses at 70 g/tonne solids resulted in suitable compressibility and hydraulic conductivity (Canadian Natural Resources Limited, 2021). Other Oil Sands Operators use doses of 900 to 1000 g PAM/tonne dry fines (Jordan Hamilton, personal communications, May 2023). Dosing requirements will likely depend on the solids content and types of tailings (fine tailings, thickened tailings, non-segregated tailings, etc.). Non-segregated tailings involve the treatment of thickened tailings with coarse tailings and a coagulant or flocculant (Canadian Natural Resources Limited, 2021). Thickened tailings are already treated with a flocculant (typically PAM) and possess a higher solids content than fine tailings (Hyndman et al., 2018). This may indicate why a lower polymer dose was required for Canadian Natural non-segregated tailings, in comparison to doses used for fluid tailings in Kearl Mine's pilot study (Canadian Natural Resources Limited, 2021; Imperial Oil Resources Limited 2021).

To obtain optimal flocculation and water release, flocculant studies have used 5 to 50 mg PAM/L tailings slurry (Xu and Cymerman 1999; Cymerman et al. 1999; Li et al., 2005; Li et al., 2009; Wang et al., 2010). More recent oil sands tailings studies determined doses of 1000 to 2000 g

PAM/tonnes solids to be suitable (Li et al., 2021; Zhang et al., 2021). Overall, dosage and pore water concentration will vary among operator and flocculation studies.

Due to the adsorption of PAM to tailings solids, the liquid concentration in the tailings pore water will not be representative of the original dosage concentration. To gain an understanding of how pore water concentration is impacted by HPAM adsorption, HPAM concentrations were measured in fine tailings and milli-Q water dosed at 1 g HPAM/L. To compare with operator and flocculation studies, this concentration is equivalent to 3119 g/tonne solids (Equation 3-1). These tailings have a 26.5% solids content and bulk density of 1.21 g/mL. Following SEC quantification, pore water concentrations in tailings treated with HPAM were 447.07 ± 46.56 mg/L. The solid phase concentration was determined to be 1874 g/tonne solids (Equations 3-2 to 3-4). In this instance, dosing tailings below 1874 g/tonne could result in complete adsorption of HPAM to the tailings solids and likely liquid phase concentrations below the SEC detection limit. However, dosing HPAM above the adsorption capacity of a system will result in detectable concentrations in the liquid phase, as seen in this trial.

Once adsorbed to soil or clay particles, PAM desorption and mobility is limited. Deng et al. (2006) found that less than 3% of adsorbed anionic PAM was removed from smectite, illite, and kaolinite after four consecutive washes. In soil systems, PAM was predominantly retained in the top 2 cm of the soil matrix with limited movement deeper than 20 cm (Lu and Wu, 2003b). Upon soil drying, Nadler et al. (1992) found that PAM became irreversibly bonded to the soil, suggesting limited mobility in terrestrial landscapes. In general, PAM is considered incapable of penetrating a soil system more than a few centimeters (Lu and Wu, 2003b; Guezennec et al., 2015). Observations from soil studies suggest that liquid concentrations are unlikely to increase once HPAM is adsorbed to the mineral component. However, the PAM monomer, acrylamide, as well as lower molecular weight polymers have higher mobility in soil systems (Sojka et al., 2007; Guezennec et al., 2015). The biodegradation of HPAM could therefore form oligomers with different chemical functionalities from their parent polymer, potentially decreasing their adsorption to tailings minerals and increasing mobility in OSPW. In low PAM concentration systems, SEC would be beneficial in detecting the formation of PAM oligomers in tailings pore water. In tailings systems with concentrations above the adsorption capacity, SEC could be used to monitor parent polymers

and oligomers above the detection limit. For this SEC method, the detection limit for A3332 and A3338 in the liquid phase was 10 mg/L.

3.3.3.2 Sample Requirements

Sample requirements for successful analysis can influence field sampling, sample preparations, volume required, and time. Centrifugation and filtration are important for removing insoluble material and suspended clays and are common practice for preparing water and oil sands tailings samples (Nollet and De Gelder, 2013; Wang et al., 2016; Cossey et al., 2021; Li et al., 2023). Depending on the analytical method, 0.2 or 0.45 µm filters can be used, however 0.45 µm was typically used for SEC (Yu et al., 2015; Li et al., 2023). For sample volume, 1 mL was required for the autosampler. Therefore, about 2 mL of tailings was required in order to ensure sufficient volume following centrifugation and filtration.

The time to prepare each sample was influenced by centrifuging and filtering time, and whether samples needed to be diluted. At 5000 rpm, centrifuging times ranged from 10-40 minutes depending on the tailings treatment and solids content. Filtering required 1-10 minutes, with longer filtering times required as HPAM concentrations increased.

An important component of sample preparation is dissolution and mobile phase compatibility, which was described in subsection 3.3.1.3. Our method had a linear response range from 10 to 500 mg/L. Concentrations higher than 500 mg/L were not investigated to limit overloading the HPLC system. If samples were suspected to have pore water concentrations higher than 500 mg/L, then dilution was necessary. Milli-Q water proved suitable for HPAM dilution and required around a week for complete dissolution. The ability to measure HPAM dissolved in OSPW or milli-Q water could assist when receiving samples from external research facilities, as reagent requirements were limited. Overall, sample requirements for analyzing HPAM in oil sands tailings pore water aligned with typical sample preparation for oil sands tailings and general water samples. This can assist when measuring various chemical parameters in the same sample or transferring techniques among labs.

3.3.3.3 Ease of Use

PAM analysis has applications in academia, research, commercial labs, and industry. The ease of use and transferability of an analytical technique is important for comparing and sharing data and training other users and labs. SEC is a common analytical technique for analyzing polymers, and

its success with measuring HPAM reinforces its use as a technique that can be adopted for various applications. Lab technicians, students, researchers, and other lab individuals that would be involved in analysis would need to be trained in mobile phase preparation, sample preparation, and HPLC operation, all of which are common practice in a research lab setting. If using the same column, other method parameters such as flow rate, injection volume, and mobile phase can be transferred.

The biodegradation of HPAM in oil sands tailings has received more interest overtime. Having a technique that is transferable and can determine various PAM molecular weights would improve knowledge sharing among academia and industry. Though the method does not provide details on the chemistry of the polymer or functional groups, it does indicate a change in the polymer structure, and its use as a carbon source. Such analysis would be beneficial for biodegradation studies in various tailings deposits, different oil sands mines, and different streams of research.

Similar to sample preparation time, the overall time it takes for analysis can influence a techniques applicability. Time requirements can include reagent preparation, gathering and preparing equipment, and analysis time. For this method, mobile phases were quick to prepare, while equipment preparation involved preparing the HPLC and ensuring the system was operating under normal conditions. Columns were typically flushed with the mobile phase in order to remove carrier or preservation solutions. This required 30 minutes to an hour. This also stabilized the chromatogram baseline. For this SEC method, run times were 20 minutes. This allowed for complete elution of a sample, as well as flushing of the column to prepare for the next sample. Another time consideration was data analysis and calculations, all of which could be performed within a day.

Overall, the SEC method was easy to use, transferable among research or industry labs, and required standard lab training. Following sample prep, SEC analysis, and data interpretations, turnover time ranged from 24-48 hours.

3.3.3.4 Detection

Overall, the method was capable of detecting various anionic and non-ionic PAM species with a level of precision suitable for analysis. This can be beneficial when monitoring a change in molecular weight or concentration for a parent PAM species. Though there were limitations when it came to measuring pore water concentrations for tailings that have been dosed under the

absorption capacity, pore water detection limit for A3332 and A3338 were suitable at 10 mg/L considering PAM is often dosed in higher concentrations (~1000 g/tonne tailings). Previous studies were able to detect PAM as low as 1-5 mg/L (Beazley 1985; Garfeh and Maradi-Araghi, 1986; Leung et al., 1987), while more recent studies obtained a detection limit of 0.1 mg/L (Xihua et al., 2007). This difference in detection limits for our SEC method compared to previous studies, suggests additional method optimization may lead to improved detection limits. Due to the strong adsorption to tailings particles, is important to note that even if PAM is not detected in the pore water, it can still exist in the solid phase. PAM analysis using the SEC method in systems dosed below the adsorption capacity may still be beneficial, especially if there is potential for desorption from biodegradation or other degradation processes.

3.3.3.5 Method Limitations

Although the SEC method demonstrated success for measuring anionic and non-ionic PAM, there are some limitations that can be addressed in future SEC method developments or PAM studies. Table 3-5 provides a summary of these limitations, as well as possible solutions.

Table 3-5 Limitations and potential solutions for the current SEC method.

Limitation		Solution
Concentration	How to measure pore water concentrations below 10 mg/L?	PAM concentration below 10 mg/L could possibly be measured using RP-HPLC (Bao et al., 2010; Liu et al., 2012; Sang et al., 2015; Yu et al., 2015; Zhao et al., 2019). Alternatively, samples could be concentrated through freeze drying and dissolved in a suitable solvent. Dissolving polymers in a buffer or ionic solution can also stabilize retention times and improve peak shape (Lu et al., 2003; Gomez-Ordonez et al., 2012).
Adsorption	Once PAM is adsorbed to the solid phase, leachability is limited. How can we measure PAM in the solid phase?	PAM can be precipitated in short-chain alcohols or acetone (Lipp and Kozakiewicz, 2000). Extractions using acetone and methanol were attempted in this study on HPAM in oil sands tailings, however this was unsuccessful.
Metabolite Identification	The SEC method cannot provide qualitative data on the chemistry of an analyte, only its molecular weight and concentration. How can we identify biodegradation products or unknown peaks detected through the SEC method?	Metabolites can potentially be identified using LC-MS and other liquid chromatography techniques (Chapter 2). The SEC method is beneficial in indicating if there is a change in the parent polymer. If a change in pore water chemistry is determined, the SEC method can indicate if this is due to changes in the polymer or another source.
Electrolyte Corrosion and Salt Build Up	Electrolytes such as NaCl possess corrosive properties and can deteriorate an HPLC system or result in salt build up. What alternative is there to NaCl?	A build up of salt can be resolved by flushing the HPLC system with warm water, followed by an alcohol. This can also limit biological growth in the HPLC. $NaNO_3$ is an electrolyte alternative to NaCl (Gomez-Ordonez et al., 2012). This reagent will require different handling procedures than NaCl.
Decreased molecular weight Precision for Samples Run on Different Days	How can retention time precision among different days be improved?	A shift in retention time was often correlated with a change in pump pressure or performance. Regular pump maintenance and parts inspection can mitigate any issues with the pump. These can be performed by a student or lab member, so long as they have received training. To minimize risks with inaccurate results, standards should be run every time.

3.4 Conclusions

The SEC method presented acceptable precision, detection of parent polymers and a range of molecular weight species (~9 to 9500 10^3 g/mol), suitability for different oil sands applications and tailings streams, and required standard lab training. RSD was 0.2-3.4% for retention time (min) and 1.4-6.2% for peak area (mAu). The method obtained a detection limit of 10 mg/L, with a linear response range from 10 to 500 mg/L. These components indicated successful method development and application. The SEC method is therefore valid for detecting and measuring HPAM in most tailings applications and can suggest a change in the parent polymer by monitoring molecular weight shifts. Challenges may arise if the pore water concentration is below the detection limit, likely due to HPAM adsorption or low polymer doses. The tailings and HPAM used in this study possessed an adsorption capacity of 1874 g HPAM/tonne solids.

Molecular weight shift would be a strong indicator of HPAM being used as a carbon source. The use of HPAM as a carbon source could potentially influence tailings structure, greenhouse gas emissions, release of toxic compounds, and other changes to water chemistry. Since the use of HPAM as a nitrogen and carbon source is contradicting based on oil sands tailings biodegradation studies, and HPAM as a carbon source is not as well understood, the SEC method could be useful to determine molecular weight shift and indicate degradation during biological studies. Most literature measuring PAM and HPAM using SEC has only recorded the initial and final molecular weights. However, there is potential for this method to monitor HPAM biodegradation throughout the experiment duration. This would indicate at what time the parent polymer begins to change, and how the rate of molecular weight shift changes overtime. Combining SEC with other analytical techniques would provide a comprehensive analysis of HPAM biodegradation.

4 BIODEGRADATION OF POLYACRYLAMIDE IN OIL SANDS TAILINGS UNDER OXIC, SULFATE-REDUCING, AND METHANOGENIC CONDITIONS.

4.1 Introduction

Surface extraction of bitumen in the Athabasca oil sands has resulted in the accumulation of over 1.3 billion m^3 of tailings (Alberta Energy Regulator, 2022a). Low flocculation and consolidation of tailings prevents the recovery and recycling of process-affected water, prolonging the presence of tailings ponds and delaying reclamation. Various tailings dewatering technologies have been established to address this environmental liability, with majority of these technologies relying on the use of polymer flocculants such as hydrolyzed polyacrylamide (HPAM). The wide use of HPAM in oil sands tailings has prompted concerns regarding its environmental fate in tailings systems, specifically its susceptibility to microbial degradation.

Previous studies have demonstrated the use of HPAM as a nutrient source for aerobic and anaerobic bacteria, either serving as a sole carbon or nitrogen source, or supporting microbial growth and biogenic gas production as a co-substrate (See Chapter 2). Potential biodegradation products for HPAM include polyacrylic acid, lower molecular weight oligomers, VFAs, ammonium (NH_4^+), CO_2, CH_4, and acrylamide (Liu et al., 2012; Dai et al., 2014; Hu et al., 2018; Berdugo-Clavijo et al., 2019; Akbar et al., 2020; Li et al., 2023). HPAM is generally considered non-toxic (Weston et al., 2009; Buczek et al., 2017), however, Clifford et al. (2022) reported hypoxemia in rainbow trout (*Oncorhynchus mykiss)* exposed to cationic polyacrylamide. The acrylamide monomer is typically of greater concern regarding acute toxicity, as it is considered a neurotoxin and carcinogen (United States Environmental Protection Agency, 2010). Health Canada is in the process of developing acrylamide drinking water guidelines, however, the World Health Organization guidelines suggest a maximum concentration of 0.5 µg/L in drinking water (World Health Organization, 2022). For aquatic invertebrates and fish, LC_{50} values range from 78 to 460 mg/L, while EC_{50} values range from 33.8 to 230 (Health Canada, 2009).

Microbial diversity and a range of redox conditions present in tailings systems suggest the potential for both aerobic and anaerobic biodegradation of HPAM. Limited studies have investigated the biodegradation of HPAM in oil sands tailings. Li et al. (2023) observed aerobic biodegradation by

tailings isolates, while Haveroen et al. (2005) found that HPAM could be used as a nitrogen source to stimulate methanogenesis. Conversely, Collins et al. (2016) found that methanogenesis was not due to the presence of HPAM, but instead nitrogen fixation. To date, no studies have investigated indigenous microbial communities involved in HPAM biodegradation. There are also limited studies monitoring a change in HPAM molecular weight throughout the incubation time.

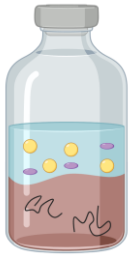
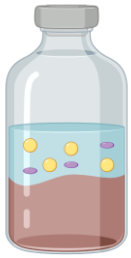
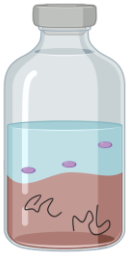
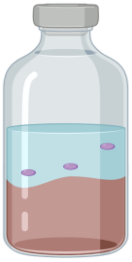
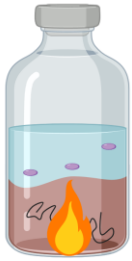
The results from this study evaluates the biodegradation of HPAM under oxic, sulfate-reducing, and methanogenic conditions, and determines HPAMs role as a nutrient source. It also determines how microbial communities in fine tailings respond to the introduction of HPAM and shift over time under different redox conditions. To obtain these results, our objectives were to (I) monitor microbial activity by measuring terminal electron acceptors, biogenic gas production, and nutrient consumption, (II) monitor HPAM pore water concentration and molecular weight, and (III) characterize tailings microbial communities. This study is the first to simultaneously monitor a shift in HPAM molecular weight and oil sands tailings microbial communities.

4.2 Experimental Design

Microcosms were established with 500 mL WHEATON^{TM} glass serum bottles (Fisher Scientific, No. 06-451-43). Based on previous PAM and HPAM biodegradation studies and current operator dosing, microcosms were amended with 400, 1000, and 2000 mg HPAM/L tailings. Cultures were prepared by adding a 1:1 ratio of fine tailings and mineral medium (subsection 4.3.2) with a headspace of helium for anoxic conditions, and atmospheric air for oxic conditions. Helium was used for anoxic conditions to limit nitrogen fixation or the use of CO_2 by methanogens (Collins et al., 2016; Stasik et al., 2016).

Each condition was established under four treatments (Table 4-1): (I) HPAM as co-substrate (P_{Co}), (II) HPAM as nitrogen source (P_N), (III) HPAM carbon source (P_C), and (IV) HPAM sole carbon and nitrogen source (P_{N+C}). Each treatment had two different HPAM doses, a no-HPAM control, and a heat-killed control. Acetate was provided as the carbon source and ammonium as the nitrogen source. Acetate was selected as it is readily consumed by bacteria, creating a positive response and indicating whether bacteria are active in the microcosms. Acetate was amended to P_{Co} and P_N, while ammonium was amended to P_{Co} and P_C

Table 4-1 Visual representation of microcosm treatments and levels.

Treatment I: HPAM as Co-Substrate (P_{Co})			
2000P_{Co}AcAm	1000P_{Co}AcAm	AcAm	1000P_{Co}AcAm Killed Control
			
Treatment II: HPAM as Nitrogen Source (P_N)			
2000P_NAc	1000P_NAc	Ac	1000P_NAc Killed Control
			
Treatment III: HPAM as Carbon Source (P_C)			
2000P_CAm	1000P_CAm	Am	1000P_CAm Killed Control
			
Treatment IV: HPAM as Nitrogen & Carbon Source (P_{N+C})			
2000P_{N+C}	1000P_{N+C}	No Amendment	1000P_{N+C} Killed Control
			

Note. Yellow circles represent acetate (Ac) and purple ovals represent ammonium (Am). Microcosms were established with a media-to-tailings ratio of 1:1.

4.3 Materials and Methods

4.3.1 Materials

4.3.1.1 Fine Tailings

Untreated fine tailings were obtained in 2021 from an Oil Sands Operator and stored at the University of Alberta at 4°C until use. The initial characterization for the fine tailings used in this study is presented in Table 4-2.

Table 4-2 Pore water chemistry and physical properties for untreated fine tailings prior to microcosms establishment. Results are presented as the mean for duplicate sampling.

Parameter	Value
Solids (%)	26.5
Water (%)	73.5
Bulk Density	1.14 (± 0.01)
pH	7.41 (± 0.00)
Redox Potential (mV)	-8.2 (± 0.00)
Dissolve Organic Carbon (mg/L)	11.58 (± 0.71)
Dissolved Inorganic Carbon (mg/L)	70.18 (± 0.49)
Total Alkalinity (mg/L $CaCO_3$)	309.16 (± 1.16)
Bicarbonate Alkalinity (mg/L)	377.18 (± 1.41)
Acetate (mg/L)	1.72 (± 0.15)
Fluoride, F^- (mg/L)	3.02 (± 0.18)
Chloride, Cl^- (mg/L)	16.93 (± 0.48)
Ammonium, NH_4^+ (mg/L)	1.12 (± 0.02)
Nitrite, NO_2^- (mg/L)	5.82 (± 0.84)
Nitrate, NO_3^- (mg/L)	0.00 (± 0.00)
Sulfate, SO_4^{2-} (mg/L)	310.53 (± 6.52)
Calcium, Ca^{2+} (mg/L)	83.77 (± 0.97)
Potassium, K^+ (mg/L)	18.10 (± 0.13)
Magnesium, Mg^{2+} (mg/L)	37.08 (± 0.36)
Sodium, Na^+ (mg/L)	280.95 (±1.61)
Sulfur, S (mg/L)	209.34 (± 0.02)

4.3.1.2 Hydrolyzed Polyacrylamide

SNF FLOPAM A3332 and A3338 were used as the polymer flocculant during the microcosms experiment (SNF Canada Ltd, Vaughan, CAN). A3332 is a linear anionic PAM while A3338 is a branched anionic PAM possessing a crosslinking agent. Both are 30% anionic with a molecular weight range of 8-10 million Daltons (g/mol). By the time of this experiment, Imperial Oil Resources Ltd. used two anionic PAMs for their thickened tailings (Imperial Oil Resources Ltd.,

2021). SNF FLOPAM A3332 was used for the thickener, and SNF FLOPAM A3338 was used for the secondary injection (in-line thickening) (Imperial Oil Resources Ltd., 2021). Microcosms were therefore amended with a 1:1 ratio of A3332 and A3338 provided by Imperial Oil Ltd. A polymer stock solution (13g/L) was created with A3332 and A3338 at a ratio of 1:1. This stock solution was mixed with the medias. HPAM doses expressed in various units are provided in Table 4-3.

Table 4-3 HPAM concentration expressed in different units.

Microcosm Condition	mg HPAM/L tailings	mg HPAM/L solution*	g HPAM/tonne solids
Oxic	1000	491	3119
	2000	964	6237
Sulfate-reducing	400	194	1247
	1000	481	3119
	2000	945	6237
Methanogenic	400	196	1247
	1000	484	3119
	2000	949	6237

*Solution refers to fine tailings and mineral media

4.3.1.3 Medias

Three different medias were prepared for the three different redox conditions: oxic, sulfate-reducing, and methanogenic. For oxic conditions, Bushnell-Haas media was prepared. The sulfate-reducing media was adapted from So and Young (1999), and the methanogenic media was adapted from Fedorak and Hrudey (1984). Separate media without NH_4 or NO_3 compounds was prepared for P_N and P_{N+C} treatments. After sterilizing media by autoclaving, an HPAM stock solution was amended to appropriate media serum bottles to obtain a concentration of 400, 1000 or 2000 mg HPAM/L (Figure 4-1). HPAM was not autoclaved to prevent thermal degradation. To allow for complete dissolution of the polymer, media bottles were left for 2 hours with 150 rpm gyratory shaking, then stored at 4°C until added to microcosms. Unless otherwise stated, all materials were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Figure 4-1 HPAM in sulfate-reducing media prior to complete dissolution. The pink colour is from residual O2 trapped within the HPAM stock solution. Once dissolved, the media turned clear indicating that any O2 had been consumed by the scavenger.

Bushnell-Haas Media

Oxic microcosms were incubated with sterile Bushnell-Haas media. 1L of media contained 1 g K_2HPO_4, 1 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.02 g $CaCl_2 \cdot 2H_2O$, 0.05 g $FeCl_3$, and 1 g NH_4NO_3 and was stored at 4°C until use.

Sulfate-reducing Media

For the sulfate-reducing media, four stock solutions were prepared. A vitamin solution was prepared containing 1 mg Vitamin B12, 20 mg biotin, 20 mg folic acid, 50 mg nicotinic acid, 50 mg p-aminobenzoic acid, 50 mg Ca pantothenate, 100 mg pyridoxine HCl, 50 mg riboflavin, 50 mg thiamine, and 50 mg thioctic acid in 1L of milli-Q water. The vitamin solution was then adjusted to a pH 7, filter sterilized, sealed, headspace flushed with helium gas, and stored at 4°C. A trace element solution was prepared containing 6 g $CoCl_2 \cdot 6H_2O$, 30 mg $CuCl_2$. $2H_2O$, 0.3 g $FeCl_2 \cdot 4H_2O$, 1.14 g H_3BO_3, 4 g $MnCl_2 \cdot 4H_2O$, 0.5 g Na_2MoO_4. $2H_2O$, 0.3 g $NiCl_2$. $6H_2O$, and 0.42 g $ZnCl_2$ in 1 L of 0.01 N HCl. The trace element solution was autoclaved and stored at 4°C. A solution containing 1 M $NaHCO_3$ and 67 mM $Na_2S \cdot H_2O$ in autoclaved milli-Q water was prepared in an anaerobic chamber. The solution was then filter sterilized, dispensed into a serum bottle, and sealed.

The sulfate-reducing media was then prepared containing 0.2 g NaCl, 1.3 g KCl, 1 g $MgCl_2 \cdot 2H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.5 g NH_4Cl, 0.2 g KH_2PO_4, 1.42 g Na_2SO_4 and 5 mL trace element solution. The media was sparged with CO_2 for 1 hour to deoxygenate the media, dispensed into serum bottles, and autoclaved. Following autoclaving, the media serum bottle headspace was flushed with helium gas for 5 minutes to maintain anoxic conditions, and each bottle received 0.1% (v/v) vitamin solution and 3% (v/v) $NaHCO_3$ and $Na_2S \cdot H_2O$ solution. Sodium sulfide ($Na_2S \cdot H_2O$) was used as an O_2 scavenger to promote anoxic conditions within the media. The vitamin solution was not added to media serum bottles for P_N, P_C, or P_{N+C} treatments.

Methanogenic Media

For the methanogenic media, three stock solutions were prepared. The first mineral solution contained 5 g NaCl, 1 g $CaCl \cdot 2H_2O$, 5 g NH_4Cl, 1 g $MgCl \cdot 6H_2O$ in 100 mL of milli-Q water. The second mineral solution contained 1 g $(NH_4)6Mo_7O_{24} \cdot 2H_2O$, 0.01 g $ZnSO_4 \cdot 7H_2O$, 0.03 g H_3BO_3, 0.15 g $FeCl_2 \cdot 4H_2O$, 1 g $CoCl_2 \cdot 6H_2O$, 0.003 g $MnCl_2 \cdot 4H_2O$, 0.003 g $NiCl_2 \cdot 6H_2O$, 0.01 $AlK(SO_4)2 \cdot 12H_2O$ in 100 mL of milli-Q water. The mineral solutions were stored at 4°C until use. A vitamin B solution containing 0.025 g pyridoxine, 0.005 g thiamine, 0.01 g nicotinic acid, 0.0025 g pantothenic acid, 0.01 g vitamin B12, and 0.005 g p-aminobenzoic acid was prepared in 100 mL of milli-Q water. The vitamin B solution was filter sterilized, sealed, and stored at 4°C.

The methanogenic media was prepared containing 14 mL mineral solution 1, 1.4 mL mineral solution 2, 14 mL resazurin, 14 mL of 50 g/L KH_2PO_4, and 5.7 g $NaHCO_3$ in 1 L boiled milli-Q water. Resazurin was used as a redox indicator, causing the media to turn pink when O_2 was present. The media was sparged with CO_2 for 1 hour and dispensed into serum bottles which were then sealed, autoclaved, and flushed with sterile helium gas for 5 minutes. Media serum bottles then received 1% (v/v) vitamin B solution and 1% (v/v) $Na_2S \cdot H_2O$ (25 g/L). Methanogenic media prior to and after sodium sulfide additions is provided in Appendix B, Figure B-1. The vitamin B solution was not added to media serum bottles for P_N, P_C, or P_{N+C} treatments.

4.3.1.4 Gases

Pure CO_2, helium, and O_2 canisters were purchased from Linde Canada (Edmonton, AB, CAN). CO_2 was used for sparging the sulfate-reducing and methanogenic medias, helium was used for

flushing microcosms headspace, and O_2 was used to maintain headspace concentrations in oxic microcosms.

4.3.2 Experimental Microcosm Set-up

Tailings were homogenized using an automatic mixer prior to transferring into sterile serum bottles. The media was tailored to meet the conditions required for each treatment and level (Table 4-4 and 4-5**)**. Microcosms were then sealed with a Bromo butyl stopper and crimped with an aluminum seal (Figure 4-2). Anoxic microcosms were then flushed with helium gas. Killed controls were autoclaved once per day for 6 consecutive days, serving as indicators of abiotic activity occurring in the microcosm.

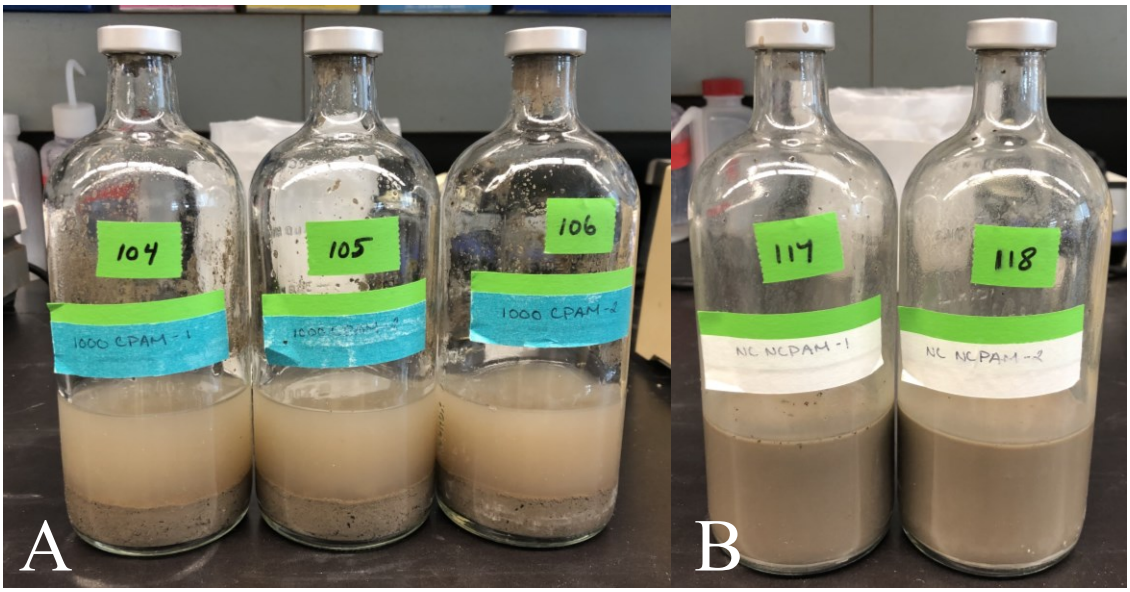


Figure 4-2 (A) Microcosms amended with 1000 mg HPAM/L tailings and (B) no-HPAM controls established under oxic conditions with a total volume of 200 mL.

4.3.2.1 Oxic Conditions

Oxic microcosms were established in 500 mL serum bottles in November 2022 and monitored until July 2023 (236 d). Each serum bottle received 100 mL of fine tailings and 100 mL of Bushnell-Haas. HPAM microcosms were amended with 2000 or 1000 mg HPAM/L tailings. P_{Co} and P_N treatments were amended with 1000 mg/L of acetate and measured weekly or biweekly throughout the incubation period. Microcosms were amended to 1000 mg/L when concentrations were depleted below 500 mg/L. Initial acetate levels in raw tailings were 1.72 (± 0.15) mg/L,

however, microcosms were amended to 1000 mg/L to ensure acetate was consistently available. Ammonium was measured every two or three weeks and amended to 50 mg/L when depleted to 5 mg/L. Microcosms were sealed under atmospheric air conditions. O_2 was monitored weekly and amended to atmospheric air conditions (21%, roughly equivalent to ~3.1 mmol O_2 depending on the headspace volume) when O_2 levels dropped to ~1.5 mmol. This ensured that headspace conditions mirrored atmospheric air without depriving the system of O_2. Microcosms were incubated in the dark at 20°C with 150 rpm gyratory shaking.

Table 4-4 Experimental design for oxic conditions. HPAM microcosms were established in triplicates, while No-HPAM controls and Heat-Killed Controls were established in duplicates.

Treatments	Levels	Amendments			Parameters Monitored
		HPAM	Acetate	Ammonium	
HPAM as Co-Substrate (P_{Co})	$2000P_{Co}$AcAm	✓	✓	✓	CO_2 O_2 Acetate HPAM Ammonium pH
	$1000P_{Co}$AcAm	✓	✓	✓	
	AcAm	-	✓	✓	
	$1000P_{Co}$AcAm Killed Control	✓	✓	✓	
HPAM as Nitrogen Source (P_N)	$2000P_N$Ac	✓	✓	-	
	$1000P_N$Ac	✓	✓	-	
	Ac	-	✓	-	
	$1000P_N$Ac Killed Control	✓	✓	-	
HPAM as Carbon Source (P_C)	$2000P_C$Am	✓	-	✓	
	$1000P_C$Am	✓	-	✓	
	Am	-	-	✓	
	$1000P_C$Am Killed Control	✓	-	✓	
HPAM as Nitrogen & Carbon Source (P_{N+C})	$2000P_{N+C}$	✓	-	-	
	$1000P_{N+C}$	✓	-	-	
	No Amendments	-	-	-	
	$1000P_{N+C}$ Killed Control	✓	-	-	

Note. Acetate and ammonium were amended at 1000 mg/L and 50 mg/L, respectively. Killed controls were sterilized through autoclaving prior to amending with 1000 mg/L of HPAM. HPAM microcosms were amended at 1000 mg/L or 2000 mg/L. (✓) indicate an amendment that was added whereas (-) indicate an amendment that was not added.

4.3.2.2 Sulfate-reducing Conditions

Sulfate-reducing microcosms were established in 500 mL serum bottles in June 2022 and monitored until August 2023 (420 d). Each serum bottle received 165 mL of fine tailings and 165 mL of sulfate-reducing media. P_{Co} and P_N treatments were amended with 1000 mg/L of acetate and measured biweekly or monthly throughout the incubation period. Microcosms were amended to 1000 mg/L when concentrations were depleted below 200 mg/L. P_{Co} and P_C were amended with ammonium as an alternative nitrogen source and measured monthly. HPAM amended cultures received either 400 or 1000 mg HPAM/L tailings. 400 mg/L microcosms were amended to 2000 mg/L on day 168 due to pore water concentrations below the detection limit.

To establish sulfate-reducing bacteria (SRB) and limit methanogenesis, microcosms were amended to 15 mM of sulfate (Lee and Ulrich, 2021). Complete inhibition of methanogenesis has been observed in the presence of 10 mM sulfate (Winfrey and Zeikus 1977), while Ramos-Padron et al. (2011) still observed some methanogenesis in tailings cultures amended with 12 mM sulfate. Depending on the tailings treatment method, current tailings deposits can contain sulfate levels as high as 11 mM (Cossey et al., 2021), while composite tailings release water have previously exhibited higher levels of sulfate from 13.5 to 83 mM (Holowenko et al., 2000). Microcosms were amended to 15 mM when concentrations were depleted below 10 mM. Microcosms were allowed to rest for two weeks at 4°C, then incubated up-side-down in the dark at 20°C.

4.3.2.3 Methanogenic Conditions

Methanogenic microcosms were established in 500 mL serum bottles in March 2022 and monitored until March 2023 (374 d). Each serum bottle received 165 mL of fine tailings and 165 mL of methanogenic media. Acetate, ammonium, and HPAM amendments aligned with sulfate-reducing conditions (subsection 4.3.2.2). 400 mg/L microcosms were amended to 2000 mg/L on day 247. Microcosms were allowed to rest for two weeks at 4°C, then incubated up-side-down in the dark at 20°C. Methanogenic microcosms for P_{Co} treatments is presented in Appendix B, Figure B-2.

Table 4-5 Experimental design for sulfate-reducing and methanogenic conditions. HPAM microcosms were established in triplicates, while No-HPAM controls and Heat-Killed Controls were established in duplicates.

Treatments	Levels	Amendments			Parameters Monitored
		HPAM	Acetate	Ammonium	
HPAM as Co-Substrate (P_{Co})	$400P_{Co}AcAm$	✓	✓	✓	CO_2 CH_4 Acetate Sulfate* HPAM Ammonium pH
	$2000P_{Co}AcAm$	✓	✓	✓	
	$1000P_{Co}AcAm$	-	✓	✓	
	AcAm	✓	✓	✓	
	$1000P_{Co}AcAm$ Killed Control				
HPAM as Nitrogen Source (P_N)	$400P_NAc$	✓	✓	-	
	$2000P_NAc$	✓	✓	-	
	$1000P_NAc$	-	✓	-	
	Ac	✓	✓	-	
	$1000P_NAc$ Killed Control				
HPAM as Carbon Source (P_C)	$400P_CAm$	✓	-	✓	
	$2000P_CAm$	✓	-	✓	
	$1000P_CAm$	-	-	✓	
	Am	✓	-	✓	
	$1000P_CAm$ Killed Control				
HPAM as Nitrogen & Carbon Source (P_{N+C})	$400P_{N+C}$	✓	-	-	
	$2000P_{N+C}$	✓	-	-	
	$1000P_{N+C}$	-	-	-	
	No Amendments	✓	-	-	
	$1000P_{N+C}$ Killed Control				

*Sulfate was only monitored in sulfate-reducing conditions.

Note. Acetate and ammonium were amended at 1000 mg/L and 50 mg/L, respectively. Killed controls were sterilized through autoclaving prior to amending with 1000 mg/L of HPAM. HPAM microcosms were initially amended at 400 mg/L or 1000 mg/L. Microcosms amended at 400 mg/L were later increased to 2000 mg/L. (✓) indicate an amendment that was added whereas (-) indicate an amendment that was not added.

4.3.3 Microcosm Measurements

4.3.3.1 Microcosm Headspace

Microbial activity was monitored by measuring biogenic gas production of CH_4 and CO_2 and consumption of O_2. CO_2 and O_2 were measured weekly in oxic conditions, CH_4 was measured biweekly or monthly in methanogenic conditions, and CO_2 was measured monthly in sulfate-reducing conditions. CH_4 was measured on Day 0 and 420 for sulfate-reducing conditions, and CO_2 was measured on day 0 and 374 for methanogenic conditions. Prior to sampling, methanogenic and sulfate-reducing microcosms were inverted to release air bubbles trapped within the sediment matrix.

O_2 was measured using a gas chromatograph equipped with a thermal conductivity detector (GC-TCD) with a molecular sieve 5A and HayeSep Q column. The injection port and detector were maintained at 120°C and oven maintained at 90°C with a current set to 90 mA. Helium was used as the carrier gas at 200 kPa. Headspace samples were manually injected at 100 µL.

CO_2 was measured using an Agilent 7890+ gas chromatograph equipped with a thermal conductivity detector (GC-TCD) and Agilent HP-PLOT/Q column (30 m x 320 µm x 0.2 µm). The oven and injection port were maintained at 50°C and the detector maintained at 200°C. Helium was used as the carrier gas with a flow rate of 4 mL/min. The flowrate of the makeup gas (helium) was set to 5 mL/min. The total run time was 1.65 min. Headspace samples were manually injected at 100 µL.

CH_4 was measured using an Agilent 7890+ gas chromatograph equipped with a flame ionization detector (GC-FID) and Agilent HP-5MS column (30 m x 250 µm x 0.25 µm). The oven and injection port were maintained at 80°C and the detector maintained at 300°C. Helium was used as the carrier gas with a flow rate of 1.5 mL/min. The flowrate of the makeup gas (N_2) was 40 mL/min, airflow was 350 ml/min, and H_2 was 35 mL/min. The total run time was 1.4 min. Headspace samples were manually injected at 100 or 50 µL.

4.3.3.2 Water Chemistry

Liquid samples were collected weekly, biweekly, or monthly depending on the redox condition. Samples were centrifuged at 5000 rpm for 20 min and filtered using 0.2 or 0.45 µm nylon filters. The pore water was analyzed for HPAM, acetate, sulfate, ammonium, and pH.

HPAM samples were prepared and measured biweekly for oxic conditions and monthly for sulfate-reducing and methanogenic conditions using the methods described in Chapter 3. HPAM degradation was calculated based on molecular weight while pore water removal was based on pore water concentrations using Equation 4-1. $HPAM_{initial}$ is the molecular weight or concentration on day 0 and $HPAM_j$ is the molecular weight or concentration on day j. Pore water concentrations were based on the parent polymer, while molecular weight was collected from the HPAM degradation products. A decrease in HPAM molecular weight would indicate degradation of the carbon chain, while a decrease in the pore water concentration could be due to degradation or adsorption.

$$\%\ degradation\ or\ removal\ = \frac{HPAM_{initial} - HPAM_j}{HPAM_{initial}} \times 100 \quad [4\text{-}1]$$

On day 168 and 247 for sulfate-reducing and methanogenic conditions, respectively, HPAM concentrations in 400 mg HPAM/L tailings microcosms were increased to 2000 mg HPAM/L tailings. To determine how much HPAM to amend, the current mass of HPAM was calculated using Equation 4-2. The current concentration (mg HPAM/L microcosm) was assumed to be the initial concentration (Table 4-3). The mass of HPAM added was then calculated using Equation 4-3 and 4-4.

$$\left(\frac{mg\ HPAM}{L\ microcosm}\right)_{current} \times\ V_{microcosm}\ =\ mg\ HPAM_{current} \quad [4\text{-}2]$$

$$\left(\frac{mg\ HPAM}{L\ microcosm}\right)_{final} \times\ V_{microcosm} =\ mg\ HPAM_{final} \quad [4\text{-}3]$$

$$mg\ HPAM_{final} -\ mg\ HPAM_{current} = mg\ HPAM_{added} \quad [4\text{-}4]$$

Acetate and sulfate were measured using a Dionex 2100 Ion Chromatography system equipped with a Dionex IonPAC AS18 column. The separation was performed using isocratic elution for the sulfate method (32 mM KOH) and gradient elution for the acetate method (10-32 mM KOH). Eluent flowrate was 0.25 mL/min. The oven temperature was maintained at 30°C while the conductivity detector was maintained at 35 °C.

To measure NH_3-N in sulfate-reducing and methanogenic conditions, samples were prepared using a HACH AmVer™ Salicylate High Range ammonia nitrogen set and following the AmVer™ Salicylate Test 'N Tube method (HACH Method 10031). Samples were then measured using the

HACH DR 900 Multiparameter Portable Colorimeter. NH_3-N in oxic conditions were measured at the Natural Resources Analytical Laboratory (NRAL) at the University of Alberta using the Salicylate-hypochlorite method and colorimetric autoanalyzer (Thermo Gallery Plus Beermaster Autoanalyzer, Thermo Fisher Scientific, Vantaa, FI). NH_3-N was then converted to NH_4 using equations 4-5 to 4-7. The fraction of aqueous NH_3 in equation 4-2 was obtained from Emerson et al. (1975) using the microcosms pH and temperature.

$$Total\ NH_3(mg/L)\ =\ NH_3 - N\ (mg/L)\ x\ \frac{17.031\ g/mol}{14.0067\ g/mol} \quad [4\text{-}5]$$

$$NH_3 = fraction\ of\ aqueous\ NH_3\ x\ Total\ NH_3 \quad [4\text{-}6]$$

$$NH_4(mg/L) = Total\ NH_3(mg/L) - NH_3(mg/L) \quad [4\text{-}7]$$

To investigate the biodegradation of HPAM, acrylamide and acrylic acid were selected as potential biodegradation products. Acrylamide and acrylic acid were measured in a pure polymer solution to determine the residual concentration prior to the biodegradation studies. Measurements were also taken on day 0 and day 236, 420, and 374 for oxic, sulfate-reducing, and methanogenic conditions, respectively. Acrylamide and acrylic acid were measured using an HPLC system equipped with an Agilent 1260 Infinity Quaternary Pump, Agilent 1260 Infinity Standard Autosampler, Agilent 1260 Infinity Thermostatted Column Compartment, and Agilent 1260 Infinity Diode Array Detector. The separation was performed using a InfinityLab Poroshell 120 Aq-C18 column (4.6 mm x 150 mm, 2.7 µm) from Agilent. The column was eluted isocratically using 10% (v/v) methanol and 90% H_3PO_4 (0.1%) at a pH of 2.5. At 20 °C and a flowrate of 0.5 mL/min, 10 uL of sample was injected and analyzed at 200 nm. The retention time for acrylamide was ~4.5 min and for acrylic acid was ~8.8 min. The detection limit for both analytes was 0.1 mg/L. No acrylic acid was detected in A3332 or A3338, however, residual acrylamide was 0.055% for A3332 and 0.047% for A3338.

Acute toxicity of pore water samples was analyzed using the Microtox® bioassay with *Aliivibrio fischeri.* The light emissions from the bioluminescent bacterium were measured using a Microtox LX and Modern Water MicrotoxLX 1.3 software. The inhibition effect was determined using the 81.9% Basic Test protocol with an incubation time of 5 min.

4.3.3.3 Microbial Communities

Microcosm samples were collected for DNA extraction and 16s rRNA sequencing on days 56, 152, and 236 for oxic conditions, days 154, 322, and 420 for sulfate-reducing conditions, and days 119, 231, and 374 for methanogenic conditions. Samples were also collected from the bulk tailings samples to represent the initial microbial community. Samples were centrifuged at 5000 rpm for 20 min and the supernatant removed. A sterilized scapula was used to collect ~250 mg solid fine tailings pellet for extraction. DNA extractions were conducted using the FastDNA^{TM} Spin Kit for Soil (MP Biomedical, Solon, USA) following the manufacturer protocol. Biological duplicates were extracted separately and pooled prior to being sent to the Molecular Biology Service Unit (MBSU) at the University of Alberta. Bacterial and archaeal 16S rRNA genes were amplified by PCR using universal primers 926F (AAACTYAAAKGAATTGRCGG) and 1392R (ACGGGCGGTGTGTRC) targeting the V6-V8 regions for both bacteria and archaea (An et al., 2013). Amplicon sequencing was performed on the Illumina MiSeq platform using the MiSeq Reagent Kit v3 (600-cycle) (Illumina, Sand Diego, US). The sequencing raw data was processed using the MetaAmp pipeline (Dong et al., 2017). A 97% identity cutoff was assigned and a 300 bp amplicon length was used.

4.3.4 Statistical Analysis

Welch's t-tests (one-tailed distribution, two-sample unequal variance) were performed using Microsoft Excel 2023 to determine the statistical significance (at $p = 0.05$) of microbial activity and HPAM biodegradation. T-tests were used to determine differences between HPAM amended cultures and no-HPAM controls or killed controls.

4.4 Results and Discussion

4.4.1 Microbial Activity in the Presence of Polyacrylamide and Biodegradation Under Oxic Conditions

The consumption of O_2, acetate, and ammonium, and production of CO_2 were monitored in oxic microcosms over 236 days to determine the use of HPAM as a nutrient source for microbial activity. Results for cumulative O_2, acetate, and ammonium consumption, and CO_2 production are provided in Table 4-6.

Table 4-6 Cumulative terminal electron acceptors and nutrients consumed and biogenic gas production for oxic conditions. Results are presented as the mean of biological replicates (2 or 3) ± one standard deviation.

Treatment	Level	O_2 Consumption (mmol)	Acetate Consumption (mmol)	Ammonium Consumption (mmol)	CO_2 Production (mmol)
HPAM as Co-Substrate (P_{Co})	$2000P_{Co}$AcAm	84.37 ± 0.22	67.51 ± 1.24	1.75 ± 0.04	17.82 ± 3.46
	$1000P_{Co}$AcAm	85.57 ± 1.67	69.76 ± 0.75	1.82 ± 0.17	15.23 ± 4.19
	AcAm	85.63 ± 1.08	68.85 ± 0.73	1.82 ± 0.03	15.06 ± 0.63
	$1000P_{Co}$AcAm Killed Control	3.03 ± 1.44	0.06 ± 0.03	-0.30 ± 0.01	0.25 ± 0.01
HPAM as Nitrogen Source (P_N)	$2000P_N$Ac	86.59 ± 0.05	64.16 ± 1.52		16.84 ± 3.99
	$1000P_N$Ac	86.2 ± 0.35	65.37 ± 1.26		12.62 ± 1.84
	Ac	86.75 ± 0.02	63.98 ± 0.64		11.78 ± 3.19
	$1000P_N$Ac Killed Control	1.60 ± 0.03	-0.44 ± 0.06		0.35 ± 0.03
HPAM as Carbon Source (P_C)	$2000P_C$Am	12.80 ± 0.44		2.29 ± 0.09	1.97 ± 0.13
	$1000P_C$Am	13.06 ± 0.75		2.32 ± 0.17	2.42 ± 0.16
	Am	13.01 ± 0.28		2.11 ± 0.21	2.55 ± 0.16
	$1000P_C$Am Killed Control	2.10 ± 0.49		0.18 ± 0.01	0.31 ± 0.01
HPAM as Nitrogen & Carbon Source (P_{N+C})	$2000P_{N+C}$	10.69 ± 0.52			1.82 ± 0.21
	$1000P_{N+C}$	9.21 ± 0.14			1.45 ± 0.14
	No Amendment	3.76 ± 0.08			0.46 ± 0.03
	$1000P_{N+C}$ Killed Control	2.27 ± 0.65			0.19 ± 0.07

HPAM pore water concentration and molecular weight were measured biweekly or monthly to monitor biodegradation. Results for pore water removal and degradation are provided in Table 4-7.

Table 4-7 HPAM pore water removal and degradation under oxic conditions.

Treatment	Level	Pore Water Removal (%)	Degradation (%)
HPAM as Co-Substrate (P_{Co})	2000P_{Co}AcAm	70	99
	1000P_{Co}AcAm	62	99
	1000P_{Co}AcAm Killed Control	42	42
HPAM as Nitrogen Source (P_N)	2000P_NAc	100	99
	1000P_NAc	100	99
	1000P_NAc Killed Control	62	36
HPAM as Carbon Source (P_C)	2000P_CAm	3	45
	1000P_CAm	74	37
	1000P_CAm Killed Control	0	25
HPAM as Nitrogen & Carbon Source (P_{N+C})	2000P_{N+C}	0	0
	1000P_{N+C}	36	13
	1000P_{N+C} Killed Control	0	0

Note. Pore water removal was calculated from the parent polymer pore water concentrations. Degradation was calculated from the decrease in molecular weight from the parent polymer to oligomers.

4.4.1.1 Hydrolyzed Polyacrylamide as a Co-Substrate

Under oxic conditions, O_2 was provided as the terminal electron acceptor and CO_2 monitored for biogenic gas production. To determine if HPAM could serve as a co-substrate, acetate and ammonium were provided as additional carbon and nitrogen sources. Nutrient consumption was also used as an indicator for microbial activity. Acetate is typically readily consumed in the presence of aerobic microorganisms, allowing it to serve as a positive response for microbial activity. Results for O_2 consumption, acetate and ammonium concentrations, CO_2 production, and HPAM molecular weight and pore water concentrations are provided in Figure 4-3. Cumulative acetate and ammonium consumption is provided in Appendix B, Figure B-5 and B-6**.**

O_2, acetate and ammonium consumption occurred without a lag phase, while CO_2 was produced after a 14-day lag phase for 2000P_{Co}AcAm, 1000P_{Co}AcAm, and AcAm. This suggests the presence of HPAM as a co-substrate did not influence how aerobic microorganisms adapted to HPAM amended systems or delayed the utilization of alternative nutrient sources. 2000P_{Co}AcAm,

1000P_{Co}AcAm, and AcAm typically required weekly acetate amendments to 1000 mg/L throughout the incubation period. On day 165 and 201 acetate could not be measured and amended the following week. Therefore, microcosms were amended to 2000 mg/L to ensure sufficient acetate availability.

Ammonium concentrations increased from 37.28 (± 1.82) on day 7, to 44.92 (± 2.06) on day 14 for 2000P_{Co}AcAm, likely due to hydrolysis of the HPAM amide group. HPAM pore water concentration had a 35-day lag phase while molecular weight decrease had a 14-day lag phase for 2000P_{Co}AcAm and 1000P_{Co}AcAm. Ammonium production and HPAM molecular weight lag phases suggested biodegradation of HPAM through amide hydrolysis prior to day 14. After day 14 the molecular weight decreased, indicating degradation of the polymer chain. A decrease in molecular weight, and therefore degradation of the polymer chain could suggest why the pore water concentration initially increased for day 14 and 35. Biodegradation could have influenced the chemical or physical properties of HPAM, thus decreasing adsorption to clay particles and increasing mobility in the pore water (Sojka et al., 2007). 1000P_{Co}AcAm and AcAm required 82 days to deplete initial ammonium, while 2000P_{Co}AcAm required 96 days, possible due to hydrolytic ammonium produced through amide hydrolysis.

Once the initial ammonium had been consumed and amended to ~50 mg/L (day 124), ammonium consumption was similar among 2000P_{Co}AcAm, 1000P_{Co}AcAm, and AcAm, possibly due to the removal of HPAM. HPAM pore water concentrations decreased below the detection limit on day 96 for 2000P_{Co}AcAm and day 110 for 1000P_{Co}AcAm. The molecular weight of the HPAM degradation products decreased below the column separation limit (<6000 g/mol) on day 152 for 2000P_{Co}AcAm and day 110 for 1000P_{Co}AcAm. The SEC column was replaced after day 124, after which the parent HPAM polymer was detected in the pore water. It is therefore likely that HPAM was still present in the pore water for 2000P_{Co}AcAm and 1000P_{Co}AcAm from day 96 to 124, only that column deterioration impacted detection sensitivity.

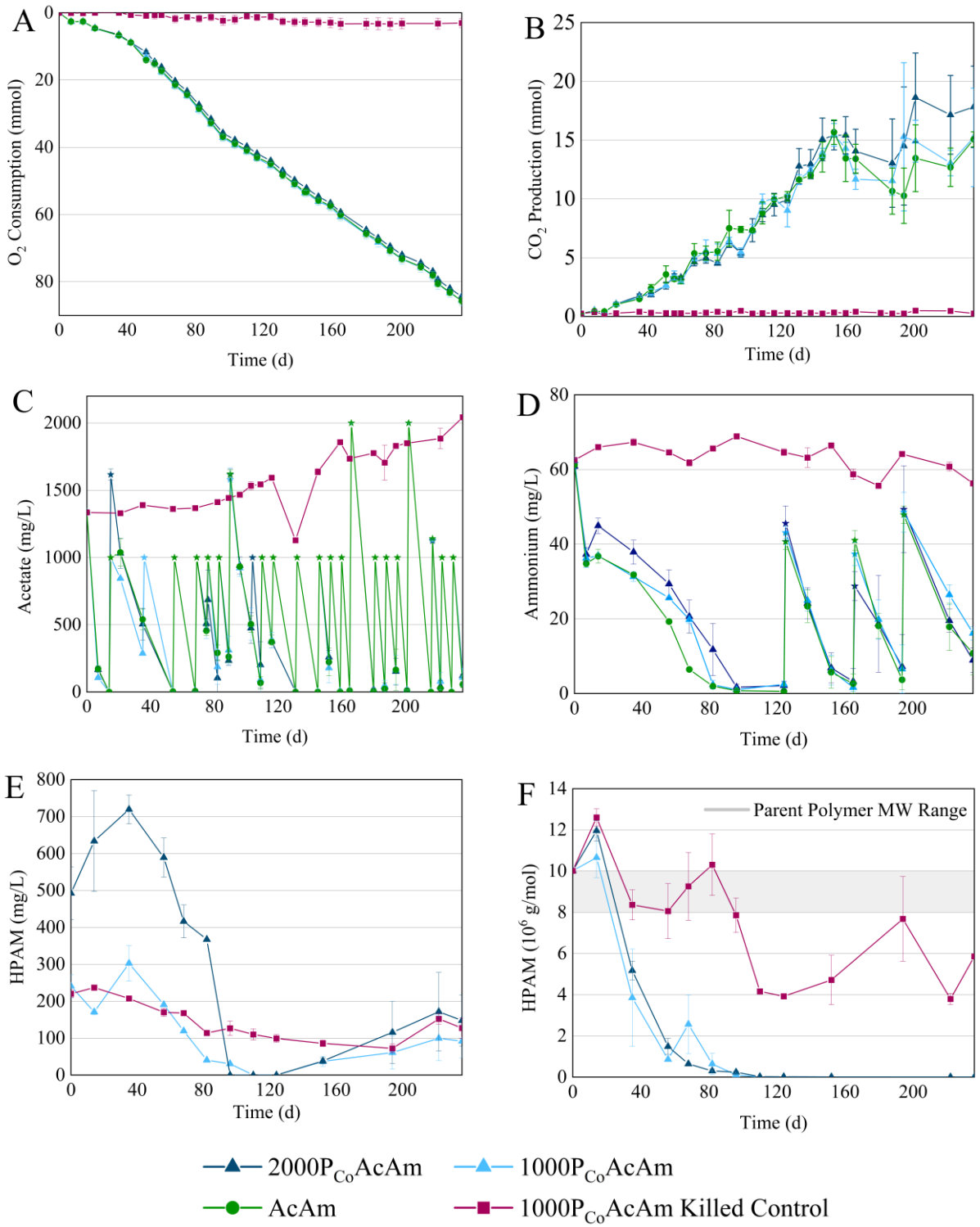


Figure 4-3 Oxic (A) O_2 consumption, (B) CO_2 production, (C) acetate concentrations, (D) ammonium concentrations, (E) HPAM pore water concentrations, and (F) HPAM molecular weights for P_{Co} treatments. Acetate and ammonium amendments are represented by stars (★). Error bars represent one standard deviation of biological replicates.

HPAM pore water concentrations were detected for the remainder of the incubation period after day 124, suggesting that a portion of HPAM is recalcitrant to biodegradation under co-metabolic conditions (Table 4-7). After day 124 two peaks were apparent. One for the parent HPAM polymer, and another for the degradation products (Appendix B, Figure B-18). Though HPAM biodegradation was evident, CO_2 production was similar among 2000P_{Co}AcAm, 1000P_{Co}AcAm, and AcAm (Table 4-6). This suggests the biodegradation of HPAM to lower molecular weight compounds, but not complete mineralization to CO_2 within the incubation period. 2000P_{Co}AcAm and 1000P_{Co}AcAm were not significantly different from AcAm ($p > 0.05$), therefore, CO_2 production was predominantly due to acetate amendments.

A decrease in pore water and molecular weight was also observed in killed controls, particularly after day 82, suggesting a portion HPAM removal or degradation can be attributed to abiotic mechanisms (Table 4-7). Microbial activity was not apparent in killed controls, indicating that sterile conditions were maintained for the incubation period. A decreased in pore water concentrations could be due to continued adsorption of HPAM to the tailings clays throughout the incubation period, while a breakdown of the carbon chain backbone (decrease in molecular weight) could be due to mechanical degradation from gyratory shaking. Mansour et al. (2014) observed 20% HPAM degradation when subjected to shear rates of 500 s^{-1}. Gyratory shaking for 236 days could possibly lead to mechanical degradation.

At the end of the incubation time, biogenic gas production, O_2 consumption, and nutrient consumption were similar among 2000P_{Co}AcAm, 1000P_{Co}AcAm, and AcAm, suggesting that the presence of HPAM did not inhibit nor enhance microbial activity when provided as a co-substrate (Table 4-6). These results however indicated that the aerobic metabolism of acetate and ammonium could potentially support HPAM biodegradation. This aligned with previous studies where the addition of glucose, yeast, or starch have been used to stimulate microorganisms for HPAM biodegradation (Matsuoka et al., 2002; Wen et al., 2010; Liu et al., 2012; Song et al., 2017). Wen et al. (2010) observed lower concentrations of glucose (100 mg/L) to be most successful for HPAM biodegradation.

Acrylamide and acrylic acid were not detected at the end of the incubation time for any of the oxic treatment, indicating that residual acrylamide was likely consumed by aerobic microorganisms. Li et al. (2023) found that residual acrylamide in the parent polymer solution was quickly consumed

by aerobic microorganisms in oil sands tailings. Acrylamide has also supported microbial growth as a carbon source in soil systems (Kay-Shoemake et al., 1998a). Biodegradation products could therefore be low molecular weight oligomers (<6000 g/mol), VFAs, or other unidentified organics.

4.4.1.2 Hydrolyzed Polyacrylamide as a Sole Nitrogen Source

To determine if HPAM could serve as a nitrogen source, acetate was provided as an additional carbon source in P_N treatments. Ammonium was monitored as an indicator for hydrolysis of the amide group in HPAM and production of hydrolytic ammonium. Results for O_2 consumption, acetate and ammonium concentrations, CO_2 production, and HPAM molecular weight and pore water concentrations are provided in Figure 4-4. Cumulative acetate consumption is provided in Appendix B, Figure B-6. O_2 and acetate were consumed without a lag phase, while CO_2 production had a 14-day lag phase for 2000P_NAc, 1000P_NAc, and Ac. Similarities between HPAM amended cultures and no-HPAM controls indicated that the presence of HPAM did not influence how aerobic microorganisms adapted to HPAM amended systems or delay microbial activity. Similar to P_{Co} treatments, acetate required weekly or biweekly amendments. Ammonium was produced after a 7-day lag phase for 2000P_NAc and 14-day lag phase for 1000P_NAc. Ammonium was also detected in 1000P_NAc Killed Control suggesting that a portion of the ammonium released may be due to abiotic mechanisms.

Ammonium concentrations peaked at day 35 for 1000P_NAc and day 124 for 2000P_NAc. A delay for 2000P_NAc may have been a result of simultaneous ammonium production and consumption by aerobic microorganisms. After day 152, ammonium concentrations were similar in HPAM amended cultures and no-HPAM controls. This could be due to complete consumption of hydrolytic ammonium released during hydrolysis of the amide group in HPAM. Liu et al. (2012) observed amide hydrolysis and the formation of hydrolytic ammonium, followed by ammonium consumption by aerobic microorganisms. Though ammonium concentrations slightly increased in 1000P_NAc Killed Control, ammonium did not fluctuate throughout the incubation time. Acetate was also not consumed in the killed controls, indicating that sterile conditions were maintained throughout the incubation period.

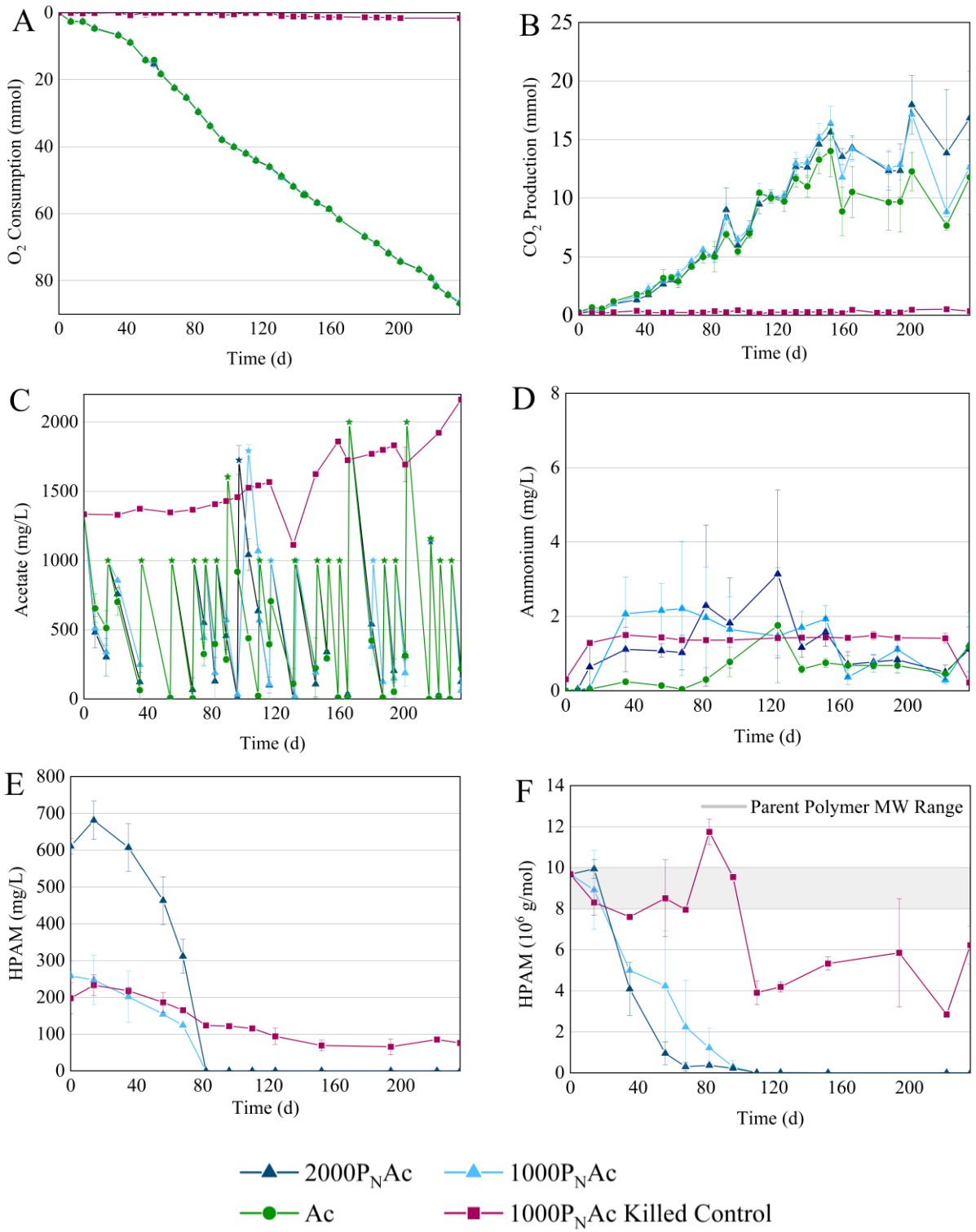


Figure 4-4 Oxic (A) O_2 consumption, (B) CO_2 production, (C) acetate concentrations, (D) ammonium concentrations, (E) HPAM pore water concentrations, and (F) HPAM molecular weights for P_N treatments. Acetate are represented by stars (★). Error bars represent one standard deviation of biological replicates.

After 236 days, acetate and O_2 consumption, and CO_2 production were similar among HPAM treated cultures and no-HPAM controls ($p > 0.05$) (Table 4-6). Aerobic microorganisms consumed acetate similarly whether HPAM was serving as a nitrogen source (2000P_NAc and 1000P_NAc) or there was no additional nitrogen in the microcosms (Ac). Comparing Ac and AcAm controls, nitrogen deficient conditions slightly decreased acetate consumption ($p = 0.04$). Activity observed in P_{Co} and P_N treatments indicated that aerobic microorganisms could consume acetate and O_2 and produce CO_2 regardless of nitrogen availability and source.

P_N treatments exhibited similar pore water and molecular weight trends to those observed for P_{Co} treatments. HPAM pore water concentration and molecular weight decrease had no lag phase for 1000P_NAc and a 14-day lag phase for 2000P_NAc. On day 82, pore water concentrations were below the detection limit for 2000P_NAc and 1000P_NAc. Molecular weights were below the column separation limit on day 152 for 2000P_NAc and day 110 for 1000P_NAc. In contrast to P_{Co} treatments, the parent HPAM polymer was not detected in the pore water after column replacement. Therefore, when HPAM was provided as the sole nitrogen source, there was 100% removal of the parent polymer in the pore water. Based on the separation limit of the SEC column, biodegradation products would be organics below 6000 g/mol. Following biodegradation, both P_{Co} and P_N exhibited removal of the flocculated tailings at the bottom of the microcosm and resuspension of tailings particles (Appendix B, Figure B-4). This suggests that HPAM biodegradation could impact tailings settlement and consolidation. 1000P_NAc Killed Control exhibited molecular weight and pore water concentration decreases, suggesting degradation due to abiotic mechanisms (described in subsection 4.4.1.1). Molecular weight primarily decreased in 1000P_NAc Killed Control after day 96. Considering changes in molecular weight and pore water concentrations for live and killed controls, biodegradation was likely the primary mechanisms prior to day 96, while abiotic degradation occurred later in the incubation period.

Release of ammonium indicated that HPAM could serve as a nitrogen source through hydrolysis of the amide group, however, did not increase microbial activity in regard to biogenic gas production. Similar to P_{Co} treatments, acrylamide and acrylic acid were not detected at the end of the incubation period. Aerobic microorganisms are capable of consuming acrylamide, so even if acrylamide was released during HPAM biodegradation, it could have served as an additional carbon source and quickly been depleted. If acrylamide was serving as a carbon source through

mineralization, CO_2 production in HPAM amended cultures would have been greater. CO_2 production in HPAM amended cultures were not significantly greater than no-HPAM controls ($p > 0.05$), suggesting that either acrylamide was not a biodegradation product of HPAM, or if it was produced it was being converted to another compound and not mineralized.

4.4.1.3 Hydrolyzed Polyacrylamide as a Sole Carbon Source

To determine if HPAM could be used as a carbon source, ammonium was provided as an additional nitrogen source for P_C treatments. Results for O_2, ammonium concentrations, CO_2 production, and HPAM molecular weight and pore water concentrations are provided in Figure 4-5. Cumulative ammonium consumption is provided in Appendix B, Figure B-5. 2000P_CAm, 1000P_CAm, and Am showed a gradual increase in O_2 consumption and CO_2 production, however, these values were less than P_{Co} and P_N treatments. Ammonium concentrations increased in 2000P_CAm and 1000P_CAm on day 7, likely due to hydrolysis of the amide group, while ammonium concentrations in Am decreased. On day 14 however, ammonium for 2000P_CAm, 1000P_CAm, and Am exhibited similar concentrations, and followed similar depletion patterns afterwards.

Initial ammonium was completely depleted in 2000P_CAm on day 138, and day 152 for 1000P_CAm and Am, after which reamendments were required every 2 weeks. Similarities in ammonium consumption between HPAM amended and no-HPAM cultures suggested that the presence of HPAM as the sole carbon source did not influence ammonium consumption (Table 4-6). Furthermore, it indicated that microbial communities could adapt to a carbon limited environment regardless of HPAM amendments. Once ammonium levels were depleted, P_C treatments required more frequent amendments compared to P_{Co} treatments. Since P_C treatments are carbon deficient, the microbial community may have shifted to more nitrogen dependent microorganisms, resulting in greater ammonium demand.

Compared to P_{Co} and P_N treatments, P_C exhibited less degradation (Table 4-7). At the end of the incubation period, the decrease in molecular weight exhibited in 2000P_CAm and 1000P_CAm was not significantly different from 1000P_CAm Killed Control ($p > 0.05$), indicating that biodegradation likely did not occur. A decrease in molecular weight was likely due to abiotic mechanisms, as was observed in P_{Co} and P_N killed controls, while a decrease in pore water concentrations could be due to continued adsorption of HPAM

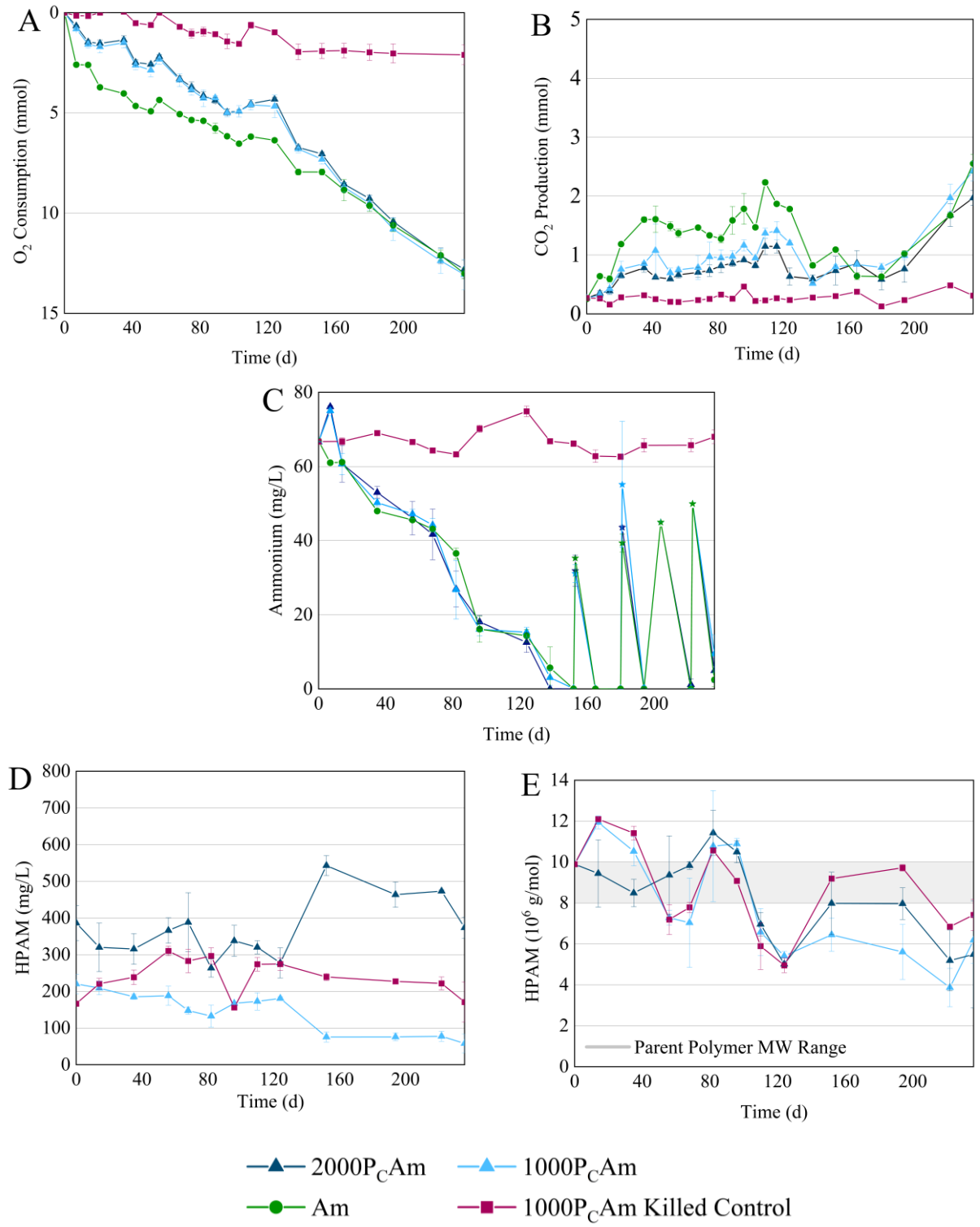


Figure 4-5 Oxic (A) O_2 consumption, (B) CO_2 production, (C) ammonium concentrations, (D) HPAM pore water concentrations, and (E) HPAM molecular weights for P_C treatments. Ammonium amendments are represented by stars (★). Error bars represent one standard deviation of biological replicates.

Ammonium and molecular weight results suggested hydrolysis of the amide group but not biodegradation of the carbon chain. CO_2 production was likely a result of mineralization of residual organics (petroleum hydrocarbon, naphthenic acids, etc.) in the fine tailings due to similar cumulative production between HPAM amended cultures and no-HPAM controls (Table 4-6). O_2 and CO_2 levels also suggested that HPAM amended as a carbon source did not enhance microbial activity. Similar results were obtained by Berdugo-Clavijo et al. (2019) where cultures amended with PAM and HPAM as the sole carbon source did not enhance CO_2 production compared to no-HPAM controls.

4.4.1.4 Hydrolyzed Polyacrylamide as a Sole Nitrogen and Carbon Source

When HPAM was provided at the sole nitrogen and carbon source, O_2 consumption and CO_2 production for $2000P_{N+C}$ and $1000PN_{+C}$ were significantly different from $1000P_{N+C}$ Killed Control (O_2 $p = 0.007$ for $2000P_{N+C}$ and 0.03 for $1000P_{N+C}$; CO_2 $p = 0.002$ for $2000P_{N+C}$ and 0.0009 $1000P_{N+C}$). For No Amendment though, these were not significantly different than the killed control ($p = 0.1$ for O_2 and 0.05 for CO_2). Differences between HPAM amended and no-HPAM controls suggest that under nitrogen and carbon limited conditions, HPAM could potentially stimulate microbial activity. Results for O_2 consumption, ammonium concentrations, CO_2 production, and HPAM molecular weight and pore water concentrations are provided in Figure 4-6.

An increase in ammonium concentrations on day 7 for $2000P_{N+C}$ and $1000PN_{+C}$ also suggested the potential for hydrolysis of the amide group. After day 7 though, ammonium concentrations decreased and followed a similar trend to No Amendment. Comparing ammonium fluctuations for P_N and P_{N+C} treatments, P_N treatments appeared to gradually increase throughout the incubation time until reaching a peak and decreasing. P_{N+C} treatments only spiked at the beginning and exhibited no fluctuations for the remainder of the incubation period. Microorganisms may have been active in the beginning due to residual carbon sources in fine tailings. However, limited nutrient availability may have prevented continued production of hydrolytic ammonium.

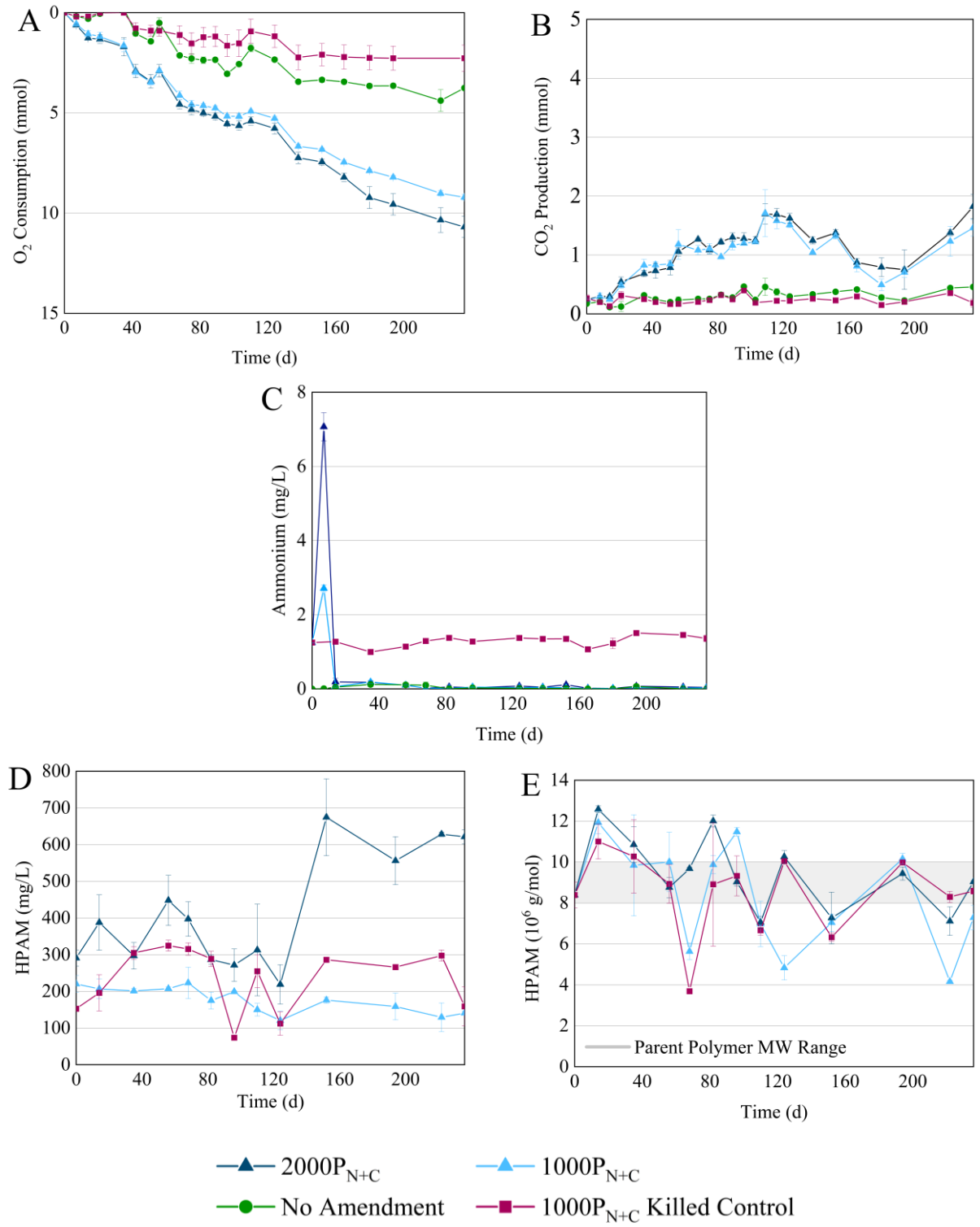


Figure 4-6 Oxic (A) O_2 consumption, (B) CO_2 production, (C) ammonium concentrations, (D) HPAM pore water concentrations, and (E) HPAM molecular weights for P_{N+C} treatments. Error bars represent one standard deviation of biological replicates.

For P_{N+C} treatments, limited HPAM biodegradation was observed (Table 4-7). Pore water concentrations only decreased in $1000P_{N+C}$, while molecular weight for HPAM amended cultures appeared to mirror killed controls throughout the incubation period. Since a decrease in molecular weight was not significant, microbial activity in HPAM amended cultures was likely due to HPAM serving a nitrogen source but not a carbon source. The degradation results from each treatment suggest that aerobic biodegradation of the carbon chain within 236 days was not possible without acetate amendments.

4.4.2 Microbial Activity in the Presence of Polyacrylamide and Biodegradation Under Sulfate-reducing Conditions

Sulfate-reducing conditions were monitored for sulfate, acetate, ammonium, and CO_2 over 420 days to determine the use of HPAM as a nutrient source for microbial activity. CH_4 was measured on day 0 and 420. Cumulative results are provided in Table 4-8.

Table 4-8 Cumulative terminal electron acceptors and nutrients consumed and biogenic gas production for sulfate-reducing conditions. Results are presented as the mean of biological replicates (2 or 3) ± one standard deviation.

Treatment	Level	Sulfate Reduction (mmol)	Acetate Consumption (mmol)	CO_2 Production (mmol)	CH_4 Production (mmol)
HPAM as Co-Substrate (P_{Co})	$2000P_{Co}$AcAm	5.63 ± 0.96	13.64 ± 0.96	0.28 ± 0.02	2.21 ± 0.34
	$1000P_{Co}$AcAm	5.51 ± 0.11	17.97 ± 1.45	0.31 ± 0.03	4.46 ± 1.79
	AcAm	7.71 ± 0.79	18.29 ± 3.05	0.29 ± 0.03	3.30 ± 0.31
	$1000P_{Co}$AcAm Killed Control	0.84 ± 0.00	1.41 ± 0.07	0.21 ± 0.03	0.10 ± 0.00
HPAM as Nitrogen Source (P_N)	$2000P_N$Ac	7.65 ± 0.50	16.91 ± 3.00	0.23 ± 0.02	2.32 ± 0.28
	$1000P_N$Ac	7.55 ± 0.08	18.44 ± 0.98	0.23 ± 0.03	1.98 ± 0.42
	Ac	3.60 ± 0.10	6.74 ± 0.20	0.17 ± 0.01	0.43 ± 0.17
	$1000P_N$Ac Killed Control	0.12 ± 0.00	2.81 ± 1.40	0.19 ± 0.02	0.10 ± 0.00
HPAM as Carbon Source (P_C)	$2000P_C$Am	0.01 ± 0.01		0.24 ± 0.04	0.09 ± 0.00
	$1000P_C$Am	0.04 ± 0.1		0.25 ± 0.00	0.09 ± 0.00
	Am	0.08 ± 0.00		0.29 ± 0.03	0.09 ± 0.00
	$1000P_C$Am Killed Control	0.07 ± 0.02		0.17 ± 0.02	0.09 ± 0.00
HPAM as Nitrogen & Carbon Source (P_{N+C})	$2000P_{N+C}$	-0.03 ± 0.06		0.23 ± 0.05	0.09 ± 0.00
	$1000P_{N+C}$	0.05 ± 0.5		0.29 ± 0.08	0.10 ± 0.00
	No Amendment	0.00 ± 0.02		0.22 ± 0.01	0.13 ± 0.04
	$1000P_{N+C}$ Killed Control	-0.04 ± 0.01		0.13 ± 0.02	0.09 ± 0.00

HPAM pore water concentration and molecular weight were measured biweekly or monthly to monitor biodegradation. Due to HPAM concentrations below the detection limit, microcosms amended to 400 mg HPAM/L tailings were increased to 2000 mg HPAM/L tailings on day 168. HPAM in microcosms amended with 2000 mg HPAM/L tailings were therefore only measured for 238 days. Results for % degradation are provided in Table 4-9.

Table 4-9 HPAM pore water removal and degradation under sulfate-reducing conditions.

Treatment	Level	Pore Water Removal (%)	Degradation (%)
HPAM as Co-Substrate (P_{Co})	2000P_{Co}AcAm	62[a]	22[a]
	1000P_{Co}AcAm	91[b]	77[b]
	1000P_{Co}AcAm Killed Control	65[b]	22[b]
HPAM as Nitrogen Source (P_N)	2000P_NAc	16[a]	2[a]
	1000P_NAc	79[b]	33[b]
	1000P_NAc Killed Control	62[b]	0
HPAM as Carbon Source (P_C)	2000P_CAm[a]	17[a]	10[a]
	1000P_CAm	98[b]	55[b]
	1000P_CAm Killed Control	85[b]	0
HPAM as Nitrogen & Carbon Source (P_{N+C})	2000P_{N+C}	35[a]	0
	1000P_{N+C}	98[b]	45[b]
	1000P_{N+C} Killed Control	63[b]	0

Note. Pore water removal was calculated from the parent polymer pore water concentrations. Degradation was calculated from the decrease in molecular weight from the parent polymer to oligomers.
[a]238-day incubation period
[b]420-day incubation period

4.4.2.1 Hydrolyzed Polyacrylamide as a Co-Substrate

To monitor activity from SRB, sulfate consumption and CO_2 production were measured biweekly or monthly over 420 days, while CH_4 production was measured at the end of the incubation period. Microcosms were flushed with helium prior to incubation, therefore initial CO_2 and CH_4 were <0.2 mmol. Results for CO_2 production, sulfate reduction, acetate and ammonium concentrations, and HPAM pore water concentrations and molecular weight for P_{Co} treatments are presented in Figure 4-7. Cumulative acetate consumption is provided in Appendix B, Figure B-7.

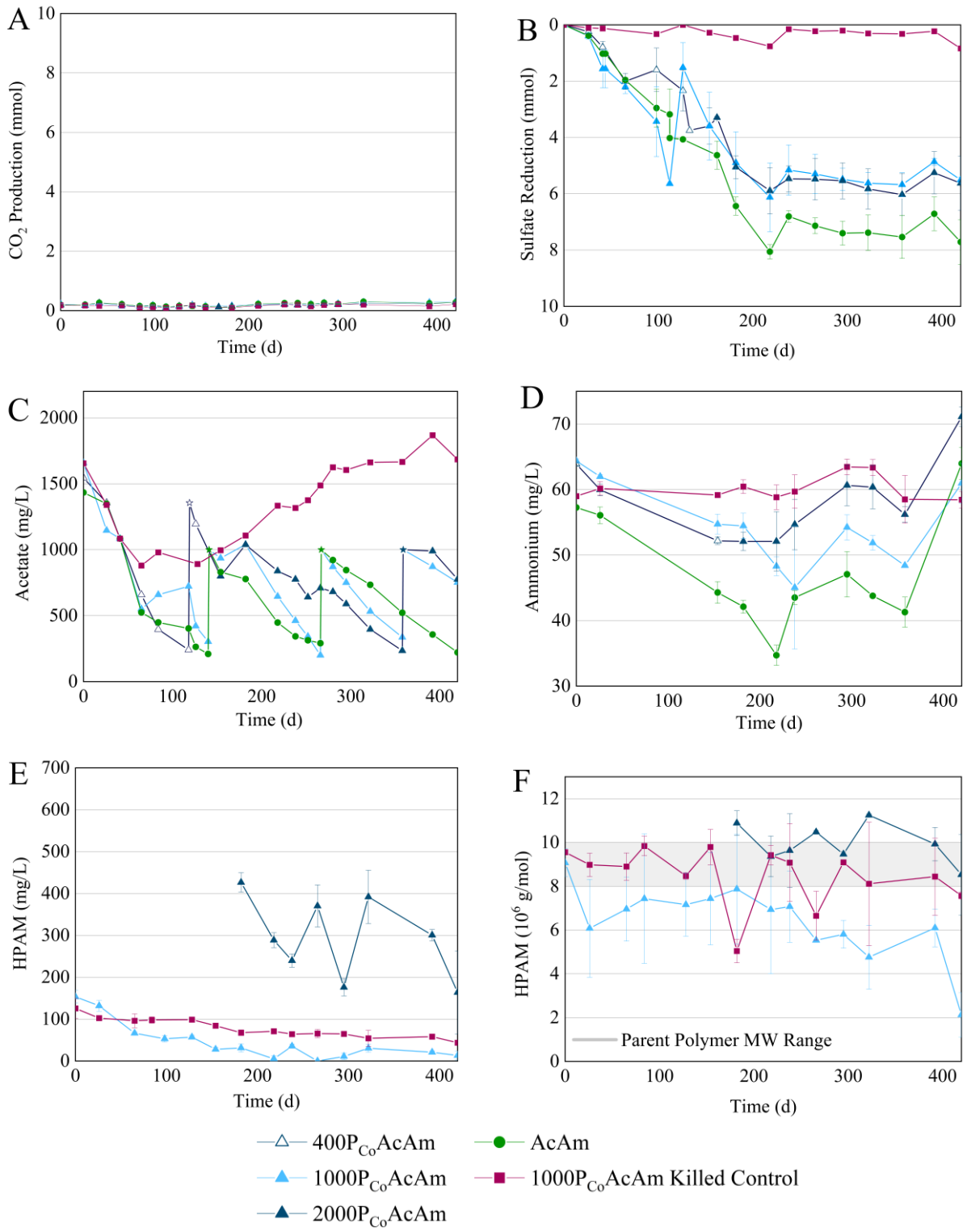


Figure 4-7 (A) CO_2 production, (B) sulfate reduction, (C) acetate concentrations, (D) ammonium concentrations, (E) HPAM pore water concentrations, and (F) HPAM molecular weights for P_{Co} treatments under sulfate-reducing conditions. Error bars represent one standard deviation of biological replicates. Acetate amendments are represented by stars (★). Acetate data points represent single measurements.

At the end of 420 days, CO_2 production in live microcosms did not differ from killed controls for all treatments. However, CH_4 production was observed in 2000P_{Co}AcAm, 1000P_{Co}AcAm, and AcAm (Table 4-8). In the presence of sulfate, acetate will be preferentially oxidized to CO_2 over fermentation by acetoclastic methanogens (Plante et al., 2015). Therefore, CO_2 production will often be higher than CH_4. The presence of sulfate and activity by SRB has frequently been shown to inhibit methanogenesis in oil sands tailings (Holowenko et al., 2000; Ramos-Padrón et al., 2011). Based on this relationship between SRB and acetoclastic methanogens, it was surprising that CO_2 production was low in P_{Co} treatments. Low CO_2 production could be explained by the conversion of CO_2 to CH_4 by hydrogenotrophic methanogens (Siddique et al., 2011). In addition to conversion to CH_4, low CO_2 level might be due to dissolution in tailings pore water, resulting in the formation of bicarbonates (Arkell et al., 2015; Stasik et al., 2016). The pH did not drop significantly indicating that either CO_2 did not dissolve in the pore water, or the presence of a buffer in the media maintained the pH (Appendix B, Figure B-10).

Sulfate was amended as the terminal electron acceptor, and acetate and ammonium as the additional carbon and nitrogen sources. Sulfate reduction occurred after a 41-day lag phase for 2000P_{Co}AcAm, 1000P_{Co}AcAm, and AcAm. Indicators of sulfate reduction included a sulfur smell and the formation of black iron sulfide precipitate (Figure 4-8). Acetate consumption occurred with a 41-day lag phase for 1000P_{Co}AcAm, and a 65-day lag phase for 400P_{Co}AcAm (later increased to 2000P_{Co}AcAm) and AcAm. This suggests that the presence of HPAM as a co-substrate did not influence the lag phase for sulfate reduction or acetate consumption.

Conversely, a lag phase was not observed for a decrease in ammonium concentration, which decreased until day 218 for 2000P_{Co}AcAm and AcAm, and day 238 for 1000P_{Co}AcAm. After these depletion periods, an increase in ammonium concentrations was observed possibly due to hydrolysis of the HPAM amide group, however, ammonium also increased in AcAm suggesting fluctuations may not be entirely related to HPAM. After 400P_{Co}AcAm was amended to 2000P_{Co}AcAm, ammonium concentrations increased, suggesting the potential for HPAM to serve as nitrogen source when provided as a co-substrate.

Figure 4-8 Sulfate-reducing microcosms after 6 months of incubation. Tailings flocculation (bottom layer) is from the addition of HPAM. The dark colour is from the formation of sulfide precipitate.

Molecular weight and pore water concentration were monitored for 420 days in 1000P_{Co}AcAm and 1000P_{Co}AcAm Killed Control and 238 days in 2000P_{Co}AcAm. No lag phase was observed for pore water concentration and molecular weight decrease. 1000P_{Co}AcAm molecular weight after 420 days was significantly lower ($p = 0.007$) than 1000P_{Co}AcAm Killed Control, suggesting that biodegradation did occur. Molecular weight for 2000P_{Co}AcAm (day 238) was not significantly different ($p > 0.05$) when compared to molecular weight for 1000P_{Co}AcAm Killed Control (day 236), therefore biodegradation likely did not occur within the measured timeframe. A direct comparison for pore water concentrations cannot be made since initial concentrations were different. A decreased in pore water concentration for 2000P_{Co}AcAm was of greater magnitude than 1000P_{Co}AcAm Killed Control, therefore, % removal in 2000P_{Co}AcAm was possibly due to abiotic and biotic mechanisms (Table 4-9). Based on molecular weight data for 2000P_{Co}AcAm and 1000P_{Co}AcAm, biodegradation of HPAM as a co-substrate within 238 days was minimal for sulfate-reducing conditions. However, within 420 days biodegradation was possible. Though HPAM pore water concentrations were below the detection limit for 400P_{Co}AcAm and molecular weight could not be measured, this does not necessarily mean that biodegradation was not occurring to the HPAM adsorbed to the tailings solids. This assumption could also be applied to 400P_{Co}AcAm and 400P_{N}Ac treatments under methanogenic conditions (subsections 4.4.3.1 and

4.4.3.2). Microbial activity in microcosms amended with 400 mg/L suggested that HPAM adsorbed to tailings solids were accessible by anaerobic microorganisms.

P_{Co} was the only treatment where acrylamide was detected at the end of the incubation period. Initial acrylamide concentrations were 272 µg/L in 1000P_{Co}AcAm and 537 µg/L in 2000P_{Co}AcAm. After 420 days, acrylamide was depleted to 201.3 ± 21.9 µg/L in 1000P_{Co}AcAm and 270.4 ± 5.6 µg/L. The presence of acrylamide could be due to its release during HPAM biodegradation, or limited use of acrylamide as a carbon source due to preferential utilization of acetate. Akbar et al. (2020) observed an increase in acrylamide from 1.8 to 7.2 mg/L after 15 days of incubation, while other anaerobic studies have not detected acrylamide accumulation (Dai et al., 2014; Sang et al., 2015; Zhao et al., 2019). The reasoning for acrylamide detected in 1000P_{Co}AcAm and 2000P_{Co}AcAm cannot be determined since acrylamide was not monitored throughout the incubation time. However, it is apparent that after 420 days acrylamide concentrations decreased for P_{Co} treatments under sulfate-reducing conditions.

4.4.2.2 Hydrolyzed Polyacrylamide as a Sole Nitrogen Source

P_N treatments were amended with HPAM as the sole nitrogen source and acetate as an additional carbon source. Similar to P_{Co} treatments, P_N treatments exhibited higher CH_4 production compared to CO_2. This treatment differed though in that CH_4 production was higher in HPAM amended cultures compared to no-HPAM controls. Greater microbial activity suggested that the presence of HPAM as a nitrogen source stimulated anaerobic activity. Results for CO_2 production, sulfate reduction, acetate and ammonium concentrations, and HPAM pore water concentrations and molecular weight are presented in Figure 4-9. Cumulative acetate consumption is provided in Appendix B, Figure B-7.

The presence of air bubbles within the flocculated tailings suggested the formation of CO_2, CH_4, or H_2S from sulfate reduction (Appendix B, Figure B-3). Sulfate reduction occurred after a 41-day lag phase for 400P_NAc and 1000P_NAc, and 133-day lag phase for Am. Acetate consumption was in concurrence with sulfate reduction and methane production, in that the lag phase and total consumption differed between HPAM amended and no-HPAM cultures. Compared to 1000P_NAc Killed Control, acetate consumption occurred with a 65-day and 118-day lag phase for 1000P_NAc and 400P_NAc (later increased to 2000P_NAc). Acetate consumption for Ac had a 126-day lag phase in only one of the duplicates, and after this duplicate was amended it did not exhibit further acetate

consumption until day 392. The other Ac duplicate had a 322-day lag phase (data not shown). Therefore, microbial activity in Ac was minimal. The difference in lag phase between HPAM amended and no-HPAM cultures suggests that the presence of HPAM as the sole nitrogen source decreased the time in which anaerobic activity could occur. These results align with previous studies that observed HPAM to support anaerobic activity when provided as a nitrogen source (Grula et al., 1994; Ma et al., 2008; Hu et al., 2018).

HPAM pore water concentrations decreased without a lag phase for 2000P_NAc and 1000P_NAc. Decreases in pore water concentrations for 1000P_NAc Killed Control suggests that pore water removal in 1000P_NAc could be a result of biotic and abiotic mechanisms (Table 4-9). For molecular weight, 1000P_NAc decreased without a lag phase, while molecular weight decrease was not apparent for 2000P_NAc. Similar to P_{Co} treatments, biodegradation of the carbon chain was not apparent within a 238-day incubation time. For all sulfate-reducing treatments that received 2000 mg HPAM/L tailings, molecular weight did not decrease below the parent polymer molecular weight range. Therefore, higher concentration of HPAM may require longer incubations time to demonstrate biodegradation for sulfate-reducing conditions. Though the molecular weight of 1000P_NAc was less than 1000P_NAc Killed Control after 420 days, this difference was not statistically significant (*$p = 0.05$)*. It is therefore inconclusive whether biodegradation of the carbon chain was possible when HPAM was provided as the sole nitrogen source. Microbial utilization of HPAM as a nitrogen source would not be reflected in the SEC results. Ammonium production suggested the potential for HPAM as a nitrogen source through hydrolysis of the amide group. Ammonium production occurred after a 154-day lag phase for 2000P_NAc, while 1000P_NAc had no lag phase. In contrast to oxic conditions, ammonium gradually accumulated in the sulfate-reducing microcosms.

These results suggest that under nitrogen limited conditions (Ac), anaerobic activity was delayed. However, when provided with HPAM as the sole nitrogen source, anaerobes under sulfate-reducing conditions were capable of acetate consumption. This indicates the potential for HPAM as a nitrogen source to stimulate SRB or other microorganisms under sulfate-reducing conditions. When compared to P_N treated under oxic conditions, nitrogen limited conditions (Ac) appeared to have a greater impact on anaerobic activity. HPAM as a nitrogen source was therefore more important for anaerobic activity compared to aerobics.

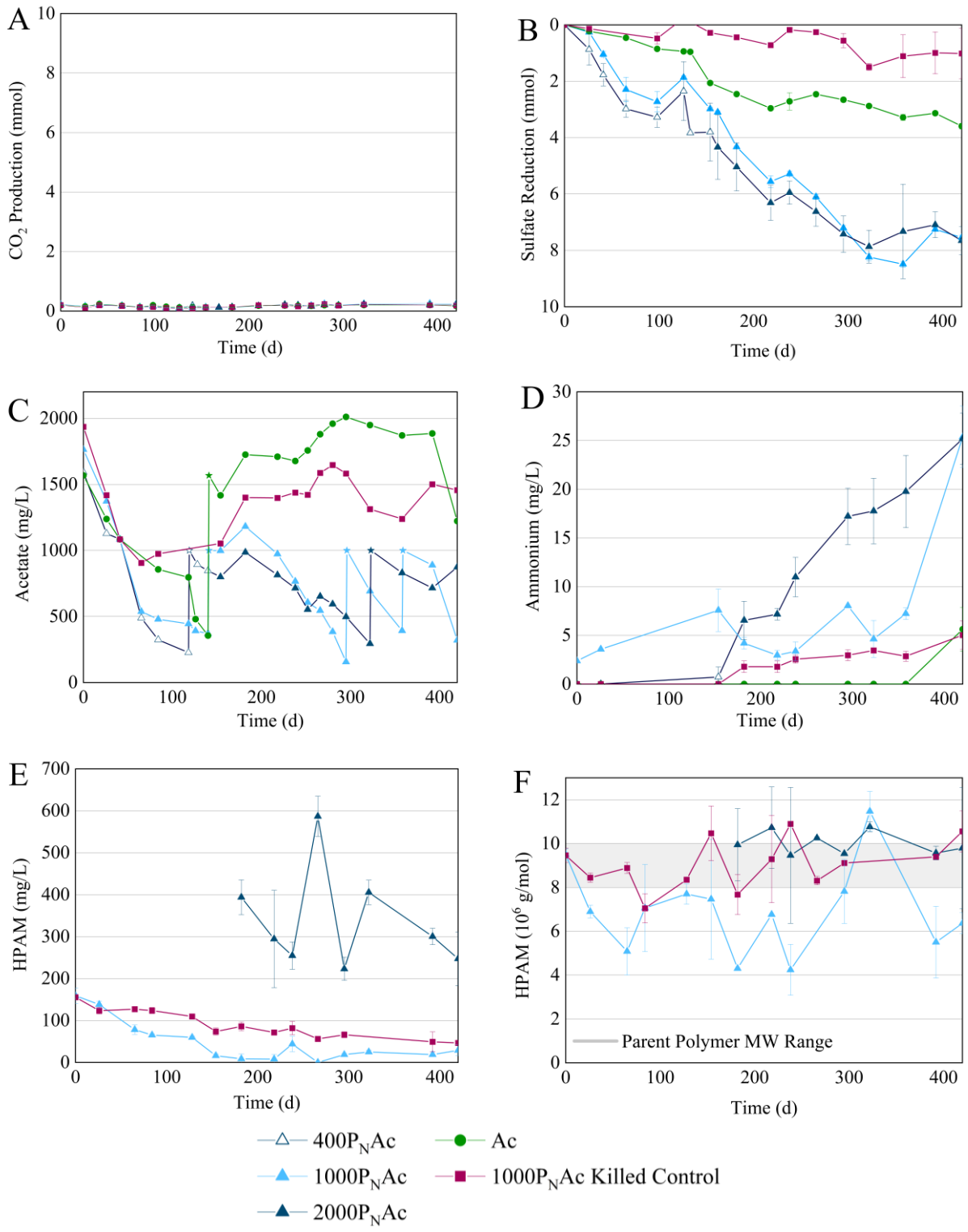


Figure 4-9 (A) CO_2 production, (B) sulfate reduction, (C) acetate concentrations, (D) ammonium concentrations, (E) HPAM pore water concentrations, and (F) HPAM molecular weights for P_N treatments under sulfate-reducing conditions. Error bars represent one standard deviation of biological replicates. Acetate amendments are represented by stars (★). Acetate data points represent single measurements.

4.4.2.3 Hydrolyzed Polyacrylamide as a Sole Carbon Source

For P_C, CO_2 and CH_4 production in live microcosms did not differ from killed controls, suggesting that HPAM could not serve as a sole carbon source to stimulate anerobic activity (Table 4-8). This also indicated that CH_4 produced in the P_{Co} and P_N treatments was not a result of HPAM mineralization, but acetate. Sulfate reduction was also not apparent in 2000P_CAm, 1000P_CAm, or Am. Results for CO_2 production, sulfate reduction, ammonium concentrations, and HPAM pore water concentrations and molecular weight are presented in Figure 4-10.

2000P_CAm and 1000P_CAm did not exhibit initial increases in ammonium as was observed for P_C treatments under oxic conditions. Ammonium concentrations were consistent for 1000P_CAm and 2000P_CAm throughout the incubation period, with any concentration fluctuations mirroring 1000P_CAm Killed Control. 2000P_CAm, 1000P_CAm, and Am did not exhibit biogenic gas production or sulfate reduction, suggesting that fluctuations in ammonium concentrations were likely not a result of microbial activity. Limited microbial activity suggests that a decrease in HPAM pore water concentrations was likely due to abiotic mechanisms. Though the molecular weight for 1000P_CAm appeared to decrease, this was not significantly different from 1000P_CAm Killed Control ($p > 0.05$) (Table 4-9). These results reinforce the conclusion that HPAM could not serve as a carbon source under sulfate-reducing conditions, and that biodegradation of HPAM was not apparent under carbon limited conditions.

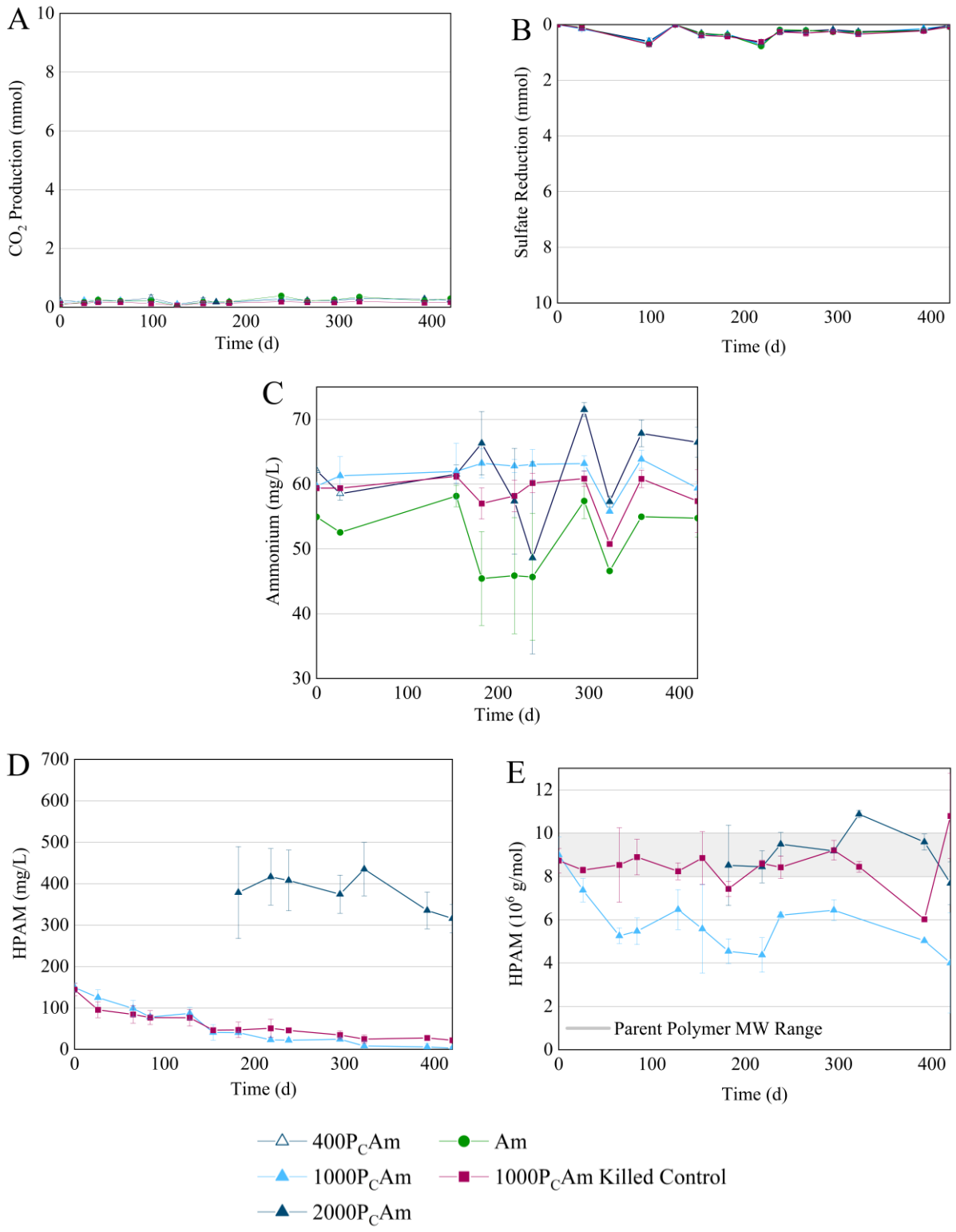


Figure 4-10 (A) CO_2 production, (B) sulfate reduction, (C) ammonium concentrations, (D) HPAM pore water concentrations, and (E) HPAM molecular weights for P_C treatments under sulfate-reducing conditions. Error bars represent one standard deviation of biological replicates.

4.4.2.4 Hydrolyzed Polyacrylamide as a Sole Nitrogen and Carbon Source

For P_{N+C} treatments, results for CO_2 production, sulfate reduction, ammonium concentrations, and HPAM pore water concentrations and molecular weight are presented in Figure 4-11. P_{N+C} treatments appeared biologically inactive based on biogenic gas production (Table 4-8). Conversely, ammonium concentrations increased with a 154-day lag phase for $2000P_{N+C}$ and no lag phase for $1000P_{N+C}$. Microorganisms could therefore be present; however, their activity was not apparent compared to other treatments. The parameters selected for monitoring and confirming microbial activity may have also been unable to capture activity occurring in these treatments. $1000P_{N+C}$ exhibited a decrease in pore water concentration and molecular weight after 420 days. Molecular weight after 420 days was significantly different from $1000P_{N+C}$ Killed Control ($p = 0.04$), however, based on limited microbial activity, degradation due to biological mechanism was inconclusive.

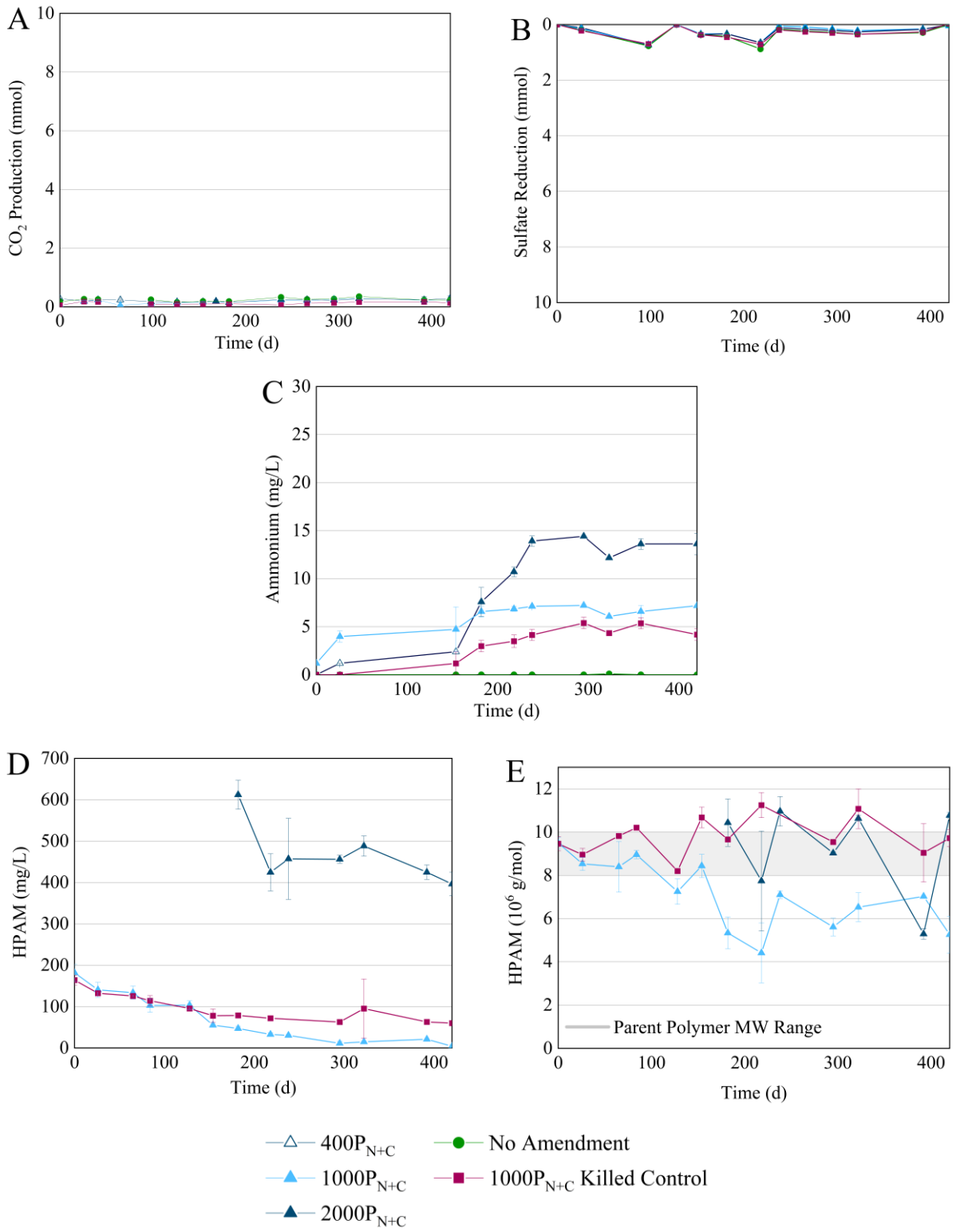


Figure 4-11 (A) CO_2 production, (B) sulfate reduction, (C) ammonium concentrations, (D) HPAM pore water concentrations, (E) HPAM molecular weights for P_{N+C} treatments under sulfate-reducing conditions. Error bars represent one standard deviation of biological replicates.

4.4.3 Microbial Activity in the Presence of Polyacrylamide and Biodegradation Under Methanogenic Conditions

Methanogenic conditions were monitored for acetate, ammonium, and CH_4 over 374 days to determine the use of HPAM as a nutrient source for microbial activity. CO_2 was measured on day 0 and 374. Cumulative results are provided in Table 4-10.

Table 4-10 Cumulative acetate consumption and biogenic gas production for methanogenic conditions. Results are presented as the mean of biological replicates (2 or 3) ± one standard deviation.

Treatment	Level	Acetate Consumption (mmol)	CH_4 Production (mmol)	CO_2 Production (mmol)
HPAM as Co-Substrate (P_{Co})	2000P_{Co}AcAm	64.27 (± 1.71)	33.67 (± 3.40)	0.90 (± 0.06)
	1000P_{Co}AcAm	65.40 (± 4.27)	38.23 (± 4.84)	1.12 (± 0.01)
	AcAm	72.24 (± 0.85)	44.73 (± 3.27)	0.94 (± 0.03)
	1000P_{Co}AcAm Killed Control	1.07 (± 0.00)	2.21 (± 2.05)	0.32 (± 0.00)
HPAM as Nitrogen Source (P_N)	2000P_NAc	43.66 (± 1.97)	24.92 (± 2.59)	0.73 (± 0.17)
	1000P_NAc	44.02 (± 0.66)	22.25 (± 1.41)	0.65 (± 0.20)
	Ac	19.47 (± 0.25)	6.44 (± 1.69)	0.82 (± 0.11)
	1000P_NAc Killed Control	2.56 (± 0.00)	1.21 (± 1.03)	0.82 (± 0.00)
HPAM as Carbon Source (P_C)	2000P_CAm		0.24 (± 0.00)	
	1000P_CAm		0.24 (± 0.00)	
	Am		0.24 (± 0.00)	
	1000P_CAm Killed Control		0.20 (± 0.00)	
HPAM as Nitrogen & Carbon Source (P_{N+C})	2000P_{N+C}		0.24 (± 0.00)	
	1000P_{N+C}		0.75 (± 0.72)	
	No Amendment		0.23 (± 0.00)	
	1000P_{N+C} Killed Control		0.20 (± 0.00)	

HPAM pore water concentration and molecular weight were measured biweekly or monthly to monitor biodegradation. Due to HPAM concentrations below the detection limit, microcosms amended to 400 mg HPAM/L tailings were increased to 2000 mg HPAM/L tailings on day 247. Killed controls were also only amended to 400 mg HPAM/L tailings initially, therefore concentrations were increased to 1000 mg HPAM/L to align with other redox conditions. HPAM in microcosms amended with 2000 mg HPAM/L tailings and killed controls were therefore only measured for 115 days. Results for % degradation are provided in Table 4-11.

Table 4-11 HPAM pore water removal and degradation under methanogenic conditions.

Treatment	Level	Pore Water Removal (%)	Degradation (%)
HPAM as Co-Substrate (P_{Co})	$2000P_{Co}AcAm$	46[a]	0
	$1000P_{Co}AcAm$	73[b]	60[b]
	$1000P_{Co}AcAm$ Killed Control	43[a]	6.49[a]
HPAM as Nitrogen Source (P_N)	$2000P_NAc$	41[a]	0
	$1000P_NAc$	53[b]	60[b]
	$1000P_NAc$ Killed Control	20[a]	0
HPAM as Carbon Source (P_C)	$2000P_CAm$	33[a]	0
	$1000P_CAm$	54[b]	34[b]
	$1000P_CAm$ Killed Control	42[a]	0
HPAM as Nitrogen & Carbon Source (P_{N+C})	$2000P_{N+C}$	8[a]	5[a]
	$1000P_{N+C}$	23[b]	31[b]
	$1000P_{N+C}$ Killed Control	32[a]	0

Note. Pore water removal was calculated from the parent polymer pore water concentrations. Degradation was calculated from the decrease in molecular weight from the parent polymer to oligomers.
[a]115-day incubation period
[b]374-day incubation period

4.4.3.1 Hydrolyzed Polyacrylamide as a Co-Substrate

Acetate consumption and methane production were used as indicators for methanogenesis. Results for CH_4 production, acetate and ammonium concentrations, and HPAM pore water concentrations and molecular weight for P_{Co} treatments are presented in Figure 4-12. Cumulative acetate consumption is provided in Appendix B, Figure B-8. Methanogenesis and acetate consumption occurred with a 28-day lag phase for $2000P_{Co}AcAm$, $1000P_{Co}AcAm$, and AcAm. Microcosms with biogenic gas production are presented in Figure 4-13. Methanogenesis and acetate consumption was significantly different for AcAm compared to $2000P_{Co}AcAm$ ($p = 0.02$ for CH_4 and $p = 0.01$ for acetate), but not for $1000P_{Co}AcAm$ ($p > 0.05$), suggesting the presences of HPAM could possibly limit microbial activity. Low CO_2 level could be due to CO_2 consumption by *Methanosarcinaceae* (acetoclastic and hydrogenotrophic methanogen) and other chemolithotrophs (Kendall and Boone, 2006). CO_2 has been identified as a significant methanogenic substrate in oil sands tailings (Stasik et al., 2016). Compared to sulfate-reducing conditions, methane production

was much greater for methanogenic P_{Co} and P_N treatments, reinforcing that sulfate inhibits methanogenesis.

Both HPAM and no-HPAM controls exhibited similar acetate consumption rates (Figure 4-12 B). Methanogenic microcosms consumed over 3 times more acetate than sulfate-reducing microcosms. These results suggested that chemoorganotrophs such as acetoclastic methanogens may be present in these cultures, as reflected in the microbial data (subsection 4.4.5.3). Acetoclastic methanogens, such as *Methanosaetaceae* and *Methanosarcinaceae* have been associated with the biodegradation of BTEX, long chain *n*-alkanes, *iso*-alkanes, and naphtha and paraffinic solvents in oil sands tailings (Mohamad Shahimin and Siddique, 2017a, 2017b; Siddique et al., 2011, 2012, 2019). Methane production was higher in AcAm microcosms compared to HPAM amended cultures, suggesting HPAM as a co-substrate did not increase methanogenic activity. High acetate concentrations (1000 mg/L in our study) may result in preferential consumption of acetate as a carbon source instead of HPAM (Wen et al. 2010).

A decrease in ammonium concentration indicated microbial activity in $400P_{Co}$AcAm, $1000P_{Co}$AcAm, and AcAm. Each treatment level exhibited a slight increase on day 28, with a greater increase for $1000P_{Co}$AcAm, possibly due to hydrolysis of the HPAM amide group. Since acetate was not consumed and methanogenesis was not observed in $1000P_{Co}$AcAm Killed Control, ammonium fluctuations in killed controls throughout the incubation period were also likely due to abiotic mechanism. After 28 days of incubation, ammonium concentrations decreased in AcAm and $1000P_{Co}$AcAm until day 246, while $400P_{Co}$AcAm decreased until day 231. Once $400P_{Co}$AcAm were amended with a higher HPAM concentrations (day 247), an increase in ammonium concentrations was observed. This increase was likely due to hydrolysis of the amide groups in HPAM and a delay in consumption of the hydrolytic ammonium. Prior to day 231, greater ammonium concentrations for $1000P_{Co}$AcAm were possibly due to a greater presence of amide groups within HPAM, compared to lower doses in $400P_{Co}$AcAm. These results suggest that HPAM could serve as an additional nitrogen source under co-metabolic conditions, with the potential for ammonium production from amide hydrolysis.

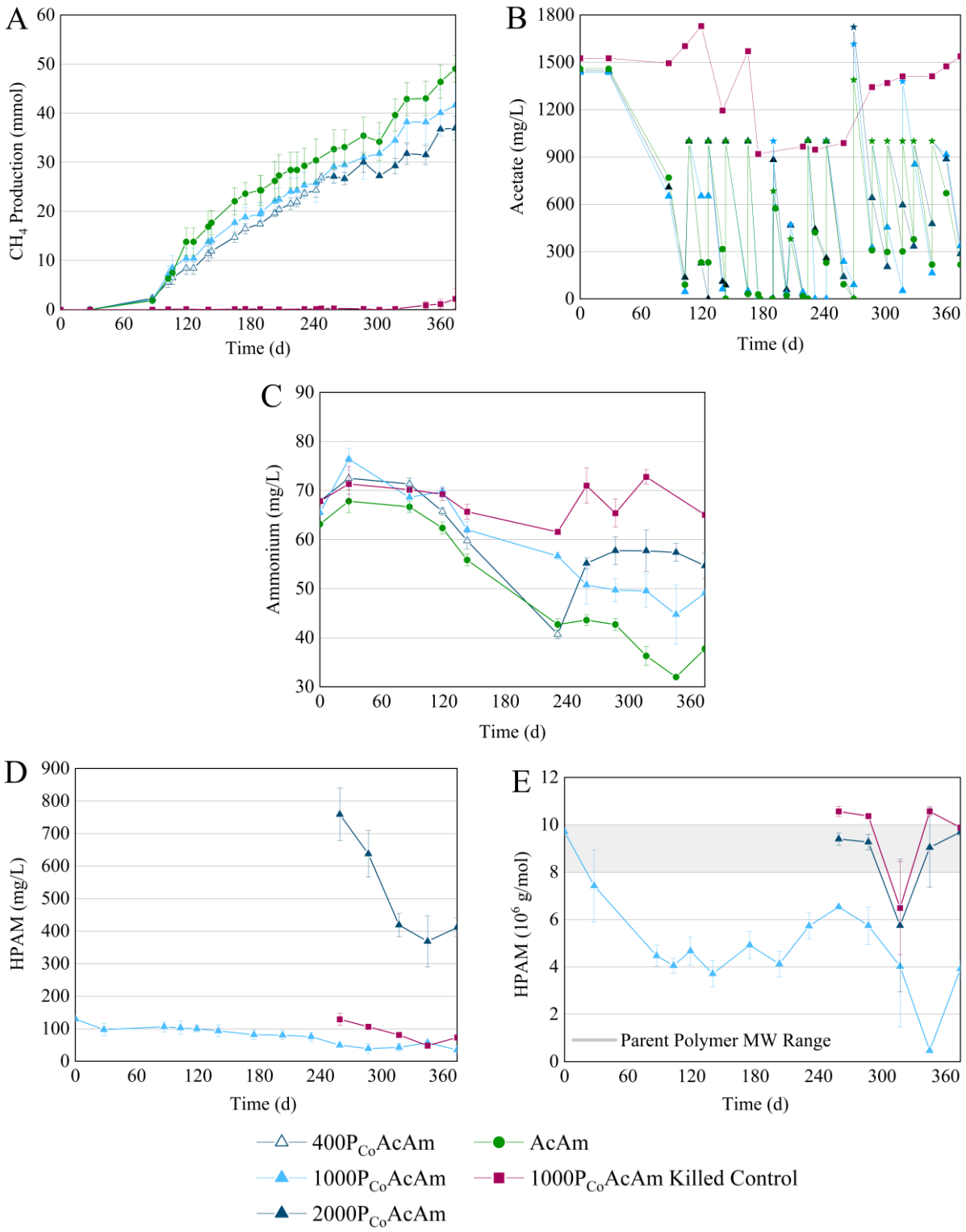


Figure 4-12 (A) CH_4 production, (B) acetate concentrations, (C) ammonium concentrations, (D) HPAM pore water concentrations, and (E) HPAM molecular weights for P_{Co} treatments under methanogenic conditions. Acetate amendments are represented by stars (★). Error bars represent one standard deviation of biological replicates.

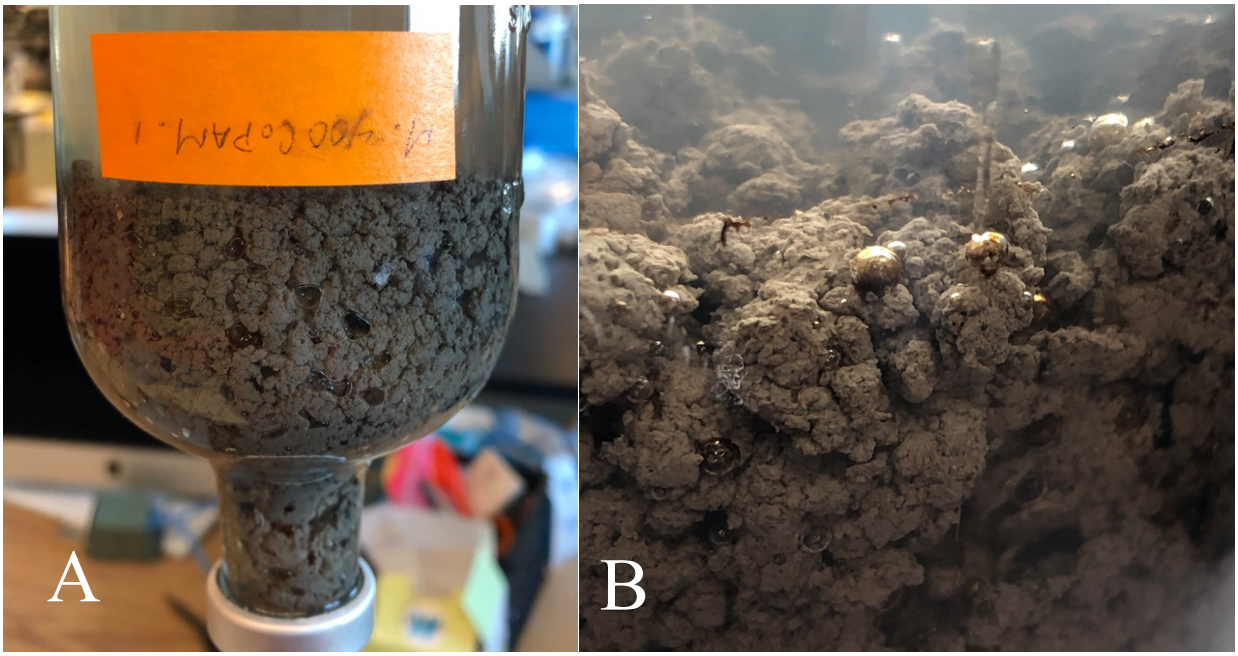


Figure 4-13 (A) Biogenic gases (likely CH_4) trapped within the pore spaces of flocculated tailings prior to inverting the microcosm for headspace analysis, and (B) biogenic gases trapped within bitumen.

Similar to sulfate-reducing conditions, microcosms dosed at 400 mg/L tailings had initial pore water concentrations below the SEC detection limit. Killed controls were increased to 1000 mg/L and live cultures increased to 2000 mg/L on day 247 to allow for the pore water concentration and molecular weight to be monitored. $2000P_{Co}AcAm$ and $1000P_{Co}AcAm$ Killed Control were therefore monitored for 115 days while $1000P_{Co}AcAm$ was monitored for 374 days. $1000P_{Co}AcAm$ had no lag phase for pore water concentration and molecular weight decrease. $2000P_{Co}AcAm$ also did not exhibit a lag phase for pore water concentration, however, since the molecular weight did not decrease throughout the incubation period, this decrease in concentration is likely due to adsorption. A similar decrease in concentration between $2000P_{Co}AcAm$ and $1000P_{Co}AcAm$ Killed Control suggested removal was likely due to abiotic mechanisms.

To compare incubation periods, after 119 days molecular weight decreased for $1000P_{Co}AcAm$ by 59.61% without a lag phase, while $2000P_{Co}AcAm$ and $1000P_{Co}AcAm$ Killed Control showed no or minimal decrease (Table 4-11). The difference in molecular weight between $1000P_{Co}AcAm$ and $1000P_{Co}AcAm$ Killed Control suggests that biodegradation occurred under co-metabolic conditions. No biodegradation observed for $2000P_{Co}AcAm$ suggests that either incubation period

or HPAM concentration influenced biodegradation. The biodegradation of HPAM as a co-substrate aligns with previous studies where glucose and urea were amended to accelerate the biodegradation process (Song et al., 2018). Residual organics in municipal and industrial wastewater, including carbohydrates, proteins and hydrocarbons are also believed to support HPAM biodegradation (Dai et al., 2015; Song et al., 2019)

4.4.3.2 Hydrolyzed Polyacrylamide as a Sole Nitrogen Source

Results for CH_4 production, acetate concentrations, ammonium concentrations, and HPAM pore water concentrations and molecular weight for P_N treatments are presented in Figure 4-14. Cumulative acetate consumption is provided in Appendix B, Figure B-8. When HPAM was provided as the sole nitrogen source, the lag phase for methanogenesis decreased from 140 days for Ac, to 28 days for $400P_NAc$ and $1000P_NAc$. These findings align with those obtained by Haveroen et al. (2005). Within 80 days methanogenesis was only observed in HPAM amended cultures, compared to no-HPAM controls (Haveroen et al., 2005). Cultures were serially transferred to dilute fixed nitrogen in the tailings samples, therefore, HPAM was the primary form of nitrogen in the microcosms (Haveroen et al., 2005). Results from Ac also indicated microorganisms were capable of methanogenesis under nitrogen deficient conditions, only more time was required for the establishment of methanogens.

Aligning with methane production, acetate consumption was greater in HPAM amended cultures. Acetate was consumed after a 28-day lag phase for $2000P_NAc$ and $1000P_NAc$, and 119-day lag phase for Ac. Similar to sulfate-reducing conditions (Figure 4-9), HPAM appeared to reduce the lag time for acetate consumption and stimulate anaerobic activity as a sole nitrogen source. Lag phases for biogenic gas production and acetate consumption indicated that nitrogen deficient conditions had a greater impact on microbial establishment under methanogenic and sulfate-reducing conditions compared to oxic conditions. Ammonium production occurred without a lag phase for $400P_NAc$ and $1000P_NAc$, likely due to hydrolysis of the amide groups in HPAM. Ammonium was depleted after 143 days for $1000P_NAc$ and 119 days for $400P_NAc$. Similar to $400P_{Co}AcAm$, once $400P_NAc$ was amended with a higher HPAM concentration ammonium concentration increased. Since ammonium also increased in $1000P_NAc$ Killed Control, it is possible that abiotic mechanisms were partially responsible for ammonium release.

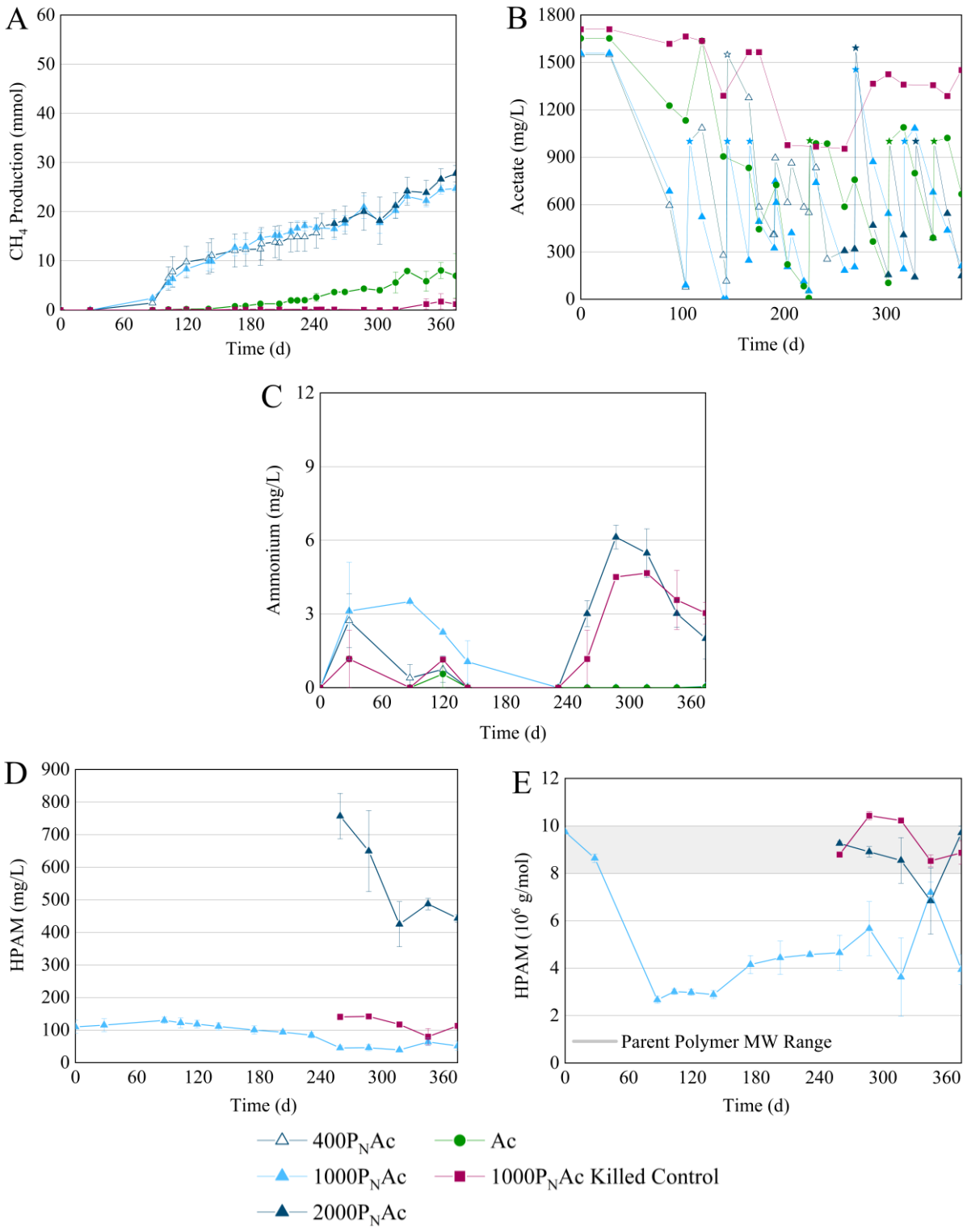


Figure 4-14 (A) CH_4 production, (B) acetate concentrations, (C) ammonium concentrations, (D) HPAM pore water concentrations, and (E) HPAM molecular weights for P_N treatments under methanogenic conditions. Acetate amendments are represented by stars (★). Error bars represent one standard deviation of biological replicates.

1000P_NAc had an 87-day lag phase for pore water concentration and no lag phase for molecular weight. 2000P_NAc had no lag phase for pore water concentration but did not exhibit a decrease in molecular weight at the end of the incubation period. 1000P_NAc exhibited the greatest decrease in molecular weight between day 28 and 87, coinciding with when methanogenesis began (Figure 4-14 A). After 87 days, HPAM molecular weight did not appear to further decrease. These results suggest that biodegradation of the carbon chain was possible when HPAM was provided as the sole nitrogen source.

4.4.3.3 Hydrolyzed Polyacrylamide as a Sole Carbon Source

Methanogenesis was not observed in P_C treatments, suggesting that HPAM could not serve as a sole carbon source (Table 4-10). Microbial activity was also not apparent based on the similarities in ammonium fluctuations between killed controls and live cultures. Results for CH_4 production, ammonium concentrations, and HPAM pore water concentrations and molecular weight are presented in Figure 4-15. Pore water and molecular weight decreased for 1000P_CAm, 2000P_CAm, and 1000P_CAm Killed Control. For pore water concentration, 2000P_CAm and 1000P_CAm Killed Control could not be directly compared due to differing initial concentrations, however, the magnitude of pore water removal was greater in 2000P_CAm. Since fluctuations in molecular weight for 2000P_CAm mirrored that of 1000P_CAm Killed Control, with no decrease in molecular weight at the end of the incubation period, it was likely that no biodegradation occurred. A lack of methanogenesis would further support this assumption that biodegradation was not possible when HPAM was provided as the sole carbon source.

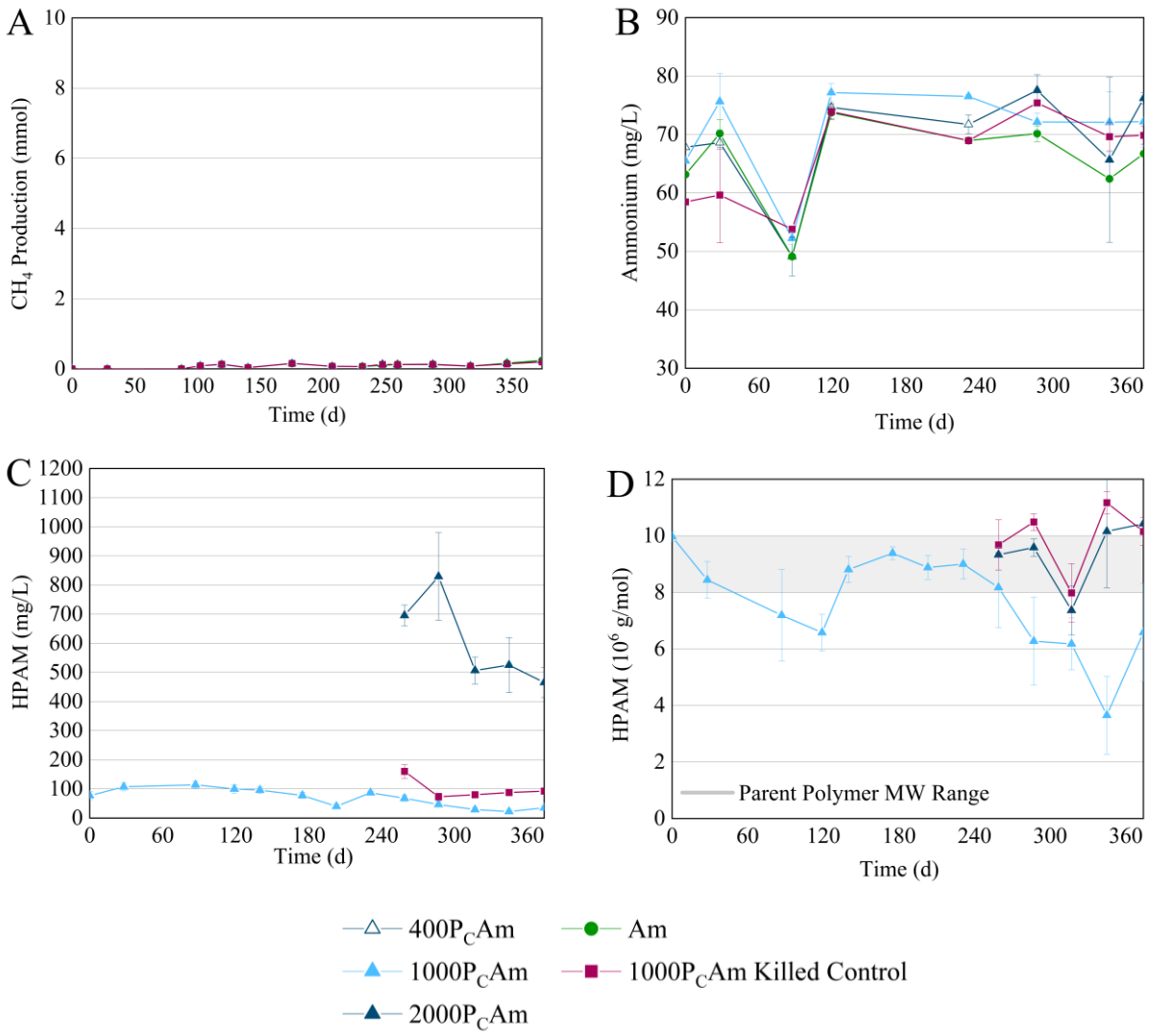


Figure 4-15 (A) CH_4 production, (B) acetate concentrations, (C) ammonium concentrations, (D) HPAM pore water concentrations, and (E) HPAM molecular weights for P_C treatments under methanogenic conditions. Error bars represent one standard deviation of biological replicates.

4.4.3.4 Hydrolyzed Polyacrylamide as a Sole Nitrogen and Carbon Source

P_{N+C} treatments were similar to P_C in that methanogenesis was not observed during the incubation time (Table 4-10). Results for CH_4 production, ammonium concentrations, and HPAM pore water concentrations and molecular weight are presented in Figure 4-16. Ammonium increased without a lag phase for $400P_{N+C}$ and $1000P_{N+C}$ and appeared higher than $1000P_{N+C}$ Killed Control. Ammonium concentrations decreased after 119 days and increased again on day 287. Release of ammonium could be due to microbial activity other than methanogenesis.

The least biodegradation of HPAM was observed in P_{N+C} treatments (Table 4-11). Given that the killed control had the greatest % removal, a decrease in pore water concentration for live cultures is likely due to abiotic mechanisms. Since microbial activity was inconclusive, biological degradation of the carbon chain cannot be confirmed as a reasoning for pore water removal or molecular weight decrease. It is therefore likely that HPAM could not serve as a carbon source, and that biodegradation was limited without additional carbon and nitrogen sources.

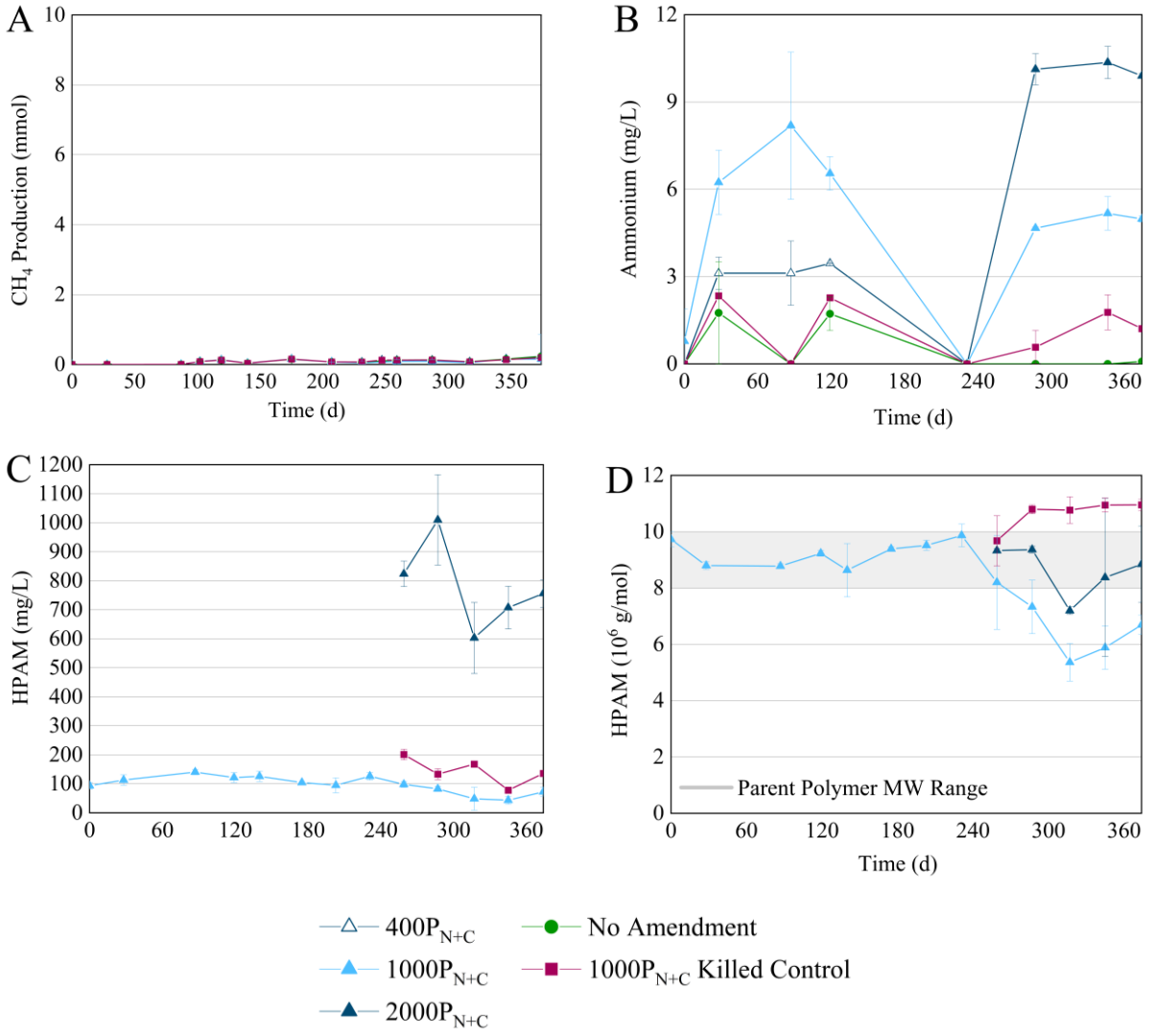


Figure 4-16 (A) CH_4 production, (B) acetate concentrations, (C) ammonium concentrations, (D) HPAM pore water concentrations, and (E) HPAM molecular weights for P_{N+C} treatments under methanogenic conditions. Error bars represent one standard deviation of biological replicates.

4.4.4 Acute Toxicity

The potential acute toxicity of oil sands tailings and OSPW has been a primary concern, especially considering the potential for release water and reclamation landscape incorporating various tailings streams. Currently there is limited research on HPAM and its metabolites following biodegradation, specifically within an oil sands context. Li et al. (2023) observed a slight increase (<10%) in the inhibition effect towards *vibrio fischeri* for pure polymer solutions and tailings augmented with HPAM degrading isolates under oxic conditions. No genotoxicity was observed. To date, no studies have investigated potential toxicity associated with HPAM biodegradation in oil sands tailings under anoxic conditions. Acute toxicity tests were performed on *A. fischeri* on initial tailings (12%) and final measurements for oxic and anoxic conditions.

4.4.4.1 Acute Toxicity Under Oxic Conditions

Acute toxicity under oxic conditions is presented in Figure 4-17. Under oxic conditions, small increases (< 8%) in the inhibition effect were observed in P_{Co} and P_{N+C} killed controls, while the inhibition effect for P_N and P_C killed controls decreased compared to initial tailings. Therefore, the presence of HPAM prior to biodegradation did not appear to influence toxicity. P_{Co} treatments had inhibition effects of 32% for 2000P_{Co}AcAm, 37% for 1000P_{Co}AcAm, and 16% for AcAm. Higher inhibition effect for 2000P_{Co}AcAm and 1000P_{Co}AcAm could potentially be due to HPAM biodegradation and the formation of lower molecular weight compounds, however, HPAM amended cultures were not significantly higher than AcAm ($p > 0.05$). P_N treatments had inhibition effects of 34% for 2000P_NAc, 37% for 1000P_NAc, and 27% for Ac, with no significant difference observed ($p > 0.05$). Acute toxicity results indicated that even with 99% degradation (decrease in molecular weight) observed in P_{Co} and P_N treatments (Table 4-7), toxicity was not significantly related to HPAM biodegradation.

For P_C and P_{N+C} treatments, no-HPAM controls exhibited inhibition effects greater than or similar to HPAM amended cultures. This further suggests that toxicity was unrelated to HPAM degradation (biological or mechanical). These results aligned with Li et al. (2023) where acute toxicity was limited in HPAM tailings systems.

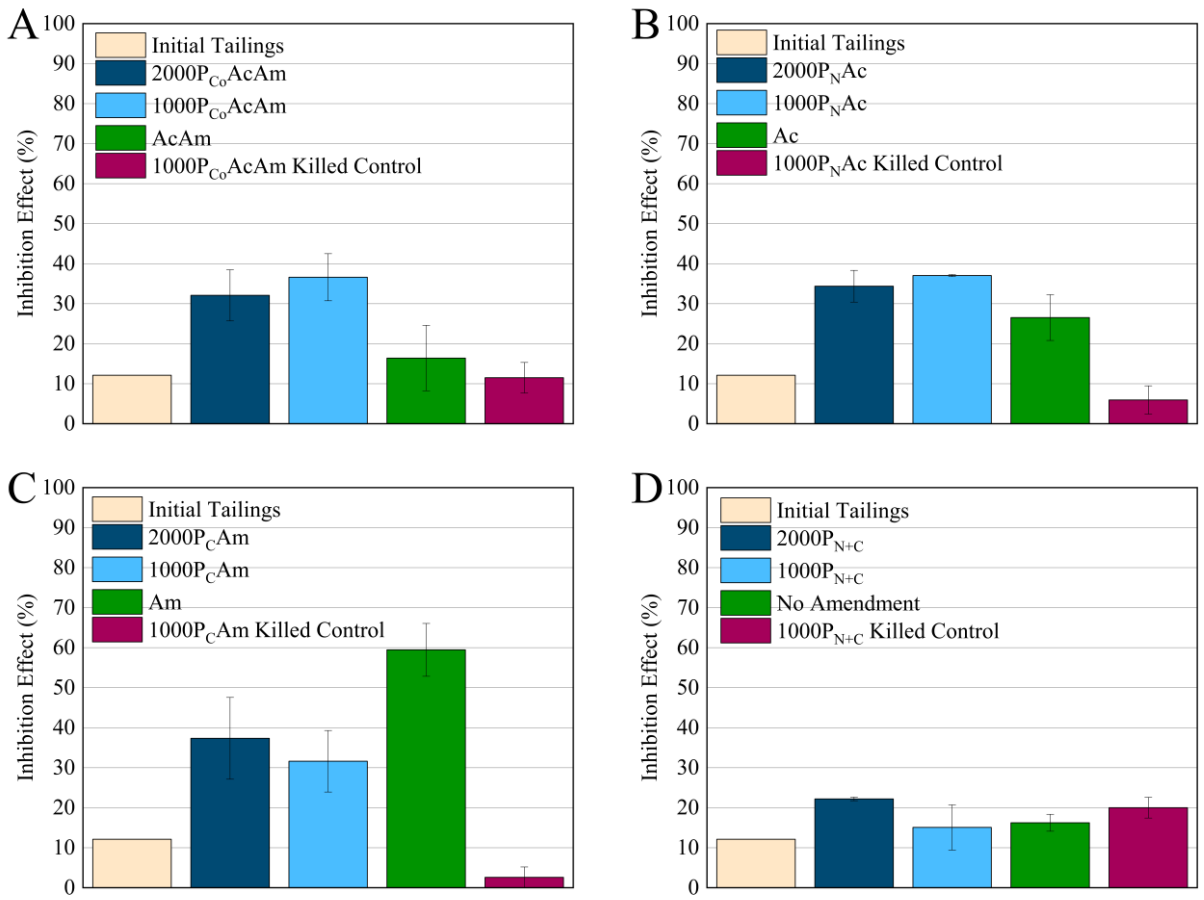


Figure 4-17 Acute toxicity under oxic conditions for (A) HPAM as co-substrate, (B) HPAM as nitrogen source, (C) HPAM as carbon source, and (D) HPAM as nitrogen and carbon source. Error bars represent one standard deviation of biological duplicates.

4.4.4.2 Acute Toxicity Under Sulfate-reducing Conditions

Acute toxicity was not observed in P_C or P_{N+C} treatments for sulfate-reducing conditions (Figure 4-18). P_{Co} treatments had inhibition effects of 20% for 2000P_{Co}AcAm, 28% for 1000P_{Co}AcAm, and 20% for AcAm. Though acrylamide was detected in 2000P_{Co}AcAm and 1000P_{Co}AcAm, concentrations were likely too low (272 and 537 µg/L) to influence inhibition effect. P_N treatments had inhibition effects of 36% for 2000P_NAc, 23% for 1000P_NAc, and 4% for Ac. 2000P_NAc inhibition effect was significantly greater ($p = 0.02$) than Ac, however, 1000P_NAc was not significantly different from Ac ($p > 0.05$). It was therefore inconclusive whether the biodegradation of HPAM potentially increased toxicity. Greater inhibition effects in 2000P_NAc and 1000P_NAc could have also been due to sulfate-reducing activity. Though samples were aerated prior to analysis, the formation of reduced sulfur compounds such as H_2S may have increased the

inhibition effect. Since Ac had lower microbial activity compared to 2000P_NAc and 1000P_NAc, it is possible that Ac had lower concentrations of reduced sulfur compounds.

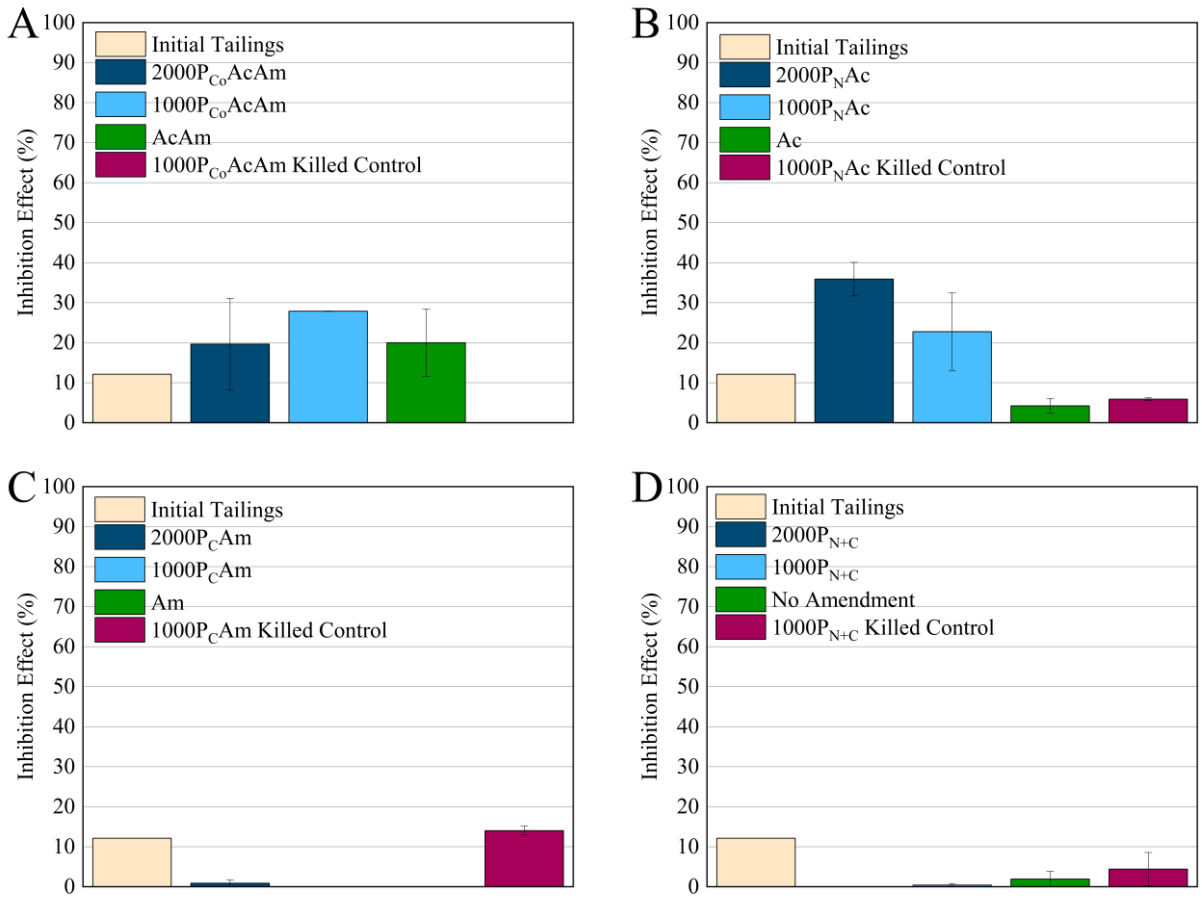


Figure 4-18 Acute toxicity under sulfate-reducing conditions for (A) HPAM as co-substrate, (B) HPAM as nitrogen source, (C) HPAM as carbon source, and (D) HPAM as nitrogen and carbon source. Error bars represent one standard deviation of biological duplicates.

4.4.4.3 Acute Toxicity Under Methanogenic Condition

Methanogenic biodegradation of HPAM did not appear to influence acute toxicity compared to no-HPAM controls (Figure 4-19). P_N treatments exhibited higher inhibition effects for HPAM amended cultures compared to no-HPAM controls, however, these levels were not significantly different ($p > 0.05$).

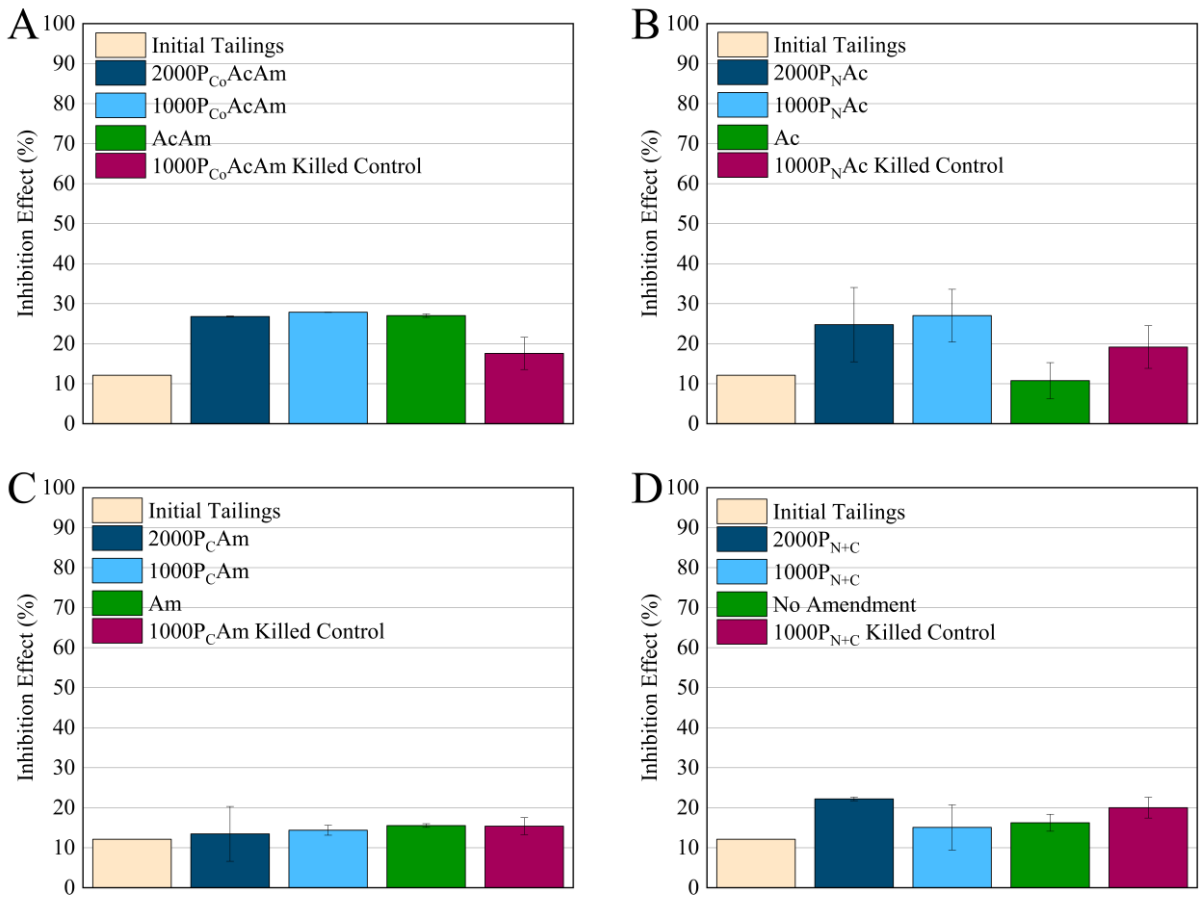


Figure 4-19 Acute toxicity under methanogenic conditions for (A) HPAM as co-substrate, (B) HPAM as nitrogen source, (C) HPAM as carbon source, and (D) HPAM as nitrogen and carbon source. Error bars represent one standard deviation of biological duplicates.

Overall, only 2000P$_N$Ac for sulfate-reducing conditions exhibited significantly greater toxicity when compared to Ac. Differences for other conditions and treatments were not statistically significant. This suggests that sulfate-reduction along with HPAM biodegradation could potentially lead to oil sands tailings toxicity. HPAM could stimulate sulfate-reduction in nitrogen limited conditions, indicating the potential for sulfate-reducing activity in HPAM and gypsum amended tailings.

4.4.5 Microbial Communities

Initial microbial communities for the oil sands tailings used in this study were comprised of the class Gammaproteobacteria (17%), orders Burkholderiales (11%) and Candidatus Kaiserbacteria (6%), and families *Syntrophaceae* (17%), *Hydrogenophilaceae* (8%), *Porticoccaceae* (3%), *Microscillaceae* (2%), and *Anaerolineaceae* (2%) (Figure 4-20).

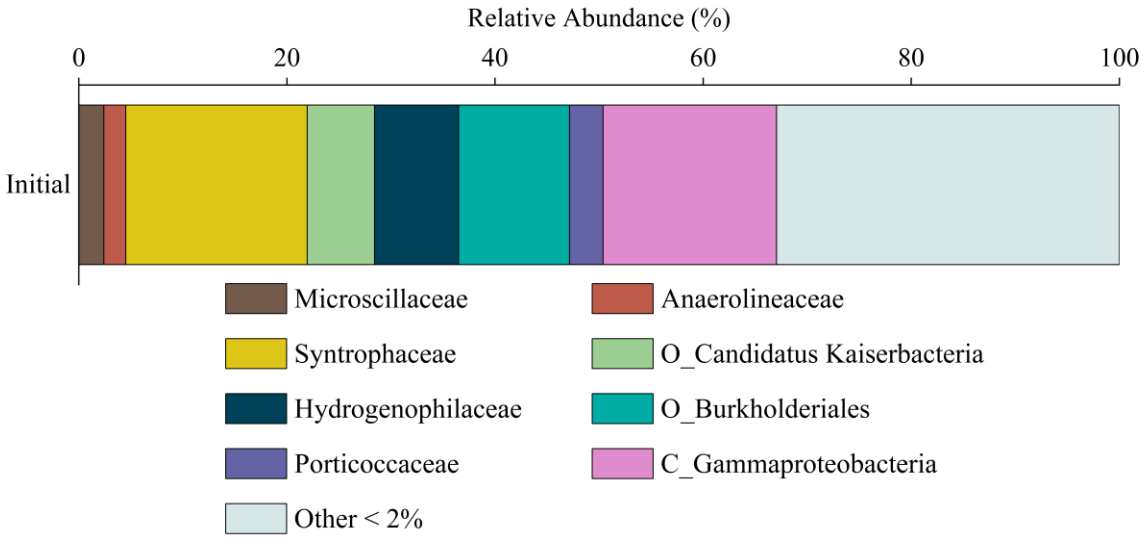


Figure 4-20 Microbial community composition at the family level for initial fine tailings. Taxa with a relative abundance < 2% were assigned to 'Other < 2%'. Taxa not identified at the family level are denoted with "O_" or "C_" to represent taxonomic ranking at the Order and Class level, respectively.

4.4.5.1 Oxic Conditions

Figure 4-21 presents the relative abundance of microbial communities on day 56, 152, and 236 for P_{Co} and P_N treatments under oxic conditions. Microbial communities for P_C and P_{N+C} were only analyzed for day 152 and 236 due to low microbial activity and limited HPAM degradation in the beginning of the incubation period (Appendix B, Figure B-12 and B-13). For P_{Co} treatments, the microbial communities most abundant in cultures amended with HPAM were *Pseudomonadaceae*, *Flavobacteriaceae,* and *Acidaminobacteraceae*.

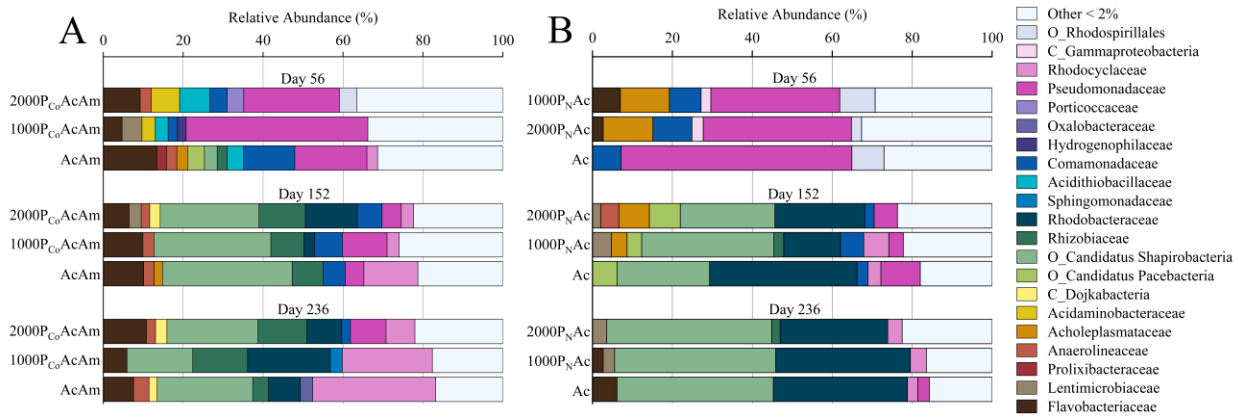


Figure 4-21 Microbial composition at the family level under oxic conditions for (A) HPAM as co-substrate (P_{Co}) and (B) HPAM as nitrogen source (P_N). Taxa with a relative abundance < 2% were asigned to 'Other < 2%". Taxa not identified at the family level are denoted with "O_" or "C_" to represent taxonomic ranking at the Order and Class level, respectively. Microbial composition for P_C and P_{N+C} are provided in Appendix B, Figure B-12 and B-13.

Pseudomonadaceae (Pseudomonadota) was most abundant in 1000P_{Co}AcAm (45%) and 2000P_{Co}AcAm (24%) on day 56, with *pseudomonas sp.* being the only taxa identified at the genus level. Greater relative abundance in 1000P_{Co}AcAm and 2000P_{Co}AcAm compared to AcAm (18%) could have been due to the presence of HPAM as an additional nitrogen and carbon source. On day 152, *Pseudomonadaceae* decreased in 1000P_{Co}AcAm (11%) and 2000P_{Co}AcAm (5%). By day 152, HPAM oligomers had undergone 99% biodegradation, with a portion of HPAM recalcitrant to biodegradation (Figure 4-3 E and F). The removal of HPAM from the pore water could suggest why *Pseudomonadaceae* had decreased. Various *Pseudomonas* strains have been identified in HPAM degrading isolates from oil sands tailings and dewatered sludge (Yu et al., 2015; Li et al., 2023). It was therefore likely that *Pseudomonadaceae* (*Pseudomonas* sp.) was involved in HPAM biodegradation. By day 236, *Pseudomonadaceae* was only present in 2000P_{Co}AcAm (9%).

Flavobacteriaceae (Bacteroidota) was observed in 1000P_{Co}AcAm, 2000P_{Co}AcAm, and AcAm on day 56, 152, and 236 (5-13%). Flavobacteriales (order) has been associated with HPAM hydrolysis (Li et al., 2015) while *Flavobacterium sp.* was observed in bacterial communities capable of HPAM biodegradation in the presence of starch as an additional carbon source (Song et al., 2017). Since *Flavobacteriaceae* was also observed in AcAm, HPAM likely did not encourage its abundance, but *Flavobacteriaceae* could have potentially been involved in HPAM biodegradation.

Acidaminobacteraceae (Firmicutes) was observed in 1000P_{Co}AcAm (3%) and 2000P_{Co}AcAm (7%) on day 56, and only appeared in HPAM-amended cultures. Members of Firmicutes that have been associated with aerobic PAM and HPAM biodegradation include *Clostridiaceae*, *Bacillaceae*, *Bacillus* sp., *Anoxybacillus* sp. (Matsuoka et al., 2002; Bao et al., 2010; Wen et al., 2010; Berdugo-Clavijo et al. 2019; Song et al., 2019; Ma et al., 2021), but *Acidaminobacteraceae* has not been previously identified. *Acidaminobacteraceae* is part of the class Clostridia which are considered anaerobic; therefore, it was surprising to observe this family under oxic conditions.

Ca. Shapirobacteria, *Ca*. Kaiserbacteria, *Rhodobacteraceae*, and *Rhodocyclaceae* were abundant in 1000P_{Co}AcAm and 2000P_{Co}AcAm on day 152 and 236, however, they were also observed in AcAm. Since HPAM had undergone 99% biodegradation by day 152 (< 6000 g/mol), and these taxa were also present in AcAm, it was likely their abundance was not associated with HPAM biodegradation.

For P_N treatments, the microbial communities most abundant in cultures amended with HPAM were *Pseudomonadaceae*, *Acholeplasmataceae,* and *Lentimicrobiaceae. Pseudomonadaceae* (*Pseudomonas* sp.) was most abundant in 1000P_NAc (37%), 2000P_NAc (32%), and Ac (58%) on day 56. *Pseudomonadaceae* abundance decreased on day 152 and disappeared in 1000P_NAc 2000P_NAc on day 236. *Pseudoxanthomonas* sp. (Pseudomonadota) was observed in wastewater sludge and hydraulic fracturing produced water cultures capable of using PAM and HPAM as a nitrogen source. Other members of Pseudomonadota associated with aerobic HPAM biodegradation include *Klebsiella* sp., *Enterobacter agglomerans*, *Azomonas macrocytogenes*, *Azotobacter beijerinckii*, *Tistrella bauzanensis*, *Brucella* sp., and *Acinetobacter* sp. (Nakamiya and Kinoshita, 1995; Nakamiya et al., 1997; Matsuoka et al., 2002; Ma et al., 2021; Zhang et al., 2023)

Acholeplasmataceae (Mycoplasmatota) was observed in 1000P_NAc (12%) and 2000P_NAc (12%) on day 56, with *Acholeplasma* sp. being the only taxa identified at the genus level. *Acholeplasma* has been found to produce dehydrogenases, which could have been responsible for HPAM biodegradation (Martini et al., 2014; Dai et al., 2015; Song et al., 2018; Zhao et al., 2019). *Acholeplasmataceae* is also part of the class Mollicutes which are recognized by the absence of a cell wall, potentially allowing for access to the large polymer. *Acholeplasmataceae* (*Acholeplasma* sp.) abundance decreased on day 152 and disappeared on day 236. Similar to P_{Co} treatments, P_N treatments were depleted of HPAM by day 152 (Figure 4-4 F), suggesting why

Acholeplasmataceae may have decreased if this family was associated with HPAM biodegradation.

Lentimicrobiaceae (Bacteroidota) was detected in 1000P_NAc (5%) and 2000P_NAc (2%) on day 152, and was still present on day 236. *Lentimicrobiaceae* (*Lentimicrobium* sp.) only appeared in HPAM amended cultures after HPAM biodegradation, suggesting a relationship with HPAM or its degradation products. Bacteroidota has been associated with HPAM biodegradation (Yan et al., 2016; Song et al., 2017), while Sphingobacteriales (class in Bacteroidota) has been detected in HPAM degrading cultures but not necessarily a major contributor (Li et al., 2015).

Ca. Shapirobacteria and *Rhodobacteraceae* were abundant in 1000P_NAc and 2000P_NAc on day 152 and 236, however, they were also observed in Ac, suggesting their presence was likely not associated with HPAM biodegradation. Under oxic conditions, the biodegradation of HPAM for P_{Co} and P_N treatments was potentially due to the presence of *Pseudomonadaceae* and *Flavobacteriaceae,* while *Lentimicrobiaceae* and *Acholeplasmataceae* were associated with the presence of HPAM as a sole nitrogen source (P_N) and possibly biodegradation.

For P_C treatments, microbial diversity was similar among HPAM amended cultures and no-HPAM controls, on day 152. On day 236, the class OLB14 (Chloroflexi) increased in 2000P_CAm and 1000P_CAm (Appendix B, Figure B-12). Members of Chloroflexi have been observed in aerobic cultures capable of HPAM biodegradation, including *Roseiflexaceae* (family), *Anaerolineaceae* (family), *Bellilinea* sp., and *Caldilinea sp.* (Yan et al., 2016; Song et al., 2017; Berdugo-Clavijo et al., 2019; Ma et al., 2021). Biodegradation was not significant in this treatment, however, the OLB14 could still be related to the presence of HPAM. For P_{N+C} treatments, microorganisms associated with HPAM were not abundant in 2000P_{N+C} and 1000P_{N+C} and thus no biodegradation occurred (Appendix B, Figure B-13).

4.4.5.2 *Sulfate-reducing Conditions*

Figure 4-22 presents the relative abundance of microbial communities on day 154, 322, and 420 for P_{Co} and P_N treatments under sulfate-reducing conditions. Microbial communities for P_C and P_{N+C} were only analyzed for day 420 due to low microbial activity and limited HPAM degradation (Appendix B, Figure B-14 and B-15**)**. For P_{Co} treatments, the microbial communities most abundant in cultures amended with HPAM were *Methanosarcinaceae,* the class

Desulfuromonadia, Anaerolineaceae, the order Peptostreptococcales-Tissierellales, and *Methanosaetaceae*.

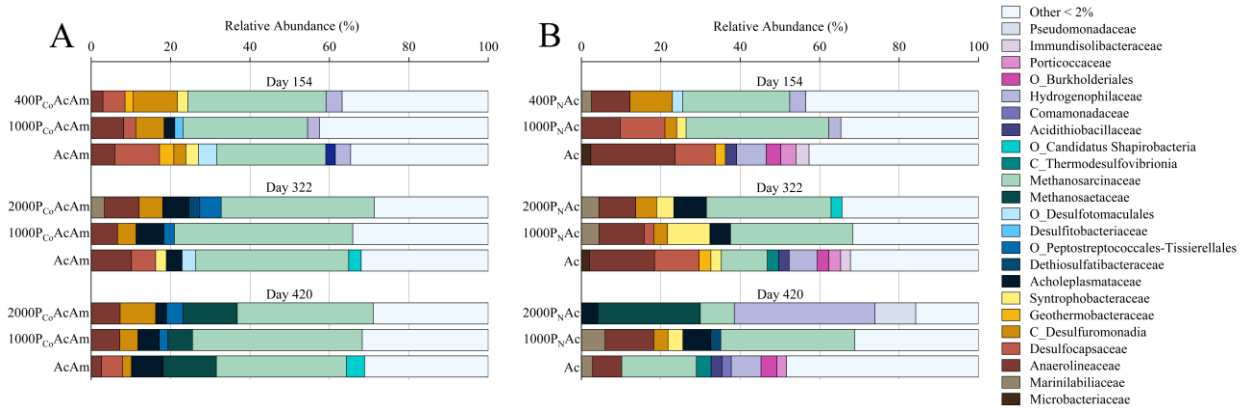


Figure 4-22 Microbial community composition at the family level under sulfate-reducing conditions for (A) HPAM as co-substrate (P_{Co}) and (B) HPAM as nitrogen source (P_N). Taxa with a relative abundance < 2% were assigned to 'Other < 2%'. Taxa not identified at the family level are denoted with "O_" or "C_" to represent taxonomic ranking at the Order and Class level, respectively. Microbial composition for P_C and P_{N+C} are provided in Appendix B, Figure B-14 and B-15.

Methanosarcinaceae (Euryarchaeota) was most abundant in 400P$_{Co}$AcAm (31%), 1000P$_{Co}$AcAm (35%), and AcAm (27%) on day 154, and remained the dominant taxa for day 322 and 420. *Methanosarcina* sp. was the only genus identified within this family. *Methanosarcinaceae* is part of the order Methanosarcinales, which has been associated with hydrolysis of HPAM (Li et al., 2015). Methanosarcinales has also been observed in oil sands tailings amended with HPAM, however, this study did not identify HPAM serving as a carbon or nitrogen source (Collins et al., 2016). Other members of Euryarchaeota that have been observed in HPAM degrading cultures include the orders Methanomicrobiales, Methanobacteriales, and Thermoplasmatales, and *Methanosaeta* sp., and *Methanofollis* sp. (Li et al., 2015; Song et al., 2018; Dai et al., 2015; Hu et al., 2018).

Desulfuromonadia (Thermodesulfobacteriota) was identified in 400PCoAcAm, 1000P$_{Co}$AcAm, and AcAm for all time points. *Geothermobacteraceae* was the only Family identified in Desulfuromonadia, however, it was not abundant in HPAM amended cultures. The remaining Families were unclassified, and therefore only the class was abundant in HPAM cultures.

Desulfuromonadia was the predominant SRB in HPAM amended cultures throughout the incubation period, suggesting their presence was potentially related to the presence of HPAM. Other members of Thermodesulfobacteriota that have been observed in HPAM degrading cultures include *Desulfovibrio* sp. and *Syntrophobacter* sp. (Grula et al., 1994; Song et al., 2017).

Anaerolineaceae (Chloroflexi) was observed in 1000P_{Co}AcAm, 2000P_{Co}AcAm, and AcAm on all sampling days (3-10%). Chloroflexi has been observed in anaerobic cultures capable of HPAM biodegradation, including *Anaerolinea* sp. and *Longilinea* sp. (Song et al., 2017; Zhao et al., 2019; Akbar et al., 2020). *Anaerolineaceae* has been associated with hydrocarbon biodegradation and syntrophic cooperation with methanogens (Sherry et al., 2013; Liang et al., 2015). *Anaerolineaceae* could therefore be involved in co-metabolism of HPAM along with syntrophic relations with *Methanosarcinacae*.

The order Peptostreptococcales-Tissierellales (Firmicutes) (including 3% *Dethiosulfatibacteraceae* in 2000P_{Co}AcAm) appeared in both 2000P_{Co}AcAm (8%) and 1000P_{Co}AcAm (3%) on day 322. Peptostreptococcales-Tissierellales was still only present in 2000P_{Co}AcAm (4%) and 1000P_{Co}AcAm (2%) on day 420. Peptostreptococcales-Tissierellales is part of the class Clostridia, which has also been associated with HPAM biodegradation in soil (family *Clostridiaceae)* (Ma et al., 2021). In oil sands tailings, Collins et al. (2016) observed an increase in Clostridia (class) in the presence of HPAM and suggested that HPAM could support the growth of aggregating microorganisms, though not necessarily as a nutrient source (Ceasar-TonThat et al., 2008). Firmicutes have been observed in anerobic cultures capable of PAM or HPAM biodegradation, including *Trichococcus* sp. (Dai et al., 2015; Akbar et a., 2020; Song et al., 2018).

This initial biodegradation of HPAM observed on day 35, was likely due to the abundance of *Anaerolineaceae* and potentially *Methanosarcinaceae* or class Desulfuromonadia depending on which enzymes were released during acetate consumption. Biodegradation observed after day 238 could potential be due to the presence of *Acholeplasmataceae*. In contrast to P_{Co} treatments, microorganisms identified and relative abundance for P_N treatments differed between HPAM amended cultures and no-HPAM controls. The microbial communities most abundant in cultures amended with HPAM were *Methanosarcinaceae,* the class Desulfuromonadia*, Anaerolineaceae, Acholeplasmataceae*, *Methanosaetaceae*, and *Hydrogenophilaceae*.

Methanosarcinaceae was most abundant in 400P_NAc (27%) and 1000P_NAc (36%) but was not present in Ac on day 154 (Figure 4-22). On day 322 *Methanosarcinaceae* was identified in Ac (12%). A delay in the presence of *Methanosarcinaceae* could possibly be to due nitrogen limited conditions. HPAM could have therefore supported *Methanosarcinaceae* as a nitrogen source, resulting in their presence on day 154 in 400P_NAc and 1000P_NAc. On day 420, *Methanosarcinaceae* was still most abundant in 1000P_NAc (34%), but decreased in 2000P_NAc (9%).

The presence of HPAM decreased the sulfate-reducing lag phase from 133 days (Ac) to 41 days (400P_NAc and 1000P_NAc). HPAM therefore likely encouraged the abundance of Desulfuromonadia, as was observed on day 154 (Figure 4-22). Since Thermodesulfobacteriota was observed in both HPAM amended cultures and no-HPAM controls for both P_{Co} and P_N, conclusions on the use of HPAM as a nitrogen source could not be made solely on microbial abundance. However, sulfate reduction (Figure 4-9 B) suggested a relationship between SRB and HPAM as a nitrogen source. Biodegradation of the carbon chain was inconclusive for P_N treatments (Figure 4-9 F)**.**

Similar to P_{Co} treatments, *Anaerolineaceae* was observed in P_N treatments for 400P_NAc (10%), 1000P_NAc (10%), and Ac (21%) on day 154. *Anaerolineaceae* was present in all cultures until day 420, where it disappeared in 2000P_NAc. *Acholeplasmataceae* (Mycoplasmatota) was only observed in 2000P_NAc and 1000P_NAc on day 322 and 420. *Acholeplasmataceae* was also believed to be associated with HPAM biodegradation under oxic conditions (subsection 4.4.5.1). *Acholeplasma* sp. are facultative anaerobes suggesting why they could be present in sulfate-reducing and oxic conditions (Martini et al., 2014).

Hydrogenophilaceae (Pseudomonadota) was most abundant in 2000P_NAc (35%) on day 420. This was observed with a simultaneous decreased in *Methanosarcinaceae* and appearance of *Methanosaetaceae* (26%). *Hydrogenophilaceae* are chemolithotrophs, relying on reduced sulfur compounds or hydrogen as electron donors (Orlygsson and Kristjansson, 2014). Consumption of H_2 by *Hydrogenophilaceae* could have created competition and resulted in a decreased in relative abundance for *Methanosarcinaceae* in 2000P_NAc. A decrease in *Methanosarcinaceae* could have led to a transition to *Methanosaetaceae* being the dominant family within Halobacterota.

Methanosaetaceae (*Methanosaeta* sp.) was the dominant archaea during HPAM biodegradation in wastewater sludge (Song et al., 2018).

Under sulfate-reducing conditions, the biodegradation of HPAM and its use as a nitrogen source was likely due to *Anaerolineaceae, Methanosarcinaceae*, *Methanosaetaceae*, and *Desulfuromonadia.* The presence of HPAM may have also supported *Acholeplasmataceae* and *Hydrogenophilaceae*. *Thiobacillus* sp. was the dominant family in the class *Hydrogenophilaceae* and is capable of iron and sulfur oxidation (Harahuc et al., 2000), as well as nitrate reduction (Bosch et al., 2012). This would suggest the potential for HPAM to support microorganisms with various metabolic functions. Deferribacteres (class of acetoclastic iron reducing bacteria) and Thermodesulfobacteriota (*Desulfovibrio* sp.) have been associated with cultures amended with HPAM and PAM as a nitrogen source (Grula et al., 1994; Hu et al., 2018). Relative abundance for Thermodesulfobacteriota (*Desulfocapsaceae*, *Desulfuromonadia*, *Geothermobacteraceae*, *Syntrophobacteraceae*) was consistently lower than Euryarchaeota (*Methanosarcinaceae*, and *Methanosaetaceae*) for both P_{Co} and P_N. This indicated the prevalence of methanogens even under sulfate-reducing conditions.

For P_C treatments, microbial communities did not appear to differ among 2000P_CAm, 1000P_CAm, and Am after 420 days (Appendix B, Figure B-14). This would reinforce the assumption that tailings microorganisms are not capable of using HPAM as a sole carbon source under sulfate-reducing conditions. For P_{N+C} treatments, the order *Ca.* Kaiserbacteria, and families *Acidithiobacillaceae,* and *Porticoccaceae* were most abundant in HPAM amended cultures (Appendix B, Figure B-15). Differences in taxa present could possibly be due to the presence of HPAM, likely as a nitrogen source or ability to create flocs that support microbial growth. Since SRB were not abundant in P_C or P_{N+C} treatments and microbial activity was limited, HPAM likely could not serve as a sole carbon source to support SRB.

4.4.5.3 Methanogenic Conditions

Figure 4-23 presents the relative abundance of microbial communities on day 119, 231, and 374 for P_{Co} and P_N treatments under methanogenic conditions. Microbial communities for P_C and P_{N+C} were only analyzed for day 374 due to low microbial activity and limited HPAM degradation (Appendix B, Figure B-16 and B-17). For P_{Co} treatments, the microbial communities most abundant in cultures amended with HPAM were *Methanosarcinaceae, Hydrogenophilaceae,*

Pseudomonadaceae, and the order Peptostreptococcales-Tissierellales. These taxa were also observed in sulfate-reducing and oxic conditions and associated with HPAM biodegradation. Overall, relative abundance and shifts in microbial communities were similar among 400P_{Co}AcAm (later 200P_{Co}AcAm), 1000P_{Co}AcAm, and AcAm.

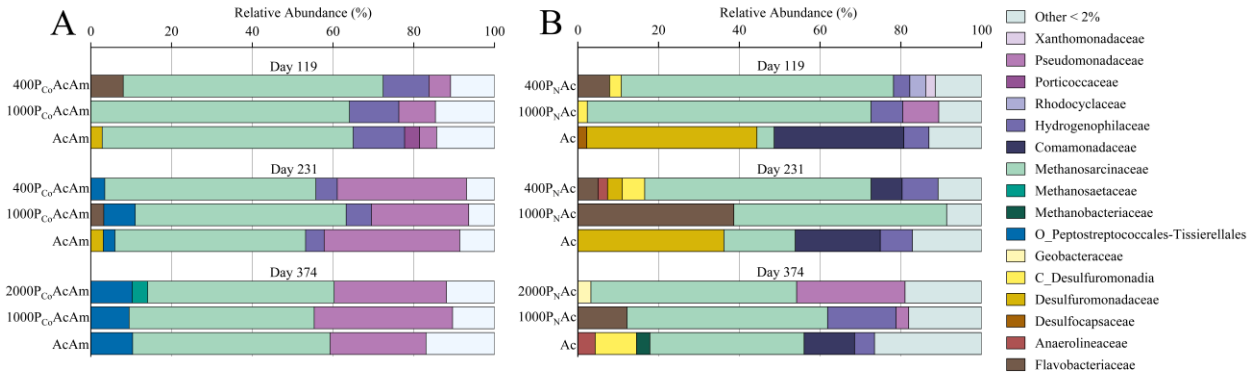


Figure 4-23 Microbial community composition at the family level under methanogenic conditions for (A) HPAM as co-substrate (P_{Co}) and (B) HPAM as nitrogen source (P_N). Taxa with a relative abundance < 2% were assigned to 'Other < 2%'. Taxa not identified at the family level are denoted with "O_" or "C_" to represent taxonomic ranking at the Order and Class level, respectively. Microbial composition for P_C and P_{N+C} are provided in Appendix B, Figure B-16 and B-17.

Methanosarcinaceae (Euryarchaeota) was most abundant in 400P_{Co}AcAm (later 200P_{Co}AcAm), 1000P_{Co}AcAm, and AcAm for sampling day. HPAM exhibited the most biodegradation prior to day 119, suggesting that *Methanosarcinaceae* (*Methanosarcina* sp.) was the primary archaea involved in the use of HPAM as a nitrogen source. With a limited understanding of enzymes involved in the biodegradation of PAM based polymers, it is inconclusive whether methanogens can utilize HPAM as a co-substrate.

Pseudomonadaceae (Pseudomonadota) was most abundant in 400P_{Co}AcAm (later 200P_{Co}AcAm), 1000P_{Co}AcAm, and AcAm on day 231 and 374. In addition to being observed in aerobic cultures (subsection 4.4.5.1) *Pseudomonadaceae* has been observed in anaerobic cultures capable of HPAM biodegradation, including *Pseudomonas* sp., (Akbar et al., 2020; Zhao et al., 2019; Dai et al., 2015; Hu et al., 218). Hydrolytic ammonium was observed after 400P_{Co}AcAm was amended to 200P_{Co}AcAm (day 247) (Figure 4-12 C), therefore, *Pseudomonadaceae* (*Pseudomonas* sp.) could have been involved in HPAM hydrolysis possibly through amidase activity (Kay-Shoemake

et al., 1998b). Similarities between HPAM amended cultures and no-HPAM controls suggested that the biodegradation of HPAM as a co-substrate did no influence relative abundance, rather, the presence of Pseudomonadota (*Pseudomonadaceae* and *Hydrogenophilaceae*) resulted in HPAM biodegradation.

P_N treatments for methanogenic conditions were similar to sulfate-reducing conditions with differences in relative abundance and microbial shifts overtime among HPAM amended cultures and no-HPAM controls. Microbial communities most abundant in cultures amended with HPAM were *Methanosarcinaceae* and *Flavobacteriaceae*. Though *Pseudomonadaceae* was not most abundant, it only appeared in HPAM amended cultures. *Methanosarcinaceae* was also observed in sulfate-reducing conditions, while *Flavobacteriaceae* was also observed in oxic conditions.

Methanosarcinaceae was most abundant in 400P_NAc and 1000P_NAc for each time point. *Methanosarcinaceae* wasn't the most abundant in Ac until day 374, though it did appear at lower levels on day 119 and 231. A delay in *Methanosarcinaceae* abundance for Ac aligns with lag times for methanogenesis (Figure 4-14**)**. When HPAM was provided as a nitrogen source, *Methanosarcinaceae* was established within 119 days (likely earlier based on methane production). Under nitrogen limited conditions (Ac) *Methanosarcinaceae* required time to become established. These results suggest that HPAM as a nitrogen source could potentially stimulate *Methanosarcinaceae* and the production of methane.

Flavobacteriaceae (Bacteroidota) appeared in 400P_NAc (8%) on day 119 and was most abundant in 1000P_NAc (38%) on day 231. Bacteroidota has been observed in anaerobic cultures capable of PAM and HPAM biodegradation, including *Bacteroides* sp. and *Paludibacter* sp., (Dai et al., 2015; Song et al., 2017; Song et al., 2018; Zhao et al., 2019; Akbar et al., 2020).

Under methanogenic conditions, *Methanosarcinacae* was potentially dependent on HPAM as a nitrogen source, while *Flavobacteriaceae* and *Pseudomonadaceae* were associated with the presence of HPAM as a sole nitrogen source (P_N) and possibly biodegradation.

Similar to sulfate-reducing conditions, P_C treatments for methanogenic conditions were similar between HPAM amended and no-HPAM controls after 374 days of incubation (Appendix B, Figure B-16). *Flavobacteriaceae* was only present in 2000P_CAm (2%) and 1000P_CAm (7%), suggesting a relationship with the presence of HPAM. No biodegradation was observed for this treatment,

suggesting that even if microorganisms associated with HPAM biodegradation are present, they may not be responsible or primarily involved in biodegradation. For P_{N+C}, *Candidatus Kaiserbacteria* (8% in 2000P_CAm, 13% in 1000P_CAm) and *Flavobacteriaceae* (5% in 2000P_CAm, 7% in 1000P_CAm) were only present in 2000P_CAm and 1000P_CAm after 374 days (Appendix B, Figure B-17). Overall, no methanogens or archaea were present in P_C or P_{N+C} and limited microbial activity was observed, suggesting that HPAM cannot serve as a carbon source to support methanogens (Figure 4-15 and 4-16).

4.5 Conclusions

This study demonstrates the potential for HPAM biodegradation (decrease in molecular weight) under various nutrient treatments and redox conditions within oil sands tailings pore water. Microbial activity indicated that the presence of HPAM was most significant when provided as a nitrogen source (P_N) under sulfate-reducing and methanogenic conditions. HPAM decreased the lag time for methane production and sulfate-reduction, indicating the potential to stimulate greenhouse gas production and reduced sulfur compounds when provided as a nitrogen source. Microbial activity was not observed when HPAM was provided as a carbon source (P_C) under methanogenic and sulfate-reducing conditions, and was minimal under oxic conditions. This suggested that HPAM could not be used as a carbon source by anaerobes. Biodegradation of HPAM was not observed in carbon limited oxic conditions, therefore microbial activity was likely due to the presence of residual organics (PHCs, NAs, etc.) with HPAM potentially serving as a nitrogen source.

HPAM experienced the greatest biodegradation under oxic conditions when HPAM was provided as a co-substrate (P_{Co}) (99%) and nitrogen source (99%). CO_2 production in HPAM amended cultures was similar to no-HPAM controls, suggesting that mineralization was not a mechanism of HPAM biodegradation, rather the breakdown of the parent polymer into oligomers (< 6000 g/mol) and other organic compounds. Acrylamide and acrylic acid were not identified as biodegradation products in this study. Microorganisms responsible for HPAM biodegradation were likely *Pseudomonadaceae* and *Flavobacteriaceae,* while *Lentimicrobiaceae* and *Acholeplasmataceae* were associated with the presence of HPAM as a nitrogen source.

For anoxic conditions, HPAM biodegradation was observed when provided as a co-substrate for methanogenic (60%) and sulfate-reducing conditions (77%), and as a nitrogen source for

methanogenic conditions (60%). The biodegradation of HPAM and its use as a nitrogen source was likely due to the presence of *Methanosarcinaceae*, *Methanosaetaceae*, Desulfuromonadia*,* *Acholeplasmataceae*, and *Hydrogenophilaceae* under sulfate-reducing conditions, and *Methanosarcinaceae*, *Flavobacteriaceae*, and *Pseudomonadaceae* under methanogenic conditions.

This study indicates that the biodegradation of HPAM would likely only occur in the presence of additional carbon sources such as acetate. The enzymes released during acetate consumption could potentially be responsible for HPAM biodegradation. The biodegradation of HPAM with additional nutrient sources indicates the potential for biodegradation in tailings systems or reclaimed landscapes, particularly those that receive influxes of carbon from oil sands operations (ex. diluent) or natural surroundings. Biodegradation of HPAM was not found to significantly impact tailings acute toxicity towards *A. fischeri*, however, the ability of indigenous oil sands microorganisms to degrade HPAM suggests further investigation into potential impacts is required.

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary of Findings

The overall purpose of this research was to investigate the potential of hydrolyzed polyacrylamide (HPAM) biodegradation under various redox conditions in oil sands tailings. To achieve this, Chapter 3 evaluated the ability of Size Exclusion Chromatography (SEC) to elucidate HPAM biodegradation and Chapter 4 monitored microbial activity and HPAM biodegradation under oxic, sulfate-reducing, and methanogenic conditions. A summary of key findings associated with each research objective and questions are provided below.

1. ***Develop a method for measuring hydrolyzed polyacrylamide using Size-Exclusion Chromatography***

Research question: Can Size-Exclusion Chromatography elucidate HPAM biodegradation in an oil sands tailings context?

The method development presented in Chapter 3 determined that SEC was capable of measuring HPAM with a molecular weight from ~9000 to 10^6 g/mol and pore water concentrations with a detection limit of 10 mg/L. The polymers used in this study (A3332 and A3338) exhibited calibration curves with $R^2 > 0.99$ and a relative standards deviation (RSD) of 1.0-3.8% for retention time and 1.4-6.2% for peak area. Molecular weight standards achieved a RSD of 0.2-3.4% for standards injected on the same day and 11.2-13.6% for standards injected on different days, which suggested standards should be run for each analysis to maintain precision. The adsorption of HPAM to tailings solids presented challenges for this study. If tailings were dosed under the adsorption capacity (1874 g HPAM/tonne solids), pore water concentrations were below the detection limit. So long as HPAM doses are above the adsorption capacity, SEC would be suitable for monitoring a change in the parent HPAM polymer.

Other analytical techniques exist that can determine the use of HPAM as a nitrogen source, identify analytes, and monitor other chemical or physical changes in the parent polymer. However, the ability of SEC to detect a range of molecular weights suggested its applicability for monitoring HPAM biodegradation of the polymer carbon chain in oil sands tailings. The ability of SEC to elucidate HPAM biodegradation was further confirmed in the biodegradation study in Chapter 4, where the parent HPAM polymer and oligomers were monitored for a shift in molecular weight.

SEC was able to indicate under which conditions biodegradation occurred and how long it took to reach complete or partial degradation.

2. ***Investigate the biodegradation of hydrolyzed polyacrylamide under methanogenic, sulfate-reducing, and oxic conditions.***

Research questions: Are indigenous oil sands tailings microorganisms capable of HPAM biodegradation under methanogenic, sulfate-reducing, and oxic conditions? Are these microorganisms capable of using HPAM as a carbon and/or nitrogen source, or degrade HPAM through co-metabolic processes? How do microbial communities in fresh tailings respond to the introduction of PAM and shift over time under different redox conditions?

Microcosm studies in Chapter 4 revealed which redox conditions could yield the greatest HPAM biodegradation (decrease in molecular weight), how HPAM is used as a nutrient source, and which microbial communities respond to the presence of HPAM and are involved in biodegradation. Based on SEC results, HPAM exhibited the most biodegradation under oxic conditions when provided as a co-substrate (99%) and nitrogen source (99%). For anoxic conditions, HPAM exhibited the most biodegradation when provided as a co-substrate under sulfate-reducing conditions (77%), followed by as a co-substrate (60%) and nitrogen source (60%) for methanogenic conditions. Degradation was observed when HPAM was provided as a carbon source as well as nitrogen and carbon source, however, due to limited microbial activity or similarities with killed controls, degradation was likely not due to biological processes. A shift in molecular weight indicated biodegradation of the polymer carbon chain, while the formation of hydrolytic ammonium indicated the use of HPAM as a nitrogen source.

When biodegradation was observed, CO_2 or CH_4 was not higher in HPAM amended cultures compared to no-HPAM controls, suggesting that if HPAM was being used as a carbon source complete mineralization was not occurring. Biodegradation was also only observed when acetate was provided, suggesting that biodegradation can only occur through co-metabolic processes or through the formation of enzymes capable of cleaving the polymer carbon chain. The presence of HPAM as a nitrogen source had the greatest effect under methanogenic and sulfate-reducing conditions (acetate was provided as the carbon source). The presence of HPAM decreased the lag times for sulfate-reducing and methane production, suggesting its potential to stimulate greenhouse gas production and reduced sulfur compounds. Hydrolytic ammonium was also

observed in almost all redox conditions and nutrient treatments, suggesting that HPAM could readily serve as a nitrogen source in oil sands tailings.

Microbial communities identified in HPAM degrading cultures included *Pseudomonadaceae Flavobacteriaceae*, *Lentimicrobiaceae*, and *Acholeplasmataceae* for oxic conditions, *Methanosarcinaceae*, *Methanosaetaceae*, *Desulfuromonadia, Acholeplasmataceae*, and *Hydrogenophilaceae* for sulfate-reducing conditions, and *Methanosarcinaceae*, *Flavobacteriaceae*, and *Pseudomonadaceae* for methanogenic conditions. Shifts in microbial communities coincided with the biodegradation of HPAM under oxic conditions. When HPAM was provided as a nitrogen source, this encouraged the presence of microbial communities that would have otherwise required longer periods of time to become established. Overall, this study indicated which redox conditions and nutrient treatments encouraged HPAM biodegradation.

5.2 Significance and Recommendations for Future Research

Oil sands tailings treated with HPAM and other polyacrylamide-based polymers will ultimately be incorporated into the reclaimed landscape. This research was therefore significant in understanding the interactions between HPAM and indigenous oil sands microorganisms, and the potential for biodegradation. Understanding the susceptibility of HPAM to microbial degradation will assist with tailings management when considering nutrient inputs and co-mixing of tailings with HPAM, sulfur compounds, or diluent, as well as guide future research to further investigate the mechanisms and implications of HPAM biodegradation.

This research indicated that biodegradation of HPAM is possible in oil sands tailings in the presence of acetate as a carbon source. Therefore, HPAM biodegradation in reclaimed landscapes that received influxes of carbon from oil sand operations or natural surroundings is possible. Future research could therefore focus on the biodegradation of HPAM with petroleum hydrocarbons as alternative carbon sources. Microcosms studies with hydrocarbons and HPAM would likely yield different biodegradation results than those amended with acetate, alluding to the biodegradation rates of oil sands tailings treated with naphtha or paraffinic diluent.

Operators are currently targeting terrestrial landscapes that will contain wetlands on HPAM treated tailings. Column or field-scale studies with HPAM amended tailings could indicate how soil organic matter, such as humic substances may influence HPAM biodegradation in a reclamation

scenario. To elucidate the mechanisms and impacts of HPAM biodegradation, future research could investigate enzymes, utilize qPCR, and refine methods for measuring biodegradation products. Oxic conditions also revealed that complete biodegradation of the parent polymer and formation of lower molecular weight oligomers could lead to resuspension of tailings particles. Therefore, future research could also investigate the impacts of HPAM biodegradation to tailings consolidation and dewatering.

In this research, ammonium and lower molecular oligomers were identified as biodegradation products. To determine the extent of HPAM biodegradation and characterize biodegradation products, future research should incorporate an additional SEC column for separating compounds less than 6000 g/mol, measure the polydispersity index of biodegradation oligomers, and utilize mass spectrometry and nuclear magnetic resonance for identifying unknown biodegradation products.

BIBLIOGRAPHY

Akbar, M., Khan, M. F. S., Qian, L., & Wang, H. (2020). Degradation of Polyacrylamide (PAM) and methane production by mesophilic and thermophilic anaerobic digestion: Effect of temperature and concentration. *Frontiers of Environmental Science and Engineering*, *14*(6).

Arkell, N., Kuznetsov, P., Kuznetsova, A., Foght, J. M., & Siddique, T. (2015). Microbial Metabolism Alters Pore Water Chemistry and Increases Consolidation of Oil Sands Tailings. *Journal of Environmental Quality*, *44*(1), 145–153.

Alberta Energy Regulator. (2022a). *State of Fluid Tailings Management for Mineable Oil Sands, 2021*.

Alberta Energy Regulator. (2022b). *Directive 85: Fluid tailings management for oil sands mining projects*.

Alberta Energy Regulator. (2023). *In Situ Recovery*. https://www.aer.ca/providing-information/by-topic/oil-sands/in-situ-recovery

Alberta Environment. 2008. *Guideline for wetland establishment on reclaimed oil sands leases (2nd edition)*. Prepared by Harris, M.L. of Lorax Environmental for the Wetlands and Aquatics Subgroup of the Reclamation Working Group of the Cumulative Environmental Management Association, For McMurray, AB. December 2007.

Alberta Environment and Parks. (2020). *Total Area of the Oil Sands Tailings Ponds over Time*. http://osip.alberta.ca/library/Dataset/Details/542

Alberta Environment and Parks. (2022). *Oil Sands Mine Reclamation and Disturbance Tracking by Year.* https://osip.alberta.ca/library/Dataset/Details/27

Allen, E. W. (2008). Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. *Journal of Environmental Engineering and Science*, *7*(2), 123–138.

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., … Voordouw, G. (2013). Metagenomics of Hydrocarbon Resource Environments Indicates Aerobic Taxa and Genes to be Unexpectedly Common. *Environmental Science & Technology*, *47*(18), 10708–10717.

Bao, M., Chen, Q., Li, Y., & Jiang, G. (2010). Biodegradation of partially hydrolyzed polyacrylamide by bacteria isolated from production water after polymer flooding in an oil field. *Journal of Hazardous Materials*, *184*(1–3), 105–110.

Beazley, P. M. (1985). Quantitative determination of partially hydrolyzed polyacrylamide polymers in oil field production water. *Analytical Chemistry*, *57*(11), 2098–2101.

Berdugo-Clavijo, C., Sen, A., Seyyedi, M., Quintero, H., O'Neil, B., & Gieg, L. M. (2019). High temperature utilization of PAM and HPAM by microbial communities enriched from oilfield produced water and activated sludge. *AMB Express*, *9*(1), 46.

BGC Engineering Inc., 2010. *Oil Sands Tailings Technology Review*. Oil Sands Research and Information Network, University of Alberta, School of Energy and the Environment, Edmonton, Alberta. OSRIN Report No. TR-1. 136 pp.

Biagi, K. M., Oswald, C. J., Nicholls, E. M., & Carey, S. K. (2019). Increases in salinity following a shift in hydrologic regime in a constructed wetland watershed in a post-mining oil sands landscape. *Science of The Total Environment*, *653*, 1445–1457.

Bolto, B., & Gregory, J. (2007). Organic polyelectrolytes in water treatment. *Water Research*, *41*(11), 2301–2324.

Bosch, J., Lee, K.-Y., Jordan, G., Kim, K.-W., & Meckenstock, R. U. (2012). Anaerobic, Nitrate-Dependent Oxidation of Pyrite Nanoparticles by Thiobacillus denitrificans. *Environmental Science & Technology*, *46*(4), 2095–2101.

Buczek, S. B., Cope, W. G., McLaughlin, R. A., & Kwak, T. J. (2017). Acute toxicity of polyacrylamide flocculants to early life stages of freshwater mussels. *Environmental Toxicology and Chemistry*, *36*(10), 2715–2721.

Burkus, Z., Wheler, J., & Pletcher, S. (2014). *GHG Emissions from Oil Sands Tailings Ponds: Overview and Modelling Based on Fermentable Substrates. Part II: Modeling of GHG Emissions from Tailings Ponds Based on Fermentable Substrates.* Alberta Environment and Sustainable Resource Development.

Butler, R. M., Mcnab, G. S., & Lo, H. Y. (1981). Theoretical studies on the gravity drainage of heavy oil during in-situ steam heating. *The Canadian Journal of Chemical Engineering*, *59*(4), 455–460.

Canadian Natural Resources Limited. (2021). Horizon Oilsands Mine and Processing Plant: 2020 Annual Fluid Tailings Management Report.

Canada's Oil Sands Innovation Alliance (COSIA). (2021). *Pit Lakes: A Surface Mining Perspective*

Caulfield, M. J., Qiao, G. G., & Solomon, D. H. (2002). Some Aspects of the Properties and Degradation of Polyacrylamides. *Chemical Reviews*, *102*(9), 3067–3084.

Caesar-TonThat, T. C., Busscher, W. J., Novak, J. M., Gaskin, J. F., & Kim, Y. (2008). Effects of polyacrylamide and organic matter on microbes associated to soil aggregation of Norfolk loamy sand. *Applied Soil Ecology*, *40*(2), 240–249.

Chalaturnyk, R. J., Don Scott, J., & Özüm, B. (2002). MANAGEMENT OF OIL SANDS TAILINGS. *Petroleum Science and Technology*, *20*(9–10), 1025–1046.

Clifford, A. M., Jasinska, E. J., Meints, J., Hanna, J., & Goss, G. G. (2022). Hypoxemia as the mechanism of acute cationic polymer toxicity in rainbow trout and prevention of toxicity using an anionic neutralizing polymer. *Aquatic Toxicology*, *248*, 106198.

Clark, K. A., & Pasternack, D. S. (1932). Hot Water Seperation of Bitumen from Alberta Bituminous Sand. *Industrial & Engineering Chemistry*, *24*(12), 1410–1416.

Collins, C. E. V., Foght, J. M., & Siddique, T. (2016). Co-occurrence of methanogenesis and N2 fixation in oil sands tailings. *Science of The Total Environment*, *565*, 306–312.

Cossey, H. L., Batycky, A. E., Kaminsky, H., & Ulrich, A. C. (2021). Geochemical Stability of Oil Sands Tailings in Mine Closure Landforms. *Minerals*, *11*(8), 830.

Cymerman, G., Kwong, T., Lord, E., Hamza, H., & Xu, Y. 1999. Thickening and disposal of fine tails from oil sand processing. *Proceedings of the 3rd UBC-McGill Bi-annual International Symposium on Fundamentals of Mineral Processing (CIM)*, 605–619.

Dai, X., Luo, F., Yi, J., He, Q., & Dong, B. (2014). Biodegradation of polyacrylamide by anaerobic digestion under mesophilic condition and its performance in actual dewatered sludge system. *Bioresource Technology*, *153*, 55–61.

Dai, X., Luo, F., Zhang, D., Dai, L., Chen, Y., & Dong, B. (2015). Waste-activated sludge fermentation for polyacrylamide biodegradation improved by anaerobic hydrolysis and key microorganisms involved in biological polyacrylamide removal. *Scientific Reports*, *5*(1), 11675.

Degenhardt, D., Van Dongen, A., Schreiber, S. G., & Bekele, A. (2023). Growth and survival of native upland and wetland species in shallow capped thickened tailings: a meso-scale greenhouse study. *Canadian Journal of Soil Science*.

Deng, Y., Dixon, J. B., & White, G. N. (2006). Adsorption of Polyacrylamide on Smectite, Illite, and Kaolinite. *Soil Science Society of America Journal*, *70*(1), 297–304.

Dompierre, K. A., Lindsay, M. B. J., Cruz-Hernández, P., & Halferdahl, G. M. (2016). Initial geochemical characteristics of fluid fine tailings in an oil sands end pit lake. *Science of The Total Environment*, *556*, 196–206.

Dong, X., Kleiner, M., Sharp, C. E., Thorson, E., Li, C., Liu, D., & Strous, M. (2017). Fast and Simple Analysis of MiSeq Amplicon Sequencing Data with MetaAmp. *Frontiers in Microbiology*, *8*.

Fedorak, P. M., & Hrudey, S. E. (1984). The effects of phenol and some alkyl phenolics on batch anaerobic methanogenesis. *Water Research*, *18*(3), 361–367.

Foght, J. M., Gieg, L. M., & Siddique, T. (2017). The microbiology of oil sands tailings: past, present, future. *FEMS Microbiology Ecology*, *93*(5).

Folta-Stogniew, E., & Williams, K. R. (1999). Determination of molecular masses of proteins in solution: Implementation of an HPLC size exclusion chromatography and laser light scattering service in a core laboratory. *Journal of Biomolecular Techniques: JBT*, *10*(2), 51–63.

Foote, L. (2012). Threshold Considerations and Wetland Reclamation in Alberta's Mineable Oil Sands. *Ecology and Society*, *17*(1), art35.

Garibaldi, A. (2009). Moving From Model to Application: Cultural Keystone Species and Reclamation in Fort McKay, Alberta. *Journal of Ethnobiology*, *29*(2), 323–338.

Gharfeh, S. G., & Moradi-Araghi, A. (1986). Determination of anionic high-molecular-weight water-soluble polymers by size-exclusion chromatography. *Journal of Chromatography A*, *366*, 343–350.

Global Safety Management Inc. (2014). *Sodium Nitrate MSDS*.

Gómez-Ordóñez, E., Jiménez-Escrig, A., & Rupérez, P. (2012). Molecular weight distribution of polysaccharides from edible seaweeds by high-performance size-exclusion chromatography (HPSEC). *Talanta*, *93*, 153–159.

Government of Alberta. (2023a). *Oil sands facts and statistics*. https://www.alberta.ca/oil-sands-facts-and-statistics

Government of Alberta. (2023b). *Oil sands 101*. https://www.alberta.ca/oil-sands-101#:~:text=Recovery-,Mining,20%25 of oil sands reserves

Grula, M. M., Huang, M.-L., & Sewell, G. (1994). Interactions of certain polyacrylamides with soil bacteria. *Soil Science*, *158*(4), 291–300.

Guezennec, A. G., Michel, C., Bru, K., Touze, S., Desroche, N., Mnif, I., & Motelica-Heino, M. (2015). Transfer and degradation of polyacrylamide-based flocculants in hydrosystems: a review. *Environmental Science and Pollution Research*, *22*(9), 6390–6406.

Harahuc, L., Lizama, H. M., & Suzuki, I. (2000). Selective Inhibition of the Oxidation of Ferrous Iron or Sulfur in Thiobacillus ferrooxidans. *Applied and Environmental Microbiology*, *66*(3), 1031–1037.

Haveroen, M. E., MacKinnon, M. D., & Fedorak, P. M. (2005). Polyacrylamide added as a nitrogen source stimulates methanogenesis in consortia from various wastewaters. *Water Research*, *39*(14), 3333–334.

Health Canada. (2009). *2-Propenamide (Acrylamide)*.

Herman, D. C., Fedorak, P. M., MacKinnon, M. D., & Costerton, J. W. (1994). Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. *Canadian Journal of Microbiology*, *40*(6), 467–477.

Holden, A. A., Donahue, R. B., & Ulrich, A. C. (2011). Geochemical interactions between process-affected water from oil sands tailings ponds and North Alberta surficial sediments. *Journal of Contaminant Hydrology*, *119*(1–4), 55–68.

Holowenko, F. M., MacKinnon, M. D., & Fedorak, P. M. (2000). Methanogens and sulfate-reducing bacteria in oil sands fine tailings waste. *Canadian Journal of Microbiology*, *46*(10), 927–937.

Hu, H., Liu, J.-F., Li, C.-Y., Yang, S.-Z., Gu, J.-D., & Mu, B.-Z. (2018). Anaerobic biodegradation of partially hydrolyzed polyacrylamide in long-term methanogenic enrichment cultures from production water of oil reservoirs. *Biodegradation*, *29*(3), 233–243.

Hyndman, A., Sawatsky, L., McKenna, G., & Vandenberg, J. (2018). Fluid Fine Tailings Processes: Disposal, Capping, and Closure Alternatives. In *Proceedings of the 6th* International *Oil Sands Tailings Conference,* Edmonton, AB, Canada, 9-12 December 2018.

Imperial Oil Resources Ltd. (2020). *Kearl Oil Sands Mine: Fluid Tailings Management Report for 2019*.

Imperial Oil Resources Ltd. (2021). *Kearl Oil Sands Mine: Fluid Tailings Management Report for 2020*.

Imperial Oil Resources Ltd. (2022). *Kearl Oil Sands Mine: Fluid Tailings Management Report for 2021*.

Jordaan, S. M. (2012). Land and Water Impacts of Oil Sands Production in Alberta. *Environmental Science & Technology*, *46*(7), 3611–3617.

Kasperski, K. L., & Mikula, R. J. (2011). Waste Streams of Mined Oil Sands: Characteristics and Remediation. *Elements*, *7*(6), 387–392.

Kay-Shoemake, J. L., Watwood, M. E., Lentz, R. D., & Sojka, R. E. (1998a). Polyacrylamide as an organic nitrogen source for soil microorganisms with potential effects on inorganic soil nitrogen in agricultural soil. *Soil Biology and Biochemistry*, *30*(8–9), 1045–1052.

Kay-Shoemake, J. L., Watwood, M. E., Sojka, R. E., & Lentz, R. D. (1998b). Polyacrylamide as a substrate for microbial amidase in culture and soil. *Soil Biology and Biochemistry*, *30*(13), 1647–1654.

Kendall, M. M., & Boone, D. R. (2006). The Order Methanosarcinales. In *The Prokaryotes* (pp. 244–256). Springer New York.

Kuznetsov, P., Kuznetsova, A., Foght, J. M., & Siddique, T. (2015). Oil sands thickened froth treatment tailings exhibit acid rock drainage potential during evaporative drying. *Science of The Total Environment*, *505*, 1–10.

Laird, D. A. (1997). Bonding between polyacrylamide and clay mineral surfaces. *Soil Science*, *162*(11), 826–832.

Lee, K., & Ulrich, A. (2021). Indigenous microbial communities in Albertan sediments are capable of anaerobic benzene biodegradation under methanogenic, sulfate-reducing, nitrate-reducing, and iron-reducing redox conditions. *Water Environment Research*, *93*(4), 524–534.

Leung, R. W. M., Pandey, R. N., & Das, B. S. (1987). Determination of polyacrylamides in coal washery effluents by ultrafiltration/size-exclusion chromatography-ultraviolet detection techniques. *Environmental Science & Technology*, *21*(5), 476–481.

Li, C., Fu, L., Stafford, J., Belosevic, M., & Gamal El-Din, M. (2017). The toxicity of oil sands process-affected water (OSPW): A critical review. *Science of The Total Environment*, *601–602*, 1785–1802.

Li, J., How, Z. T., & Gamal El-Din, M. (2023). Aerobic degradation of anionic polyacrylamide in oil sands tailings: Impact factor, degradation effect, and mechanism. *Science of The Total Environment*, *856*, 159079.

Li, Y., Kaminsky, H., Gong, X. Y., Sun, Y. S., Ghuzi, M., & Sadighian, A. (2021). What Affects Dewatering Performance of High Density Slurry? *Minerals*, *11*(7), 761.

Li, H., Long, J., Xu, Z., & Masliyah, J. H. (2005). Synergetic Role of Polymer Flocculant in Low-Temperature Bitumen Extraction and Tailings Treatment. *Energy & Fuels*, *19*(3), 936–943.

Li, C. Y., Li, J. Y., Mbadinga, S. M., Liu, J. F., Gu, J. D., & Mu, B. Z. (2015). Analysis of bacterial and archaeal communities along a high-molecular-weight polyacrylamide transportation pipeline system in an oil field. *International Journal of Molecular Sciences*, *16*(4), 7445–7461.

Li, H., Long, J., Xu, Z., & Masliyah, J. H. (2005). Synergetic Role of Polymer Flocculant in Low-Temperature Bitumen Extraction and Tailings Treatment. *Energy & Fuels*, *19*(3), 936–943.

Li, H., O'Shea, J., & Franks, G. V. (2009). Effect of molecular weight of poly(N -isopropyl acrylamide) temperature-sensitive flocculants on dewatering. *AIChE Journal*, *55*(8), 2070–2080.

Liang, B., Wang, L.-Y., Mbadinga, S. M., Liu, J.-F., Yang, S.-Z., Gu, J.-D., & Mu, B.-Z. (2015). Anaerolineaceae and Methanosaeta turned to be the dominant microorganisms in alkanes-dependent methanogenic culture after long-term of incubation. *AMB Express*, *5*(1), 37.

Lipp, D., & Kozakiewicz, J. (2000). Acrylamide Polymers. In *Kirk-Othmer Encyclopedia of Chemical Technology*. John Wiley & Sons, Inc.

Liu, J., Ren, J., Xu, R., Yu, B., & Wang, J. (2016). Biodegradation of partially hydrolyzed polyacrylamide by immobilized bacteria isolated from HPAM-containing wastewater. *Environmental Progress & Sustainable Energy*, *35*(5), 1344–1352.

Liu, L., Wang, Z., Lin, K., & Cai, W. (2012). Microbial degradation of polyacrylamide by aerobic granules. *Environmental Technology*, *33*(9), 1049–1054.

Long, J., Li, H., Xu, Z., & Masliyah, J. H. (2006). Role of colloidal interactions in oil sand tailings treatment. *AIChE Journal*, *52*(1), 371–383.

Lu, J. H., & Wu, L. (2001). Spectrophotometric Determination of Polyacrylamide in Waters Containing Dissolved Organic Matter. *Journal of Agricultural and Food Chemistry*, *49*(9), 4177–4182.

Lu, J., & Wu, L. (2003a). Polyacrylamide quantification methods in soil conservation studies. *Soil and Water Conservation*, *58*(5), 270–275.

Lu, J., & Wu, L. (2003b). Polyacrylamide Distribution in Columns of Organic Matter–Removed Soils following Surface Application. *Journal of Environmental Quality*, *32*(2), 674–680.

Lu, J., Wu, L., & Gan, J. (2003). Determination of Polyacrylamide in Soil Waters by Size Exclusion Chromatography. *Journal of Environmental Quality*, *32*(5), 1922–1926.

Ma, L., Hu, T., Liu, Y., Liu, J., Wang, Y., Wang, P., Zhou, J., Chen, M., Yang, B., & Li, L. (2021). Combination of biochar and immobilized bacteria accelerates polyacrylamide biodegradation in soil by both bio-augmentation and bio-stimulation strategies. *Journal of Hazardous Materials*, *405*, 124086.

Ma, F., Wei, L., Wang, L., & Chang, C. C. (2008). Isolation and identification of the sulphate-reducing bacteria strain H1 and its function for hydrolysed polyacrylamide degradation. *International Journal of Biotechnology*, *10*(1), 55.

Mansour, A. M., Al-Maamari, R. S., Al-Hashmi, A. S., Zaitoun, A., & Al-Sharji, H. (2014). In-situ rheology and mechanical degradation of EOR polyacrylamide solutions under moderate shear rates. *Journal of Petroleum Science and Engineering*, *115*, 57–65.

Martini, M., Marcone, C., Lee, I.-M., & Firrao, G. (2014). The Family Acholeplasmataceae (Including Phytoplasmas). In *The Prokaryotes* (pp. 469–504). Springer Berlin Heidelberg.

Masliyah, J., Zhou, Z. J., Xu, Z., Czarnecki, J., & Hamza, H. (2008). Understanding Water-Based Bitumen Extraction from Athabasca Oil Sands. *The Canadian Journal of Chemical Engineering*, *82*(4), 628–654.

Matsuoka, H., Ishimura, F., Takeda, T., & Hikuma, M. (2002). Isolation of polyacrylamide-degrading microorganisms from soil. *Biotechnology and Bioprocess Engineering*, *7*(5), 327–330.

Mohamad Shahimin, M. F., & Siddique, T. (2017a). Sequential biodegradation of complex naphtha hydrocarbons under methanogenic conditions in two different oil sands tailings. *Environmental Pollution*, *221*, 398–406.

Mohamad Shahimin, M. F., & Siddique, T. (2017b). Methanogenic biodegradation of paraffinic solvent hydrocarbons in two different oil sands tailings. *Science of The Total Environment*, *583*, 115–122.

Moody, G. (2007). Polymeric Flocculants. In *Handbook of industrial water soluble polymers* (pp. 134–173). Blackwell Pub.

Nadler, A., Malik, M., & Letey, J. (1992). Desorption of polyacrylamide and polysaccharide polymers from soil materials. *Soil Technology*, *5*(1), 91–95.

Nakamiya, K., & Kinoshita, S. (1995). Isolation of polyacrylamide-degrading bacteria. *Journal of Fermentation and Bioengineering*, *80*(4), 418–420.

Nakamiya, K., Sakasita, G., Ooi, T., & Kinoshita, S. (1997). Enzymatic degradation of polystyrene by hydroquinone peroxidase of Azotobacter beijerinckii HM121. *Journal of Fermentation and Bioengineering*, *84*(5), 480–482.

Nollet, L. M. L., & De Gelder, L. S. P. (Eds.). (2013). *Handbook of Water Analysis*. CRC Press.

Nyyssölä, A., & Ahlgren, J. (2019). Microbial degradation of polyacrylamide and the deamination product polyacrylate. *International Biodeterioration and Biodegradation*, *139*(October 2018), 24–33.

Oil Sand Magazine. (2023). *In situ bitumen extraction*. https://www.oilsandsmagazine.com/technical/in-situ

Oil Sands Tailings Consortium (OSTC) & Canada's Oil Sands Innovation Alliance (COSIA). (2012). *Technical Guide for Fluid Fine Tailings Management*.Peukert, W., Kaspereit, M., Hofe, T., & Gromotka, L. (2022). Size exclusion chromatography (SEC). In *Particle Separation Techniques* (pp. 409–447). Elsevier.

Orlygsson, J., & Kristjansson, J. K. (2014). The Family Hydrogenophilaceae. In *The Prokaryotes* (pp. 859–868). Springer Berlin Heidelberg.

Penner, T. J., & Foght, J. M. (2010). Mature fine tailings from oil sands processing harbour diverse methanogenic communities. *Canadian Journal of Microbiology*, *56*(6), 459–470.

Plante, A. F., Stone, M. M., & McGill, W. B. (2015). The Metabolic Physiology of Soil Microorganisms. In *Soil Microbiology, Ecology and Biochemistry* (pp. 245–272). Elsevier.

Podzimek, S. (2011). Size Exclusion Chromatography. In *Light Scattering, Size Exclusion Chromatography and Asymmetric Flow Field Flow Fractionation* (pp. 99–206). Wiley.

Prosser, J. I., Head, I. M., & Stein, L. Y. (2014). The Family Nitrosomonadaceae. In *The Prokaryotes* (pp. 901–918). Springer Berlin Heidelberg.

Reid, M. L., & Warren, L. A. (2016). S reactivity of an oil sands composite tailings deposit undergoing reclamation wetland construction. *Journal of Environmental Management*, *166*, 321–329.

Ramos-Padrón, E., Bordenave, S., Lin, S., Bhaskar, I. M., Dong, X., Sensen, C. W., Fournier, J., Voordouw, G., & Gieg, L. M. (2011). Carbon and Sulfur Cycling by Microbial Communities in a Gypsum-Treated Oil Sands Tailings Pond. *Environmental Science & Technology*, *45*(2), 439–446.

Ripmeester, J. A., Kotlyar, L. S., & Sparks, B. D. (1993). 2H NMR and the sol—gel transition in suspensions of colloidal clays. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *78*, 57–63.

Samad, A., Degenhardt, D., Séguin, A., Morency, M.-J., Gagné, P., & Martineau, C. (2023). Microbial community structural and functional differentiation in capped thickened oil sands tailings planted with native boreal species. *Frontiers in Microbiology*, *14*.

Sanford, E. C. (1983). Processibility of athabasca oil sand: Interrelationship between oil sand fine solids, process aids, mechanical energy and oil sand age after mining. *The Canadian Journal of Chemical Engineering*, *61*(4), 554–567.

Sang, G., Pi, Y., Bao, M., Li, Y., & Lu, J. (2015). Biodegradation for hydrolyzed polyacrylamide in the anaerobic baffled reactor combined aeration tank. *Ecological Engineering*, *84*, 121–127.

Sherry, A., Gray, N. D., Ditchfield, A. K., Aitken, C. M., Jones, D. M., Röling, W. F. M., Hallmann, C., Larter, S. R., Bowler, B. F. J., & Head, I. M. (2013). Anaerobic biodegradation of crude oil under sulphate-reducing conditions leads to only modest enrichment of recognized sulphate-reducing taxa. *International Biodeterioration & Biodegradation*, *81*, 105–113.

Siddique, T., Kuznetsov, P., Kuznetsova, A., Arkell, N., Young, R., Li, C., Guigard, S., Underwood, E., & Foght, J. M. (2014a). Microbially-accelerated consolidation of oil sands tailings. Pathway I: changes in porewater chemistry. *Frontiers in Microbiology*, *5*.

Siddique, T., Kuznetsov, P., Kuznetsova, A., Li, C., Young, R., Arocena, J. M., & Foght, J. M. (2014b). Microbially-accelerated consolidation of oil sands tailings. Pathway II: solid phase biogeochemistry. *Frontiers in Microbiology*, *5*.

Siddique, T., Penner, T., Klassen, J., Nesbø, C., & Foght, J. M. (2012). Microbial Communities Involved in Methane Production from Hydrocarbons in Oil Sands Tailings. *Environmental Science & Technology*, *46*(17), 9802–9810.

Siddique, T., Penner, T., Semple, K., & Foght, J. M. (2011). Anaerobic Biodegradation of Longer-Chain n -Alkanes Coupled to Methane Production in Oil Sands Tailings. *Environmental Science & Technology*, *45*(13), 5892–5899.

Siddique, T., Stasik, S., Mohamad Shahimin, M. F., & Wendt-Potthoff, K. (2019). Microbial Communities in Oil Sands Tailings: Their Implications in Biogeochemical Processes and Tailings Management. In *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology* (pp. 251–283). Springer International Publishing.

Small, C. C., Cho, S., Hashisho, Z., & Ulrich, A. C. (2015). Emissions from oil sands tailings ponds: Review of tailings pond parameters and emission estimates. *Journal of Petroleum Science and Engineering*, *127*, 490–501.

So, C. M., & Young, L. Y. (1999). Isolation and characterization of a sulfate-reducing bacterium that anaerobically degrades alkanes. *Applied and Environmental Microbiology*, *65*(7), 2969–2976.

Sojka, R. E., Bjorneberg, D. L., Entry, J. A., Lentz, R. D., & Orts, W. J. (2007). *Polyacrylamide in Agriculture and Environmental Land Management* (pp. 75–162).

Song, T., Li, S., Ding, W., Li, H., Bao, M., & Li, Y. (2018). Biodegradation of hydrolyzed polyacrylamide by the combined expanded granular sludge bed reactor-aerobic biofilm reactor biosystem and key microorganisms involved in this bioprocess. *Bioresource Technology*, *263*(168), 153–162.

Song, T., Li, S., Lu, Y., Yan, D., Sun, P., Bao, M., & Li, Y. (2019). Biodegradation of hydrolyzed polyacrylamide by a Bacillus megaterium strain SZK-5: Functional enzymes and antioxidant defense mechanism. *Chemosphere*, *231*, 184–193.

Song, W., Zhang, Y., Gao, Y., Chen, D., & Yang, M. (2017). Cleavage of the main carbon chain backbone of high molecular weight polyacrylamide by aerobic and anaerobic biological treatment. *Chemosphere*, *189*, 277–283.

Striegel, A. M. (2009). *Modern size-exclusion liquid chromatography: practice of gel permeation and gel filtration chromatography* (2nd ed.).

Stasik, S., & Wendt-Potthoff, K. (2016). Vertical gradients in carbon flow and methane production in a sulfate-rich oil sands tailings pond. *Water Research*, *106*, 223–231.

Suncor Energy Operating Inc. (2022). *2021 FORT HILLS FLUID TAILINGS MANAGEMENT REPORT*.

Sutton, O. F., & Price, J. S. (2020). Soil moisture dynamics modelling of a reclaimed upland in the early post-construction period. *Science of The Total Environment*, *718*, 134628.

Trathnigg, B. (2000). Size-Exclusion Chromatography of Polymers. In *Encyclopedia of Analytical Chemistry*. John Wiley & Sons, Ltd.

United States Environmental Protection Agency. (2010). *Toxicological Review of Acrylamide In Support of SUmmary Information on the Integrated Risk Information System (IRIS)*.

United States Environmental Protection Agency. (2010). *Toxicological Review of Acrylamide In Support of Summary Information on the Integrated Risk Information System (IRIS)*.

Vedoy, D. R. L., & Soares, J. B. P. (2015). Water-soluble polymers for oil sands tailing treatment: A Review. *The Canadian Journal of Chemical Engineering*, *93*(5), 888–904.

Wang, X. (2016). Review of characterization methods for water-soluble polymers used in oil sand and heavy oil industrial applications. *Environmental Reviews*, *24*(4), 460–470.

Wang, X. T., Feng, X., Xu, Z., & Masliyah, J. H. (2010). Polymer aids for settling and filtration of oil sands tailings. *The Canadian Journal of Chemical Engineering*.

Warren, L. A., Kendra, K. E., Brady, A. L., & Slater, G. F. (2016). Sulfur Biogeochemistry of an Oil Sands Composite Tailings Deposit. *Frontiers in Microbiology*, *6*.

Wen, Q., Chen, Z., Zhao, Y., Zhang, H., & Feng, Y. (2010). Biodegradation of polyacrylamide by bacteria isolated from activated sludge and oil-contaminated soil. *Journal of Hazardous Materials*, *175*(1–3), 955–959.

Westcott, F., & Watson, L. (2007). *End Pit Lakes Technical Guidance Document*. Clearwater Environmental Consultants for CEMA End Pit Lakes Subgroup, Project 2005-61

Weston, D. P., Lentz, R. D., Cahn, M. D., Ogle, R. S., Rothert, A. K., & Lydy, M. J. (2009). Toxicity of Anionic Polyacrylamide Formulations when Used for Erosion Control in Agriculture. *Journal of Environmental Quality*, *38*(1), 238–247.

Winfrey, M. R., & Zeikus, J. G. (1977). Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments. *Applied and Environmental Microbiology*, *33*(2), 275–281.

World Health Organization. (2022). *Guideliens for drinking-water quality: fourth edition incorporating the first and second addenda, Geneva.*Xihua, S., Qingfang, Z., Mingbo, W., & Yansheng, G. (2007). Preparation and application of silica-based perfusion packing of size exclusion chromatography for quantitation of polyacrylamide in enhanced oil recovery systems. *Petroleum Science*, *4*(2), 82–90.

Xu, Y., & Cymerman, G. (1999). Flocculation of fine oil sand tails. *Polymers in Mineral Processing*. *Proceedings of the 3rd UBC-McGill Bi-annual International Symposium on Fundamentals of Mineral Processing (CIM)*, 591-604.

Yan, M., Zhao, L., Bao, M., & Lu, J. (2016). Hydrolyzed polyacrylamide biodegradation and mechanism in sequencing batch biofilm reactor. *Bioresource Technology*, *207*, 315–321.

Yaws, C. L. (2003). Yaws' handbook of thermodynamic and physical properties of chemical compounds : physical, thermodynamic and transport properties for 5,000 organic chemical compounds. Knovel.

Yu, F., Fu, R., Xie, Y., & Chen, W. (2015). Isolation and Characterization of Polyacrylamide-Degrading Bacteria from Dewatered Sludge. *International Journal of Environmental Research and Public Health*, *12*(4), 4214–4230.

Zhang, D., Abraham, T., Dang-Vu, T., Xu, J., Gumfekar, S. P., & Thundat, T. (2021). Optimal floc structure for effective dewatering of polymer treated oil sands tailings. *Minerals Engineering*, *160*, 106688.

Zhang, X., Li, H., Li, F., Dong, X., Guo, Z., Han, H., & Wang, J. (2023). Two strains of bacteria from oilfield slickwater and their performance in partially hydrolyzed polyacrylamide biodegradation. *Geoenergy Science and Engineering*, *228*, 211962.

Zhao, L., Song, T., Han, D., Bao, M., & Lu, J. (2019). Hydrolyzed polyacrylamide biotransformation in an up-flow anaerobic sludge blanket reactor system: key enzymes, functional microorganisms, and biodegradation mechanisms. *Bioprocess and Biosystems Engineering*, *42*(6), 941–951.

APPENDIX A: CHAPTER 3 SUPPLEMENTARY INFORMATION

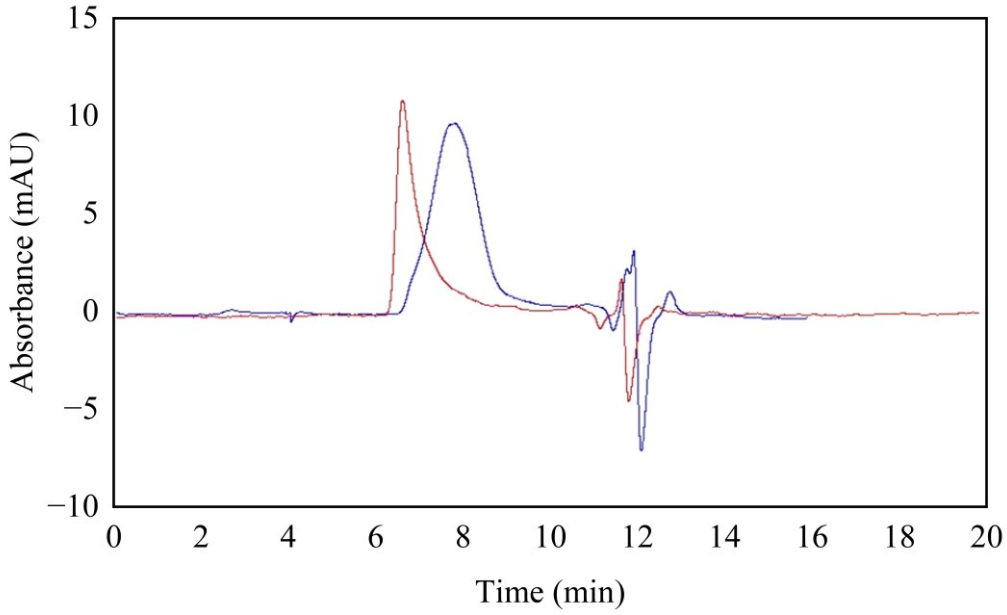


Figure A-1 Effect of injection volume on peak resolution for hydrolyzed polyacrylamide. The injection volume was 20 uL for the red chromatogram and 30 uL for the blue chromatogram.

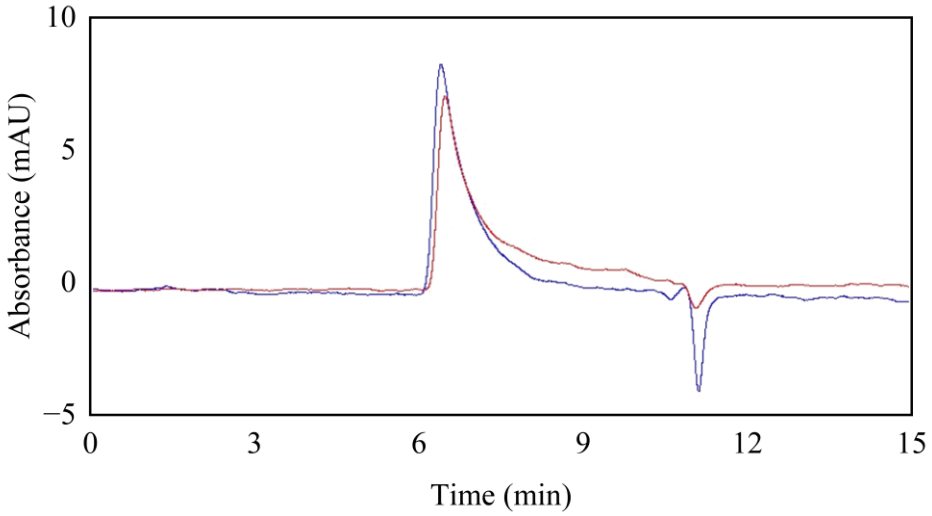


Figure A-2 Effect of electrolyte addition on peak tailings for hydrolyzed polyacrylamide. The red chromatogram represents polymer elution with electrolyte while the blue chromatogram was eluted with the addition of 0.1 M NaCl.

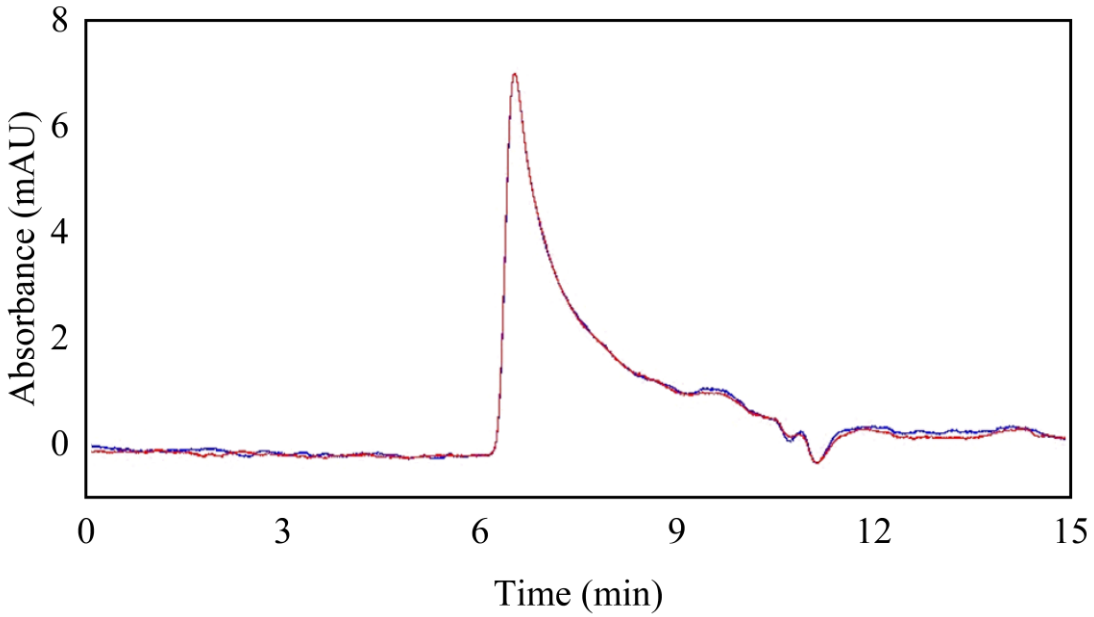


Figure A-3 Effect of filtering with 0.45 µm filters on hydrolyzed polyacrylamide dissolved in milli-Q water. The red chromatogram depicts sample preparation with centrifugation and filtering, while the blue chromatogram did not receive any sample preparation.

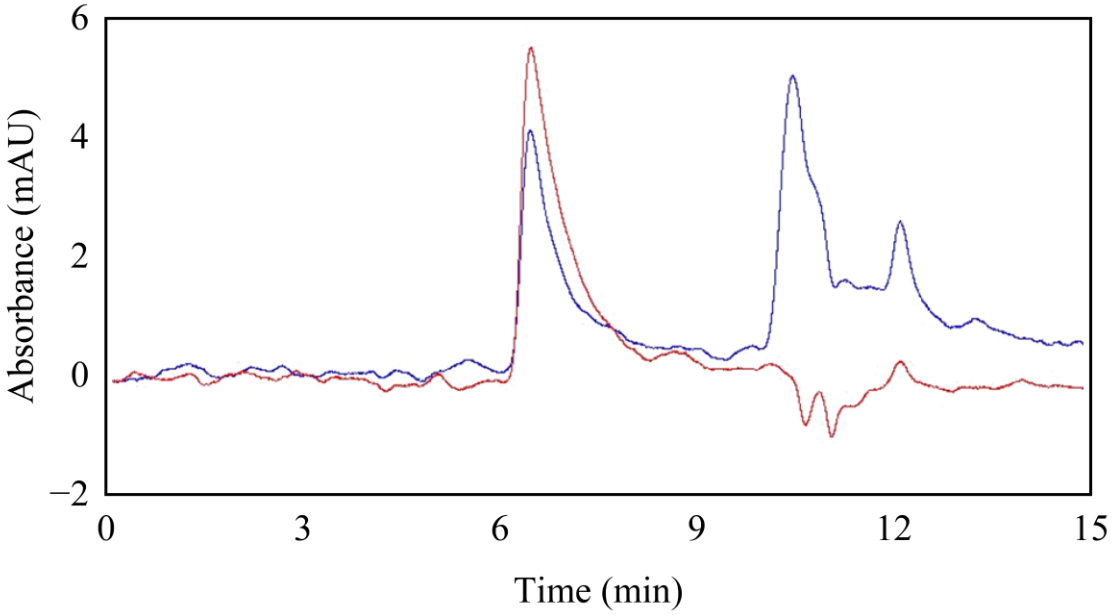


Figure A-4 Chromatograms from hydrolyzed polyacrylamide dissolved in milli-Q water (red) and oil sands process-affected water (blue).

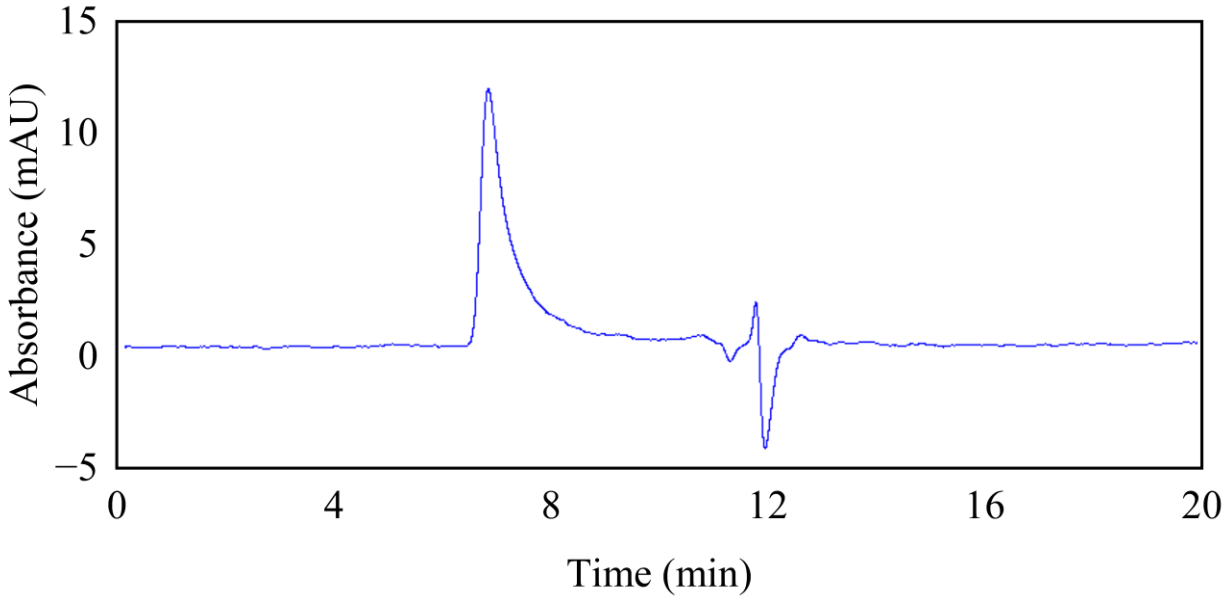


Figure A-5 Chromatogram of 200 mg/L HPAM for finalized SEC method.

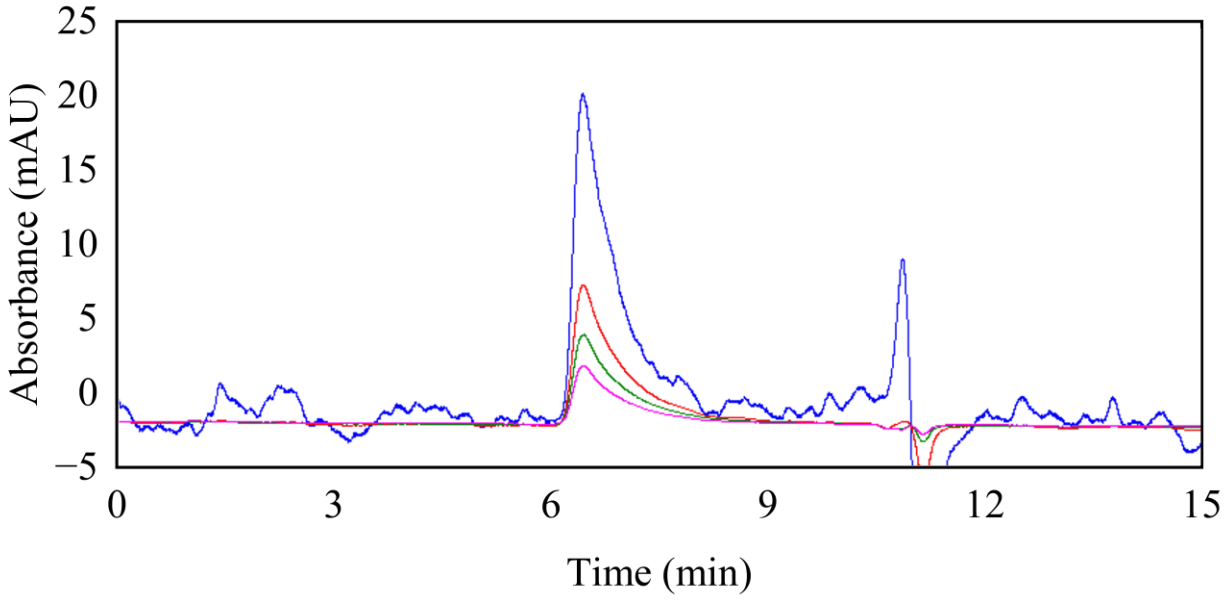


Figure A-6 Chromatograms for HPAM at 200 (blue), 210 (red), 215 (green), and 220 (pink) nm using a Diode Array Detector. 210 nm was selected as the method wavelength.

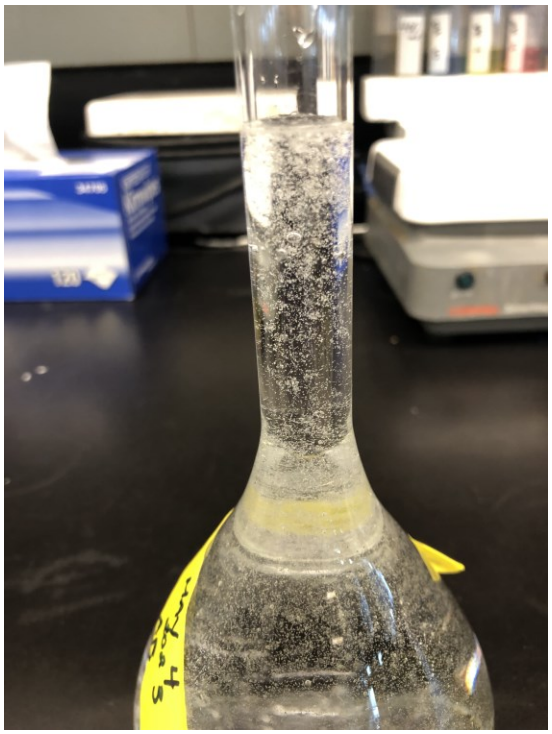


Figure A-7 Incomplete dissolution of 500 mg/L hydrolyzed polyacrylamide in milli-Q water.

Table A-1: Molecular weight and polydispersity index for polyacrylamide standards.

Standards	Molecular Weight (g/mol)	Polydispersity Index
PAAM9000K	9450000	2.12
PAAM7000K	7300000	3.47
PAAM6000K	5352000	10.00
PAAM1000K	1145000	2.45
PAAM500K	539700	2.50
PAAM350K	381700	2.60
PAAM80K	81200	1.80
PAAM60K	59000	1.60
PAAM20K	22600	1.60
PAAM9K	8980	1.69

APPENDIX B: CHAPTER 4 SUPPLEMENTARY INFORMATION

Figure B-1 Media serum bottles for methanogenic conditions. The pink colour is due to residual O_2 present in the bottles. Once sodium sulfide (O_2 scavenger) was added the media turned black and then eventually clear indicating anoxic conditions had been achieved.

Figure B-2 Methanogenic microcosms for P_{Co} treatments. The killed controls (9 and 10) remained pink for the entirety of the experiment indicating that no O_2 had been consumed by initial microbial communities and therefore sterile conditions were maintained.

Figure B-3 Air bubbles trapped within flocculated oil sands tailings prior to inverting the microcosm for headspace analysis. Air bubbles could possibly consist of CO_2, CH_4, H_2S, or a mixture.

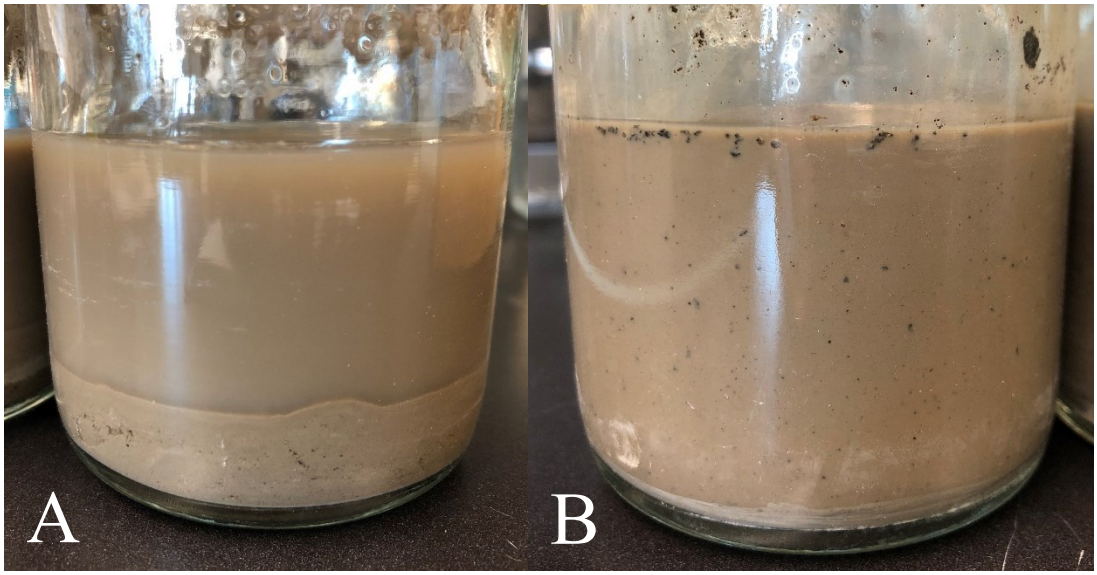


Figure B-4 HPAM amended oil sands tailings (A) before and (B) after biodegradation under oxic conditions.

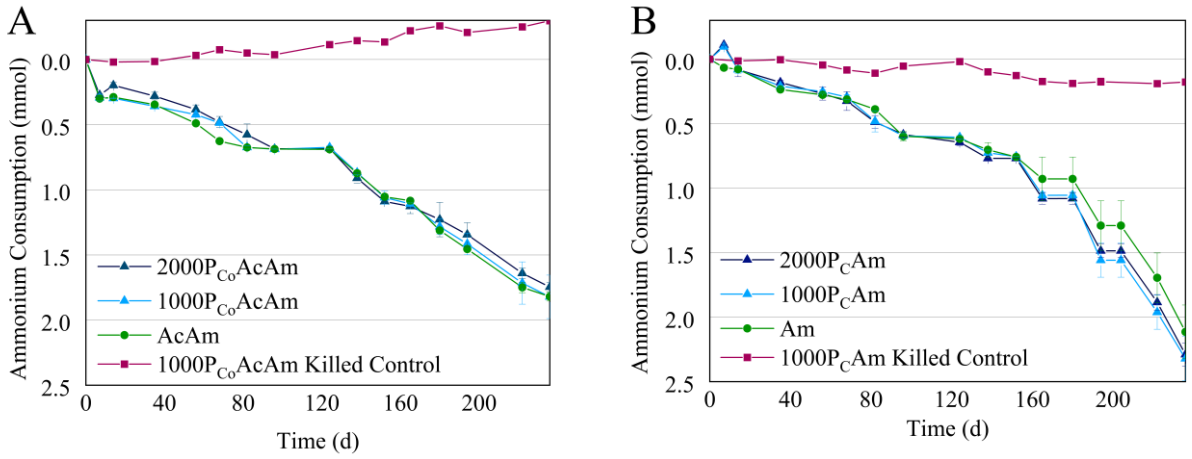


Figure B-5 Cumulative ammonium consumption in (A) HPAM as co-substrate and (B) HPAM as carbon source under oxic conditions. Error bars represent one standard deviation of biological replicates.

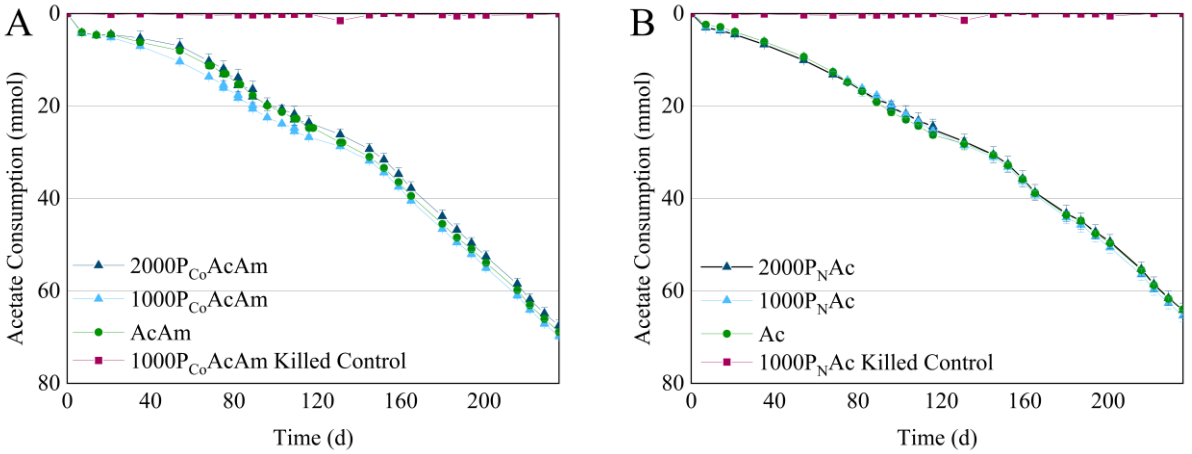


Figure B-6 Cumulative acetate consumption in (A) HPAM as co-substrate and (B) HPAM as nitrogen source under oxic conditions. Error bars represent one standard deviation of biological replicates.

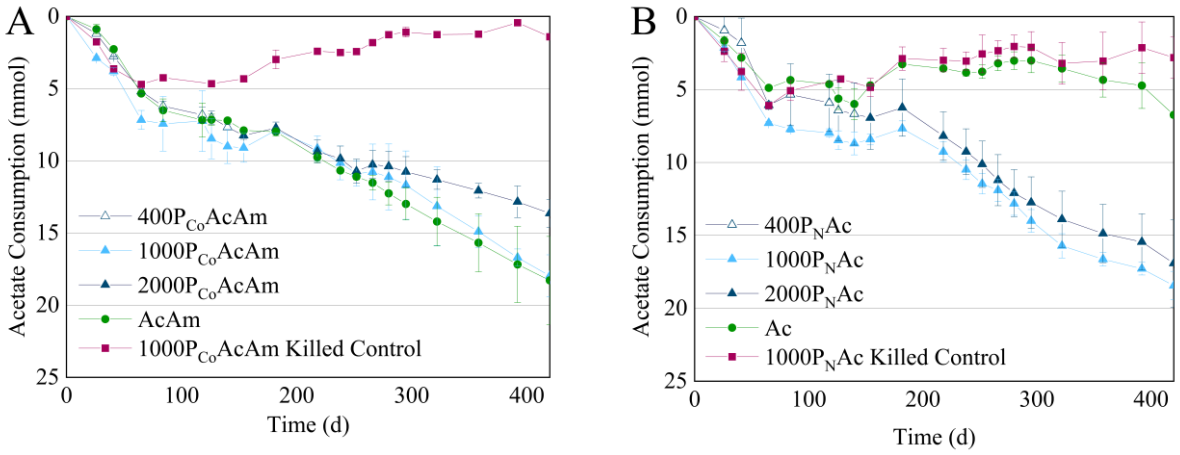


Figure B-7 Cumulative acetate consumption in (A) HPAM as co-substrate and (B) HPAM as nitrogen source under sulfate-reducing conditions. Error bars represent one standard deviation of biological replicates.

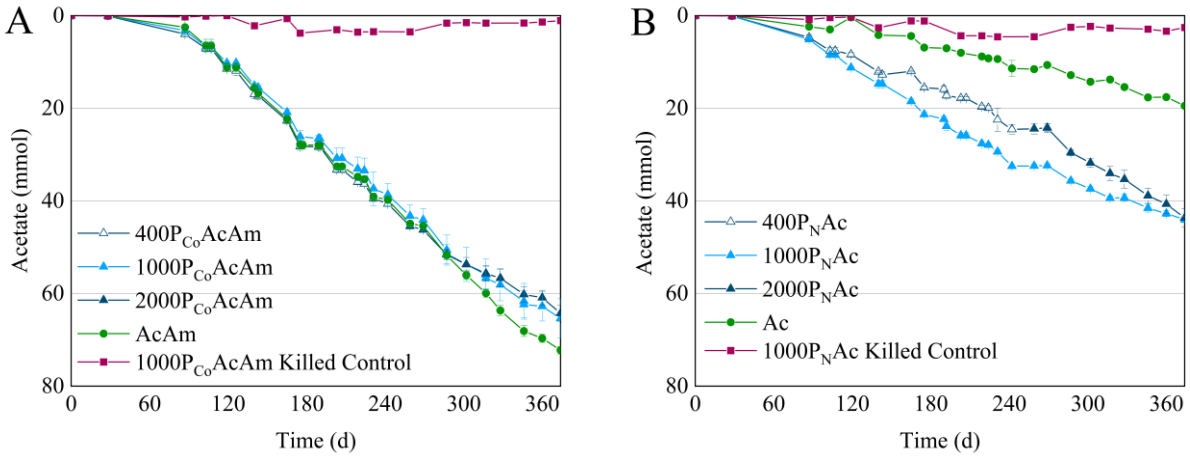


Figure B-8 Cumulative acetate consumption in (A) HPAM as co-substrate and (B) HPAM as nitrogen source under methanogenic conditions. Error bars represent one standard deviation of biological replicates.

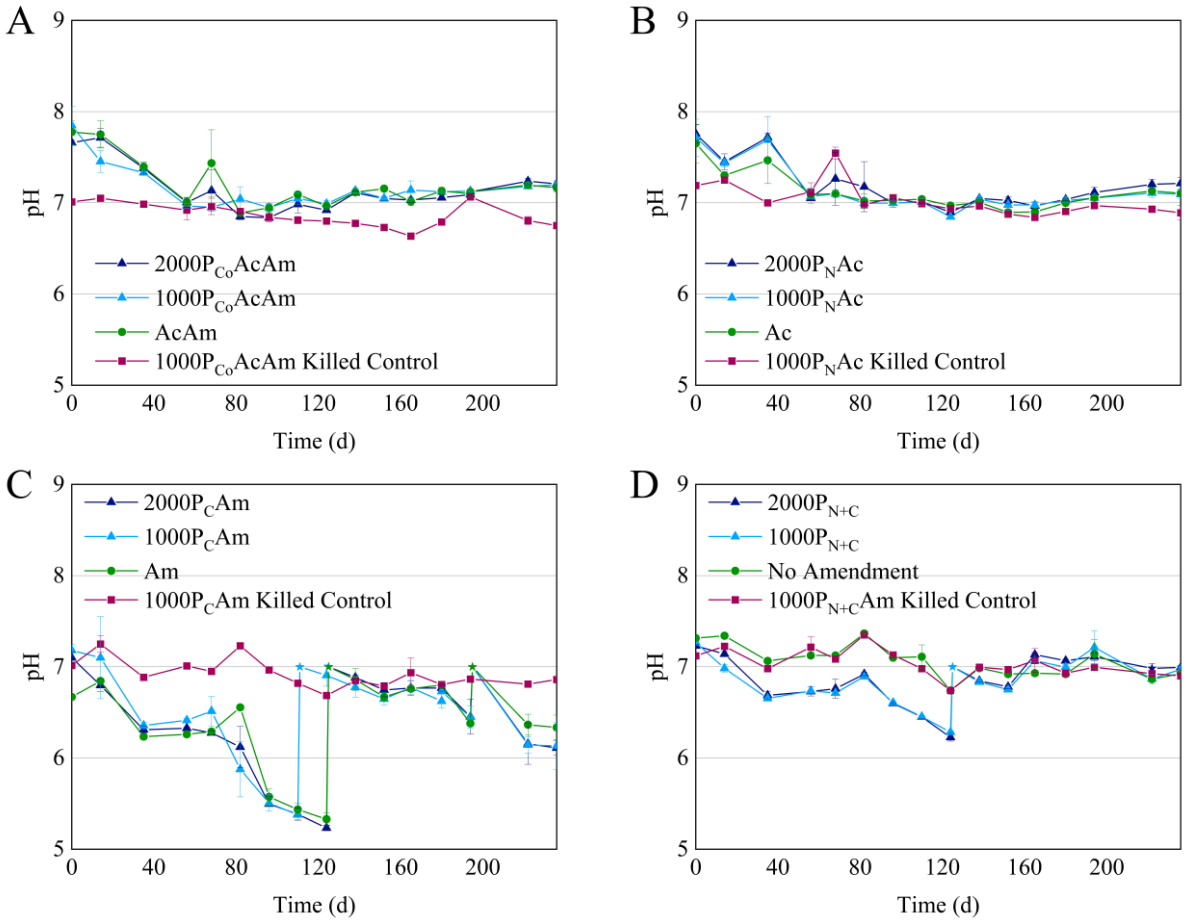


Figure B-9 pH in (A) HPAM as co-substrate, (B) HPAM as nitrogen source, (C) HPAM as carbon source, and (D) HPAM as nitrogen and carbon source under oxic conditions. pH adjustments are represented by stars (★). Error bars represent one standard deviation of biological replicates.

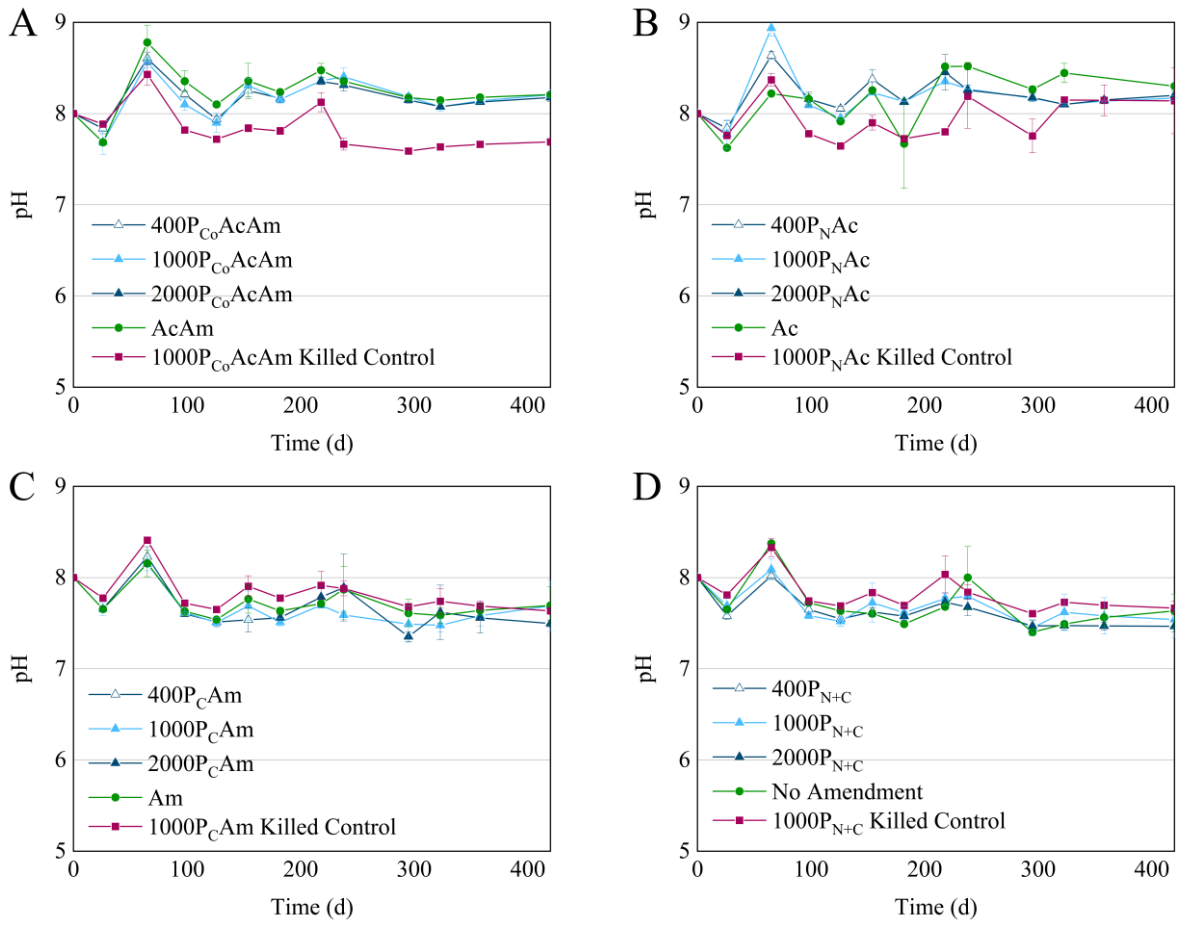


Figure B-10 pH in (A) HPAM as co-substrate, (B) HPAM as nitrogen source, (C) HPAM as carbon source, and (D) HPAM as nitrogen and carbon source under sulfate-reducing conditions. Error bars represent one standard deviation of biological replicates.

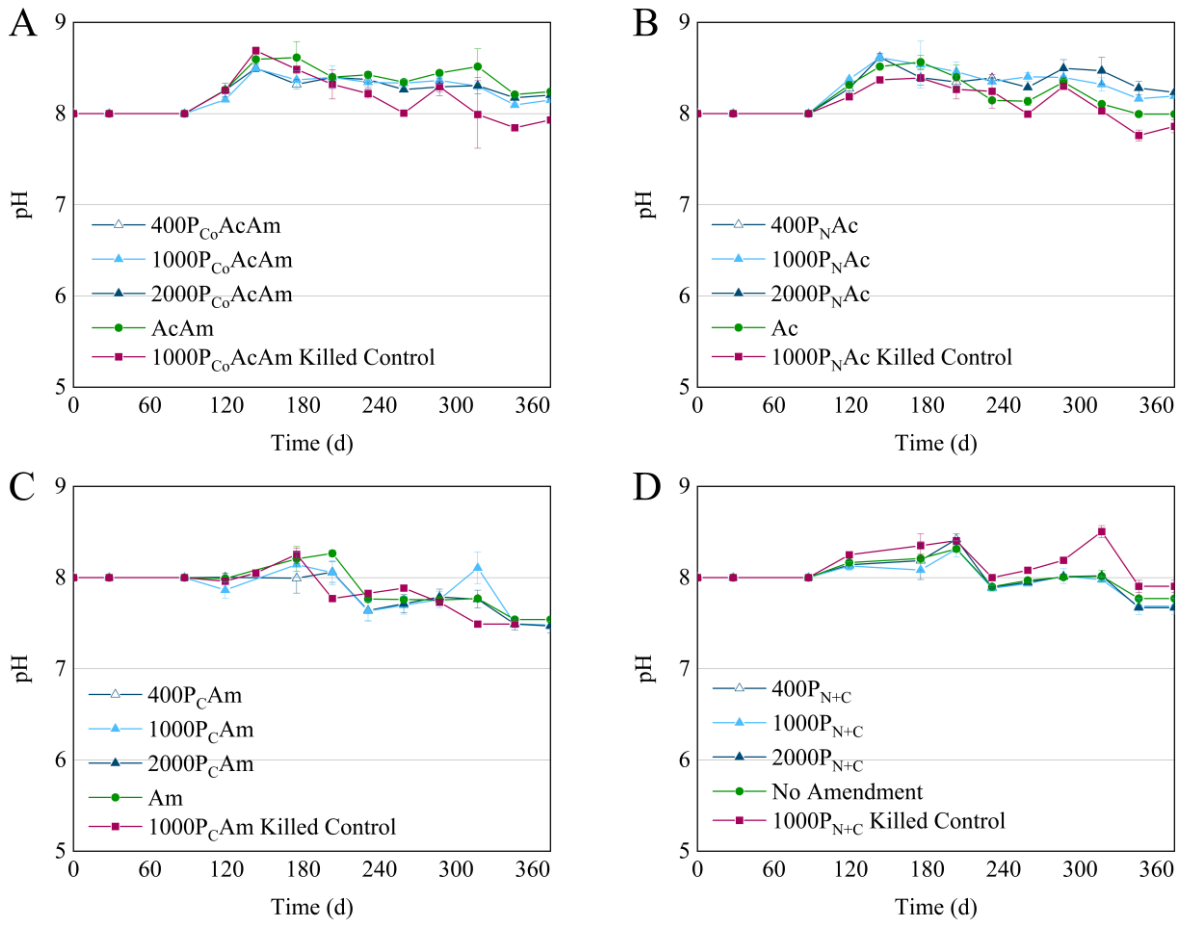


Figure B-11 pH in (A) HPAM as co-substrate, (B) HPAM as nitrogen source, (C) HPAM as carbon source, and (D) HPAM as nitrogen and carbon source under methanogenic conditions. Error bars represent one standard deviation of biological replicates.

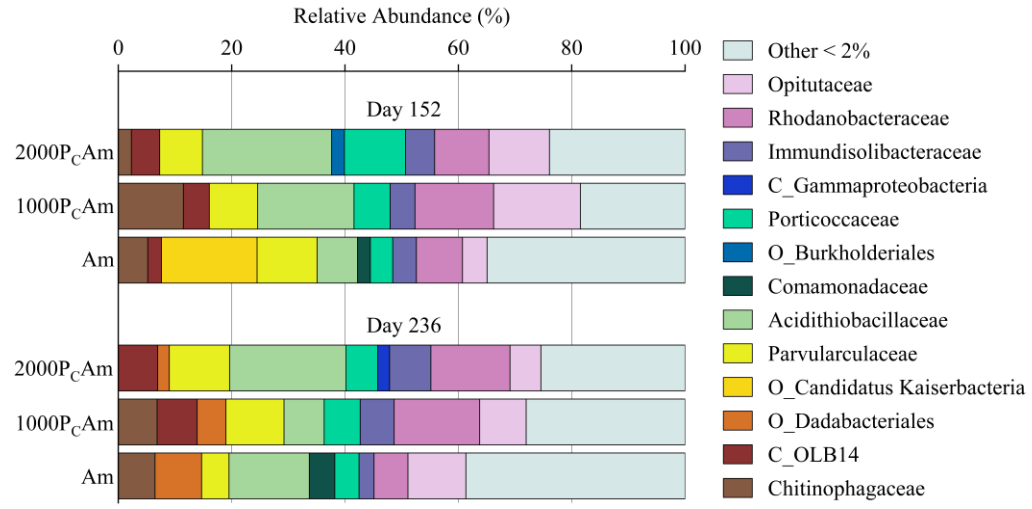


Figure B-12 Microbial community composition at the family level for HPAM as carbon source (P_C) under oxic conditions for. Taxa with a relative abundance < 2% were assigned to 'Other < 2%'. Taxa not identified at the family level are denoted with "O_" or "C_" to represent taxonomic ranking at the Order and Class level, respectively.

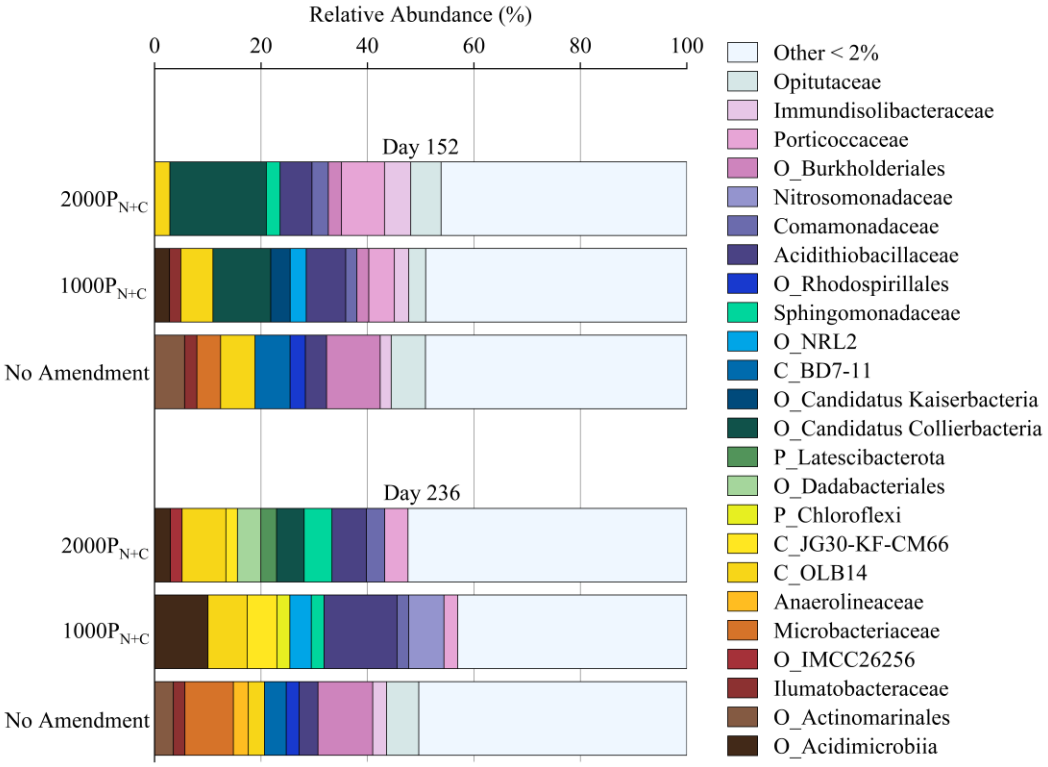


Figure B-13 Microbial community composition at the family level for HPAM as nitrogen and carbon source (P_{N+C}) under oxic conditions for. Taxa with a relative abundance < 2% were assigned to 'Other < 2%'. Taxa not identified at the family level are denoted with "O_", "C_", or "P_" to represent taxonomic ranking at the Order, Class, and Phylum level, respectively.

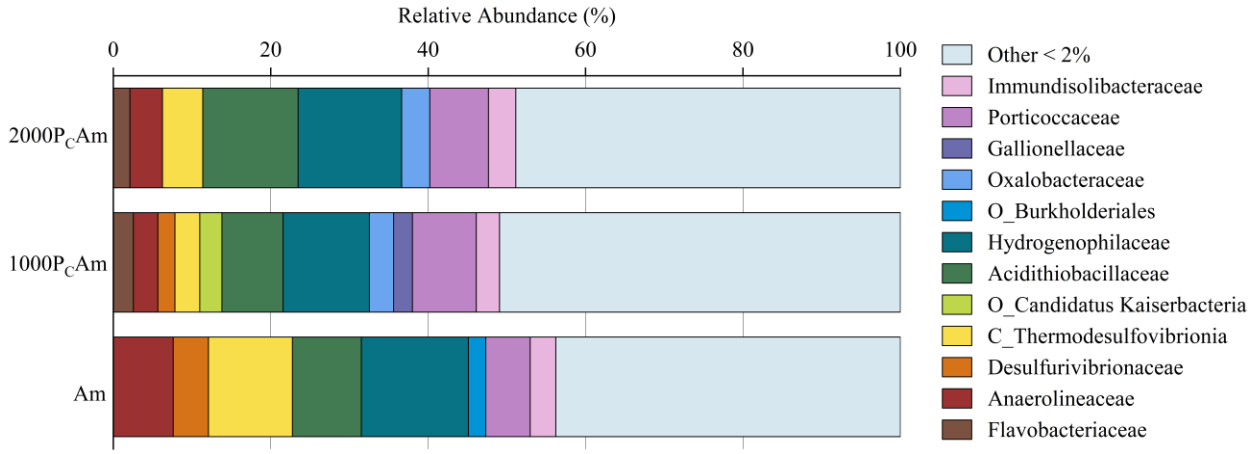


Figure B-14 Microbial community composition at the family level for HPAM as carbon source (P_C) under sulfate-reducing conditions on day 420. Taxa with a relative abundance < 2% were assigned to 'Other < 2%'. Taxa not identified at the family level are denoted with "O_" or "C_" to represent taxonomic ranking at the Order and Class level, respectively.

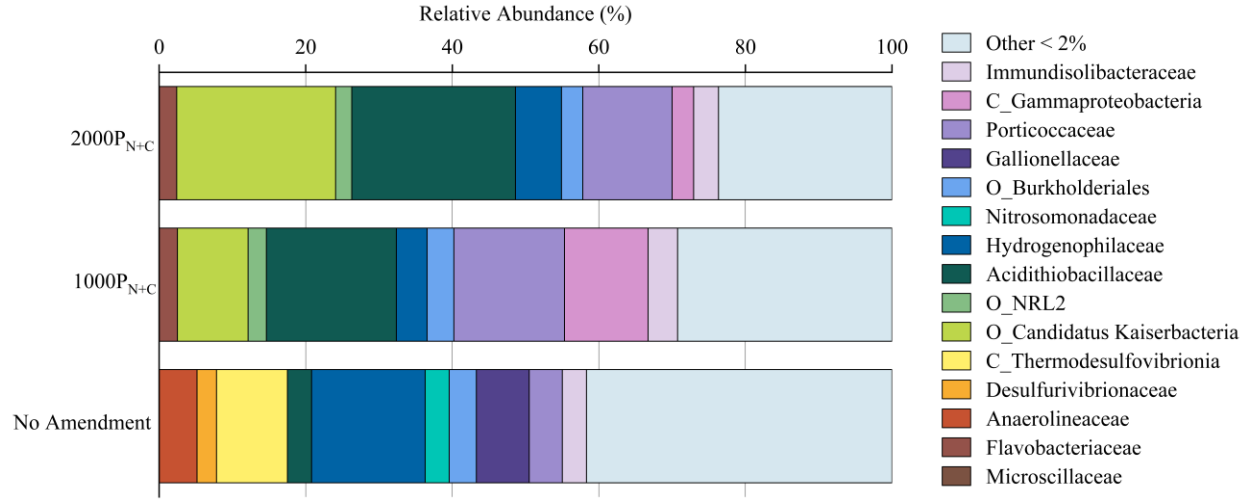


Figure B-15 Microbial community composition at the family level for HPAM as nitrogen and carbon source (P_{N+C}) under sulfate-reducing conditions on day 420. Taxa with a relative abundance < 2% were assigned to 'Other < 2%'. Taxa not identified at the family level are denoted with "O_" or "C_" to represent taxonomic ranking at the Order and Class level, respectively.

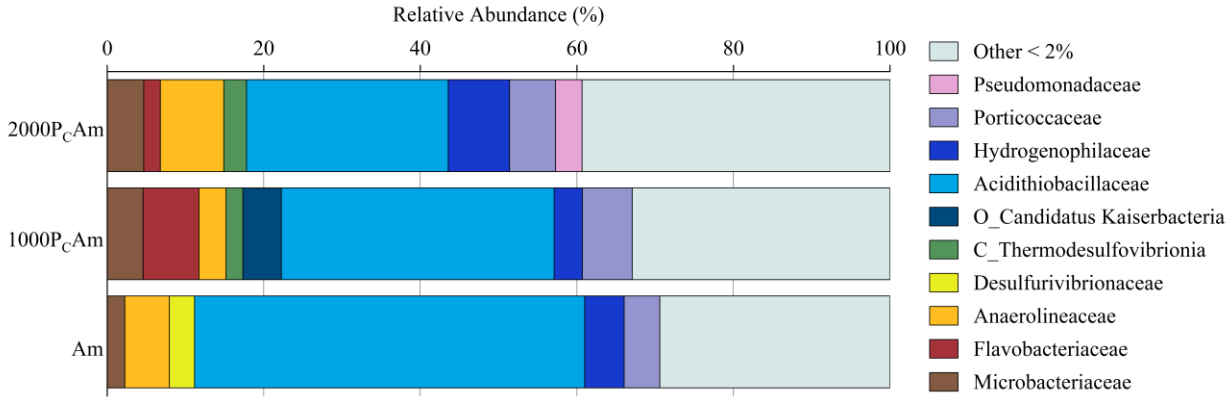


Figure B-16 Microbial community composition at the family level for HPAM as carbon source (P_C) under methanogenic conditions on day 374. Taxa with a relative abundance < 2% were assigned to 'Other < 2%'. Taxa not identified at the family level are denoted with "O_" or "C_" to represent taxonomic ranking at the Order and Class level, respectively.

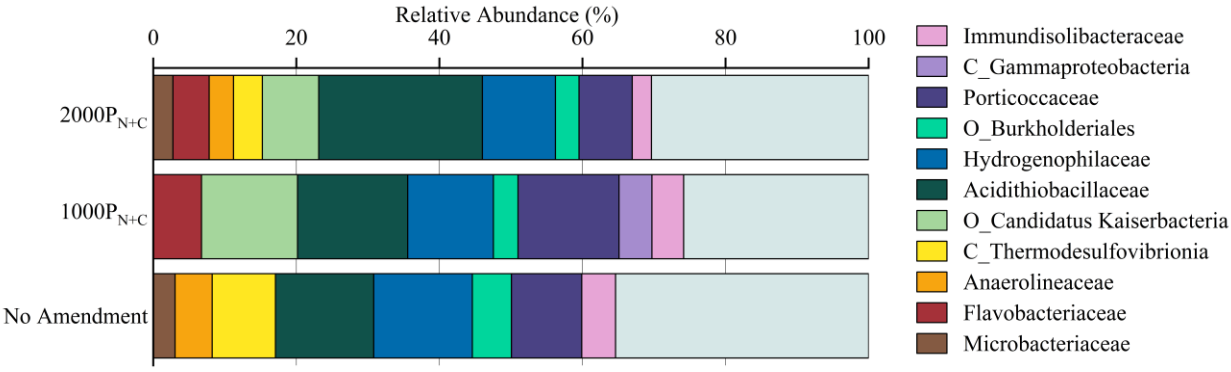


Figure B-17 Microbial community composition at the family level for HPAM as nitrogen and carbon source (P_{N+C}) under methanogenic conditions on day 374. Taxa with a relative abundance < 2% were assigned to 'Other < 2%'. Taxa not identified at the family level are denoted with "O_" or "C_" to represent taxonomic ranking at the Order and Class level, respectively.

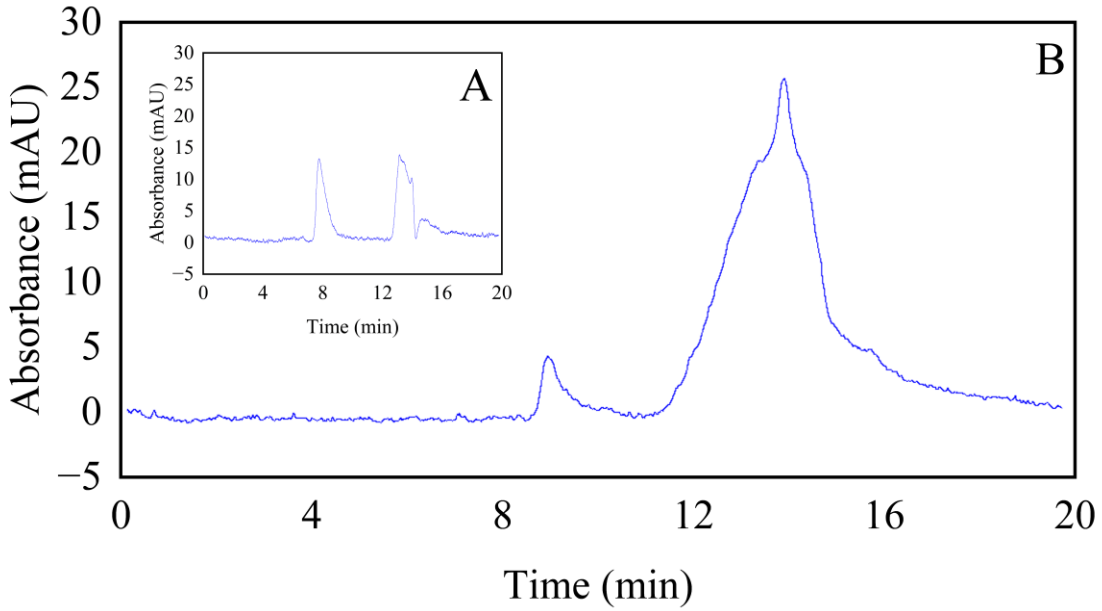


Figure B-18 Aerobic HPAM (A) before and (B) after biodegradation on day 236 under P_{Co} treatments. Before biodegradation, HPAM appeared at ~8 minutes and residual organics in oil sand tailings appeared at ~13 minutes. After biodegradation there was residual HPAM (~9 minutes) as well as biodegradation oligomers (13-16 minutes).