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Mitigation of Methane Emissions from Oil Sands Tailings by Redox Amendment: Mathematical Modeling of Empirical Observations

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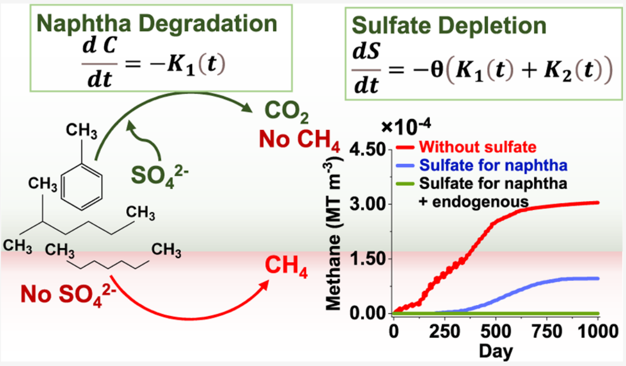

ABSTRACT: Anaerobic biodegradation of fugitive diluent hydrocarbons in oil sands fine tailings (FT) sustains CH_4 emissions from tailings facilities and potentially from pit lakes, which impact the climate and effective tailings reclamation. We investigated the effectiveness of sulfate as a redox amendment to mitigate CH_4 production from FT containing ∼0.2% naphtha. FT were collected from four different locations (two methanogenically more active and two less active) in a tailings-containing pit lake. Microcosms incubated for ∼800 d suggested that labile hydrocarbons (∼35−38% of naphtha, supporting methanogenesis), including monoaromatics, *n*-alkanes, and *iso*-alkanes, were biodegraded under sulfate-reducing conditions in all FT with no significant CH_4 production. Although the extent of hydrocarbon biodegradation was similar, *iso*-alkanes were biodegraded faster in FT from sampling locations that were methanogenically less active in situ. A phenomenological model developed using zero-order kinetics predicted well naphtha biodegradation and sulfate reduction in microcosms. Using reported unrecovered naphtha input to an active tailings facility (Mildred Lake Settling Basin), the model suggested that sulfate amendment could reduce predicted CH_4 production from the labile naphtha fraction by ∼51−85%, potentially reaching 95−100% if sulfate reduction supported by other endogenous substrates was also considered. These findings can inform potential methane mitigation solutions for diluent (naphtha) affected tailings.

KEYWORDS: *methane mitigation, petroleum hydrocarbons, biodegradation, sulfate reduction, modeling of microbial processes*

1. INTRODUCTION

In Alberta, Canada, the oil sands industry produces ∼75 million metric tonnes of greenhouse gases (GHG) per year, constituting ∼26% of total emissions from the province (https://www.alberta.ca/climate-oilsands-emissions). Additionally, oil sands tailings facilities contribute 2.8 million metric tonnes CO_2 equiv per year to the total emissions from Alberta.[1] Bitumen extraction from surface-mined oil sands generates waste, termed tailings. If the extraction process includes froth treatment with a hydrocarbon diluent (e.g., naphtha or paraffinic solvent), unrecovered diluent becomes part of the froth treatment tailings stream.[2] Tailings are temporarily deposited in tailings facilities where solids segregate upon deposition, resulting in the formation of fine tailings (FT), which is a suspension of fine clays in water with <3% bitumen and <0.5% diluent.[2] Oil sands tailings facilities that receive diluent-affected tailings become methanogenic because indigenous complex microbial communities biodegrade labile diluent hydrocarbons to methane (CH_4).[3−5] Currently, the volume of stored tailings has increased to >1.39 billion m^3.[6] To reclaim this FT inventory, one reclamation strategy is to establish pit lakes by depositing FT in mined-out pits with an overlying water layer (fresh water or a mixture of fresh and oil sands process-affected water).[7] However, methanogenesis can contribute to the flux of bitumen and chemicals from tailings to the water layer, which influences pit lake ecological performance.[8−10] Therefore, investigating potential CH_4 mitigation strategies becomes important for tailings reclamation and the environment.

Biodegrading diluent hydrocarbons under alternative redox conditions by providing a competitive advantage to specific microorganisms over methanogens can be a potential strategy to mitigate CH_4 emissions. Some oil sands operators treat tailings with coagulants such as gypsum or alum to accelerate consolidation, which results in high dissolved sulfate concentrations in treated tailings and, eventually, microbial sulfate reduction.[11,12] Base Mine Lake, the first demonstration

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pit lake containing tailings treated through self-weight consolidation rather than a chemical amendment, also received a single alum treatment in 2016 to improve surface water quality by decreasing turbidity. This resulted in elevated sulfate concentrations in the water cap which increased sulfate reduction in the seasonal anoxic zone at the tailings/water interface and FT; this suggests persistent activity by sulfate reducers in Base Mine Lake.[13] Despite the evidence of the sulfate-reduction process in oil sands tailings facilities and a pit lake,[5,13−15] the range of hydrocarbons that biodegrade to CH_4 has not been fully tested for biodegradability under sulfate-reducing conditions with the objective of CH_4 suppression. Our few previous studies report biodegradation of only selected hydrocarbons such as toluene, a few *n*-alkanes, and 2-methylpentane under sulfate-reducing conditions.[16−19] Though the potential of sulfate amendment in inhibiting methanogenesis from oil sands tailings has been suggested previously, evidence for hydrocarbon biodegradation under sulfate-reducing conditions was not provided in those studies.[12,20,21] Even in studies conducted using enrichment cultures from other hydrocarbon-contaminated environments such as soil or sediments, biodegradation of selected hydrocarbons such as polycyclic aromatics, particularly naphthalene, phenanthrene, and pyrene,[22,23] *n*-alkanes including propane, pentane,[24] and a few longer-chain alkanes ($>C_{15}$),[25] and monoaromatics[26,27] has been reported under sulfate-reducing conditions. Exploring the effectiveness of sulfate as an alternative electron acceptor for the biodegradation of a broad range of petroleum hydrocarbons from a CH_4 mitigation perspective is required.

Therefore, we conducted laboratory experiments using FT data collected from four different sampling locations in Base Mine Lake to investigate the biodegradation of naphtha hydrocarbons under sulfate-reducing conditions to suppress CH_4 production. The naphtha used by Syncrude Canada Ltd. is a relatively narrow distillate ($\sim C_6-C_{10}$) comprising >200 compounds including monoaromatics, *n*- and *iso*-alkanes, and cycloalkanes.[28] FT cultures received naphtha as a carbon source and sodium sulfate to establish the sulfate-reducing conditions. We hypothesized that FT from a methanogenically less active sampling location would become enriched with sulfate reducers faster upon sulfate amendment and maintain this redox condition, while the methanogenically more active FT might exhibit simultaneous occurrence of methanogenesis and sulfate reduction. In the current study, ~41% of naphtha compounds known to be predominant contributors to CH_4 production by FT[4,28] were investigated for their biodegradability under sulfate-reducing conditions. Using laboratory results, a phenomenological model has been developed to predict sulfate depletion corresponding to naphtha biodegradation and estimate sulfate requirements for CH_4 mitigation. Finally, using model parameters derived from observations in Base Mine Lake FT, we predicted the effectiveness of sulfate amendment for suppressing CH_4 in situ in Mildred Lake Setting Basin (MLSB) because Base Mine Lake tailings were partially derived from MLSB, supporting the applicability of the model to MLSB. Globally, these results are important considerations for the remediation of other hydrocarbon-contaminated anaerobic environments.

2. METHODOLOGY

2.1. Chemicals and Materials. FT used to conduct experiments was collected from four locations of Base Mine Lake by Syncrude Canada Ltd. in 2021. FT from sampling location D17 (UTM Coordinates 12N 0461221 E 6316889 N; sampled from depth 11.6 m; solid contents, ~45 wt %) and P2 (UTM Coordinates 12N 0462888 E 6319753 N; sampled from depth 14.4 m; solid contents, ~49 wt %) were methanogenically more active, while the other two locations D04 (UTM Coordinates 12N 0461201 E 6318598 N; sampled from depth 11.4 m; solid contents, ~39 wt %) and P1 (UTM Coordinates 12N 0462150 E 6318878 N; sampled from depth 13.9 m; solid contents, ~42 wt %) were methanogenically less active. According to in situ CH_4 measurements in Base Mine Lake, platforms P3 (close to D17) and P2 produce more CH_4 than platform P1 (close to D04)[10] suggesting different aspects of methanogenic activity in situ. Naphtha[28] was used as a source of hydrocarbons in the experiment and was provided by Syncrude Canada Ltd.

2.2. Establishment of FT Cultures. For experiments, FT (50 mL) collected from Base Mine Lake (D04, D17, P1, or P2 sampling locations) was mixed with 50 mL of Coleville synthetic brine medium[29] in 158 mL serum bottles (microcosms), flushed with 30% CO_2 balance N_2 gas to establish anaerobic conditions, and incubated at room temperature for 1 week for microbial acclimation before amendment, as described previously.[30] Three treatments were prepared using FT from each sampling location: (i) live amended cultures that were amended with 1 mmol of Na_2SO_4 and spiked with 0.2% v/v naphtha after preincubation (i.e., active sulfate-reducing conditions), (ii) sterile amended cultures that were prepared parallel to live-amended but autoclaved three times before amendment to observe abiotic depletion of hydrocarbons and Na_2SO_4, and (iii) live unamended cultures that received 1 mmol of Na_2SO_4 but no naphtha, to observe sulfate depletion relevant to the oxidation of any endogenous substrates in FT (e.g., partially oxidized labile hydrocarbons, recalcitrant hydrocarbons, dead biomass). Notably, live amended and unamended cultures received Na_2SO_4 multiple times periodically during incubation to alleviate sulfate depletion. Two additional treatments were established for FT from location D17 and D04 (in situ methanogenically more and less active, respectively); these included (iv) live amended cultures, which received 0.2% v/v naphtha but no Na_2SO_4 (i.e., methanogenic conditions) and (v) unamended baseline cultures that did not receive either naphtha or Na_2SO_4 amendments, representing Base Mine Lake in situ chemical conditions. All live amended treatments were prepared in triplicate, whereas sterile amended and unamended treatments were prepared in duplicate (see the experimental design in Table S1). After amendment, microcosms were incubated statically at room temperature overnight in the dark to allow for chemical equilibration and then were subjected to initial (day 0) and periodical analyses for residual hydrocarbons and CH_4 in the headspace and sulfate in the liquid phase during ~800 d incubation.

2.3. Chemical Analyses. Biodegradation of the labile fraction of naphtha hydrocarbons (~41 wt % that could be identified using external standards) was determined periodically by taking ~100 μL of headspace sample and injecting it into a gas chromatograph connected to a mass selective detector (GC−MS; Thermo Fisher Scientific) as described by Afzal et al.[31] To calculate depletion, peak areas of individual labile hydrocarbons and 1,1,3-trimethyl cyclohexane (a recalcitrant hydrocarbon used as an internal standard that constitutes ~1.5 wt % of naphtha) were determined in live naphtha-amended and sterile amended cultures. The percent-age of hydrocarbon that remained after biodegradation in live naphtha-amended cultures was calculated by using eq 1.

$$\%\text{ remaining of HC} = \mathrm{RA}_{(\text{Amended})}/\mathrm{RA}_{(\text{Sterile Amended})} \times 100 \tag{1}$$

HC indicates the hydrocarbon, and RA is the relative peak area of the individual hydrocarbon (relative to the internal standard). Knowing the naphtha composition,[28,31] the initial mass of each hydrocarbon at day 0 was calculated. The percent remaining in the cultures during incubation was presented as mass (mmol). Zero- and first-order kinetics of hydrocarbon biodegradation were determined per Siddique et al.[32]

CH_4 production was monitored periodically by analyzing ~50 μL of headspace sample, using a GC equipped with a flame ionization detector (GC-FID; Thermo Fisher Scientific) as described previously.[33] Theoretical CH_4 was calculated using the Symons and Buswell equation as explained by Mohamad Shahimin and Siddique.[34] In sulfate-amended cultures, soluble sulfate concentrations were determined following a turbidimetric method[35] as follows: FT slurry samples of ~0.3 mL were taken aseptically using an airtight sterile syringe, placed in 1.5 mL microcentrifuge tubes, and separated into solid and liquid phases using a microfuge (Eppendorf 5424 Centrifuge) at 5000 rpm. The liquid phase was mixed with barium chloride solution and conditioning reagent (a mixture of sodium chloride, glycerol, concentrated hydrochloric acid, and ethanol in water) at a ratio of 1:5:9. The resultant turbid barium sulfate suspension was analyzed by an UV/visible spectrophotometer (Optizen POP) at $\lambda = 420$ nm. Notably, during optimization of this method, samples with known sulfate concentrations were submitted to the Natural Resources Analytical Laboratory (NRAL) at the University of Alberta, Canada, for analysis for cross-reference. The data indicated that the turbidimetric method was as accurate as the method (EPA 375.4) used by NRAL, suggesting an accuracy of ~85−99%. Therefore, we used a rapid turbidimetric technique for routine sulfate analysis of cultures. Theoretical sulfate reduction corresponding to hydrocarbon biodegradation was also calculated based on equations[36] (Text S1), assuming that hydrocarbons were biodegraded completely to CO_2 under sulfate-reducing conditions.

2.4. Model Development for Hydrocarbon Biodegradation under Sulfate-Reducing Conditions.

Biodegradation of naphtha hydrocarbons under methanogenic conditions can be modeled using the zero-order kinetics model.[32] Biodegradation of hydrocarbons under sulfate-reducing conditions can also be modeled using the same model, whose integral form is given as

$$C_i(t) = C_i(0) - k_0^i t, \qquad \text{for } t \geq \lambda_s^i \text{ and } C_i(t) = C_i(0) \text{ otherwise} \tag{2}$$

where $C_i(0)$ is the initial mass of ith (individual) hydrocarbon (at day 0), $C_i(t)$ is the mass of ith individual hydrocarbon at time t (days), in mmol to facilitate stoichiometric calculations, or conventionally in mg, k_0^i is the degradation rate constant (mmol day^{-1}), and λ_s^i is the lag period (days) (zero-order kinetics parameters are provided in Tables S2 and S3). The zero-order model (under sulfate-reducing or methanogenic conditions) can be useful for predictions by also applying $C_i(t) = 0$ when $t \geq C_i(0)/k_0^i$, i.e., when the ith hydrocarbon is depleted, ensuring non-negative values and realistic outputs. However, for this work, we simplified the zero-order model for the biodegradation of labile naphtha hydrocarbons under sulfate-reducing conditions by a single function that captures the aggregate hydrocarbon mass degradation dynamics over time (eq 4). To do this, we first determined the zero-order biodegradation kinetics for individual hydrocarbons under sulfate-reducing conditions by fitting eq 2 to our data. We then aggregated these kinetics during the monitoring period, i.e., summing all zero-order kinetics for $\lambda_s^i \leq t \leq C_i(0)/k_0^i$ for each ith hydrocarbon and fitting an exponential decay function ($K_1(t)$) to describe the overall naphtha hydrocarbons biodegradation rate (eq 3). The function $K_1(t)$ was selected as an exponential decay function for simplicity and because it accurately describes the aggregate zero-order kinetics over time when sufficient sulfate is available. Finally, we established our zero-order kinetics-based model for hydrocarbon degradation under sulfate-reducing conditions as in eq 4 (integral form shown in eq 5).

$$K_1(t) = a\,\mathrm{e}^{-bt} \tag{3}$$

$$\frac{\mathrm{d}C}{\mathrm{d}t} = -K_1(t) \tag{4}$$

$$C(t) = C(0) - \frac{a}{b}(1 - \mathrm{e}^{-bt}) \tag{5}$$

Here, $C(0)$ is the total mass (mg) of hydrocarbons (representative of labile naphtha) at day 0, $C(t)$ is the total mass (mg) at time (t), a is the maximum degradation rate (mg day^{-1}), and b is the exponential decay constant (day^{-1}).

For the sulfate data modeling, we first calculated total sulfate from experimental data and assumed that sulfate was added as a single amendment (at day 0) rather than periodic additions to simplify our model. The zero-order rate constants during each sulfate amendment period were determined (Table S4) and used during that specific amendment period to model sulfate depletion over time ($S_T(t)$). The stoichiometric sulfate depletion corresponding to aggregate hydrocarbon biodegradation was computed using the zero-order model (eq S2), and so the additional sulfate reduction (relevant to other endogenous substrates in FT, which were neither identified nor quantified) could also be estimated (eq S4). For our new simplified sulfate model, we first estimated a dimensionless parameter indicating proportional sulfate consumption (θ) based on the hydrocarbon degradation kinetics in eq 5 and the corresponding sulfate depletion for all degradable hydrocarbons (see Text S2). We then assumed that endogenous substrates in FT are also exponentially degraded under sulfate-reducing conditions, such as $K_2(t) = g\,\mathrm{e}^{-dt}$, where g (mg day^{-1}) and d (day^{-1}) are parameters. These were estimated by considering $S_T(t)$, sulfate depletion corresponding to the degradation of hydrocarbons and endogenous substrates, assuming the same sulfate consumption factor θ for both (see Text S2 and Table S5). Hence, the simplified sulfate depletion model is proposed in eq 6 (solution is given in eq 7).

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -\theta(K_1(t) + K_2(t)) \tag{6}$$

$$S(t) = S(0) - \theta\left(\frac{a}{b}(1 - \mathrm{e}^{-bt}) + \frac{g}{d}(1 - \mathrm{e}^{-dt})\right) \tag{7}$$

Here $S(0)$ represents the total sulfate amendment (mg) theoretically added at day 0, $S(t)$ is the sulfate at time (t), and $K_2(t)$ represents the endogenous organics degradation rate

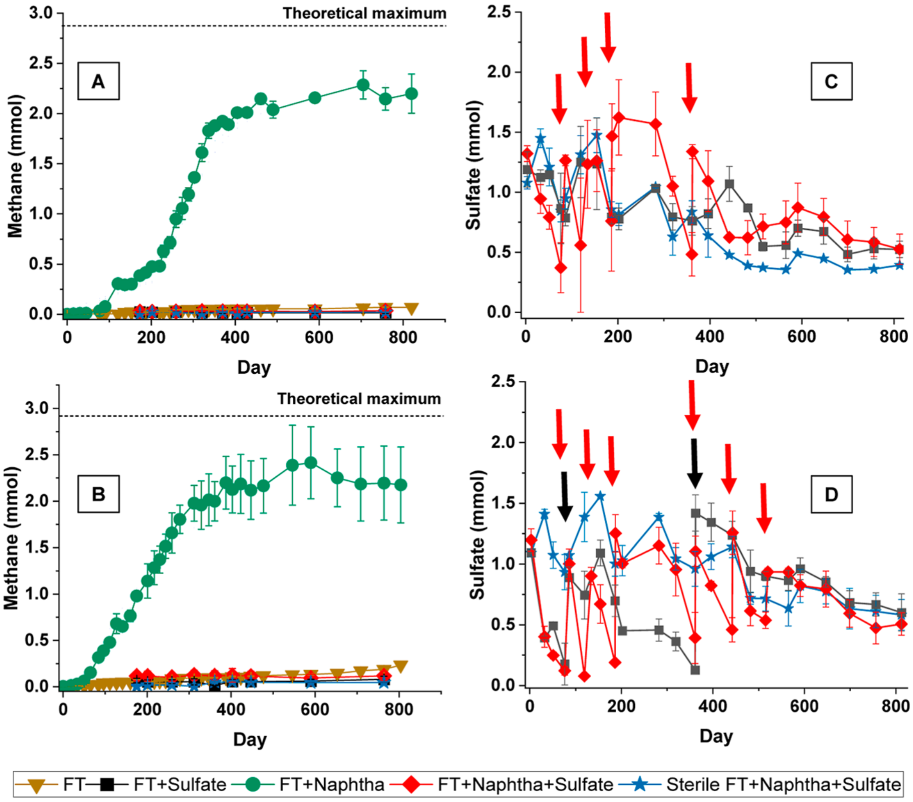

Figure 1. CH_4 production and liquid phase sulfate depletion over time in FT cultures from locations D04 (panel A, C) and D17 (panel B, D); see Figure S2 for sulfate data in P1 and P2 FT cultures. Panels A and B represent cumulative CH_4 production data for D04 and D17 FT cultures, respectively. The dashed line represents an average of the theoretical maximum CH_4 production calculated based on the mass of hydrocarbon biodegraded in naphtha-amended FT with and without sulfate. Panels C and D present soluble sulfate data in D04 and D17 FT cultures, respectively. The arrows indicate times of sulfate reamendments in live FT cultures (sterile cultures were not reamended). Each measured observation is the average of triplicates, and error bars indicate one standard deviation.

(parameters are provided in Table S5, and a comparison of new model-derived predictions with zero-order predictions is given in Table S6).

Finally, eq 7 and parameters provided in Table S5 made it possible to estimate the sulfate requirement for suppressing CH_4 production from biodegradation of labile components of 0.2% v/v naphtha, while also considering sulfate depletion linked with other endogenous substrates. Hence, the final simple equation to calculate sulfate requirements is presented as follows (eq 8):

$$S(0) = \theta\left(\frac{a}{b} + \frac{g}{d}\right) \tag{8}$$

Model development was performed by using MATLAB 2023. More details regarding modeling are provided in the Supporting Information (Texts S1 and S2).

2.5. In Situ Quantitative Assessment of Model Predictions for CH_4 Mitigation in Mildred Lake Settling Basin (MLSB). The effectiveness of sulfate amendment and model predictions were evaluated in the context of potential CH_4 mitigation in MLSB, a tailings facility at Syncrude Canada Ltd. in northern Alberta that consistently receives froth-treatment tailings that contain naphtha. Data for naphtha input (reported in m^3) to MLSB on a monthly basis for the year 2021 were retrieved from online sources[37] https://static.aer.ca/prd/documents/sts/ST39-2021.pdf. From the naphtha data, we estimated the tailings volume entering MLSB every month for the year 2021 assuming that naphtha constitutes 0.2% v/v of tailings (Table S7), a common naphtha range found in froth treatment tailings.[2] To project sulfate effectiveness in CH_4 mitigation from fresh tailings deposited in MLSB in 2021, we considered three scenarios: (i) deposition without any sulfate addition, (ii) monthly sulfate amendment proportional to degradation of labile components of naphtha present in fresh tailings, and (iii) monthly sulfate amendment proportional to biodegradation of both naphtha and endogenous organics in tailings deposited in MLSB every month for 2021. In the first scenario (strictly methanogenic conditions), we considered $S(0) = 0$ and predicted naphtha biodegradation and cumulative CH_4 production over time using the zero-order kinetics model per Siddique et al.[32] In the second $S(0) = \theta(a/b)$ and third $S(0) = \theta(a/b + g/d)$ scenarios representing sulfate-reducing conditions, naphtha biodegradation and sulfate reduction were predicted using our new model eqs 5 and 7, respectively (derived for D04 and D17 FT). Methane production in scenarios two and three was modeled in the same way as explained for scenario one. We modeled naphtha biodegradation and CH_4 mitigation on a monthly basis for all scenarios and presented the outcome as cumulative results (explained in Results and Discussion).

3. RESULTS AND DISCUSSION

Indigenous complex microbial communities in oil sands tailings biodegrade diluent hydrocarbons leading to CH_4 emissions[38] that may affect tailings reclamation by causing chemical mass loading to cap water.[8−10] Since sulfate-reducing microorganisms have been identified in oil sands tailings,[5,15,39] the current study investigated the effectiveness of sulfate in supporting the biodegradation of naphtha hydrocarbons to mitigate CH_4 emissions from tailings. We used FT from four locations in Base Mine Lake to understand how methanogenically active microbial communities (from methanogenically more active locations D17 and P2) representing current/legacy tailings facilities would respond to sulfate amendment versus sampling locations having less active methanogenic communities (D04 and P1) simulating diluent-exhausted legacy repositories. Data from D04 and D17 cultures are included in the main text, whereas observations for the other two FT sets (P1 and P2) can be found in the Supporting Information. The current study focuses on the following main aspects: (i) the effectiveness of establishing sulfate-reducing conditions in the context of CH_4 mitigation, (ii) the extent and kinetics of naphtha hydrocarbon biodegradation under sulfate-reducing conditions, (iii) the development of a model to predict sulfate reduction corresponding to naphtha biodegradation, and (iv) the application of the developed model using in situ naphtha loss data to MLSB to predict sulfate amendment efficacy for CH_4 mitigation. In complementary studies, we are characterizing the periodic shifts in microbial community composition under sulfate-reducing and methanogenic conditions during hydrocarbon biodegradation and investigating sulfur biogeochemistry and partitioning in FT (gas, liquid, and solid phases) to ameliorate any negative consequences of sulfate addition (manuscript in preparation).

3.1. Effectiveness of Sulfate Amendment for CH_4 Mitigation.

To estimate the extent of CH_4 suppression by sulfate addition to a methanogenic environment, cumulative CH_4 production was monitored in the headspace of all microcosms (unamended, live amended, and sterile amended cultures) established with sulfate using FT from four sampling locations (D04, D17, P1, and P2) and without sulfate for two FT sets (D04 and D17). Unamended cultures established under methanogenic conditions did not produce significant CH_4 during the incubation, suggesting that FT from both sampling locations was depleted in labile naphtha when the experimental cultures were established. Meanwhile, cultures amended with naphtha and established under methanogenic conditions produced substantial CH_4 ($\sim$2.19 ± 0.19 mmol in D04 FT and $\sim$2.17 ± 0.40 mmol in D17 FT) by 800 d of incubation (Figure 1A,B) during the metabolism of labile components of naphtha (monoaromatics, *n*- and *iso*-alkanes) (Figure S1). D17 FT, being more methanogenic in situ, exhibited a shorter lag time (19 d) than D04 FT ($\sim$45 d) before the onset of significant CH_4 production in naphtha-amended cultures, but the final extent of CH_4 production was similar. Cumulative CH_4 production values were $\sim$75–80% of the theoretical maximum CH_4 based on the stoichiometry of hydrocarbon mass depletion. This corresponds with previous reports of $\sim$68–79% of theoretical CH_4 production from naphtha.[31,34] Methanogenic hydrocarbon biodegradation patterns and results observed in the current study were anticipated based on our previous extensive research on hydrocarbon biodegradation by oil sands tailings incubated under methanogenic conditions.[3,4,38]

In contrast, FT cultures from all sampling locations, both unamended and naphtha-amended, that received sulfate as an alternate terminal electron acceptor did not produce significant CH_4 during 800 d incubation (Figure 1A,B for D04 and D17 FT, respectively; data for P1 and P2 FT not shown). Instead, continuous substantial sulfate depletion was observed in these cultures (cumulatively $\sim$0.5–2 mmol of sulfate depletion in unamended and $\sim$4 to 5.8 mmol in naphtha-amended FT over 800 days; Figure 1C,D for D04 and D17 FT and Figure S2A,B for P1 and P2 FT), suggesting that labile hydrocarbons in amended microcosms plus endogenous metabolites in unamended microcosms were metabolized concomitant with sulfate reduction. Notably, sulfate was added in multiple doses rather than one-time amendment to live cultures at day 0 because soluble sulfide, the end product of sulfate reduction, might inhibit microbial activity at higher mass if not attenuated.[40,41] In the current study, periodic soluble sulfide analysis in the liquid phase of FT cultures and mass balance calculations suggested that $\sim$60–70% of total depleted sulfate was present as soluble sulfide. The remaining might be present as gaseous H_2S or might have precipitated with indigenous chalcophile elements, mainly Fe^{II}, as Base Mine Lake tailings have appreciable Fe contents.[42] To decrease the soluble sulfide concentration, we added $ZnCl_2$ in live FT cultures to precipitate soluble sulfide with Zn as ZnS (data not shown). Sulfate depletion in heat-treated amended FT cultures after $\sim$300 d incubation implies gradual recovery of microbial activity, similar to observations in our previous methanogenic experiments.[34,43] FT consists of complex solids, making it challenging to completely sterilize the matrix and maintain abiotic conditions. Additionally, several sulfate reducers, such as *Desulfosporosinus* species, are spore formers[44−47] that may recover their activity despite autoclaving. Therefore, we later added the chemical inhibitor sodium nitroprusside[48] to maintain abiotic conditions for hydrocarbon calculations. Overall, our observations of CH_4 production and sulfate reduction suggest that establishing sulfate-reducing conditions can inhibit CH_4 production from naphtha hydrocarbon biodegradation in oil sands tailings. We had expected that FT from methanogenically active locations (D17 and P2) might exhibit co-occurrence of sulfate reduction and methanogenesis in the presence of sufficient organic substrates upon naphtha amendment, as noted previously during the study of carbon flow gradients in sulfate-rich tailings.[39] However, insignificant CH_4 production by sulfate-amended FT from all locations in the current study suggests that methanogenesis can be inhibited completely if there is stoichiometrically sufficient sulfate for sulfate reducers to outcompete the methanogenic community for hydrocarbon degradation, as suggested previously.[49]

3.2. Hydrocarbon Biodegradation under Sulfate-Reducing Conditions.

The naphtha-influenced tailings deposited into MLSB contain 19 major hydrocarbons previously determined to be labile under methanogenic conditions, including monoaromatics (toluene, *o*-xylene, and *m,p*-xylenes), *n*-alkanes (pentane, hexane, heptane, octane, nonane, and decane), and *iso*-alkanes (2-methylpentane, 2-methylhexane, 3-methylhexane, 2-methylheptane, 3-methylheptane, 4-methylheptane, 2-methyloctane, 3-methyloctane, and 2-methylnonane);[28,34] these hydrocarbons are the main contributors to CH_4 emissions from oil sands tailings.[50] Of these, 16 compounds constituting $\sim$41 wt % of naphtha (excluding pentane, decane, and 2-methylnonane due to their small wt % in naphtha) were detected in the current experiment and monitored for biodegradation under sulfate-reducing conditions. Calculated masses of residual individual compounds over 800 days of incubation in FT sets from D04, D17, and P1 and P2 sampling locations are shown in Figures 2

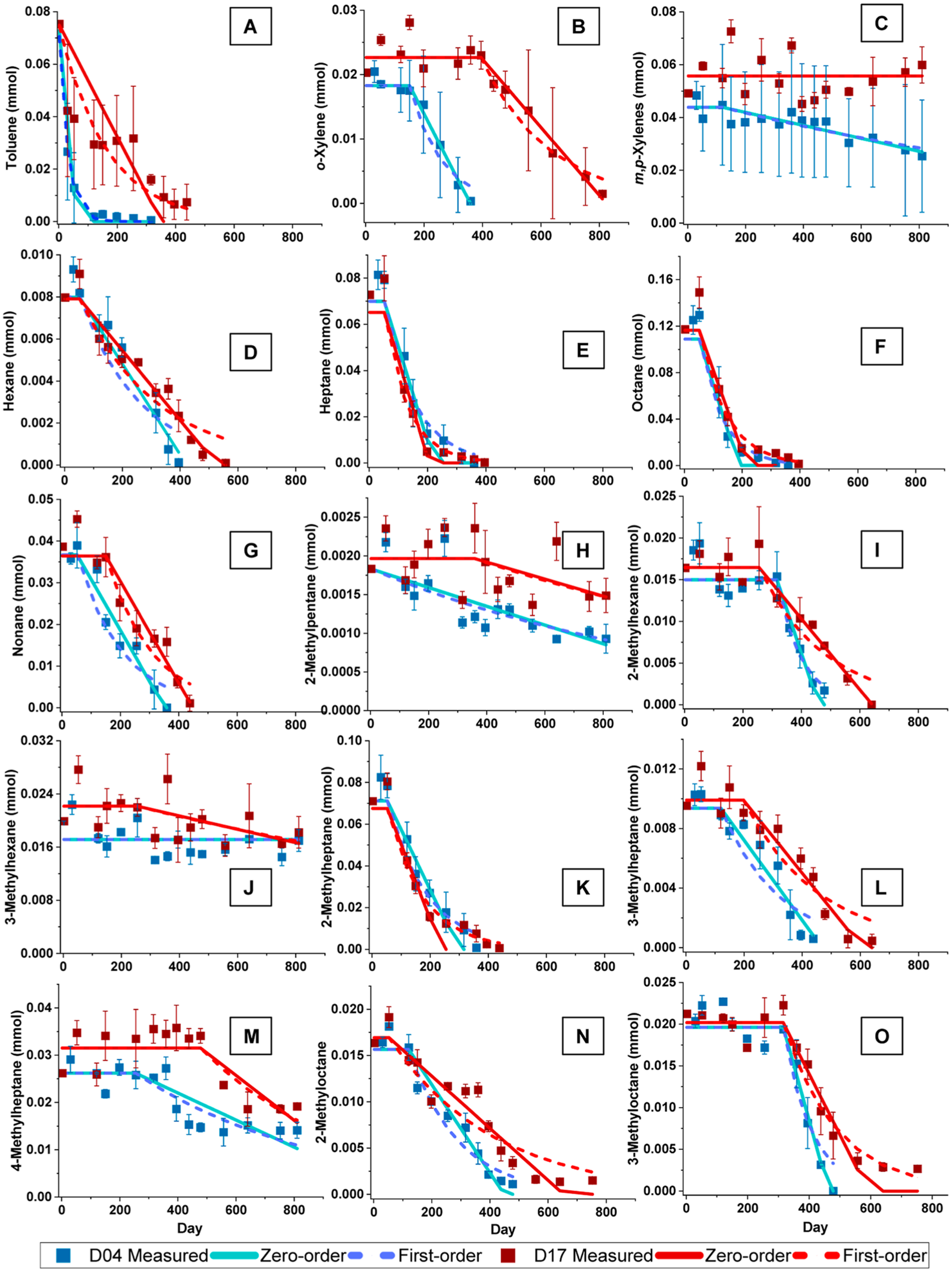

Figure 2. Biodegradation kinetics of individual hydrocarbons in FT from location D04 (blue) and D17 (red); see Figure S3 for sites P1 and P2. Panels A−C are monoaromatics, panels D−G are *n*-alkanes, and panels H−O are *iso*-alkanes. Data points represent experimental measurements, solid lines represent hydrocarbon depletion predicted by zero-order kinetics, and dashed lines indicate depletion predicted by first-order kinetics. Each measured observation is the average of triplicates, and error bars indicate one standard deviation.

and S3, respectively. Individual compound biodegradation kinetics modeled using zero- and first-order kinetics are also presented in Figures 2 and S3.

3.2.1. Extent of Naphtha Hydrocarbon Biodegradation.

Among the major 16 labile hydrocarbons (~41 wt % of naphtha), ~35−38 wt % including some monoaromatics

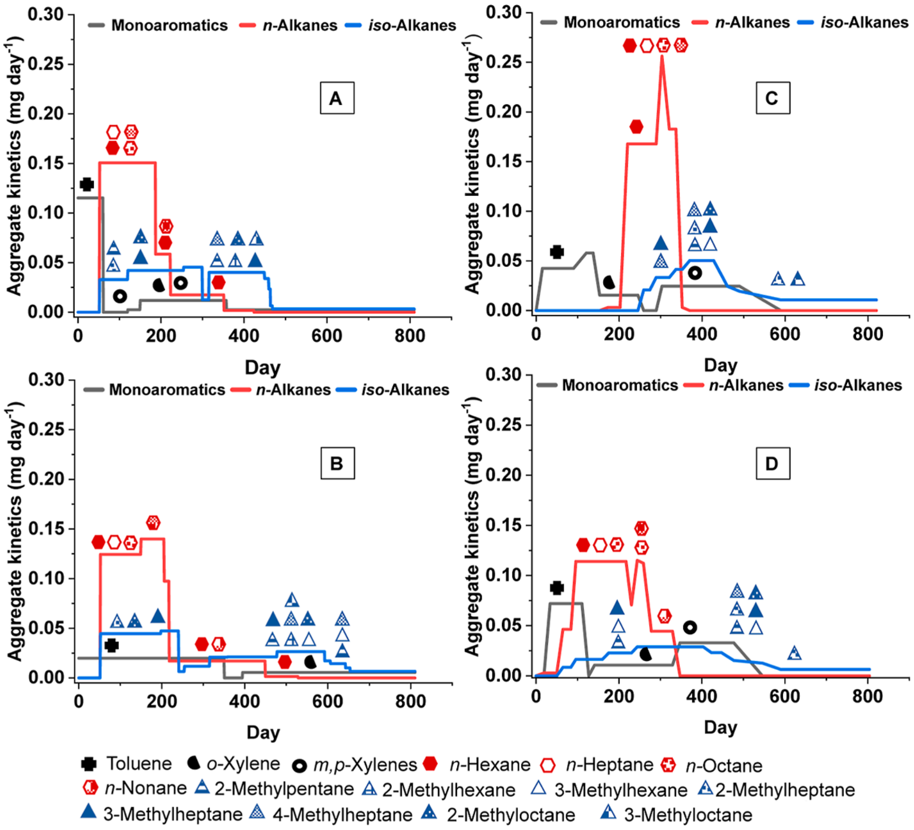

Figure 3. Aggregate zero-order biodegradation rates of hydrocarbons (mg day^{-1}) presented in three groups: monoaromatics, *n*-alkanes, and *iso*-alkanes to compare overall lag phases, biodegradation rates, and pattern of biodegradation under sulfate-reducing and methanogenic conditions. Panels A and B represent aggregate kinetics of biodegradation under sulfate-reducing conditions for locations D04 and D17, respectively (see Figure S4 for P1 and P2 FT sets). Panels C and D represent aggregate kinetics of biodegradation under methanogenic conditions for locations D04 and D17, respectively. Circular symbols represent monoaromatics, hexagons represent *n*-alkanes, and triangles are *iso*-alkanes. Their placement at specific positions in graphs represents their inferred contribution to cumulative kinetics at that specific time point.

[toluene, *o*-xylene, and *m*,*p*-xylenes (only in D04 FT)], all *n*-alkanes (C_6−C_9) and most *iso*-alkanes (2-methylpentane, 2-methylhexane, 2-methylheptane, 3-methylheptane, 4-methylheptane, 2-methyloctane, and 3-methyloctane) were biodegraded under sulfate-reducing conditions in all cultures (Figure 2 for D04 and D17 and Figure S3 for P1 and P2) irrespective of in situ methanogenesis. Previously, a few individual hydrocarbons such as toluene, *n*-alkanes (C_6−C_{10}), and 2-methylpentane were found biodegradable under sulfate-reducing conditions in oil sands tailings.[16−18] The current study suggests the potential of sulfate-reducing microorganisms to biodegrade a broader range of naphtha hydrocarbons, including *iso*-alkanes, than previously appreciated.

3.2.2. Lag Phases and Kinetics of Hydrocarbon Biodegradation.

Lag phases before hydrocarbon biodegradation onset under sulfate-reducing conditions were either similar or even shorter for few analytes such as *n*-alkanes (30−52 d) than under methanogenic conditions (50−301 d) in all FT sets (Figure 2 and Figure S3 for sulfate-reducing and Figure S1 for methanogenic conditions) with little or no apparent difference attributable to in situ methanogenic activity of the FT source. This suggests that FT from all locations maintained the potential to biodegrade hydrocarbons under sulfate-reducing conditions. Sulfate reducers might still be present as members of methanogenic microbial consortia even when sulfate is limiting, as potential sulfate reducers have previously been reported to be involved in the methanogenic degradation of hydrocarbons.[38] Hence, the addition of the energetically more favorable electron acceptor sulfate provided them competitive advantage,[51,52] enabling initiation of biodegradation with minimal time delay.

Kinetics of hydrocarbon biodegradation under both redox conditions were compared for groups, monoaromatics, *n*-alkanes, and *iso*-alkanes, rather than for individual compounds to gain more meaningful information. For this, zero-order kinetics for compounds within each group were summed up and plotted as aggregate kinetics over time as presented in Figure 3 for D04, D17 FT (A, B for sulfate-reducing and C, D for methanogenic conditions, respectively) and Figure S4A,B for P1, P2 FT, respectively. Zero-order kinetics showed relatively greater normalized mean square error (NMSE) values (≥0.9) for most hydrocarbons, supporting its use for kinetics comparison and model development (Section 3.3). Overall, aggregate kinetics in all FT sets for sulfate-reducing and methanogenic conditions exhibited faster kinetics for monoaromatics and *n*-alkanes known to be labile[28,30] than for relatively recalcitrant *iso*-alkanes.[4] For *n*-alkanes, aggregate biodegradation kinetics was almost similar in all FT sets under sulfate-reducing conditions and in the D17 FT set under methanogenic conditions. Interestingly, the D04 FT exhibited relatively faster rates of methanogenic biodegradation for *n*-alkanes (Figure 3C). This suggests that D04 FT (though less methanogenic in situ) retained biodegradation capability and developed a competent methanogenic microbial community

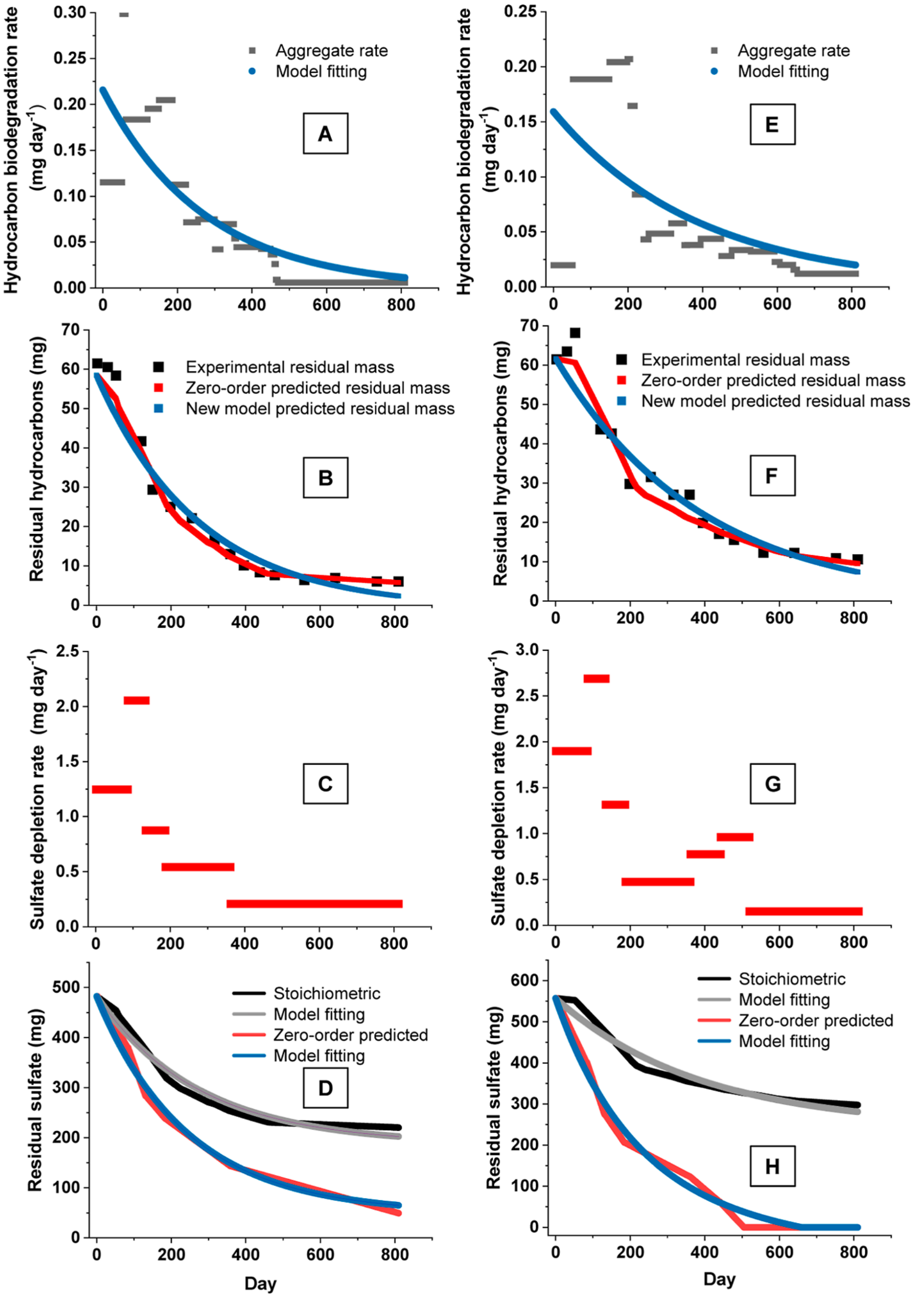

Figure 4. Measurement of model parameters and comparison of experimental observations with model predictions for naphtha biodegradation and sulfate reduction in D04 (panels A–D) and D17 FT cultures (panels E–H); see Figure S5 for P1 and P2 FT sets. Panels A and E: Adjusted model function for capturing the trend of aggregate hydrocarbon biodegradation zero-order kinetics over time. Panels B and F: Comparison of naphtha depletion predicted by the new model with experimentally measured depletion and zero-order model-predicted depletion to assess the effectiveness of the new model. Panels C and G: Zero-order rates of sulfate depletion calculated from experimental observations. Panels D and H: Comparison of sulfate depletion values predicted by the new model with stoichiometrically calculated and zero-order-predicted total sulfate depletion over time.

upon naphtha amendment, leading to faster degradation. Focusing on sulfate-reducing conditions, aggregate biodegradation kinetics for *iso*-alkanes was slower after ~250–300 d of incubation in D17 and P2 FT sets (Figures 3B and S4B, respectively) compared to D04 and P1 FT (Figures 3A and S4A, respectively), whereas monoaromatics did not show obvious trends with locations. It is possible that FT from methanogenically less active sampling locations developed competent *iso*-alkane-degrading sulfate-reducing microbial communities relatively quickly, which resulted in faster kinetics and earlier complete biodegradation (by ~500 d) of *iso*-alkanes in D04 and P1 FT cultures, versus ~700 d for complete degradation by D17 and P2 FT cultures.

3.2.3. Pattern of Hydrocarbon Biodegradation. Hydrocarbon biodegradation under sulfate-reducing conditions followed the same sequential pattern (monoaromatics = *n*-alkanes > *iso*-alkanes) as observed under methanogenic conditions in the current study (Figure 3) and in previous studies.[28,31,34] Similar to methanogenic conditions, preferential biodegradation within monoaromatics (toluene > *o*-xylene > *m*,*p*-xylenes) was observed in all sulfate-reducing FT cultures with *m*,*p*-xylenes biodegradation occurring only in some D04

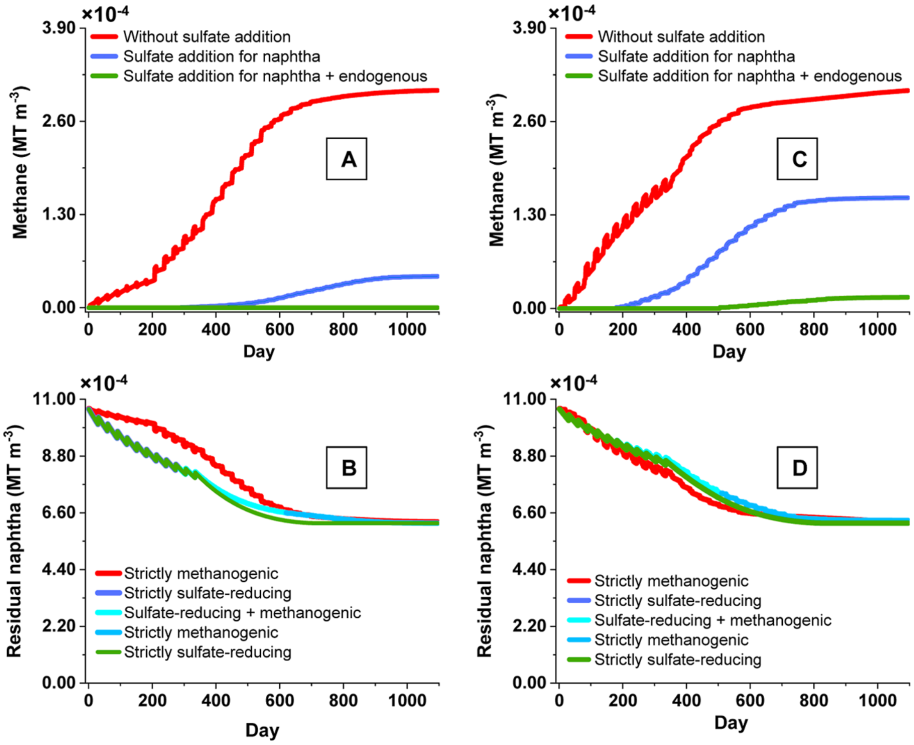

Figure 5. Application of the new model to predict the efficacy of sulfate in mitigating CH_4 production from naphtha biodegradation in Mildred Lake Settling Basin (MLSB) as a case scenario using in situ 2021 naphtha loss data (see Table S7) and assuming two locations representative of methanogenically less active FT (D04) (panels A and B) and methanogenically more active FT (D17) (panels C and D). Projections have been made assuming three scenarios: (i) without sulfate addition, (ii) sulfate addition estimated based on the requirement to biodegrade 35−38% naphtha, and (iii) sulfate addition estimated based on the requirement to biodegrade 35−38% naphtha plus other unidentified and unquantified endogenous organics. Panels A and C: Projection of CH_4 production corresponding to naphtha biodegradation under all three scenarios. Panels B and D: Naphtha biodegradation prediction under all three scenarios, with scenario (i) representing strict methanogenic conditions; scenario (ii) representing a shift in redox conditions depending upon the depletion of sulfate from strictly sulfate-reducing conditions followed by simultaneous sulfate reduction plus methanogenesis, then finally strict methanogenic conditions; and scenario (iii) representing strict sulfate-reducing conditions.

replicates over 800 d (Figure 3A,B for D04, D17 FT and Figure S4A,B for P1, P2 FT, respectively). Dou et al.[27] also reported a similar biodegradation pattern for aromatics in contaminated soil under sulfate-reducing conditions with slower *p*-xylene biodegradation. Within *n*-alkanes, heptane (C_7) and octane (C_8) were degraded preferentially, followed by the biodegradation of remaining compounds under sulfate-reducing conditions. Again, this preferential pattern is similar to methanogenic conditions where longer-chain hydrocarbons were preferentially biodegraded over shorter-chain hydrocarbons ($C_{10} > C_8 > C_7 > C_6 > C_5$) in FT from tailings ponds operated by Syncrude Canada Ltd. and other operators that use naphtha diluent, probably due to acclimation of indigenous microorganisms to utilizing components of broad range (C_5−C_{10}) naphtha.[30,53] Regarding *iso*-alkanes, no obvious preferential degradation pattern was observed under sulfate-reducing conditions except early biodegradation of 2-methylheptane (simultaneously with *n*-alkanes), which could be related to enzyme specificity.[54]

3.3. Modeling of Naphtha Biodegradation and Sulfate Reduction. Masses (mg) of naphtha hydrocarbon biodegraded and sulfate consumed (on average) in live triplicate microcosms (sulfate added naphtha amended and unamended) in all FT sets were used in developing a model that would predict oxidation (biodegradation) of naphtha and other endogenous substrates with sulfate reduction over time (Figure 4A−D for D04 FT and E−H for D17 FT and Figure S5A−D for P1 FT and E−H for P2 FT). To develop a model for naphtha biodegradation, first zero-order kinetics of biodegradation for individual hydrocarbons were determined and used to predict depletion of labile components of naphtha (collectively) over time, which matched well with experimentally measured depletion of labile naphtha in FT from all locations. This zero-order modeling could be laborious because it requires the calculation of zero-order kinetics for each hydrocarbon separately. For simplicity in our new model, we summed up zero-order kinetics of all labile hydrocarbons and plotted them as aggregate rate over 800 days to observe the trend of aggregate rate over time. Then this trend was captured by adjusting a function $K_1(t)$, which indicated an exponential decay in kinetics over time (Figures 4A,E and S5A,E). This decay function represents our new model, which minimizes the need to calculate zero-order kinetics for individual hydrocarbons but still captures individual kinetics. To check the validity of the model, when labile naphtha biodegradation was predicted using the decay function, the model-derived values matched well with experimentally measured naphtha depletion and zero-order predicted naphtha depletion in FT from all locations (Figures 4B,F and S5B,F) which supports the effectiveness of the newly developed simplified model for predicting naphtha degradation under sulfate-reducing conditions.

Before model development for sulfate reduction, total sulfate depletion over time was modeled using zero-order rates of depletion during each amendment period in all FT sets (zero-order rates are provided in Table S4 and shown graphically in

Figures 4C,G and S5C,G). Additionally, sulfate depletion was calculated stoichiometrically based on the mass of hydrocarbons degraded over time. Later, when sulfate depletion over time was predicted using the model developed for sulfate reduction (eq 7), it matched well with stoichiometrically calculated values (when only $K_1(t)$ was considered, representative of 35−38% of naphtha) and zero-order kinetics predicted total sulfate depletion values (when $K_1(t)$ and $K_2(t)$ were considered, representative of naphtha and endogenous substrates) (Figures 4D,H and S5D,H). This suggests the effectiveness of eq 7 in predicting sulfate depletion. Model predictions indicated that sulfate reduction was greater (∼1.39−1.99-fold) than the mass required for biodegradation of 35−38% of 0.2% v/v naphtha (∼250−312 mg of sulfate), which can be attributed to the oxidation of endogenous organic substrates and degradation of other unidentified hydrocarbons, knowing that naphtha has >200 compounds that warrant detailed analysis. Interestingly, sulfate depletion was more pronounced in D17 and P2 FT (∼1.90-fold on average higher than stoichiometrically required) compared to D04 and P1 FT (∼1.43-fold greater on average). Relatively greater in situ concentrations of CH_4 and CO_2 from P3 (close to D17) and P2 locations[10] suggest the presence of greater concentrations of unidentified endogenous organic substrates, which might have resulted in relatively greater reduction of sulfate in current laboratory FT cultures from these locations.

Based on the model for sulfate reduction corresponding to oxidation of labile components of 0.2% v/v naphtha plus unknown endogenous organics, the final simple mathematical eq 8 (parameters in Table S5) may be used to calculate the potential sulfate requirement to suppress CH_4 production from naphtha diluent-affected tailings.

3.4. Application of the Developed Model to CH_4 Suppression in Mildred Lake Settling Basin (MLSB): A Case Scenario. The developed model based on current laboratory findings in FT collected from Base Mine Lake was applied to the MLSB for predicting efficacy in CH_4 suppression in situ. Reasons for using MLSB as a case scenario and its relevance to Base Mine Lake include the following: (1) Base Mine Lake contains tailings from MLSB; therefore, there are similar microbiomes at both locations[55] suggesting the likelihood of similar microbial processes; (2) Base Mine Lake has not received any tailings inputs since 2012, whereas MLSB is an active tailings facility that receives diluent-affected tailings input constantly, supporting continuous CH_4 production from MLSB, as reported online.[37] To predict CH_4 suppression, naphtha input values were required for model application; (3) both MLSB and Base Mine Lake have more- and less-methanogenically active locations. Therefore, model parameters derived for the D04 and D17 FT sets could project suppression of CH_4 emissions from MLSB depending upon activity. Notably, the MLSB scenario in the current study has been assumed to be simple (similar to the laboratory experimental conditions) for model application, whereas in situ variability may limit model application as discussed in Section 3.5.

CH_4 measurements from MLSB suggested that 4667 metric tonnes of CH_4 was produced in 2021, when ∼2000−4000 m^3 of diluent (naphtha) was deposited (in fresh tailings) per month in tailings facility (Table S7).[37] Using parameters from our model and considering one year naphtha input to the tailings pond, assuming 0.2% v/v naphtha in freshly deposited tailings, we predicted CH_4 production from MLSB under three scenarios (Figure 5) so that the effectiveness of the new model could be assessed for projecting CH_4 suppression by sulfate amendment: (i) without sulfate amendment, representative of strictly methanogenic conditions; (ii) with sulfate amendment, required for biodegradation of only labile (35−38%) naphtha hydrocarbons; (iii) with sulfate amendment, assuming requirements for biodegradation of naphtha plus unknown endogenous substrates. In the first scenario representing no sulfate addition to naphtha-containing tailings entering MLSB in 2021, CH_4 production was predicted per Siddique et al.[32] considering methanogenic biodegradation of 41% of naphtha hydrocarbons, which calculated $\sim 3 \times 10^{-4}$ metric tonnes m^{-3} CH_4 production by ∼1000 d (Figure 5A,C for proposed methanogenically less active site D04 and the more active location [D17], respectively). To compare model-predicted values with in situ CH_4 emissions, we estimated in situ CH_4 production for 2021 in metric tonnes m^{-3} from the data reported in metric tonnes.[37] The model predicted that total CH_4 production by 365 d (1.2×10^{-4} metric tonnes m^{-3}) is comparable with the in situ estimate of CH_4 emissions from MLSB in 2021 (1.9×10^{-4} metric tonnes m^{-3}), supporting the proposal that the labile components of naphtha are major contributors to CH_4 production over the short-term. The difference between in situ CH_4 estimates and model-predicted CH_4 can be attributed to the contribution from naphtha input over previous years due to long hydrocarbon biodegradation times and from endogenous organics that have been neither identified nor quantified and, therefore, were not accounted for in model predictions. In the second and third scenarios with sulfate addition to fresh tailings before deposition, projections of CH_4 suppression are interesting in the following aspects: (1) Considering the sulfate requirement for biodegradation of labile (35−38%) naphtha components, the model calculated that ∼51 to 85% of total predicted CH_4 could be suppressed in situ with sulfate addition. Complete CH_4 suppression did not occur according to model predictions due to sulfate depletion resulting from consumption (sulfate reduction) by unknown endogenous substrates (possibly more in methanogenically more active tailings represented by location D17 in the current laboratory study), which could possibly shift the redox conditions from strictly sulfate-reducing to simultaneous sulfate-reducing plus methanogenic to strictly methanogenic conditions (Figure 5B,D). (2) When sulfate reduction associated with other endogenous substrates was also taken into account, the model calculated suppression of ∼95−100% of predicted CH_4, irrespective of the difference in initial methanogenic activity. Model predictions based on data for the year 2021 suggest that if freshly produced diluent naphtha-affected tailings are mixed with sulfate (calculated using model eq 8) before deposition in the tailings facility, it may suppress methane from that deposition site.

3.5. Limitations of Model Applications. Our new simple zero-order kinetics-based phenomenological model developed to predict naphtha biodegradation and sulfate reduction projects substantial CH_4 suppression by sulfate amendment in naphtha-affected tailings. This model equation is simple enough that it does not require numerical solvers and provides sulfate estimations for CH_4 suppression from naphtha-affected tailings. However, considering the differences between laboratory cultures under ideal conditions and field nonideal conditions, the current model has limitations for in situ application that need to be addressed and require further experimentation. (1) The current model was developed using biodegradation kinetics for labile naphtha components, which limits its application to only naphtha-affected tailings (more specifically, to Base Mine Lake/MLSB). Other operators use diluents of different compositions[56] and, hence, tailings facilities exhibit different microbiomes.[55] Therefore, further experimentation using different diluents is required to improve the flexibility of the current model. (2) Current laboratory FT cultures were amended with naphtha at a concentration of 0.2% v/v. Experimentation using different concentrations of naphtha is required as higher concentrations may have some inhibitory effect on microbial activity[28] that may affect kinetics. The current model does not account for the effect of this variation in concentration. (3) Laboratory FT cultures were supplemented with a nutrient medium that does not simulate in situ conditions and has not been accounted for in the current model. Supply of trace elements may enhance the hydrocarbon biodegradation rates. Additional studies using laboratory cultures that are closer to in situ conditions might test the strength of the current model. (4) In current laboratory cultures, sulfate was added in multiple amendments to maintain sufficient soluble sulfate concentrations; however, for model development, we assumed that sulfate addition would occur as a one-time amendment in freshly produced tailings before deposition, from an in situ application perspective. The effect of one-time sulfate amendment on microbial behavior in the context of biodegradation is unknown and requires experimentation. (5) In laboratory experiments, repeated sampling mixes FT, which reduces the development of microenvironments devoid of nutrients, naphtha, and sulfate due to slow diffusion, as compared to in situ conditions, which are more heterogeneous at the microbial scale. This microheterogeneity was not considered during model development. (6) Hydrocarbon biodegradation under sulfate-reducing conditions is carried out by specific microbial communities that may take time to develop depending upon tailings age such as in mature tailings (represented by Base Mine Lake or bottom layers in MLSB) versus fresh tailings. Significant research would be required to integrate this aspect into the model. Hence, further refinement of the current phenomenological model or development of a mechanistic model with more flexibility may broaden its applicability.

Although sulfate amendment is shown here to suppress CH_4 production from naphtha hydrocarbon biodegradation, it is important to highlight that sulfate reduction produces reduced sulfur compounds, predominantly sulfides ($H_2S/HS^-/S^{2-}$)[14,57] that are toxic and may have environmental and tailings management implications. Soluble sulfide may precipitate with chalcophile elements, whereas gaseous sulfide (H_2S) may migrate in upper layers of tailings facilities or Base Mine Lake where it may escape to the atmosphere (posing a serious concern because of its acute toxicity, foul odor, and potential for corrosion) or be reoxidized to sulfur/sulfate in the presence of oxygen. Previously, sulfide production from gypsum-treated tailings generated by a different oil sands operator increased environmental concerns.[12] In the current study, when sodium sulfate was used as a sulfate source, we observed sulfide (mainly soluble sulfide HS^-/S^{2-}) as the dominant end product of the sulfate reduction process that can be precipitated. Therefore, we are investigating alternate sulfate amendments such as ferric sulfate, predicting that reduced iron (Fe^{II}) would immobilize the produced sulfide species in FT solids, thereby reducing the implications associated with sulfate amendment (studies in progress).

Overall, the current laboratory study clearly explains the inhibition of CH_4 production from naphtha diluent-affected oil sands tailings through redox amendment. It also provides oil sands operators with a simple model for sulfate amendment calculations, although at present, its application is limited to Base Mine Lake/MLSB tailings. These findings also broaden our understanding of hydrocarbon biodegradation processes in anaerobic conditions, which is important for the management of other hydrocarbon-contaminated environments such as soils, estuaries, and wetlands.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestengg.5c00448.

Detailed description of the model development for hydrocarbon biodegradation and sulfate depletion; tables containing information on experimental design and hydrocarbon biodegradation kinetics parameters under sulfate-reducing and methanogenic conditions for different sites (D04, D17, P1, and P2), sulfate depletion kinetics parameters, model parameters, and estimations of tailings volume from naphtha loss data in Mildred Lake Settling Basin for in situ predictions; and figures presenting hydrocarbon biodegradation, sulfate depletion, and model validation data for sites P1 and P2 (PDF)

Stoichiometric sulfate calculation for hydrocarbon biodegradation, zero-order kinetics of hydrocarbon biodegradation and sulfate depletion, fitting of exponential decay function to aggregate zero-order kinetics of hydrocarbon biodegradation, and zero-order and new model predicted data of hydrocarbon biodegradation and sulfate depletion (XLSX)

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Notes

The authors declare no competing financial interest.

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Supporting Information

Mitigation of Methane Emissions from Oil Sands Tailings by Redox Amendment: Mathematical Modeling of Empirical Observations

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Text S1: Zero-order Model for Hydrocarbon Degradation under Sulfate-reducing Conditions

We denoted $C(t)$ as the aggregate naphtha hydrocarbons mass (known labile hydrocarbons in naphtha), and $C_i(t)$ as each individual hydrocarbon mass. The mass units are usually expressed in mmol for stoichiometric reasons or mg for convenience. To develop the zero-order kinetics model for hydrocarbon degradation under sulfate-reducing conditions, we used the slightly modified zero-order kinetics model given as

$$\frac{d\,C_i}{dt} = \begin{cases} 0 & if\ t < \lambda_s \\ -k_0^i, & otherwise, \end{cases} \tag{S1}$$

or $C_i(t) = C_i(0) - k_0^i t$ for $t \geq \lambda_s$, were $C_i(0)$ is the initial hydrocarbon mass (mmol or mg), λ_s is the lag phase (day), k_0^i is the zero-order degradation rate (mass day^{-1}), and we assumed $C_i(t) = 0$ for $C_i(t) < 0$ $(t \geq C_i(0)/k_0^i)$, i.e., when the *i*-th hydrocarbon is depleted, ensuring non-negative values and realistic outputs. This model neglects cell growth and captures degradation kinetics with a degradation rate constant. The zero-order kinetics were estimated by minimizing the Root Mean Square Error (RMSE) function

$$RMSE = \sqrt{\frac{1}{N}\,||x - y||^2}\,,$$

where N is the number of elements of a vector, x is the data vector and y is the model prediction. Once the zero-order kinetics parameters were determined, we calculated the goodness of fit to measure the performance of the model. For this, we used the Normalized Mean Square Error ($NMSE$) given as

$$NMSE = 1 - \frac{||x - y||^2}{||x - \bar{x}||^2}$$

where || .|| indicates the 2-norm of a vector and $\bar{x}$ is the mean of the actual data. The $NMSE$ output is between $(-\infty, 1)$, where $-\infty$ indicates a bad fit and 1 a perfect fit. The parameters and $NMSE$ estimated for the monitored hydrocarbons are given in Tables S2 for D04 and D17 FT and Table S3 for P1 and P2 FT.

Stoichiometric sulfate calculations for each hydrocarbon (θ_i) were based on the following equations:

***n*-Hexane, 2-Methylpentane**

$$C_6H_{14} + 4.5SO_4^{2-} + 2.5H^+ \longrightarrow 6CO_2 + 4.5HS^- + 6H_2O$$

***n*-Heptane, 2-Methylhexane, 3-Methylhexane**

$$C_7H_{16} + 5.25SO_4^{2-} + 3.25H^+ \longrightarrow 7CO_2 + 5.25HS^- + 7H_2O$$

***n*-Octane, 2-Methylheptane, 3-Methylheptane, 4-Methylheptane**

$$C_8H_{18} + 6SO_4^{2-} + 4H^+ \longrightarrow 8CO_2 + 6HS^- + 8H_2O$$

***n*-Nonane, 2-Methyloctane, 3-Methyloctane**

$$C_9H_{20} + 6.75SO_4^{2-} + 4.75H^+ \longrightarrow 9CO_2 + 6.75HS^- + 9H_2O$$

Toluene

$$C_7H_8 + 4.5SO_4^{2-} + 4.5H^+ \longrightarrow 7CO_2 + 4.5HS^- + 4H_2O$$

Xylenes

$$C_8H_{10} + 5.25SO_4^{2-} + 5.25H^+ \longrightarrow 8CO_2 + 5.25HS^- + 5H_2O$$

Using Table S2, Table S3 and sulfate stoichiometry (θ_i), sulfate depletion over time for each hydrocarbon was calculated as $S_i(t) = \theta_i(C_i(0) - C_i(t))x\frac{Sulfate\ molar\ mass}{molar\ mass\ i{-}th\ HC}$. Then, the total sulfate uptake to biodegrade 16 hydrocarbons ($S_{HC}(t)$) was given as follows (Equation S2)

$$S_{HC}(t) = \sum S_i(t) \tag{S2}$$

To calculate the experimental total sulfate depletion ($S_e(t)$), we first determined the zero-order rate of sulfate depletion between sulfate amendment periods per equation S3.

$$\frac{d\,S_e}{dt} = -k_0^s, \tag{S3}$$

or $S_e(t) = S_s(0) - k_0^s t$. We defined $S_e(t) = 0$ for $S_e(t) < 0$ for the model to be biologically relevant. The zero-order kinetics for the sulfate depletion between amendments were computed by minimizing the Root Mean Square Error (*RMSE*) (Table S4).

Using zero-order kinetics for each amendment period as presented in Table S4, it was possible to model the total sulfate depletion ($S_T(t)$) assuming that all sulfate was added at the beginning of the experiment ($S(0)$). In this way, additional sulfate depletion (more than what was required for biodegradation of ~35-38% of naphtha) was estimated ($S_{additional}(t)$) using equation S4.

$$S_{additional}(t) = S(0) - S_T(t) - S_{HC}(t) \tag{S4}$$

Text S2: Simple Zero-order Kinetics-based New Model for Hydrocarbon Degradation and Sulfate Depletion

New zero-order derived model for hydrocarbon degradation and sulfate depletion was developed under sulfate-reducing conditions. For this, the zero-order kinetics of biodegradation for each hydrocarbon (Table S2 and Table S3) was added and plotted as aggregate kinetics over 800 d. Then we adjusted an exponential decay function to model the aggregate zero-order kinetics trend over time for FT from all sets (equation S5):

$$K_1(t) = ae^{-b\,t} \tag{S5}$$

where a (mg day^{-1}) is the maximum degradation rate and b (day^{-1}) is the exponential decay constant. This function approximates the aggregate hydrocarbon degradation rate, and the parameters were calculated by minimizing the $RMSE$, approximating the sum of each zero-order degradation rate constant with variation over time. Hence, the new model for hydrocarbon degradation under sulfate-reducing conditions is given as:

$$\frac{d\,C}{dt} = -\,K_1(t) \tag{S6}$$

The integral form is shown in equation S7

$$C(t) = C(0) - \frac{a}{b}(1 - e^{-b\,t}) \tag{S7}$$

The estimated parameters are given in Table S5. Since equation S7 describes accurately the hydrocarbon degradation over time under sulfate-reducing conditions, we determined the dimensionless parameter indicating proportional sulfate consumption when equation S6 was used, i.e., we determined θ (see Table S5) by solving

$$\min_{\theta} \left\| \theta\big(C(0) - C(t)\big) - S_{HC}(t) \right\|^2$$

To model the degradation of additional substrates (endogenous organics or naphtha components other than 16 analytes), we proposed $K_2(t)$ as

$$K_2(t) = g e^{-d\,t} \tag{S8}$$

Here, g (mg day^{-1}) and d (day^{-1}) are model parameters.

Then, sulfate uptake was modeled as

$$\frac{dS}{dt} = -\theta\big(K_1(t) + K_2(t)\big) \tag{S9}$$

and the integral form is shown in equation S10

$$S(t) = S(0) - \theta\left(\frac{a}{b}(1 - e^{-b\,t}) + \frac{g}{d}(1 - e^{-d\,t})\right) \tag{S10}$$

To estimate the parameters for $K_2(t)$, we fitted equation S10 using the parameters found for $K_1(t)$ and θ with $S_T(t)$. The parameters are given in Table S5 with the $NMSE$. To estimate the sulfate required for naphtha hydrocarbon degradation and endogenous organics, we used equations S2 and S4 for large time ($t \to \infty$), respectively. The sulfate required can also be estimated using equation S10 for large time ($t \to \infty$) (see Table S6).

Based on equation S10, and parameters given in Table S5, it is possible to calculate the sulfate required for biodegrading labile components of 0.2 %v/v naphtha as well as endogenous substrates as follows:

$$\lim_{t\to\infty} S(t) = 0 = S(0) - \theta\left(\frac{a}{b} + \frac{g}{d}\right)$$

$$S(0) = \theta\left(\frac{a}{b} + \frac{g}{d}\right) \tag{S11}$$

Table S1. Experimental design to investigate naphtha hydrocarbon biodegradation under sulfate-reducing conditions in FT from locations exhibiting different methanogenic activity *in situ* in Base Mine Lake; D04 and P1 (methanogenically less active *in situ*) and D17 and P2 (methanogenically more active *in situ*) locations.

Treatment [Replicates]	**Base Mine Lake FT sampling locations for laboratory experiments**			
	D04	**D17**	**P1**	**P2**
FT + Nutrient medium (Baseline control) [3]	√	√		
FT + Nutrient medium + Sulfate [2]	√	√	√	√
FT + Nutrient medium + (0.2 %v/v) Naphtha [3]	√	√		
FT + Nutrient medium + (0.2 %v/v) Naphtha + Sulfate [3]	√	√	√	√
Sterile FT + Nutrient medium + (0.2 %v/v) Naphtha + Sulfate [2]	√	√	√	√

Table S2: Zero-order biodegradation kinetics parameters of labile components of naphtha under sulfate-reducing conditions in FT cultures for location D04 (methanogencally less active *in situ*) and D17 (methanogenically more active *in situ*). Mass for each hydrocarbon was calculated from the %wt of the individual hydrocarbon in naphtha.

Hydrocarbon	**% wt. in naphtha**	**Sampling location D04 FT**			**Sampling location D17 FT**		
		Lag time λ_s (d)	**$k_0{}^i$ (mg d^{-1})**	**$NMSE$**	**Lag time λ_s (d)**	**$k_0{}^i$ (mg d^{-1})**	**$NMSE$**
Toluene	4.63	0	0.115	0.97	0	0.0198	0.66
o-Xylene	1.44	150	0.009	0.99	395	0.0056	0.93
m/p-Xylenes	3.49	120	0.003	0.75	ND	ND	ND
Hexane	0.46	52	0.0018	0.98	52	0.0014	0.97
Heptane	4.86	52	0.041	0.97	52	0.0426	0.97
Octane	8.93	52	0.0921	0.97	52	0.0804	0.96
Nonane	3.30	52	0.0157	0.98	150	0.0156	0.95
2-methylpentane	0.11	0	0.0001	0.64	359	0.00009	0.19
2-methylhexane	1.11	316	0.0104	0.95	255	0.0043	0.97
3-methylhexane	1.33	ND	ND	ND	255	0.0010	0.29
2-methylheptane	5.41	52	0.0329	0.98	52	0.0409	0.95
3-methylheptane	0.73	120	0.0031	0.97	198	0.0028	0.95
4-methylheptane	2.03	255	0.0033	0.72	478	0.0054	0.67
2-methyloctane	1.39	120	0.0061	0.97	52	0.0036	0.94
3-methyloctane	1.83	316	0.0171	0.98	316	0.0094	0.96

NMSE: Normalized Mean Square Error
ND: Not Degraded

Table S3: Zero-order biodegradation kinetics parameters of labile components of naphtha under sulfate-reducing condition in FT sets for location P1 (methanogencally less active *in situ*) and P2 (methanogenically more active *in situ*). Mass for each hydrocarbon was calculated from %wt of individual hydrocarbon in naphtha.

Hydrocarbon	**% wt. in naphtha**	**Sampling location P1 FT**			**Sampling location P2 FT**		
		Lag time λ_s (d)	**$k_0{}^i$ (mg d^{-1})**	**$NMSE$**	**Lag time λ_s (d)**	**$k_0{}^i$ (mg d^{-1})**	**$NMSE$**
Toluene	4.63	0	0.0965	0.60	0	0.1954	0.81
o-Xylene	1.44	198	0.0075	0.96	120	0.0092	0.98
m/p-xylenes	3.49	ND	ND	ND	ND	ND	ND
Hexane	0.46	0	0.0019	0.94	30	0.0020	0.89
Heptane	4.86	0	0.0419	0.99	0	0.0418	0.99
Octane	8.93	30	0.0699	0.98	30	0.0748	0.99
Nonane	3.30	52	0.0157	0.99	120	0.0149	0.92
2-methylpentane	0.11	198	0.00008	0.40	ND	ND	ND
2-methylhexane	1.11	255	0.0069	0.97	359	0.0047	0.90
3-methylhexane	1.33	198	0.0008	0.21	ND	ND	ND
2-methylheptane	5.41	0	0.0306	0.98	0	0.0335	0.97
3-methylheptane	0.73	255	0.0063	0.99	255	0.0024	0.93
4-methylheptane	2.03	316	0.0036	0.56	478	0.0044	0.65
2-methyloctane	1.39	120	0.0056	0.98	120	0.0039	0.90
3-methyloctane	1.83	255	0.0133	0.95	316	0.0070	0.87

NMSE: Normalized Mean Square Error
ND: Not Degraded

Table S4: Zero-order kinetics rate of sulfate depletion during each amendment period in naphtha-amended (with sulfate) FT cultures from locations D04, D17, P1 and P2.

FT sampling location	**Period (d)**	k_0^s **(mg d^{-1})**	$RMSE$
D04	[3-85]	1.249	4.46
	[86 - 133]	2.055	0
	[134 - 186]	0.876	6.76
	[187 - 361]	0.543	9.11
	[362 - 812]	0.209	16.53
D17	[3 - 85]	1.901	20.61
	[86 - 133]	2.689	0
	[134 - 186]	1.315	1.37
	[187 - 361]	0.475	9.85
	[362 - 442]	0.776	0.21
	[443 - 519]	0.962	5.71
	[520 - 812]	0.153	2.77
P1	[3 - 85]	1.268	21.09
	[86 - 133]	1.697	0
	[134 - 186]	1.200	7.20
	[187 - 361]	0.503	11.39
	[362 - 812]	0.100	7.47
P2	[3 -85]	1.517	29.12
	[86 - 133]	2.619	0
	[134 - 186]	1.224	0.21
	[187 - 519]	0.267	12.91
	[520 - 812]	0.248	5.58

RMSE: Root Mean Square Error

Table S5: Estimated parameters based on the new model of naphtha hydrocarbon biodegradation and sulfate depletion (Equation S10).

FT sampling location	**a (mg d^{-1})**	**b (d^{-1})**	**g (mg d^{-1})**	**d (d^{-1})**	$\boldsymbol{\theta}$	***NMSE***
D04	0.2155	0.0036	0.1447	0.0052	4.99	0.993
D17	0.1592	0.0026	0.3563	0.0059	5.09	0.987
P1	0.2546	0.0046	0.1358	0.0062	4.94	0.990
P2	0.2762	0.0055	0.1751	0.0043	4.96	0.974

Table S6: Sulfate depletion estimations using zero-order model (Equations S2 and S4) and zero-order kinetics-based new model (Equation S10). Here θ represents proportional sulfate consumption determined by solving the minimization problem (**Text S2**).

Sulfate calculations		**FT sampling locations**			
		D04	**D17**	**P1**	**P2**
Zero-order model calculations	Sulfate required for degradation of selected (35-38%) hydrocarbons (mg)	262	260	257	251
	Additional sulfate depletion (mg)	171	297	122	222
	Total (mg)	433	557	379	472
New model calculations	Proportional sulfate consumption for selected (35-38%) hydrocarbon biodegradation (mg) $[\theta\frac{a}{b}]$	299	312	273	249
	Additional sulfate depletion (mg) $[\theta\frac{g}{d}]$	139	307	108	202
	Total (mg)	437	619	382	451

Table S7: Estimation of tailings volume using ***in situ*** naphtha loss data for one year (2021) in Mildred Lake Settling Basin (MLSB) https://static.aer.ca/prd/documents/sts/ST39-2021.pdf [1].

Month	**Naphtha flared/ wasted (m^3)**	**Density of diluent (MT m^{-3})**	**Naphtha flared/ wasted (MT)**	**Volatilization factor ***	**Mass of naphtha after volatilization (MT)**	**Estimated tailings volume 0.2% v/v (L)**	**Estimated tailings volume 0.2% v/v (m^3)**	**% (v/v) naphtha**
Jan	5,362.30	0.76	4,075.35	0.7	2,852.74	2.68E+09	2681150	**0.2**
Feb	4,995.00		3,796.20		2,657.34	2.5E+09	2497500	**0.2**
Mar	4,459.50		3,389.22		2,372.45	2.23E+09	2229750	**0.2**
Apr	2,724.50		2,070.62		1,449.43	1.36E+09	1362250	**0.2**
May	2,212.20		1,681.27		1,176.89	1.11E+09	1106100	**0.2**
Jun	2,862.00		2,175.12		1,522.58	1.43E+09	1431000	**0.2**
Jul	4,746.50		3,607.34		2,525.14	2.37E+09	2373250	**0.2**
Ago	4,210.20		3,199.75		2,239.83	2.11E+09	2105100	**0.2**
Sep	3,757.00		2,855.32		1,998.72	1.88E+09	1878500	**0.2**
Oct	4,537.00		3,448.12		2,413.68	2.27E+09	2268500	**0.2**
Nov	4,617.50		3,509.30		2,456.51	2.31E+09	2308750	**0.2**
Dec	4,361.60		3,314.82		2,320.37	2.18E+09	2180800	**0.2**
Total	48,845.3		37,122.43		25,985.70	2.44E+10	24422650	

* The volatilization factor shows the volatilization-induced weight loss of volatile hydrocarbons from naphtha [2]. It was estimated as 0.7 according to Henry's Law for the most abundant naphtha compounds.

Table S8: Zero-order biodegradation kinetics parameters of labile components of naphtha under methanogenic conditions for FT from location D04 (methanogencally less active *in situ*) and D17 (methanogenically more active *in situ*). Mass for each hydrocarbon was calculated from %wt of individual hydrocarbon in naphtha.

Hydrocarbon	**% wt. in naphtha**	**Sampling location D04 FT**			**Sampling location D17 FT**		
		Lag time λ_i (d)	**$k_0{}^i$ (mg d^{-1})**	**$NMSE$**	**Lag time λ_i (d)**	**$k_0{}^i$ (mg d^{-1})**	**$NMSE$**
Toluene	4.63	0	0.0424	0.95	19	0.0719	0.97
o-Xylene	1.44	120	0.0154	0.99	139	0.0107	0.99
m/p-Xylenes	3.49	301	0.0245	0.98	330	0.0328	0.99
Hexane	0.46	153	0.0032	0.97	0	0.0029	0.98
Heptane	4.86	208	0.0734	0.99	50	0.0434	0.99
Octane	8.93	208	0.0911	0.99	84	0.0679	0.99
Nonane	3.30	301	0.0884	0.97	230	0.0444	0.97
2-methylpentane	0.11	278	0.0007	0.98	50	0.0003	0.97
2-methylhexane	1.11	ND	ND	ND	ND	ND	ND
3-methylhexane	1.33	278	0.0119	0.95	50	0.0059	0.98
2-methylheptane	5.41	362	0.0089	0.79	160	0.0063	0.79
3-methylheptane	0.73	249	0.0049	0.97	50	0.0023	0.97
4-methylheptane	2.03	249	0.0158	0.98	84	0.0078	0.97
2-methyloctane	1.39	323	0.0081	0.95	230	0.0063	0.97
3-methyloctane	1.83	458	0.0017	0.36	ND	ND	ND

NMSE: Normalized Mean Square Error
ND: Not Degraded

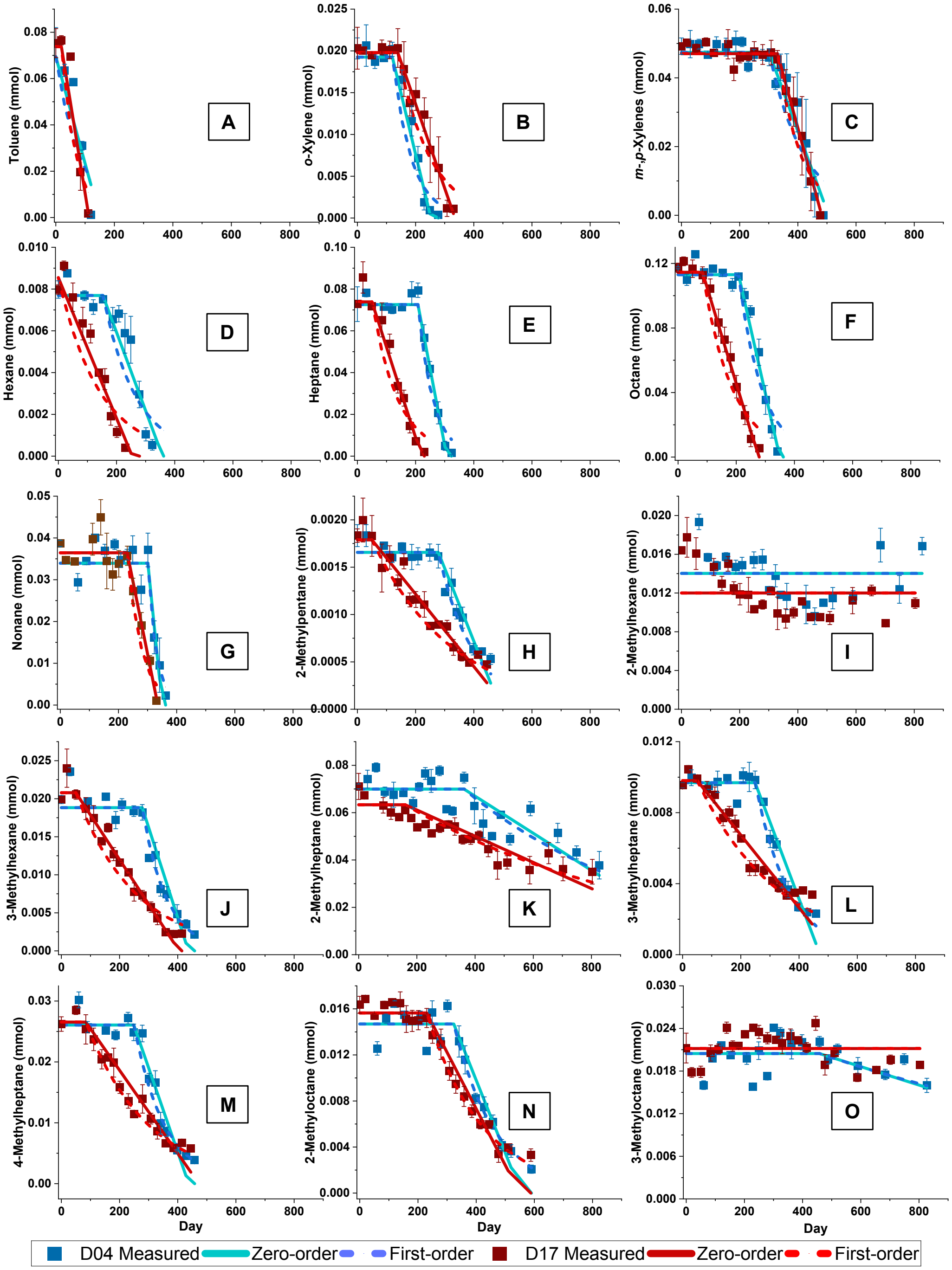

Figure S1 Biodegradation kinetics of individual hydrocarbons in FT from location D04 (blue) and D17 (red) under methanogenic conditions. **Panels A-C** are monoaromatics, **Panels D-G** are *n*-alkanes, and **Panels H-O** are *iso*-alkanes. Data points represent experimental measurements, solid lines represent hydrocarbon depletion predicted by zero-order kinetics, and dashed lines indicate depletion predicted by first-order kinetics. Each measured observation is the average of triplicates, and error bars indicate one standard deviation.

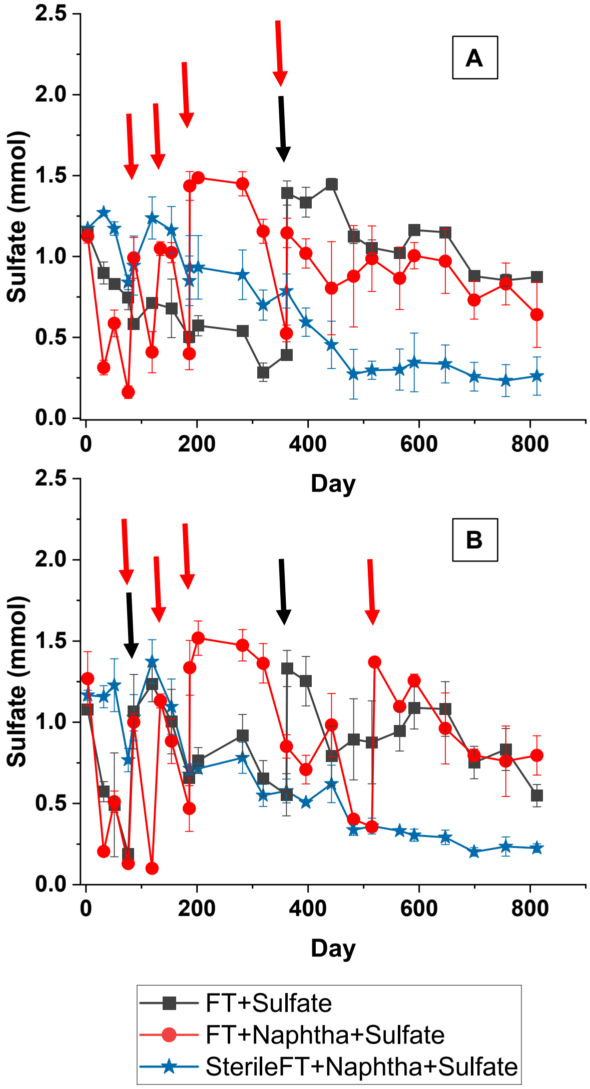

Figure S2 Liquid phase sulfate depletion over time in unamended (FT + Sulfate), live amended (FT + Naphtha + Sulfate) and sterile amended (Sterile FT + Naphtha + Sulfate) cultures for location P1 (**Panel A**) and P2 (**Panel B**). Each measured observation is the average of triplicates, and error bars indicate one standard deviation. The arrows indicate times of sulfate re-amendments in live FT cultures (sterile cultures were not re-amended).

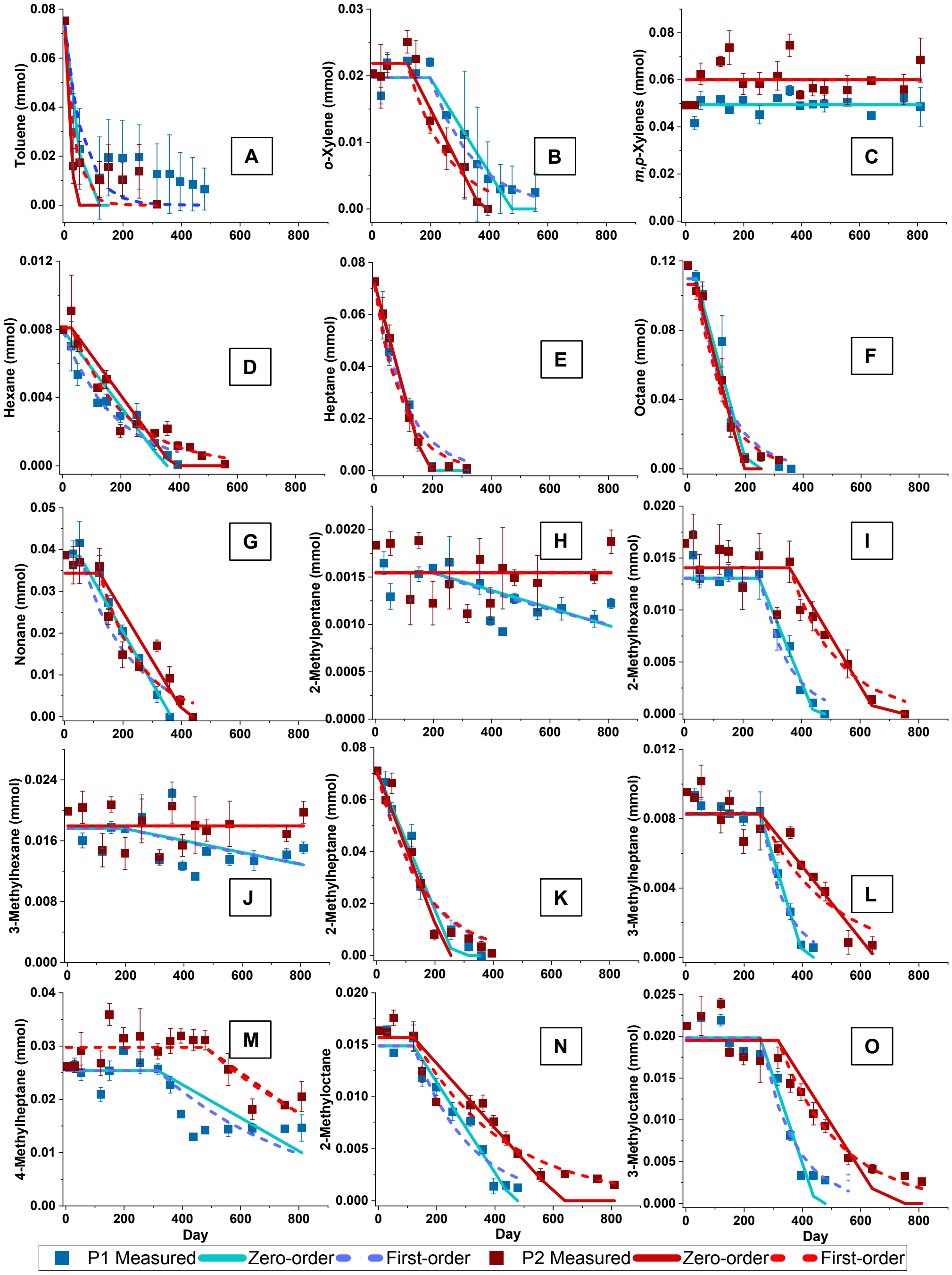

Figure S3 Biodegradation kinetics of individual hydrocarbons in FT from location P1 (blue) and P2 (red) under sulfate-reducing conditions. **Panels A-C** are monoaromatics, **Panels D-G** are *n*-alkanes, and **Panels H-O** are *iso*-alkanes. Data points represent experimental measurements, solid lines represent hydrocarbon depletion predicted by zero-order kinetics, and dashed lines indicate depletion predicted by first-order kinetics. Each measured observation is the average of triplicates, and error bars indicate one standard deviation.

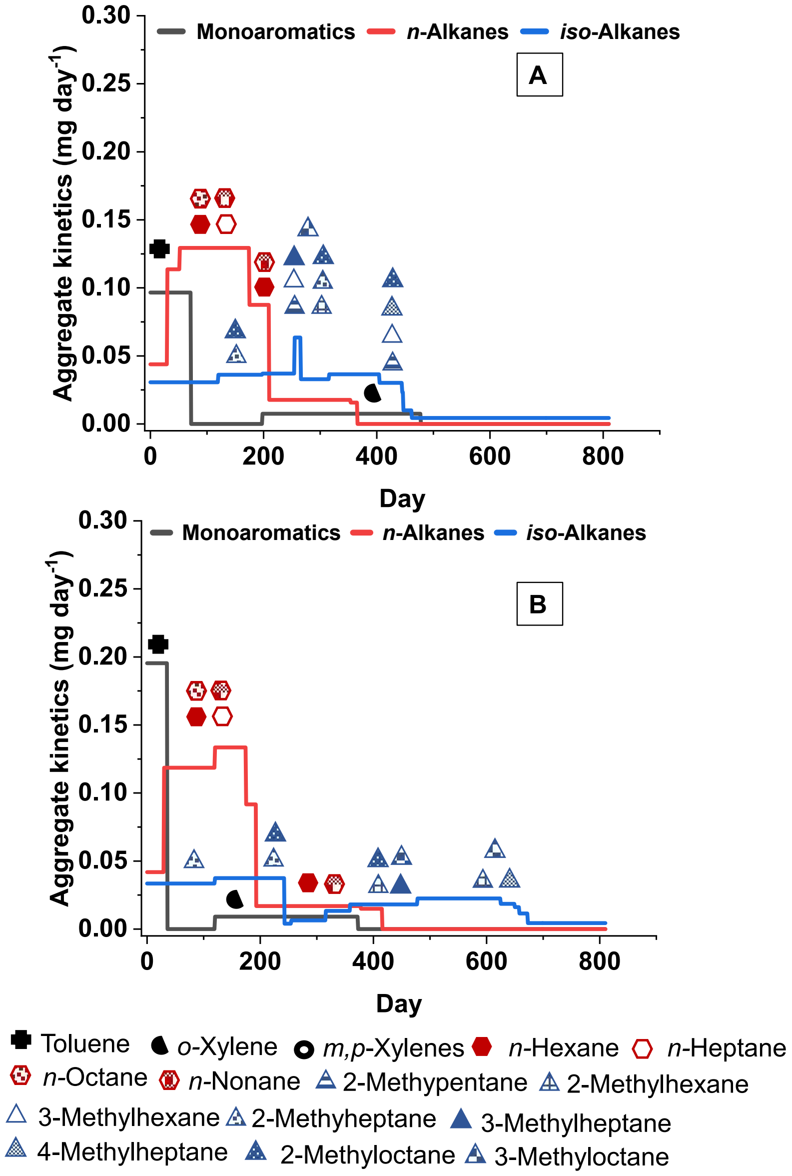

Figure S4 Aggregate zero-order biodegradation rates of hydrocarbons (mg d^{-1}) presented in three groups: monoaromatics, *n*-alkanes, and *iso*-alkanes to observe lag phases, biodegradation rates and pattern of biodegradation. **Panels A and B** represent aggregate kinetics of biodegradation under sulfate-reducing conditions for locations P1 and P2 FT, respectively. Interpretations of different symbols are the same as in Figure 3 (main text).

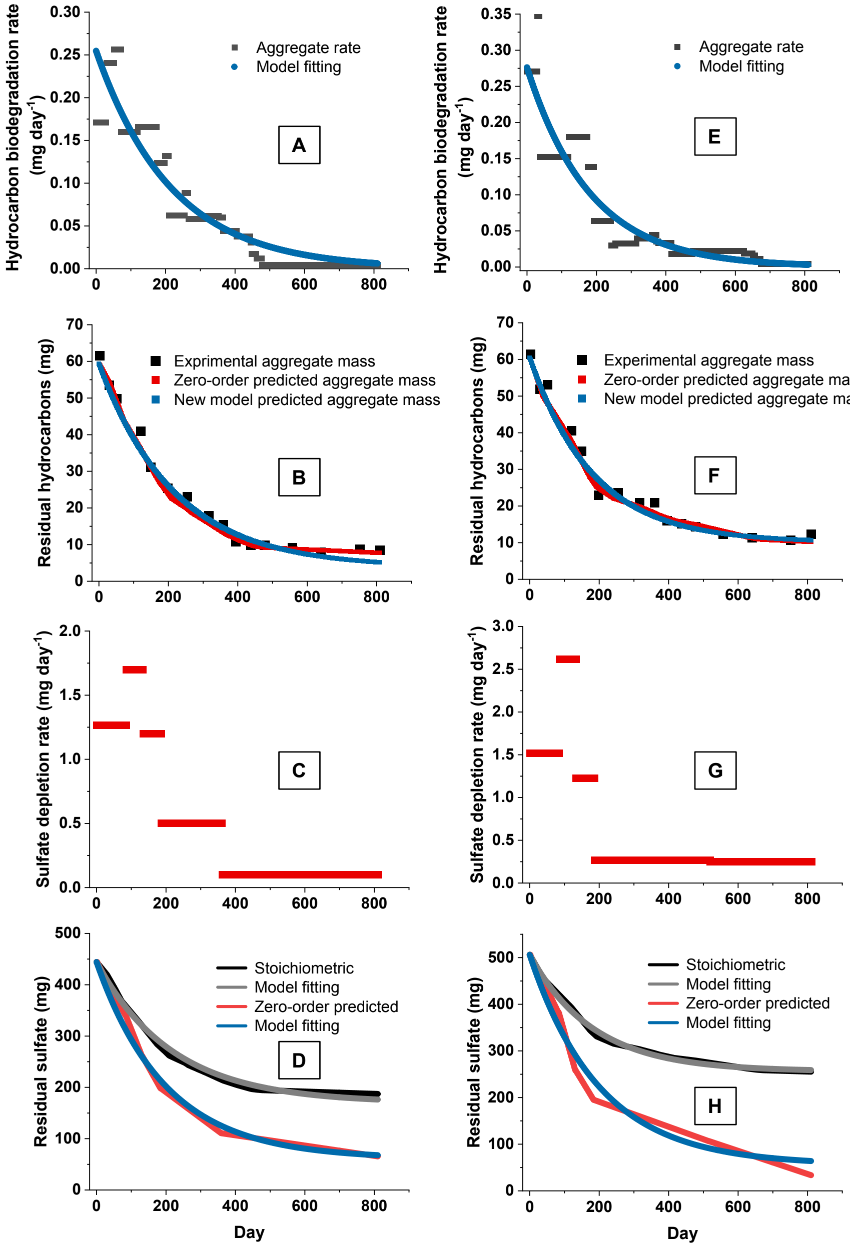

Figure S5 Measurement of model parameters and comparison of experimental observations with model predictions for naphtha biodegradation and sulfate reduction in location P1 **(Panels A-D)** and P2 FT cultures (**Panels E-H**). **Panels A and E:** Adjusted model function for capturing the trend of aggregate hydrocarbon biodegradation zero-order kinetics over time. **Panels B and F**: Comparison of naphtha depletion predicted by the new model with experimentally measured depletion and zero-order model predicted depletion to assess the effectiveness of the new model. **Panels C and G:** Zero-order rates of sulfate depletion calculated from experimental observations. **Panels D and H**: Comparison of sulfate depletion values predicted by the new model with stoichiometrically calculated and zero-order-predicted total sulfate depletion over time.

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