

# FINAL REPORT ADDENDUM

Perchlorate Destruction and Potable Water Production  
Using Membrane Biofilm Reduction  
and Membrane Filtration

ESTCP Project ER-200541

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## EXECUTIVE SUMMARY

### Background

This report summarizes the findings of research carried out at Arizona State University (ASU) in parallel with a field pilot study led by CDM Smith in cooperation with APTwater and reported in its own final report. The objective of the overall project was biological treatment of a groundwater contaminated with two oxidized contaminants: nitrate ( $\text{NO}_3^-$ ) and perchlorate ( $\text{ClO}_4^-$ ). Dissolved oxygen ( $\text{O}_2$ ) and sulfate ( $\text{SO}_4^{2-}$ ) are oxidized compounds that also were present. The treatment goal was to use the  $\text{H}_2$ -based Membrane Biofilm Reactor (MBfR) to achieve microbial reduction of  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  into innocuous substances ( $\text{N}_2$ ,  $\text{H}_2\text{O}$ , and  $\text{Cl}^-$ ), while not promoting  $\text{SO}_4^{2-}$  reduction to sulfide.

The MBfR promotes the growth of autotrophic bacteria capable of using hydrogen gas ( $\text{H}_2$ ) as their electron donor by its diffusion through bubbleless gas-transfer membranes. A microbial community develops as a biofilm attached to the membrane's outer surface; the biofilm normally is comprised of a diverse spectrum of autotrophic and heterotrophic microorganisms embedded in a matrix composed of extracellular polymeric substances (EPS). "Side-reactors" were established as part of the pilot MBfRs to study the biofilm and its microbial community structure. The ASU team carried out in-depth analyses of the biofilms from the side-reactors, as well as from two pilot modules. These analyses included microbial ecology, microscopy, and chemical analyses.

The ASU team also carried out a series of experiments with bench-scale MBfR and developed mechanistic mathematical models of the biofilms. The goal of these studies was to gain fundamental understanding of the kinetic, transport, and ecological mechanisms controlling the performance of the pilot MBfRs. Thus, the over-arching goal of the ASU research was to provide a fundamental, mechanistic foundation for understanding the results obtained in the pilot study and for identifying strategies to improve performance for future applications.

The report is divided into nine chapters plus the cited references. The first five chapters address ASU's analyses for the pilot MBfRs. Chapters 6 to 8 report on the bench-scale and modeling research. Chapter 9 then gives a global synthesis of all of the results. Much of the work reported here has been published in peer-review journals, and these primary sources are identified throughout the report.

### Performance and characteristics of the biofilms from the pilot MBfRs

The field pilot study showed that, although the two-stage MBfR system was effective for reducing  $\text{NO}_3^-$  and  $\text{ClO}_4^-$ , it did not achieve one of the goals: an effluent  $\text{ClO}_4^-$  concentration less than 6  $\mu\text{g/L}$ . Attempts to reduce the loading rates of the electron acceptors by lowering the flow rate did not lead to the desired outcome of  $\text{ClO}_4^- < 6 \mu\text{g/L}$  for sustained operation, but led to  $\text{SO}_4^{2-}$  reduction, an undesired outcome. Regularly switching the lead and lag positions also did not bring about the desired result. The ASU Team focused on uncovering why the two-stage MBfR system did not achieve the 6- $\mu\text{g/L}$  goal, even though it performed well in other ways. The ASU team performed chemical, ecological, and kinetic evaluations to gain comprehensive insight into MBfR performance.

The biofilms in the side reactors and the reactor modules in the MBfR vessels had consistent characteristics that reflect the operating conditions at the pilot site. While the biofilm thickness varied, it typically was about 200  $\mu\text{m}$ . The biofilm was only about 10% inorganic, which means that precipitation was prevented well by the pH-control system. Although the biofilm contained 40 – 50% extracellular polymeric substances (EPS), the cells were predominantly live, particularly near the membrane substratum. For all MBfRs, perchlorate-reducing bacteria (PRB) always made up the smallest fraction of the active bacteria, sulfate-reducing bacteria (SRB) always were present, and SRB became more important when  $\text{SO}_4^{2-}$  reduction was the a major sink for  $\text{H}_2$ .

Analysis of the fluxes of the four electron acceptors (i.e.,  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ ,  $\text{O}_2$ , and  $\text{SO}_4^{2-}$ ) showed that  $\text{H}_2$  availability and acceptor fluxes determined the degree of success with  $\text{ClO}_4^-$  removal and minimizing  $\text{SO}_4^{2-}$  reduction. The lead MBfR was responsible of reducing 70-90% of the  $\text{NO}_3^-$  loading and > 99% of the  $\text{O}_2$  loading. A  $\text{NO}_3^- + \text{O}_2$  surface loading of  $\geq 0.3 \text{ g H}_2/\text{m}^2\text{-day}$  suppressed  $\text{SO}_4^{2-}$  reduction in the lead MBfR. With most  $\text{NO}_3^-$  and all  $\text{O}_2$  removed by the lead MBfR, the low surface loading of  $\text{NO}_3^-$  and  $\text{O}_2$  influent to the lag MBfR allowed the onset of  $\text{SO}_4^{2-}$  reduction in the lag MBfR. Quantitatively, a  $\text{NO}_3^-$  flux  $< \sim 0.1 \text{ g H}_2/\text{m}^2\text{-day}$  allowed  $\text{SO}_4^{2-}$  reduction in the lag MBfR when  $\text{H}_2$  delivery was not limiting. (In order to compare all fluxes, they are expressed as  $\text{H}_2$  consumption flux in  $\text{g H}_2/\text{m}^2\text{-day}$ .)

$\text{ClO}_4^-$  respiration occurred in the lead and lag MBfRs, but the highest  $\text{ClO}_4^-$  reduction occurred at the lag MBfR, where competition from  $\text{NO}_3^-$  and  $\text{O}_2$  respirations was minimized. However,  $\text{ClO}_4^-$  reduction flux in the lag MBfR was inversely correlated to the  $\text{SO}_4^{2-}$  flux. A  $\text{SO}_4^{2-}$  flux of  $0.003 \text{ g H}_2/\text{m}^2\text{-day}$  or more seemed to slow  $\text{ClO}_4^-$  reduction. A  $\text{SO}_4^{2-}$  flux of  $0.001 \text{ g H}_2/\text{m}^2\text{-day}$  allowed the highest  $\text{ClO}_4^-$  flux.

Operational conditions applied to the pilot MBfRs affected the microbial communities in ways that corresponded to acceptor fluxes. When acceptor surface loadings were high due to a high flow rate, the pilot MBfRs (especially the lead MBfR) experienced a limitation of  $\text{H}_2$  delivery, and DB were dominant. Increasing the HRT, which decreased the surface loading for all acceptors, eliminated  $\text{H}_2$  limitation, but at the expense of favoring SRB, whose metabolic versatility helped them remain in the biofilm regardless of  $\text{SO}_4^{2-}$  reduction activity.

## **Bench-scale experiments and modeling**

The ASU team carried out a series of bench-scale experiments designed to elucidate critical kinetic and ecological mechanisms that control the performance of the MBfR when  $\text{ClO}_4^-$  reduction to  $\mu\text{g/L}$  concentrations is the goal. Of over-arching importance is that multiple electron acceptors were present in the influent water:  $\text{O}_2$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{ClO}_4^-$ . Typically and in the case of the field study,  $\text{ClO}_4^-$  is present at by far the lowest concentration. This means that PRB must compete for the common electron donor ( $\text{H}_2$ ) and space in the biofilm with bacteria that reduce the other acceptors. The competition can be an important factor controlling whether or not the PRB are able to reduce  $\text{ClO}_4^-$  to very low concentrations.

In a series of experimental studies, the ASU team evaluated competition for  $H_2$  and space among PRB, DB, and SRB. Three of the studies focused specifically on the ability to reduce  $ClO_4^-$  to  $\mu g/L$  concentrations, while three others provided foundation information needed to interpret the studies about  $ClO_4^-$  reduction.

In parallel to the bench-scale experiments, the ASU team developed and applied a series of mechanistic mathematical models. The primary objective of the mathematical modeling was to integrate and quantify the many ecological, kinetic, and transport mechanisms occurring when  $H_2$  is delivered to a biofilm of bacteria exposed to multiple electron acceptors. Two models addressed different combinations of electron acceptors, but with a common framework. One model described  $NO_3^-$  and  $ClO_4^-$  reductions occurring in a  $H_2$ -based biofilm. The second model represented  $NO_3^-$  and  $SO_4^{2-}$  reductions.

Bench-scale experiments were able to achieve  $< 6 \mu g ClO_4^-/L$  under proper conditions, and modeling results provided mechanistic understanding about how the fluxes of  $NO_3^-$ ,  $O_2$ , and  $SO_4^{2-}$  need to be managed to allow for complete  $ClO_4^-$  reduction. Taken together, the bench-scale and modeling results, which are consistent with pilot results, lead to the follow guidance on managing electron-acceptor surface loadings.

First, a moderate flux of  $NO_3^- + O_2$  is a key to helping promote PRB and  $ClO_4^-$  reduction by giving the PRB three electron acceptors ( $ClO_4^-$ ,  $NO_3^-$ , and  $O_2$ ) without causing too much competition for  $H_2$  and space. A  $NO_3^- + O_2$  flux of  $\leq \sim 0.21 g H_2/m^2\text{-day}$  promotes  $ClO_4^-$  reduction, while a flux  $\geq \sim 0.36 g H_2/m^2\text{-day}$  begins to cause serious inhibition to  $ClO_4^-$  reduction. For comparison, the pilot MBfRs gave the highest  $ClO_4^-$  flux in the lag MBfR when the  $NO_3^- + O_2$  flux was  $\sim 0.17 g H_2/m^2\text{-day}$ .

Second, the  $NO_3^- + O_2$  flux should be held in a moderate range to suppress  $SO_4^{2-}$  reduction. A target flux for  $NO_3^- + O_2$  is  $\sim 0.3 g H_2/m^2\text{-day}$ . For comparison,  $NO_3^- + O_2$  fluxes  $> 0.3 g H_2/m^2\text{-day}$  stopped  $SO_4^{2-}$  reduction in the pilot lead MBfR, but lower  $NO_3^- + O_2$  fluxes allowed  $SO_4^{2-}$  reduction in the lag MBfR.

### **Interpreting the pilot results in light of modeling and bench-scale results**

Factors specific to the operation of the pilot MBfRs affected performance for  $ClO_4^-$  reduction. The ASU team performed special modeling runs to gain insight into the observed performance and to define possibilities for achieving better performance in the future. The team also made direct comparisons with the bench-scale experiments.

Modeling the pilot conditions suggested that external mass-transport resistance may have been greater in the pilot MBfRs than in the bench-scale MBfRs. This interpretation was supported by the observation that a large amount of biomass accumulated between the spacers in the side reactors. The special modeling runs also point to the possibility that the pilot biofilms selected for different and less-efficient PRB.

A difference between the pilot and bench-scale MBfRs is that the influent of the lag MBfRs was oxygenated for the bench-scale experiments, but not for the pilot. Oxygenation

made it possible to control the  $\text{NO}_3^- + \text{O}_2$  surface loading to the lag MBfR, keeping it in the optimal range even though most of the  $\text{NO}_3^-$  was removed in the lead MBfR. Inter-stage oxygenation is a good option to build into MBfR systems designed for reducing  $\text{ClO}_4^-$  and  $\text{NO}_3^-$ .

Another difference is that the lead and lag MBfRs were switched regularly in the field pilot, while they were maintained as lead or lag for the bench-scale studies and special modeling analysis. The switching definitely caused the biofilm communities to be similar between lead and lag MBfRs, although they were distinct in the bench-scale MBfRs. Switching also may have played a role in accentuating accumulation of SRB by making the biofilm thicker in the lag MBfR.

In summary, the modeling and bench-scale results show no intrinsic roadblock for achieving a very low  $\text{ClO}_4^-$  concentration when the influent water contains much higher concentrations of  $\text{NO}_3^-$ ,  $\text{O}_2$ , and  $\text{SO}_4^{2-}$ . One clear key is managing the  $\text{NO}_3^- + \text{O}_2$  loading so that it promotes PRB while simultaneously suppressing  $\text{SO}_4^{2-}$  reduction. The two-stage strategy is well tuned for this goal, and particular attention has to be paid to the  $\text{NO}_3^- + \text{O}_2$  loading to the lag MBfR.

## Introduction and Organization

This report summarizes the findings of research carried out at Arizona State University (ASU) in parallel with a field pilot study led by CDM Smith in cooperation with APTwater. All of the results of the pilot study are reported in a separate final report (Evans et al., 2013). The objective of the overall project was biological treatment of a groundwater contaminated with two oxidized contaminants: nitrate ( $\text{NO}_3^-$ ) and perchlorate ( $\text{ClO}_4^-$ ). Dissolved oxygen ( $\text{O}_2$ ) and sulfate ( $\text{SO}_4^{2-}$ ) are oxidized compounds also present. Table 1 synthesizes the concentrations of these compounds in the groundwater from West Valley Water District Well 22 in Rialto, CA. The treatment goal was to use the  $\text{H}_2$ -based Membrane Biofilm Reactor (MBfR) to achieve microbial reduction of  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  into innocuous substances ( $\text{N}_2$ ,  $\text{H}_2\text{O}$ , and  $\text{Cl}^-$ ) (Rittmann 2007), while not promoting  $\text{SO}_4^{2-}$  reduction to sulfide.

The MBfR promotes the growth of autotrophic bacteria capable of using hydrogen gas ( $\text{H}_2$ ) as their electron donor by its diffusion through bubbleless gas-transfer membranes. A microbial community develops as a biofilm attached to the membrane's outer surface; the biofilm normally is comprised of a diverse spectrum of autotrophic and heterotrophic microorganisms embedded in a matrix composed of extracellular polymeric substances (EPS).

**Table 1.** Concentrations of oxidized compounds in the groundwater treated at Rialto, CA

| Electron Acceptor             | Concentration           |
|-------------------------------|-------------------------|
| Nitrate, $\text{NO}_3^-$      | 8-9 mg N/L              |
| Perchlorate, $\text{ClO}_4^-$ | 160-200 $\mu\text{g/L}$ |
| Oxygen, $\text{O}_2$          | ~9 mg/L                 |
| TCE, $\text{C}_2\text{HCl}_3$ | 50-60 $\mu\text{g/L}$   |
| Sulfate, $\text{SO}_4^{2-}$   | 20-22 mg/L              |

Figure 1a shows one of the “side-reactors” that were established as part of the pilot MBfRs to study the biofilm and its microbial community structure. Figure 1b shows biofilm development on the fiber sheet of side reactors. The side-reactors were removed and shipped to ASU at the end of each phase (i.e., start-up, optimization, and challenge) of testing at Rialto, CA (Evans et al., 2013). While CDM Smith and APTwater focused on the operational conditions for the pilot MBfR vessels, ASU was in charge of elaborating in-depth analyses of the side-reactors established at Rialto, CA. During operation of the pilot MBfR vessels, some pilot-reactor modules were taken down due to leaking problems. These broken reactor modules also were sent to ASU to perform the same analysis done to the side-reactors.





**Figure 1a** Side reactor shipped to ASU. **1b** Biomass development on the surface of fibers in the side reactor.

The ASU report summarizes the findings of the molecular, microscopy, and chemical analysis that the ASU team carried out with biofilm samples from the side-reactors, as well as from membranes removed from broken reactor modules in the MBfR vessels. The report also summarizes our results from bench-scale MBfRs operated at ASU and mathematical modeling to help us understand the microbial community function and structure during nitrate, perchlorate, and sulfate reductions. The over-arching goal of the ASU research was to provide a fundamental, mechanistic foundation for understanding the results obtained in the pilot study and for extrapolating the pilot finding to improve performance for future application.

We divide this report into ten chapters, of which the first five address our analyses for the Rialto pilot MBfRs:

1. Chemical fluxes from Rialto pilot data
2. Community function and structure
3. Solids and EPS in the biofilm
4. Microscopic analysis on distribution of live and dead portions within the biofilm
5. Synthesis for the side-reactors and reactor modules in the MBfR vessels.
6. Exploring mechanisms and performance with bench-scale experiments
7. Exploring mechanisms and performance with mechanistic modeling
8. Special modeling of the pilot MBfRs
9. Global synthesis
10. References

Materials, methods, results, and interpretations are provided in each of Chapters 1 through 8, and Chapter 9 synthesizes all of the results in a way that interprets them in the context of the pilot experiments.

## Chapter 1. Chemical Fluxes from Rialto Data

### 1.1 Operational conditions at the Time of Biomass Sampling.

Experimental results from the pilot reactors (e.g., influent and effluent concentrations) used for the ASU analyses were presented in detail in the ESTCP Demonstration Final Report (Evans et al., 2013). We selected six time points to establish the  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{O}_2$  concentrations and removal fluxes; they correspond to the sampling dates for reactor modules and side-reactors sent to ASU from Rialto. Table 2 summarizes the shipment numbers, phases, and other operating conditions. Cells shaded in blue correspond to the shipments from module reactors, while cells shaded in red correspond to the shipments of side-reactors. Flow rates were provided to ASU by APTwater, and we assumed that they were constant leading up to the sampling times. For shipment 5, we received two sets of side-reactors, which are named 5 and 5\*.

**Table 2.** Synthesis of the field-MBfR operational conditions at the time of the module shipments

| Shipment | Phase        | Shipment date | H <sub>2</sub> pressure (psig) |      | Flow rate (GPM) |     |
|----------|--------------|---------------|--------------------------------|------|-----------------|-----|
|          |              |               | Lead                           | Lag  | Lead            | Lag |
| 1        | Start up     | 6/20/2011     | 17.1                           | 11.4 | 12              | 12  |
| 2        | Start up     | 8/15/2011     | 26.6                           | 19.0 | 18              | 18  |
| 3        | Optimization | 10/3/2011     | 14.3                           | 12.4 | 10              | 10  |
| 4        | Optimization | 11/28/2011    | 17.1                           | 14.3 | 8               | 8   |
| 5<br>5*  | Challenge    | 1/9/2012      | 15.6                           | 8.6  | 6               | 6   |
| 6        | Challenge    | 1/9/2012      | 15.6                           | 8.6  | 6               | 6   |

Red corresponds to side reactors, and blue corresponds to field modules.

### 1.2 Fluxes

We use fluxes of  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{O}_2$  as performance metrics. Flux is defined as the mass of electron acceptor reduced per unit membrane surface area per unit time. For interpreting the results with the side-reactors, all chemical concentrations were from the two MBfR vessels (lead/lag configuration). We made two important assumptions when linking the field results to the results with the side-reactors:

- The operational conditions ( $\text{H}_2$  pressure and temperature) and chemical concentrations were the same for the side-reactors and reactor modules in the MBfR vessels.
- Biofilms developed in side-reactors were representative for biofilms in the reactor modules in the MBfR vessels.

The calculations of  $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{O}_2$  fluxes were based on these conditions:

- All fluxes were calculated individually for lead and lag MBfRs as  $J = Q(S_{\text{in}} - S_{\text{eff}})/A$ , in which  $J$  = the substrate flux (g substrate/ $\text{m}^2$ -d),  $Q$  = the influent flow rate to the MBfR pilot module ( $\text{m}^3/\text{d}$ ),  $S_{\text{in}}$  = influent concentration ( $\text{g}/\text{m}^3$ ),  $S_{\text{eff}}$  = effluent concentration ( $\text{g}/\text{m}^3$ ), and  $A$  = the biofilm surface area ( $\text{m}^2$ ).

- b. For the surface area of the field MBfRs, we considered 4 modules per MBfR vessel. Total fiber surface area of field MBfR was then  $576 \text{ m}^2$  (4 field modules =  $144 \text{ m}^2 \times 4$ ).
- c. The flux for each substrate was converted to a  $\text{H}_2$  flux ( $\text{g H}_2/\text{m}^2\text{-day}$ ) using the stoichiometry in Zhao et al. (2011) and Ontiveros-Valencia et al. (2012).
- d. The effluent from a lead MBfR was the influent to its lag MBfR.
- e. Because sulfide concentrations always were zero in the lead reactor and were detected only from the lag MBfR, we assumed  $\text{SO}_4^{2-}$  was reduced only in the lag MBfR.
- f. The lead and lag MBfRs switched positions every three days.

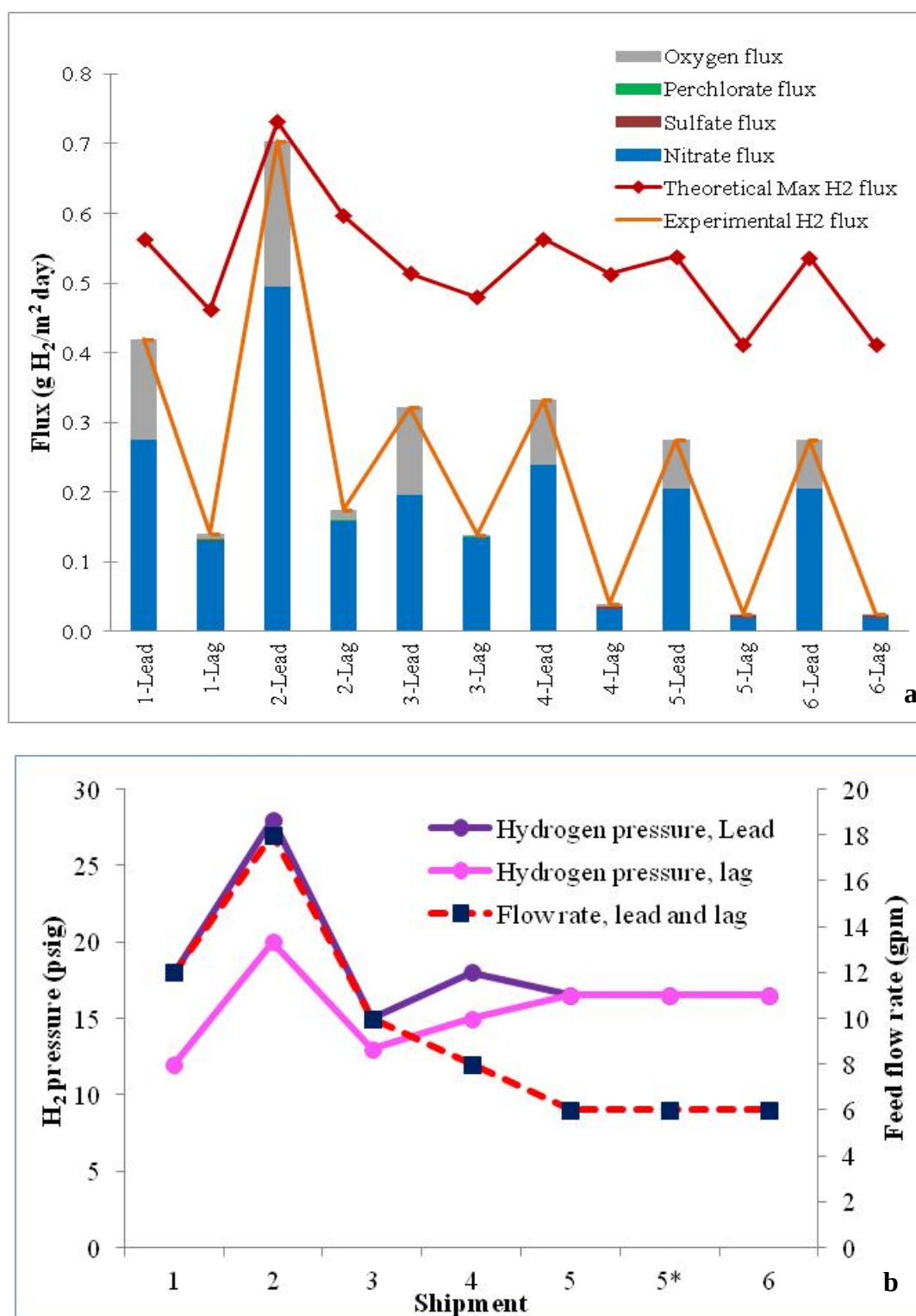
We present in Figure 2a a general overview of the consumption of  $\text{H}_2$  by each electron acceptor and a comparison of the experimental  $\text{H}_2$  fluxes (calculated by the method of Ontiveros-Valencia et al. (2012)) with the maximum-possible  $\text{H}_2$  flux as calculated by Tang et al. (2012) for the polypropylene fibers and the operating  $\text{H}_2$  pressures. The comparison between the experimental and maximum-possible  $\text{H}_2$  flux points out that  $\text{H}_2$  delivery was sufficient at the time of shipments 1, 3, 4, 5, and 6 (a difference of at least  $0.19 \text{ g H}_2/\text{m}^2\text{-d}$ ). However, the difference between experimental and maximum  $\text{H}_2$  fluxes was small for shipment 2 ( $0.03 \text{ g H}_2/\text{m}^2\text{-d}$ ), which suggests that  $\text{H}_2$  limitation was possible at the time of shipment 2, particularly for the lead MBfR. To complement the picture, Figure 2b shows the  $\text{H}_2$  pressures and influent flow rates applied over the course of time (corresponding to the different shipments to ASU) to the pilot MBfRs. Particularly important is the reduction of flow rate right after shipment 2 (August 15, 2011), which eliminated any limitation from  $\text{H}_2$  delivery, as shown in Figure 2a.

The major sink for electrons was denitrification, followed by  $\text{O}_2$ ,  $\text{SO}_4^{2-}$ , and  $\text{ClO}_4^-$  reductions. The fluxes for  $\text{SO}_4^{2-}$  and  $\text{ClO}_4^-$  were significantly smaller than the fluxes for  $\text{NO}_3^-$  and  $\text{O}_2$ , and they cannot be distinguished in Figure 1. Therefore, we plot the  $\text{ClO}_4^-$  and  $\text{SO}_4^{2-}$  fluxes separately in Figure 3a.

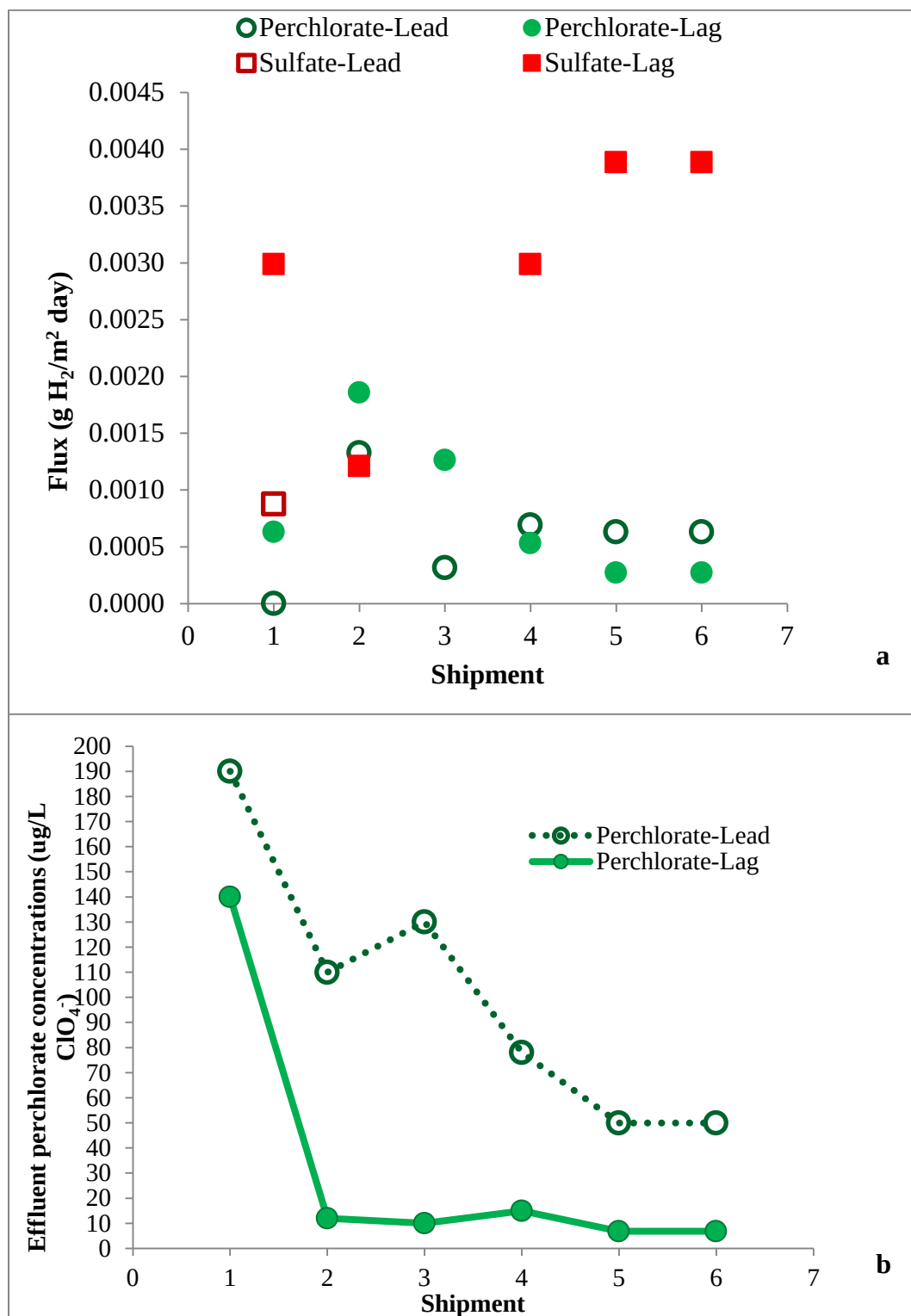
The lead MBfRs were responsible for  $\sim 99\%$  of the  $\text{O}_2$  respiration, 70-90% of the denitrification, and a small loss of  $\text{ClO}_4^-$ . In the lead MBfR, the  $\text{NO}_3^- + \text{O}_2$  flux was greater than  $\sim 0.3 \text{ g H}_2/\text{m}^2\text{-day}$ , and  $\text{SO}_4^{2-}$  reduction was completely suppressed. The excellent removals of  $\text{NO}_3^-$  and  $\text{O}_2$  in the lead MBfR created a scenario in which  $\text{NO}_3^-$  (with an average of  $2.4 \text{ mg N/L}$ ) and  $\text{SO}_4^{2-}$  (average concentration of  $\sim 20 \text{ mg/L}$ ) were the dominant electron acceptors entering the lag MBfR, but with a total acceptor loading much less than for the lead MBfR. This allowed greater  $\text{ClO}_4^-$  reduction in the lag MBfR (Figure 3b), the desired outcome, due to reduced competition for  $\text{H}_2$  and space in the biofilm. For the same reason, it also favored  $\text{SO}_4^{2-}$  reduction, an undesired outcome. Comparing Figures 2 and 3 shows that the  $\text{SO}_4^{2-}$  flux (in the lag MBfR) was inversely proportional to the  $\text{NO}_3^- + \text{O}_2$  flux. Specifically, the lag MBfR had significant  $\text{SO}_4^{2-}$  reduction, particularly for shipments 4-6, when the total electron acceptor loading was  $< 0.1 \text{ g H}_2/\text{m}^2\text{-day}$ .

For the lag MBfR, the  $\text{ClO}_4^-$  and  $\text{SO}_4^{2-}$  fluxes were linked to the  $\text{ClO}_4^-$  and  $\text{SO}_4^{2-}$  loadings, which co-varied with the flow rate (Figure 2a). However, the  $\text{ClO}_4^-$  flux was higher when the  $\text{SO}_4^{2-}$  flux was low. At the time of the second sample (20 days from the end of the start up phase), the  $\text{ClO}_4^-$  flux in the lag MBfR increased from  $0.0006 \text{ g H}_2/\text{m}^2 \text{ day}$  (1<sup>st</sup> shipment) to  $0.0019 \text{ g H}_2/\text{m}^2 \text{ day}$ , while the  $\text{SO}_4^{2-}$  flux was reduced from  $0.003 \text{ g H}_2/\text{m}^2 \text{ day}$  to  $0.001 \text{ g H}_2/\text{m}^2 \text{ day}$ . Shipment 2 was the only point at which the  $\text{ClO}_4^-$  flux exceeded the  $\text{SO}_4^{2-}$  flux, and that

was the time when H<sub>2</sub> delivery was limiting. Around the second shipment, the influent flow rate to the MBfR system was 18 gpm (Figure 2b), which was the highest value throughout the operational period. Therefore, a relatively high surface loading of all electron acceptors, combined with some H<sub>2</sub>-delivery limitation, seems to have caused decreased SO<sub>4</sub><sup>2-</sup> reductions and increased ClO<sub>4</sub><sup>-</sup> removal for the time of the second shipment.



**Figure 2a** Electron acceptor fluxes (as H<sub>2</sub> consumption), along with experimental and maximum total H<sub>2</sub> fluxes for all shipments of lab and field modules. **2b** Corresponding operational conditions: H<sub>2</sub> pressure and flow rate (red dashed line).



**Figure 3a.** Comparison of  $\text{ClO}_4^-$  and  $\text{SO}_4^{2-}$  fluxes for the pilot MBfRs at the times of module shipments. We lacked a  $\text{SO}_4^{2-}$  influent concentration for shipment 3, which explains the absence of  $\text{SO}_4^{2-}$  removal flux for that point. **3b.** Effluent  $\text{ClO}_4^-$  concentrations for lead and lag pilot MBfRs.

## Chapter 2. Community Function and Structure

In this section, we synthesize the results of the quantitative Polymerase Chain Reaction (qPCR) analysis we did for the biofilm samples sent to ASU from the Rialto pilot site. We present results for the shipments of 5 side-reactors and 11 biofilm samples from broken reactor modules in the MBfR vessels, and we correlate them to the chemical fluxes reported in the previous section.

The procedures of DNA extraction and qPCR protocols are explained in detail in Ontiveros-Valencia et al. (2012) and Zhao et al. (2011). For RNA extraction, we directly cut a section of the fiber sheet and processed it (vortexing and centrifuging) while using "RNA protect bacteria reagent" all of the time. After collecting 1 mL of solution in a micro-centrifuge tube, we followed the manufacturer's protocol of the RNeasy Mini Kit (QIAGEN, USA). We then did 2 DNase treatments for 5  $\mu$ L of RNA each, and we ran PCR with 16S rDNA primers to confirm the absence of DNA contamination in the RNA samples. After successful DNase treatment, we performed reverse transcription PCR using an Omniscript RT kit to generate cDNA for downstream qPCR analysis, which followed the methods of Ontiveros-Valencia et al. (2012) and Zhao et al. (2011).

We normalized the qPCR data from gene copy numbers to cells by using the following conversion factors:

- 1 *dsrA* gene per cell of sulfate-reducing bacteria (SRB) (Kondo et al., 2004)
- 1 *nirK* gene per cell of denitrifying bacteria (DB) (Phillipot, 2006)
- 2 *nirS* genes per cell of DB (Coates et al., 2001)
- 1 *pcrA* gene per cell of perchlorate-reducing bacteria (PRB) (Coates et al., 2001)
- 4 16S rDNA genes per microbe cell. We took the average number of 16S rDNA genes found in Proteobacteria phylum, which is the expected dominant phylum in our samples, as reported by Kapellenbach et al. (2001) and Lee et al. (2008).

Figure 4 combines the electron-acceptor removal fluxes with the biomass distributions (cells/cm<sup>2</sup> of biofilm) for the lead MBfR (Figure 4a) and lag MBfR (Figure 4b) side-reactors. The lead MBfR was mostly utilizing the electrons donated by H<sub>2</sub> to reduce NO<sub>3</sub><sup>-</sup> and O<sub>2</sub> (Fig. 2a). Since the lead and lag positions were switched every 3 days, the microbial communities from both MBfRs had similar biomass distributions. Thus, most of the changes were related to the changes in overall operating conditions, not to being in the lead or lag position.

DB comprised the largest fraction in biofilm samples from both MBfRs for shipments 1, 2, and 4. However, SRB became the highest microbial fraction for shipments 5 and 5\*, when SRB had slightly higher cells/cm<sup>2</sup> than for total bacteria. This apparent discrepancy might be explained by the presence of *Archaea* that contain the *dsrA* gene (Muyzer and Stams; 2008). The reduction of flow rate (after shipment 2, Figure 2b) dramatically favored SRB.

DB assayed by the functional gene *nirS* gene were more abundant than DB assayed with the functional gene *nirK* gene (data not shown here). The abundance of *nirS* also was significantly higher in studies by Zhao et al. (2011) and Ontiveros-Valencia et al. (2012) in denitrifying MBfR biofilms. A single DB with both genes has not been reported. Ontiveros-

Valencia et al. (2012) discussed that DB with *nirK* are more sensitive to nutrient limitation, a situation that might favor the abundance of DB with *nirS* in autotrophic biofilms.

SRB (assayed by the functional gene *dsrA*) significantly increased their abundance for shipment 5 (Figure 4a&b). In terms of operational conditions, shipment 5 (challenge phase) showed the lowest flow rate tested (6 gpm) for the field MBfR (Figure 2b) and the largest  $\text{SO}_4^{2-}$  flux for the lag MBfR (Figure 4a&b). A reduction of flow rate for the field reactor increased the hydraulic retention time (HRT), which lowered the electron-acceptor surface loadings, a situation that favored the slow-growing SRB, which became the major biomass fraction in the biofilm for shipments 5 and 5\*.

In both MBfRs, PRB (assayed by the functional gene *pcrA*) showed an increasing trend of their abundance over time, and they increased with the lower flow rate (Figures 4a&b and 2b), most noticeably for shipment 5. Of particular note is that PRB were the third largest fraction of the biofilm community, being out-numbered by SRB and DB. However, PRB cannot be clearly separated from DB, since some DB are capable of respiring  $\text{ClO}_4^-$  either by using a nitrate-reductase or by having the *pcrA* gene along with the *nirS* or *nirK* gene. Also, most PRB can reduce  $\text{NO}_3^-$ .

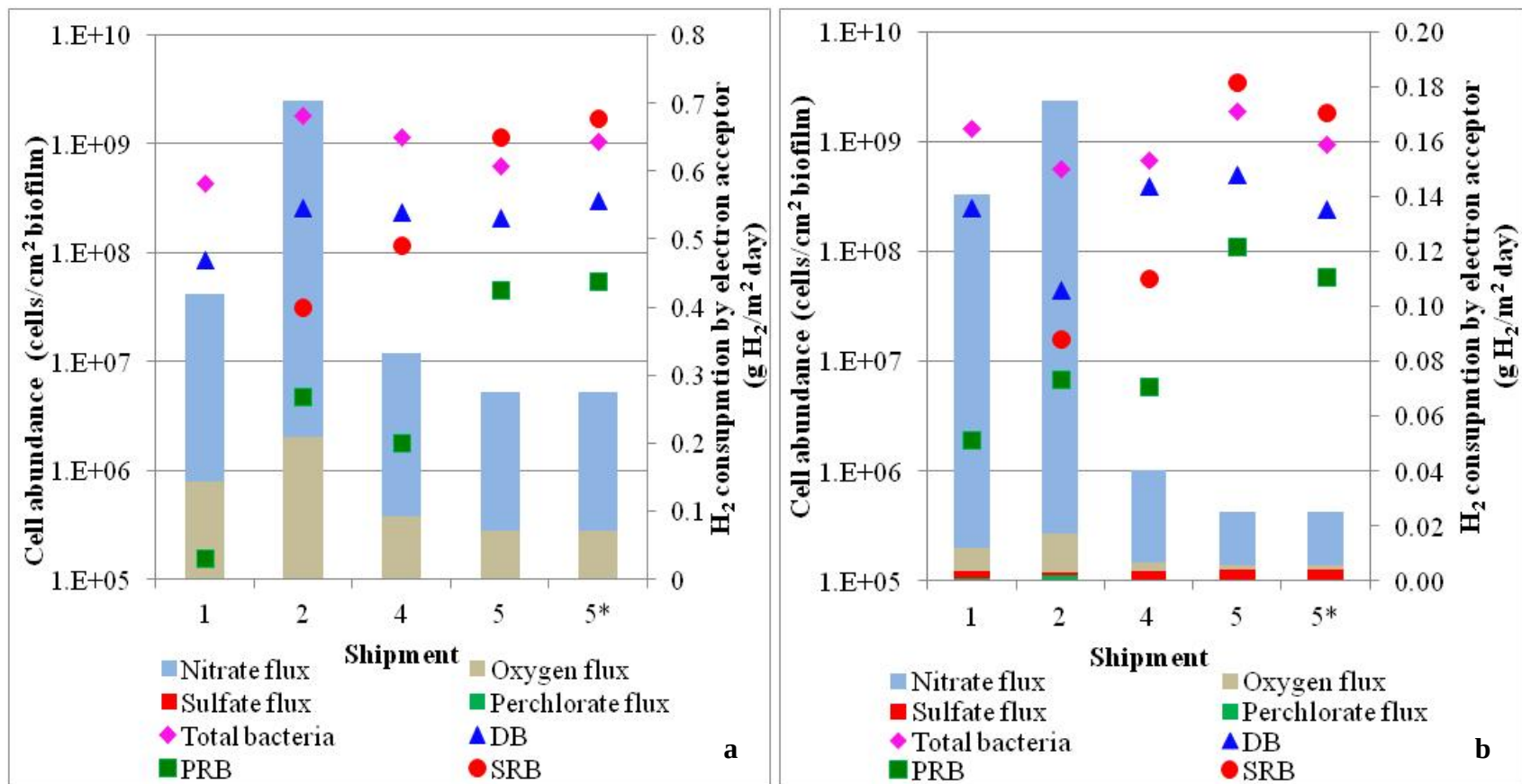
Figure 5 synthesizes the mRNA transcripts for all the shipments corresponding to lead (Figure 5a) and lag (Figure 5b) side-reactors. A comparison between Figures 4 and 5 shows higher numbers of transcripts than the number of cells for general bacteria/cm<sup>2</sup>, which implies a highly active biofilm. The abundance of the transcripts for the lead and lag lab modules verifies that DB had the greatest activity for the first two samples, but SRB were similar to or even greater than DB for samples 4, 5, and 5\*. Also, *pcrA* transcripts were significantly enriched in shipment 5 and 5\*, although the difference between *pcrA* and *nirS/K* transcripts was lower than an order of magnitude. Lastly, *dsrA* transcripts were slightly higher than *pcrA* transcripts, but we should be cautious when interpreting differences lower than an order of magnitude with a logarithmic method such as qPCR.

Figure 6 shows results from DNA samples from the broken reactor modules in the MBfR vessels for shipments 3 (optimization phase) and 6 (challenge phase). The results are normalized to cells/cm<sup>2</sup> of biofilm. Samples 1-9 were sent to ASU as shipment 3, while samples APT 107 and APT 185 were sent to ASU as shipment 6. Table 2 provides the operational conditions for these two shipments. The abundance of general bacteria was not homogenously distributed for all 9 samples for shipment 3. This might indicate an uneven distribution of biofilm across the field MBfR or some loss of biomass when handling the samples. Despite some variability, it is clear that DB and SRB established a strong competition for space within the biofilm community. DB were slightly higher for sample 3, but SRB were slightly higher for sample 6. PRB clearly were the smallest fraction within the microbial communities. These trends are consistent with the results for the side-reactors, and (as will be discussed in depth later) the biomass distributions correspond to the removal fluxes of the electron acceptors  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{ClO}_4^-$ . In particular, the flow rate was lowered between shipments 3 and 6, and this lead to SRB becoming the largest portion within the biofilm community.

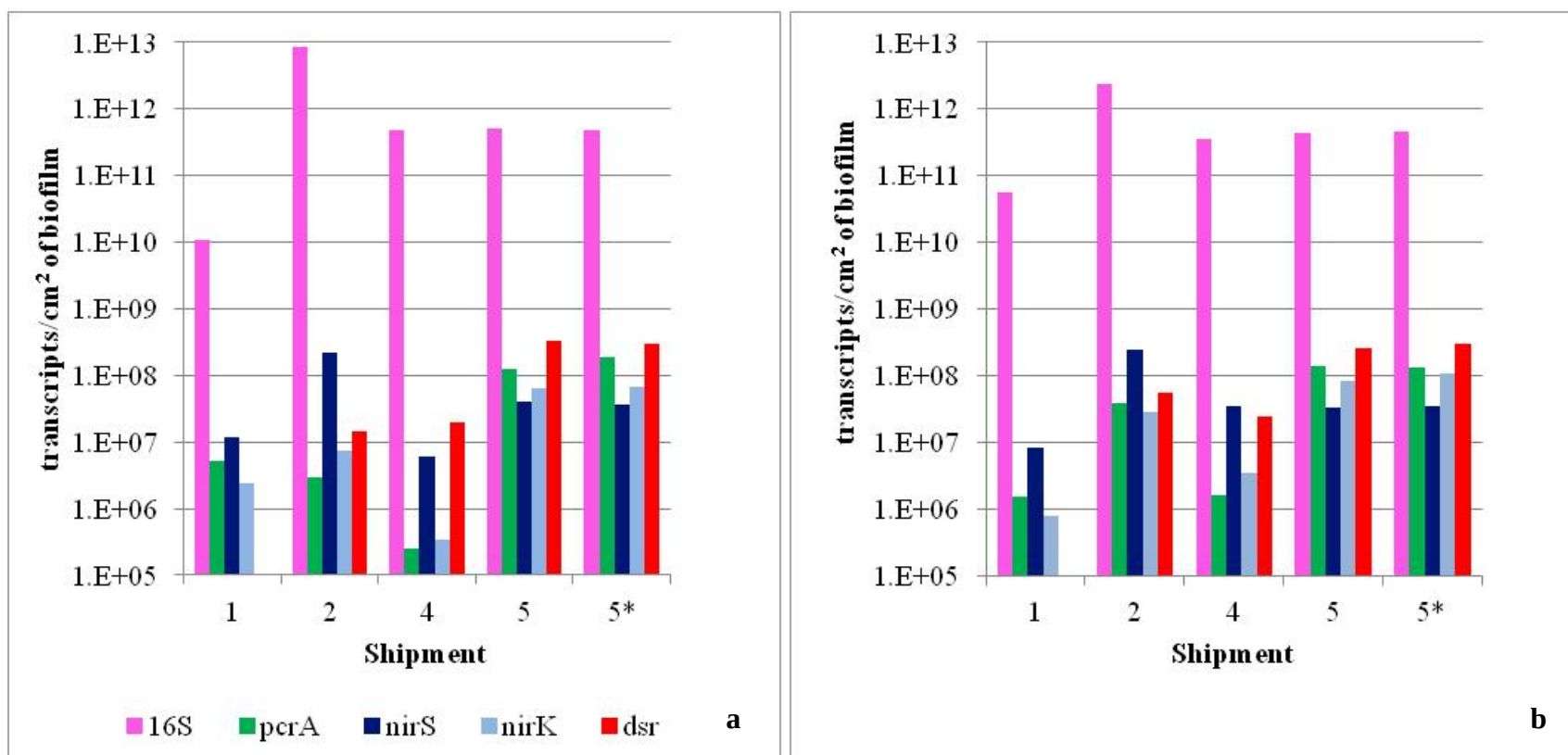


Figure 7 reports the mRNA transcripts for general bacteria and functional genes for the 9 samples of shipment 3 and 2 samples of shipment 6. Worth noting is that the biofilms were patchy in the reactor modules. This patchiness might correspond to the location of the biofilm sample within the reactor module (e.g., inner membrane sheet, top, or bottom of the reactor module). In parallel with the side-reactors, the number of transcripts for 16S rRNA were significantly higher than the number of genes for 16S rDNA, indicating that the biofilm was highly active. The number of transcripts for the functional genes *nirS*, *nirK*, and *dsrA* were smaller than the number of genes, while the number of transcripts for *pcrA* were slightly higher than the number of genes. Perhaps the most significant finding is the large increase of *dsrA* transcripts from samples for shipment 3 to samples for shipment 6. This reinforces that SRB were favored by the reduction of the flow rate and subsequent decrease on total electron acceptor surface loading (Table 2).

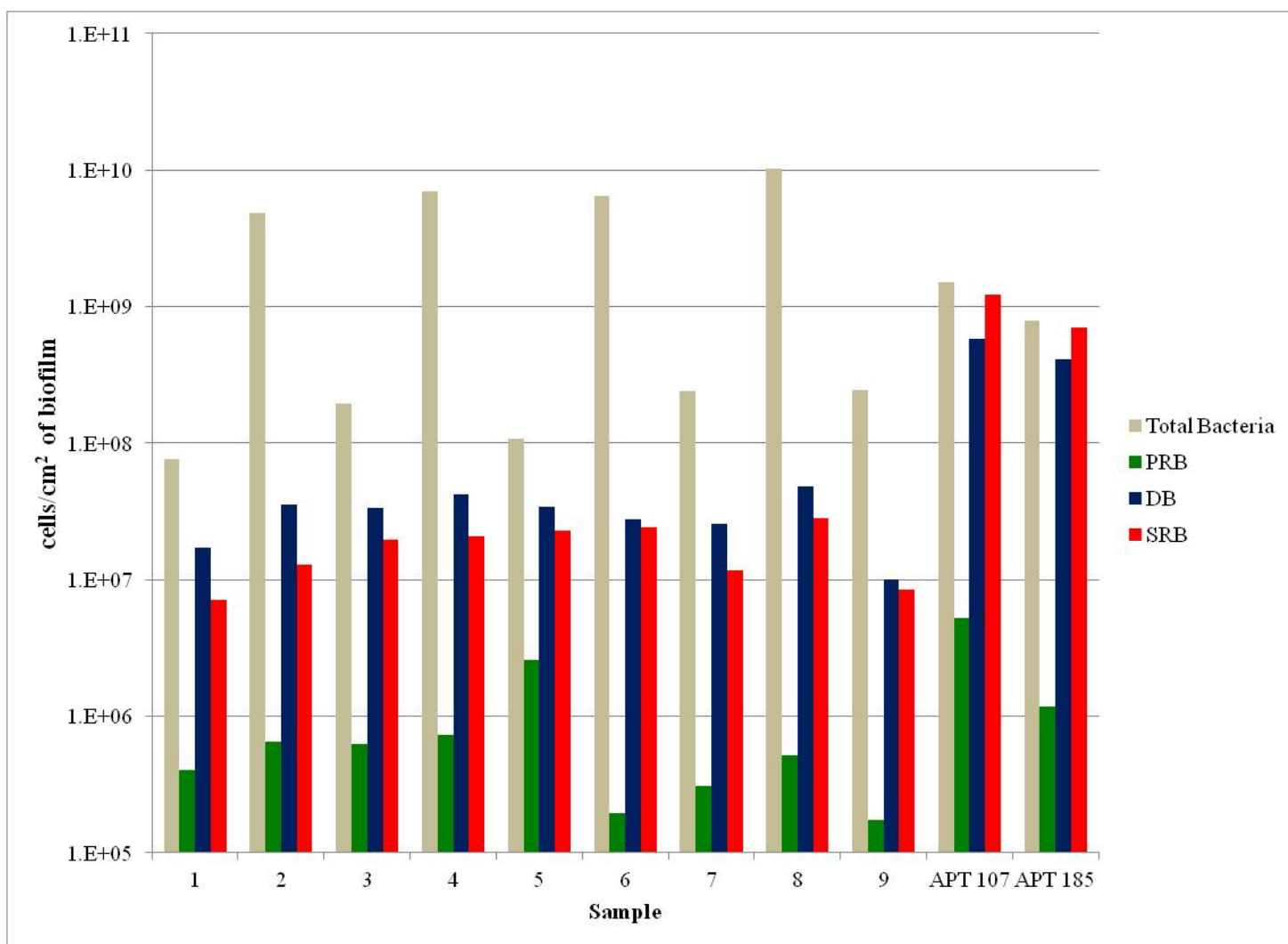
Figure 8 combines the electron-acceptor fluxes with the biomass distributions (in cells/cm<sup>2</sup>) for the field MBfRs. We report average values for each of shipments 3 and 6; this practice is supported by the similar biomass distributions we saw between lead and lag side-reactors. Figure 8 shows trends similar to those in Figures 6 and 7: DB, PRB, and SRB showed an increasing trend over time, but the increase was larger for the broken reactor modules in the MBfR vessels. Likewise, SRB out-numbered DB and PRB for shipment 6, which had a longer HRT and lower total-acceptor and NO<sub>3</sub><sup>-</sup> fluxes for a significant amount of time (Table 2 and Figure 2b), even though the NO<sub>3</sub><sup>-</sup> flux was much higher than the SO<sub>4</sub><sup>2-</sup> flux. Ontiveros-Valencia et al. (2012) also observed high abundance of SRB in denitrifying MBfR biofilms regardless of SO<sub>4</sub><sup>2-</sup> reduction activity. Clearly, the biofilm samples of side-reactors (Figure 4a&b) and reactor modules in the MBfR vessels (Figure 8) had many more SRB than PRB, and this seemed to favor incomplete ClO<sub>4</sub><sup>-</sup> reduction in the lag MBfR. As interpreted by Ontiveros-Valencia et al. (2012), this high fraction of SRB could be active in O<sub>2</sub> respiration, fermentation of organics, and even NO<sub>3</sub><sup>-</sup> respiration. Therefore, the metabolic diversity of SRB makes it not surprising that SRB had high abundance despite small or no SO<sub>4</sub><sup>2-</sup> reduction. Regardless of the specific activity of SRB within the biofilm, they surely competed for electron donor and space situations with PRB.



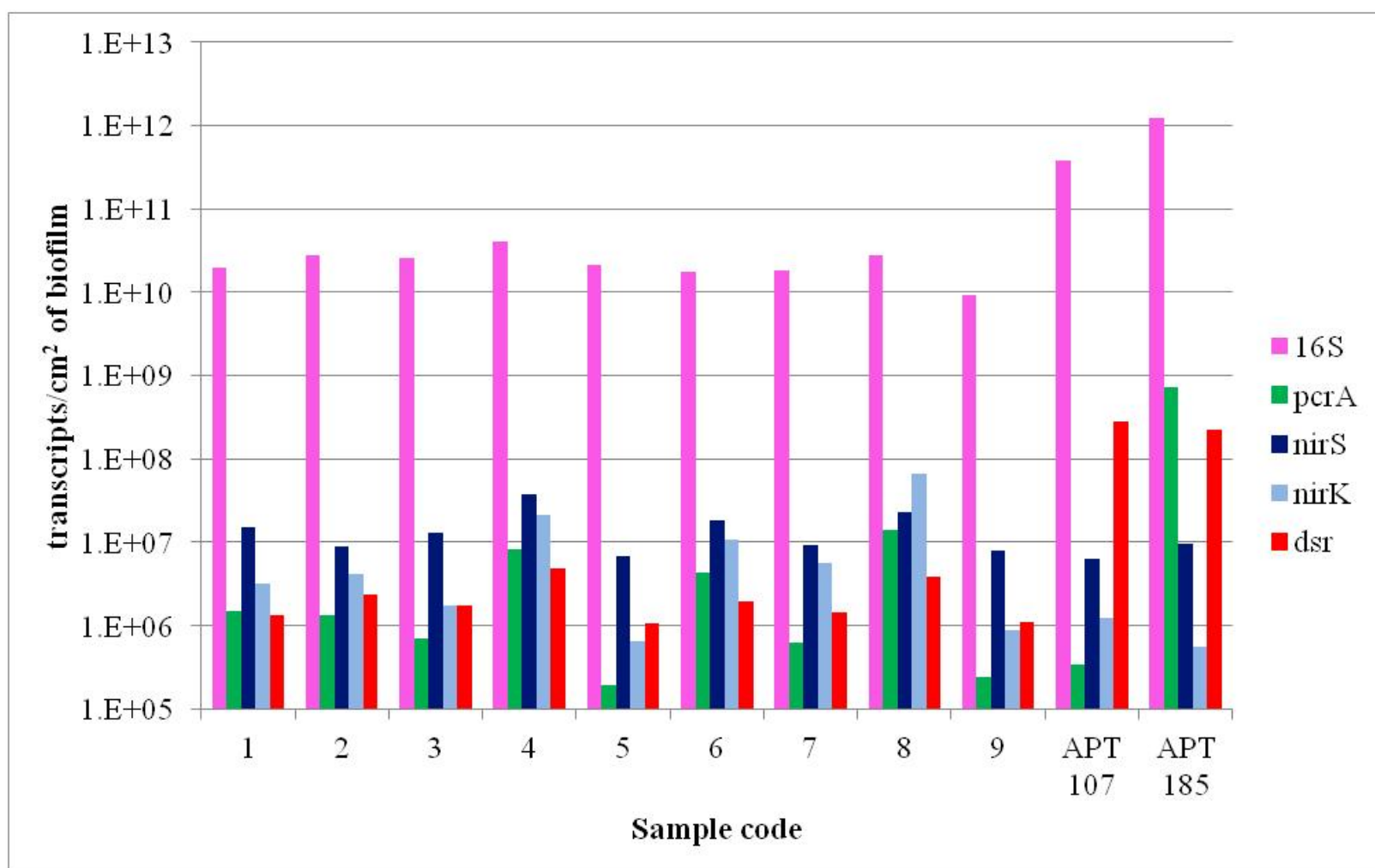
**Figure 4.** Microbial groups within the biofilm samples and corresponding acceptor fluxes (as H<sub>2</sub> consumption by each electron acceptor) for lead (4a) and lag (4b) side-reactors.



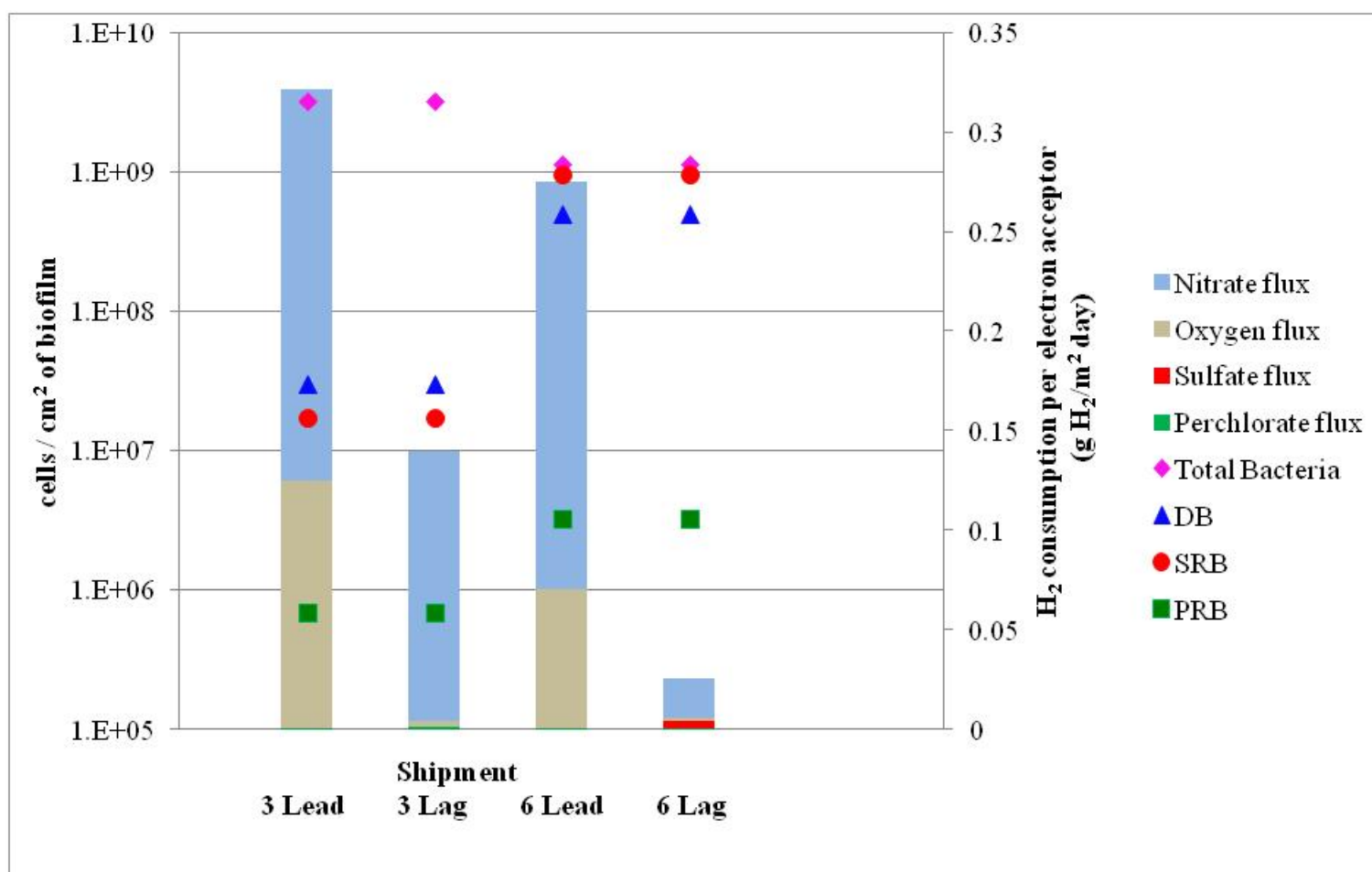
**Figure 5.** Abundances of total bacterial and functional transcripts (RNA samples) for lead (5a) and lag (5b) side-reactors.



**Figure 6.** Biomass distributions for broken reactor modules in the MBfR vessels. Samples 1-9 correspond to shipment #3, while samples 107 & 185 correspond to shipment #6.



**Figure 7.** Abundances of total bacteria and functional transcripts for the broken reactor modules in the MBfR vessels. Samples 1-9 correspond to shipment #3, while samples 107 & 185 correspond to shipment #6.



**Figure 8.** Microbial groups within the biofilm and acceptor fluxes (as H<sub>2</sub>) for lead and lag field reactor modules in the MBfR vessels. The perchlorate fluxes are too small to see in three cases.

## Chapter 3. Solids and EPS in the Biofilm

### 3.1 Solids analysis

Except for the first side-reactor, we collected biomass only from the fibers. The reason was that the significant agglomeration of biomass covering spacers in the side reactors had characteristics clearly distinct from the biomass on the fibers, where the H<sub>2</sub>-based reductions took place. Some of these distinctions were color, viscosity, and texture. Also, when dismantling the side-reactors, we observed that the biomass associated with the spacers was separated from the fiber surface, further supporting that the biomass on the spacers was not receiving H<sub>2</sub> in the same manner as did the biofilm solids. Hence, we assumed that the biomass on the spacers was not representative of the biofilm carrying out H<sub>2</sub>-based reductions, and we sampled only biofilm material for samples 2 – 6.

We measured TCOD, SCOD, TSS, VSS, TS, VS, and extracellular polymeric substances (EPS) for solids samples. We normalized the solids mass to the area of the corresponding fiber sheet. TCOD and SCOD were analyzed using Hach<sup>®</sup> COD vials (product #2415915), and TSS, VSS, TS, and VS were measured using *Standard Methods* 2540 A, 2540 B, 2540 D, and 2540 E, respectively (AWWA; APWA; WEF, 2011). Methods for EPS are summarized in the section that reports the EPS results. All biomass masses were normalized to the area of the corresponding fiber sheet. We applied a typical dense-biofilm VSS concentration of 50,000 g/m<sup>3</sup> (Rittmann & McCarty, 2001) to estimate the thickness of the biofilm.

#### 3.1.1 Solids analysis results

The average solids-analysis results for lead and lag MBfRs are summarized in Table 3. We differentiate the origin of biofilm samples as either from side-reactor or reactor modules in the MBfR vessels. For side-reactors, the solids from sample 1 were much higher than the others, a sign that they included suspended solids retained by the spacers. The solids of shipment 2 were by far the lowest among all samples. Although this correlates to H<sub>2</sub> limitation in the MBfRs (Figure 1), another explanation is that biomass continued to accumulate over time for the later shipments, although shipment 5 had relatively lower biomass. For the reactor modules in the MBfR vessels, the solids of shipment 6 were generally higher than those for shipment 3. A possible explanation is that the biofilm for shipment 6 was more metabolically active in respiring all electron acceptors, a situation enhanced by a significant increase in the HRT and an ample supply of H<sub>2</sub> as electron donor.

All samples had high %VSS/TSS and %VS/TS ratios, around 90% in all cases. This indicates low mineral precipitation due to successful pH control (Evans et al., 2013). The estimated biofilm thicknesses were around 200 µm when using a biomass concentration of

50,000 g VS/m<sup>3</sup>. The  $L_f$  values were not consistently different for the lead and lag MBfRs, the expected outcome of regular switching of their positions.

**Table 3.** Results of solids analyses for biofilm samples from side-reactors (red-shaded cells) and reactor modules in the MBfR vessels (blue-shaded cells). The solids in sample 1 were exaggerated due to including suspended solids trapped by spacers.

| Parameter<br>Shipment         | 1st  |      | 2nd  |     | 3rd          | 4th  |      | 5th  |      | 6th          |
|-------------------------------|------|------|------|-----|--------------|------|------|------|------|--------------|
|                               | Lead | Lag  | Lead | Lag | Lead/<br>Lag | Lead | Lag  | Lead | Lag  | Lead/<br>Lag |
| TCOD [g TCOD/m <sup>2</sup> ] | 20   | 77   | 7.1  | 4.0 | 16.9         | 16.7 | 12.4 | 14.5 | 19.5 | 28.3         |
| SCOD [g SCOD/m <sup>2</sup> ] | 1.0  | 1.0  | 0.7  | 0.2 | 0.4          | 1.1  | 1.1  | 0.8  | 0.9  | 0.6          |
| TSS [g TSS/m <sup>2</sup> ]   | 32   | 58   | 5.3  | 3.1 | 13           | 14   | 7.9  | 10   | 13   | 20           |
| VSS [g VSS/m <sup>2</sup> ]   | 28   | 51   | 5.0  | 2.9 | 12.5         | 13.4 | 7.7  | 9.8  | 13.0 | 19           |
| VSS/TSS [%]                   | 87   | 87   | 94   | 94  | 95           | 94   | 97.2 | 95   | 97   | 96           |
| TS [g TS/m <sup>2</sup> ]     | 13.0 | 41.0 | 6.8  | 6.0 | 14.5         | 16.8 | 13.0 | 9.9  | 12.5 | 21.1         |
| VS [g VS/m <sup>2</sup> ]     | 12.0 | 33.0 | 5.4  | 3.3 | 13.6         | 14.8 | 10.0 | 8.7  | 11.5 | 19.2         |
| VS/TS [%]                     | 92   | 81   | 79   | 55  | 94           | 88   | 78   | 87   | 92   | 91           |
| $L_f$ , $\mu\text{m}$         | 560  | 1060 | 100  | 58  | 250          | 270  | 150  | 200  | 260  | 390          |

$L_f$  was computed from the g VSS/m<sup>2</sup> and with an assumed biofilm VSS concentration of 50,000 gVSS/m<sup>3</sup>.

### 3.2 EPS analysis

Extracellular polymeric substances (EPS) are solid-phase products of bacterial metabolism that the cells use for aggregation, including attachment as biofilms. EPS offer other benefits to the microorganisms, such as formation of protective layer against harsh environmental conditions, protection against predation, and energy and carbon storage (Liu & Fang, 2002).

EPS are composed of various organic polymers that can be categorized roughly as carbohydrates, proteins, humics, and nucleic acids (Liu & Fang, 2002; Frølund et al., 1996; Sutherland and Kennedy, 1996; Cescutti et al., 1999; Veiga et al., 1997). Information on the four major components in EPS in biofilms is limited in general (Liu & Fang, 2002) and absent for MBfR biofilms.

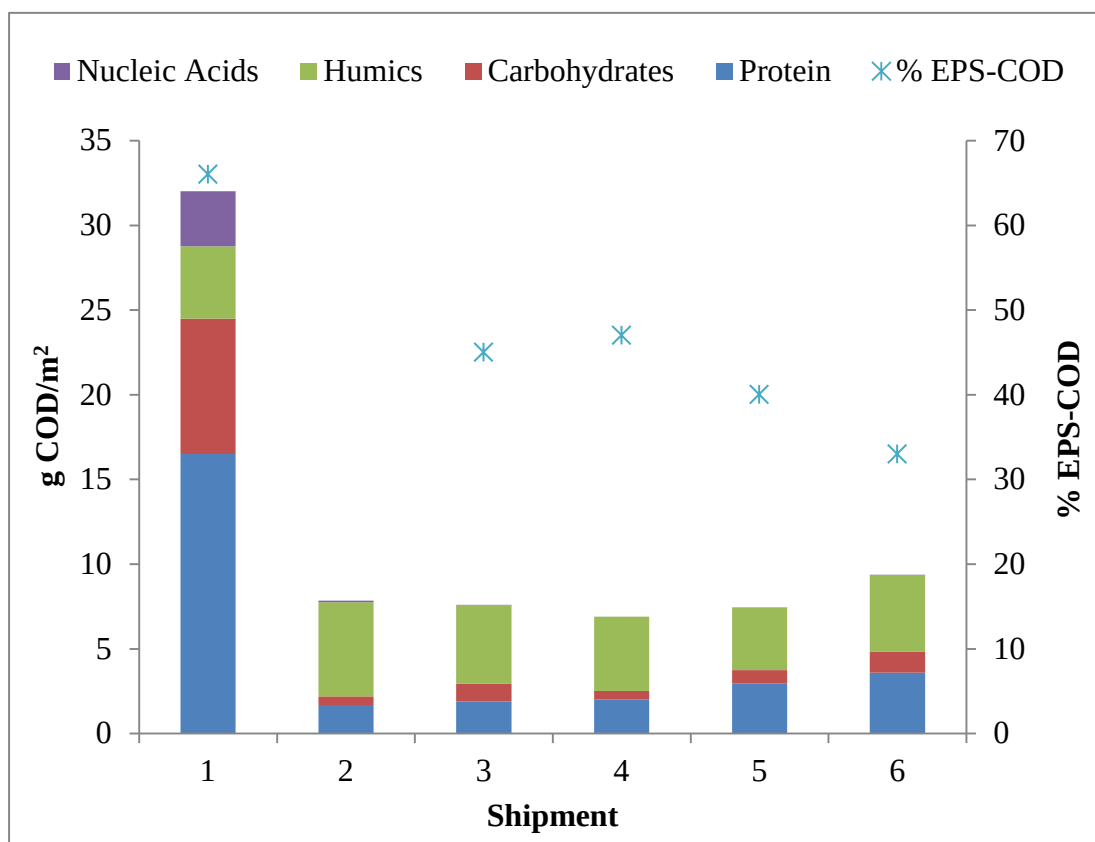
We analyzed EPS components for every biofilm sample. We first extracted EPS using the NaOH + formaldehyde method, since it gives highest yield reported in literature (Liu & Fang, 2002). Then, we used the phenol-sulfuric acid method to measure carbohydrates (Dubois, et al., 1951), the Folin method to measure humics (Box, 1983), and the BCA method for protein



analysis (Thermo Fisher Scientific Inc., 2011). We converted all EPS component values into g COD/m<sup>2</sup> based on the conversion factors for protein (1 g protein = 1.5 g COD), carbohydrates (1 g carbohydrate = 1.1 g COD), humic substances (1 g humics = 1.34 g COD), and nucleic acids (1 g nucleic acids = 1.1 g COD) (Rittmann & McCarty, 2001).

Figure 9 synthesizes the average amount of EPS for the side-reactors and reactor modules in the MBfR vessels. Most EPS measurements were in the range of 7 to 9 g COD/m<sup>2</sup>; however, EPS results for shipment 1 were significantly higher (~32 g COD/m<sup>2</sup>). The high value for sample 1 corresponds to the inclusion of biomass from the spacers in the extraction of EPS for shipment 1. We standardized biofilm sampling for shipments 2 through 6 to capture only biofilm from fibers. Except for shipment 2, the EPS-COD constituted 36 – 52% of the TCOD. Thus, a substantial portion of the biofilm was EPS, not active cells.

For the side-reactors and reactor modules in the MBfR vessels for shipments 2 - 6, humic substances accounted for the highest portion of the EPS, followed by proteins and carbohydrates. The distribution was quite different for shipment 1, another sign that it contained solids other than bacteria. We do not have a conclusive understanding about the role of humic substances in the biofilm development and its EPS. Perhaps the most logical reason for the relatively large build up of humics is that they are hard to biodegrade (Blondeau, 1988; Masahiro et al., 2008). Clearly, more research would be needed to know why humics comprised the largest portion in the MBfR biofilms. Lastly, the low values of nucleic acids suggest that EPS extraction occurred with minimum cell lysis, an indication that the analysis was conducted correctly.



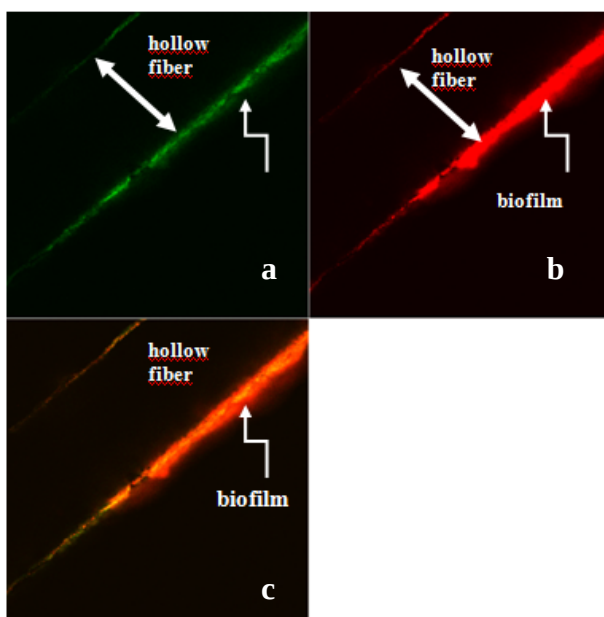
**Figure 9.** Breakdown of EPS components for the six shipments, as well as the percentage of the biofilm's TCOD that is EPS-COD. Samples 1, 2, 4, and 5 correspond to lab modules, while samples 3 and 6 correspond to field MBfRs.

#### Chapter 4. Microscopic Analysis on Distribution of Live and Dead Portions within the Biofilm

For shipments 2 - 6, a subset of samples from the fiber sheet was taken for confocal laser scanning microscopy (CLSM) analysis, a technique used to visualize biofilms at high resolution with depth selectivity. With CLSM, we can observe specimens one-level depth at a time, while the computer software provided with the CLSM equipment can aggregate these individual observations at a particular depth to construct a final three-dimensional image. For visualization, we used the Live/Dead staining kit from Invitrogen (USA). Cells with undamaged membranes are stained green with SYTO9 dye at a wavelength of 488 nm, while cells with damaged membranes are stained red with propidium iodide at a wavelength of 543 nm. Green zones are considered live portions of the biofilm, while the red zones are considered dead. The kit we used to perform the CLSM analysis just captures cells membranes and is unable to quantify EPS within the biofilm.

Here, we report key findings across the different shipments, and we only include figures that highlight the key conclusions.

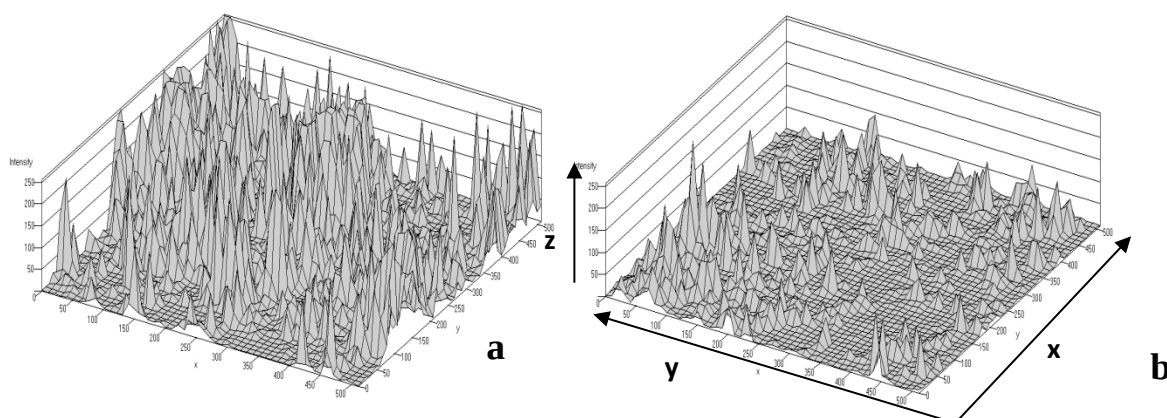
For shipments 2 and 3, we took individual fibers from the fiber sheets. Figure 10 shows representative images from shipment 3. The fluorescent green and red zones from one fiber sample from the lab module are shown in three ways: live (10a), dead (10b), and combined (10c). Due to possible artifacts from removing individual fibers from the sheet before CLSM, the thicknesses are not necessarily representative of the biofilm on the sheet or the absolute amounts of live or dead cells. In contrast, the chemical analysis performed with intact fiber sheets should give more representative thickness values for the entire sheet. Perhaps more important is that the biofilm used for CLSM appeared to be uneven. A possible explanation for unevenness is biofilm loss during shipping and processing the fiber sample. Thus, to reduce the amount of biofilm detachment, we decided to analyze a sample of  $\sim 0.5$  cm x 0.5 cm of fiber sheet for further shipments.



**Figure 10.** Live and dead portions in the biofilm from side-reactor in the lag position at time of shipment 3. Images were taken with 40X resolution. The fiber is in the middle, with biofilm on either side. The biofilm thickness shown here is  $\sim 20$   $\mu$ m. The panels are: live (10a), dead (10b), and combined (10c).

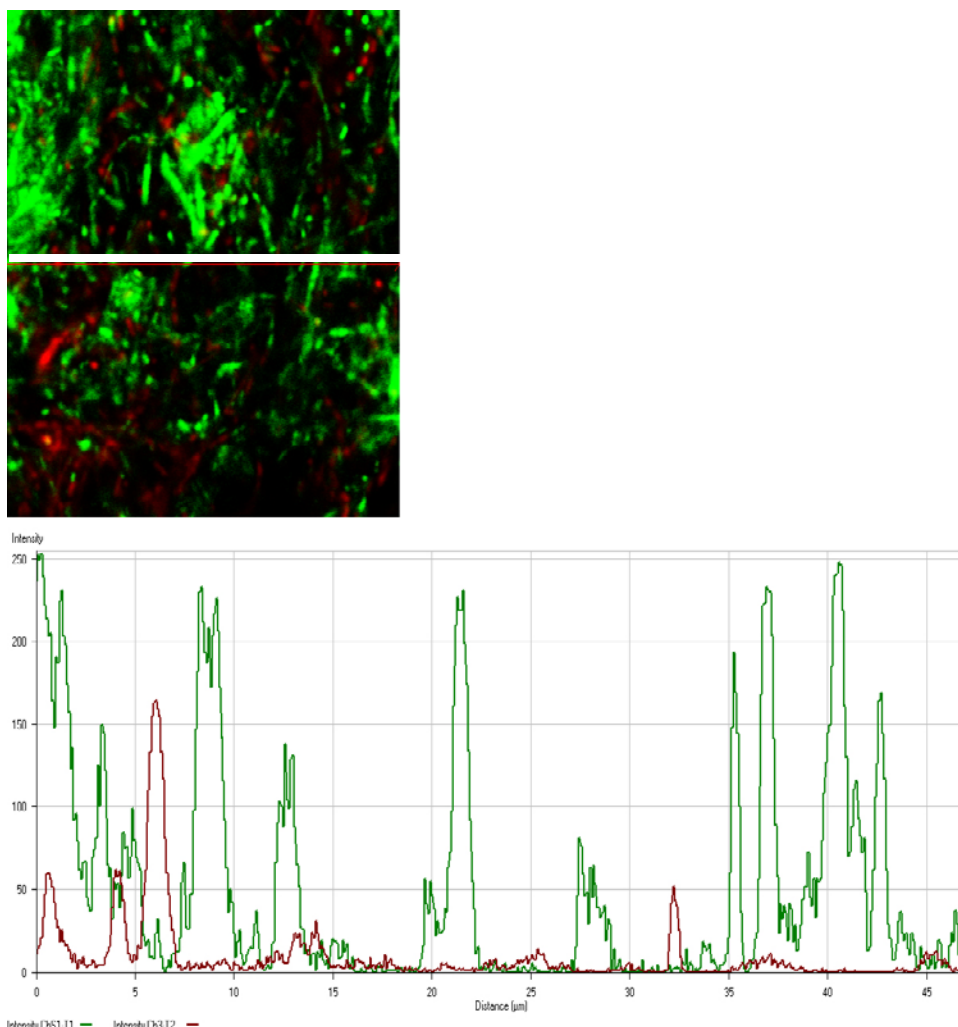
The uneven distribution of the biofilm is well illustrated with a 3D view of the biofilm from side-reactor samples corresponding to shipment 5, shown in Figure 11. Figure 12 shows the intensities captured by the green and red channel for the same sample, but it is a 2D representation accompanied by a visualization of the biofilm at the top. Higher intensity for the green or red channel correlates to higher abundance of live or dead cells, respectively. Across

the biofilm sample, the intensity received by the green channel was higher (Figure 11a and 12) than the intensity received by the red channel (Figure 11b and 12); however, the live/dead assay should not be interpreted quantitatively due to possible artifacts caused by sample shipping and handling. While the living cells were more evenly distributed than the dead cells, the overall interpretation may be that the biofilm had an uneven distribution for bacterial cells, which corresponds to the visually patchy nature of the biofilms. The CLSMs do not show the distribution of EPS, which may make the biofilm more physically homogeneous than for the bacterial cells.



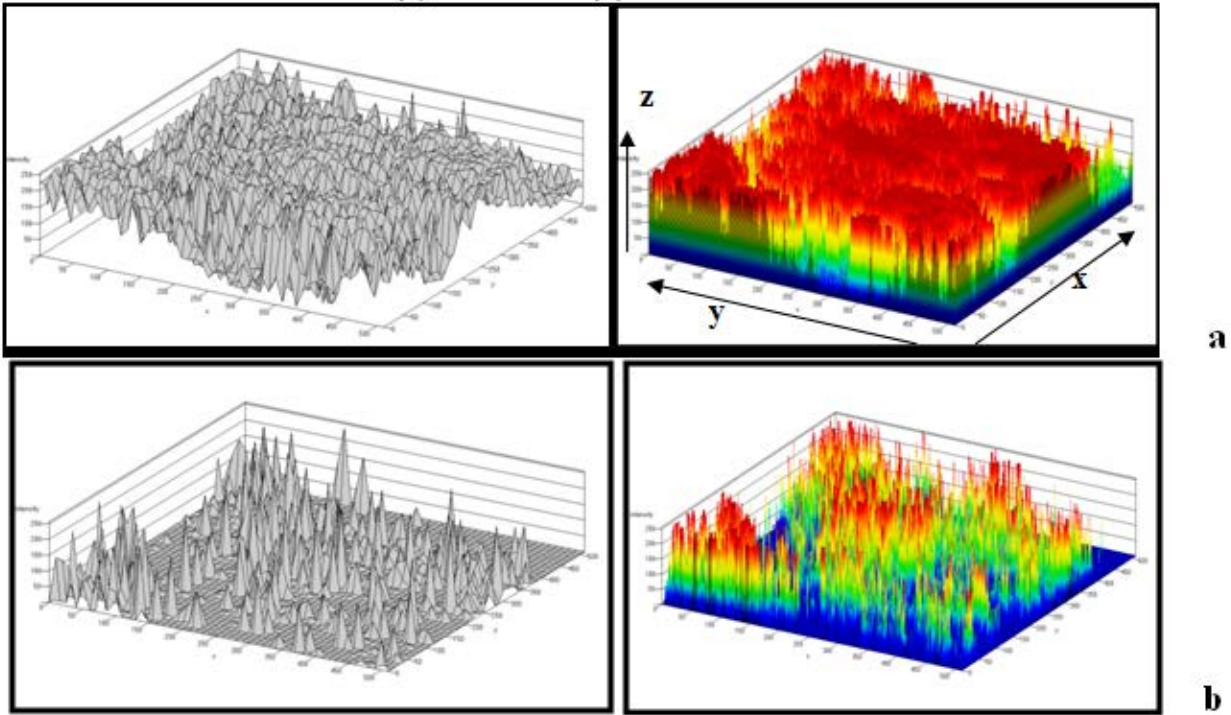
**Figure 11.** 3D representation of biofilm from lab-module R100D. The portions of the biofilm stained green (a) and red (b) are illustrated according to an x-y-z vector graph, where x and y are the length and width of sample analyzed, and z is the intensity of the fluorescent signal. A higher z intensity represents a higher abundance of either live cells (left) or dead cells (right).

The apparent differences between Figure 10 and Figure 11 probably are explained by the sampling procedures before CLSM. While Figure 10 shows a single fiber, Figure 11 shows a portion of a fiber sheet sample (~0.5 cm X 0.5 cm). Thus, less biofilm detachment probably occurred for the sample illustrated in Figure 11 than for Figure 10. If this is true, then the relative proportions between live and dead cells are better represented by Figure 11, and indicates that live cells are more significant than dead cells within the biofilm.

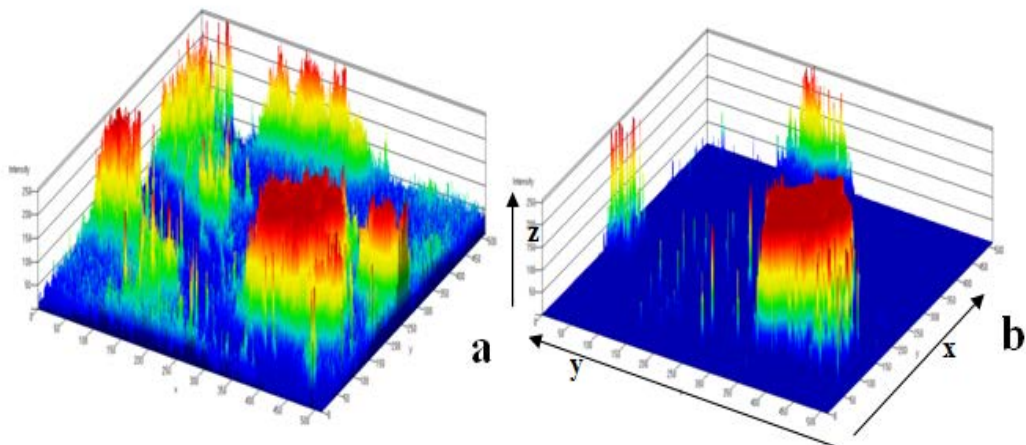


**Figure 12.** 2D graph of the intensities caught by the green and red channels. The top shows the 2D image, while the bottom shows the intensities along the transect indicated by the red line in the top image.

To investigate further if the biofilm was mostly populated with living areas rather than dead areas, we took several images of the same biofilm sample from a fiber sheet, but from different areas. Figure 13 shows the 3D representations of the intensities received by the green channel (Figure 13a) and red channel (Figure 13b) for the biofilm from the field sample coded as APT107 (shipment 6). The 3D representation in gray scale of the same sample for the green and red channel is also included to help understanding the location and coverage of living and dead portions within the biofilm. The 3D images make it clear that the biofilm had substantially more live biomass than dead biomass. Furthermore, the live biomass was more uniform.



**Figure 13.** 3D black (left) and color (right) representation of biofilm from field sample coded as APT107, first spot. The portions of the biofilm that stained green (a) and red (b) are illustrated according to a x-y-z vector graph, where x and y are the length and width of sample analyzed, and z is the intensity of the fluorescent signal. A higher intensity represents a higher abundance of either live cells (a) or dead cells (b).

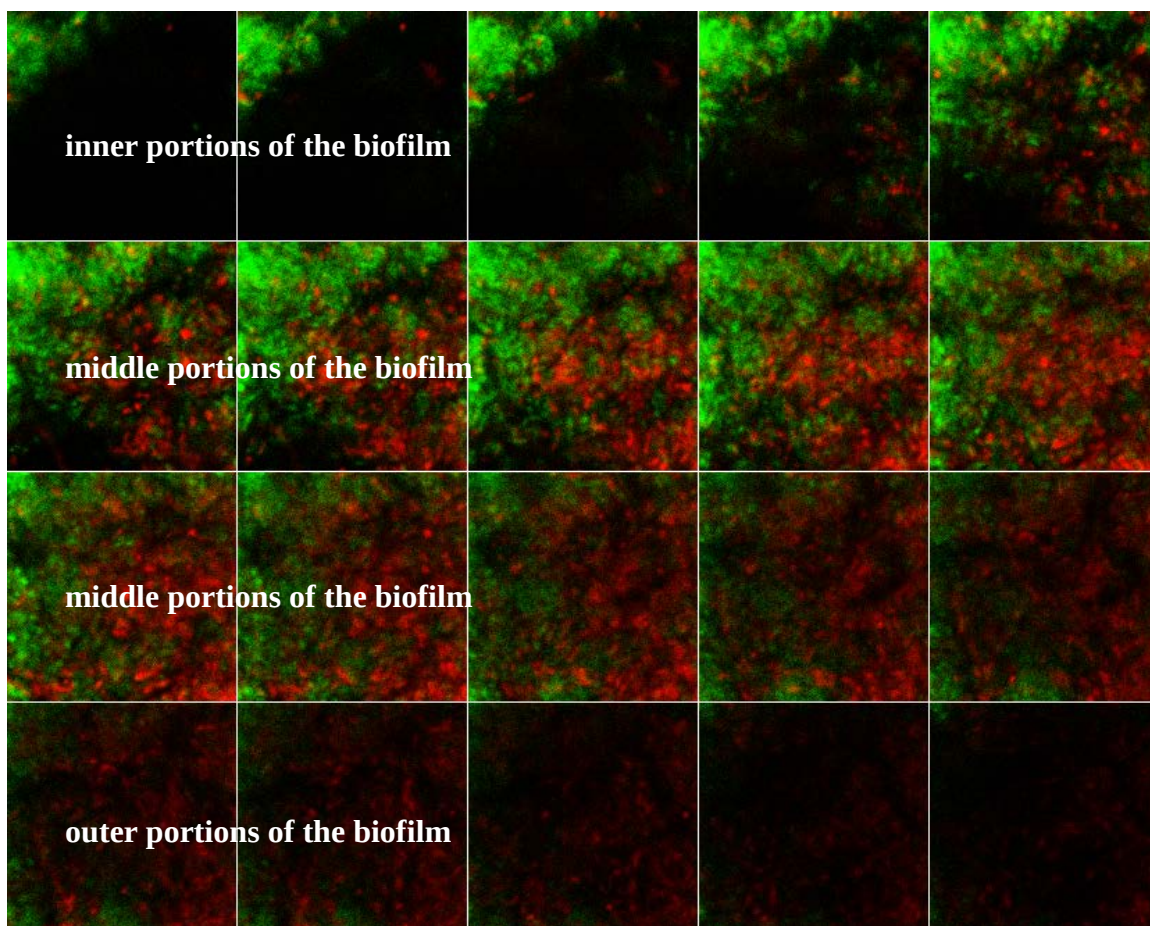


**Figure 14.** 3D color-representation of biofilm from field sample APT107, second spot. The portions of the biofilm that stained green (a) and red (b) are illustrated according to a x-y-z vector graph, where x and y are the length and width of sample analyzed, and z is the intensity of the fluorescent signal. A higher intensity represents a higher abundance of either live cells (a) or dead cells (b).

Figure 14 is from the biofilm of sample APT107, but for a second spot. It shows the same pattern of dominant living zones as in Figure 13, but all of the biomass appears patchier for this spot.

Another important observation that can be taken from CLSM imaging is illustrated well in Figure 15, which shows a gallery of Z-scans from the attachment substratum to the outer surface of the biofilm. The living portions (green zones) are closest to the fiber. When  $H_2$  is limiting, the active biomass tends to grow best close to the fiber, where it has better access to  $H_2$ . This trend is consistent with modeling results presented later. Reinforcing this trend is that water flowing past the biofilm creates forces that help remove the outer layer of the biofilm, which reduces the total biofilm thickness. Since the active biomass is farthest from the liquid, the most active biofilm is relatively protected from detachment. This usually is good for MBfR performance, because a thin and highly active biofilm promotes solute mass transport from the bulk liquid to the active portion of the biofilm. Substrates (e.g.,  $NO_3^-$ ) must diffuse into the biofilm to promote growth, and alkalinity must diffuse out of the biofilm to maintain a suitable pH. These complementary trends, which tend to keep the MBfR biofilm active, also are reflected in the clear trends that the biomass is much more active than dead (e.g., Figures 10 – 14) and has little inorganic solids (Table 3).





**Figure 15.** Combined overlay at 40X of biofilm from the pilot reactor for shipment #3. Live cells stain green, while dead cells stain red. This figure is a gallery of the Z-scan function provided by the software of the CLSM, and the 20 panels represent a depth of view of ~150  $\mu\text{m}$ , which is consistent with the typical biofilm thickness in Table 3. The Z-scan function layers the specimen at different depths. Each square is the dissection procedure done by the confocal laser at different depths in the specimen. The spatial distribution of the live and dead cells is clear. The inner layers (closest to the fibers) are almost totally active, while the outer layers (near the liquid) have a predominance of dead cells.

## Chapter 5. Synthesis for the Side-Reactors and Reactor Modules in the MBfR Vessels

We conclude with a synthesis of the major findings for the acceptor fluxes, microbial community structure, EPS composition, and CLSM imaging for the side-reactors and reactor modules in the MBfR vessels at Rialto. We offer preliminary interpretations about why the pilot MBfRs failed to meet consistently the 6- $\mu\text{g/L}$  goal for  $\text{ClO}_4^-$ , even though they performed well for acceptor removal overall. These interpretations are tested and refined by the laboratory experiments and modeling summarized in the next sections.



a) The relative magnitudes of the surface loadings for the electron acceptors allowed denitrification and  $O_2$  respiration to control the flow of electrons from  $H_2$  in the lead MBfR. The lead MBfR was responsible of reducing 70-90% of the  $NO_3^-$  loading and 99% of the  $O_2$  loading. The  $NO_3^- + O_2$  surface loading of  $\geq 0.3 \text{ g } H_2/m^2\text{-day}$  suppressed  $SO_4^{2-}$  reduction.

b) The  $NO_3^-$  influent concentration for the lag MBfR was 0.2-4.4 mg N/L, which allowed the onset of  $SO_4^{2-}$  reduction in the lag MBfR. Quantitatively, a  $NO_3^-$  flux  $< \sim 0.1 \text{ g } H_2/m^2\text{-day}$  allowed  $SO_4^{2-}$  reduction in the lag MBfR when  $H_2$  delivery was not limiting.

c)  $ClO_4^-$  respiration was observed in the lead and lag MBfRs, but the highest  $ClO_4^-$  reduction occurred at the lag MBfR, where competition from  $NO_3^-$  and  $O_2$  respirations was minimized.

d)  $ClO_4^-$  reduction flux in the lag MBfR was inversely correlated to the  $SO_4^{2-}$  flux. A  $SO_4^{2-}$  flux of  $0.003 \text{ g } H_2/m^2\text{-day}$  or more seemed to slow  $ClO_4^-$  reduction. A  $SO_4^{2-}$  flux of  $0.001 \text{ g } H_2/m^2\text{-day}$  allowed the highest  $ClO_4^-$  flux.

e) Operational conditions applied to field MBfRs affected the microbial communities in ways that corresponded to acceptor fluxes. When acceptor surface loadings were high due to a high flow rate, the field MBfRs (especially the lead MBfR) experienced a limitation of  $H_2$  delivery, and DB were dominant. Increasing the HRT, which decreased the surface loading for all acceptors, eliminated  $H_2$  limitation, but at the expense of favoring SRB, whose metabolic versatility helped them remain in the biofilm regardless of  $SO_4^{2-}$  reduction activity.

f) The chemical and physical characteristics of the biofilm were similar for all conditions. All samples had a high ratio of %VS/TS and %VSS/TSS ( $\sim 90\%$ ), indicating minimum mineral precipitation. The biofilm thickness was around  $200 \mu\text{m}$ , EPS was a large portion of the biofilm mass ( $\sim 40\text{-}50\%$ ), and EPS was dominated by humics, with proteins also important. Based on rRNA copies, mRNA transcripts, and CLSM visualization, the biofilms were highly active, particularly near the membrane substratum. Finally, the biofilms had uneven distributions of live and dead cells.

In summary, while the two-stage pilot system had an active biofilm and was effective for reducing  $NO_3^-$  and  $ClO_4^-$ , it did not reliably achieve the  $6\text{-}\mu\text{g/L } ClO_4^-$  goal because lowering the  $ClO_4^-$  surface loading also lowered the loading of all acceptors. In particular, low surface loading of  $NO_3^-$  in the lag MBfR led to  $SO_4^{2-}$  reduction, which had a negative impact on the rate of  $ClO_4^-$  reduction. For instance, shipments 4-6 had significantly higher HRT and thus smaller surface loadings for all acceptors (Evans et al., 2013); this situation allowed SRB to outcompete DB and PRB in the lag MBfR.

To control the overgrowth of SRB and achieve a very low effluent  $ClO_4^-$  concentration, the  $NO_3^- + O_2$  surface loading has to be significant, but it should not be too large to cause excessive competition from denitrification and DB. Based on the pilot results, the  $NO_3^- + O_2$  surface loading for the lag MBfR should be in the range of  $0.1 - 0.3 \text{ g } H_2/m^3\text{-day}$ . This strategy is addressed in some of the bench-scale experiments reported in the following sections.

## Chapter 6. Exploring Mechanisms and Performance with Bench-Scale Experiments

The ASU team carried out a series of bench-scale experiments designed to elucidate critical kinetic and ecological mechanisms that control the performance of the MBfR when  $\text{ClO}_4^-$  reduction to  $\mu\text{g/L}$  concentrations is the goal. Of over-arching importance is that multiple electron acceptors are present in the influent water:  $\text{O}_2$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{ClO}_4^-$ . Typically and in the case of the Rialto field study,  $\text{ClO}_4^-$  is present at by far the lowest concentration. This means that PRB must compete for the common electron donor ( $\text{H}_2$ ) and space in the biofilm with bacteria that reduce the other acceptors. The competition can be an important factor controlling whether or not the PRB are able to reduce  $\text{ClO}_4^-$  to very low concentrations.

In a series of experimental studies, the ASU team evaluated competition for  $\text{H}_2$  and space among PRB, DB, and SRB. Three of the studies focused specifically on the ability to reduce  $\text{ClO}_4^-$  to  $\mu\text{g/L}$  concentrations, while three others provided foundation information needed to interpret the studies about  $\text{ClO}_4^-$  reduction. All bench scale-related work was developed in 60-mL (total volume) MBfRs, as illustrated in Figure 16. This section provides a succinct summary of each experimental study and how they relate to the pilot performance. Each study resulted in a published or accepted manuscript that is listed at the beginning of each summary.



**Figure 16.** Bench scale MBfR showing the biofilm development on the fiber's surface.

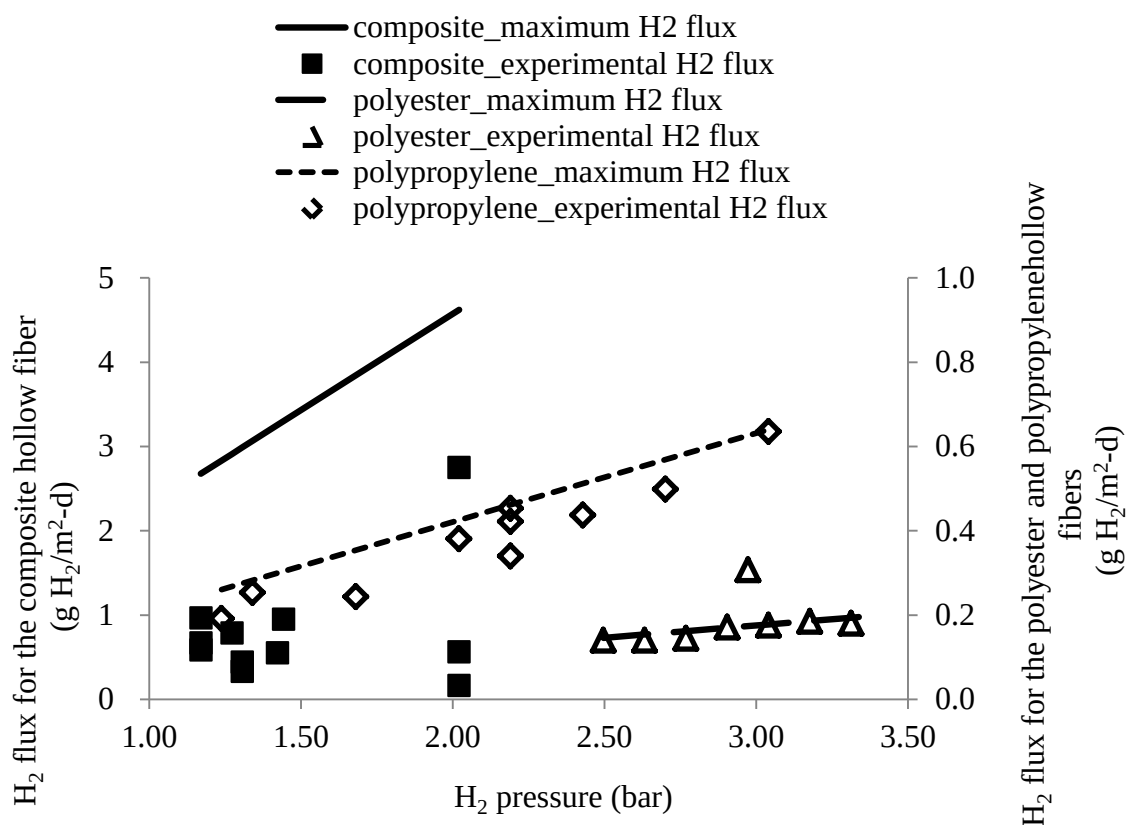
### **6.1 Permeation Rates for Hydrogen Gas Through MBfR Fibers**

Tang, Y., C. Zhou, S. Van Ginkel, A. Ontiveros, J. Shin, and B.E. Rittmann (2012b). Hydrogen-permeabilities of the fibers used in a H<sub>2</sub>-based membrane biofilm reactor. *J. Membrane Sci.* **407-408**: 176 – 183.

The capacity to deliver the H<sub>2</sub> electron donor by diffusion through the walls of the gas-transfer fibers is fundamental information needed to interpret all of the experimental results, as well as to model and design MBfRs. In this published study, we developed and used steady-state permeation tests and a mathematical model to determine the H<sub>2</sub> permeabilities of the three hollow fibers commonly used in the H<sub>2</sub>-based MBfR: composite from Mitsubishi-Rayon, polypropylene from Teijin, and polyester from Teijin. The H<sub>2</sub> permeabilities spanned a wide range:  $1.6 \times 10^{-6}$ ,  $1.8 \times 10^{-7}$ , and  $6.6 \times 10^{-8}$  m<sup>3</sup> H<sub>2</sub> @ standard temperature and pressure • m membrane thickness / (m<sup>2</sup> hollow fiber surface area • d • bar) for the composite, polypropylene,

and polyester hollow fibers, respectively; this represents a ratio of the maximum H<sub>2</sub> flux for composite: polypropylene: polyester hollow fibers = 32: 3.3: 1.

The H<sub>2</sub> permeabilities were then used to correlate hollow-fiber type to contaminant-removal flux from a wide range of previous MBfR experiments. As shown in Figure 17, only the experiments with composite hollow fibers were not routinely limited by H<sub>2</sub> delivery. However, the composite fiber was susceptible to fouling by mineral precipitates in its outer-layer micropores, and this lowered the H<sub>2</sub> flux in some cases with the composite fibers. The Teijin polypropylene fibers, which have no micropores and were used in the Rialto pilots, did not seem to be affected adversely by mineral precipitation. Having the permeability values made it possible to determine whether or not the bench-scale or pilot-scale experiments were limited by the H<sub>2</sub>-delivery capacity. For the field-pilot studies, the H<sub>2</sub> delivery capacity was substantially greater than the H<sub>2</sub>-consumption rate for most of the experimental program, except for the start up phase when side-reactor shipment 2 was delivered.



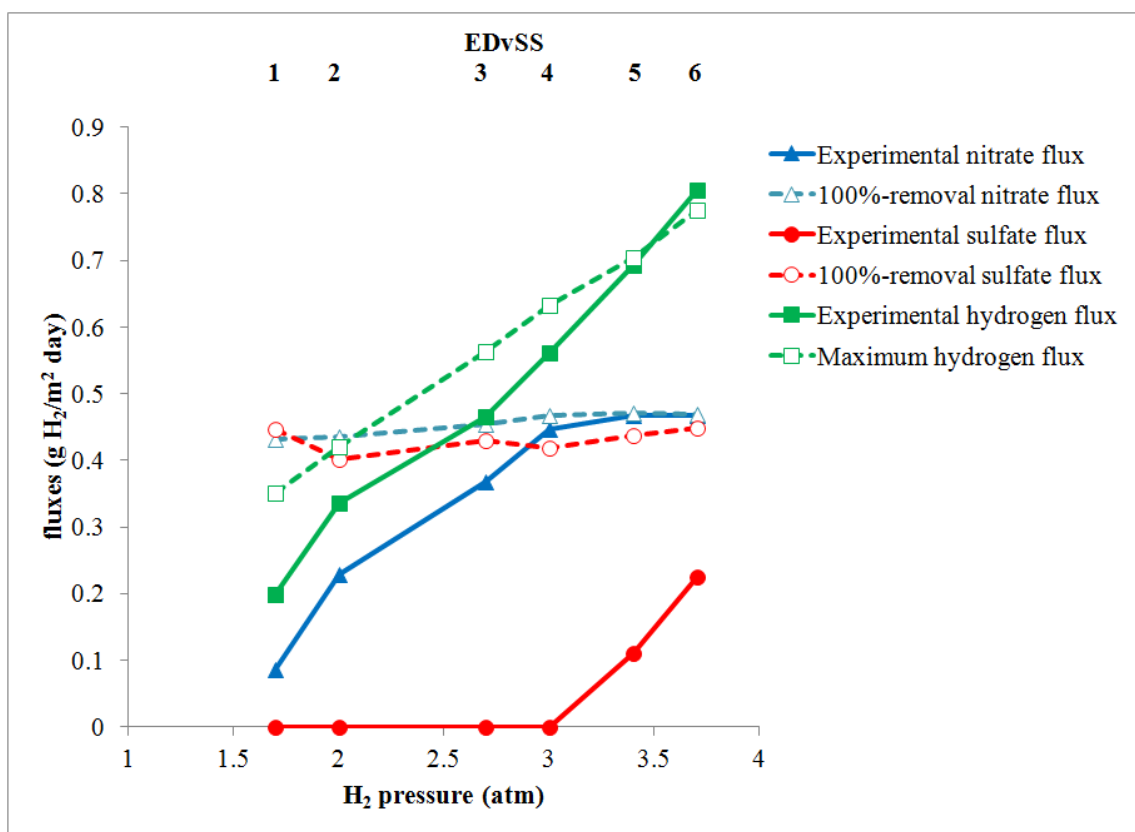
**Figure 17.** Comparison of maximum-possible H<sub>2</sub> fluxes and experimentally measured H<sub>2</sub> fluxes in MBfR experiments. The maximum-possible flux occurs when the water-phase concentration of H<sub>2</sub> is zero.

## 6.2 Ecological Interactions Between DB and SRB in the MBfR Biofilm

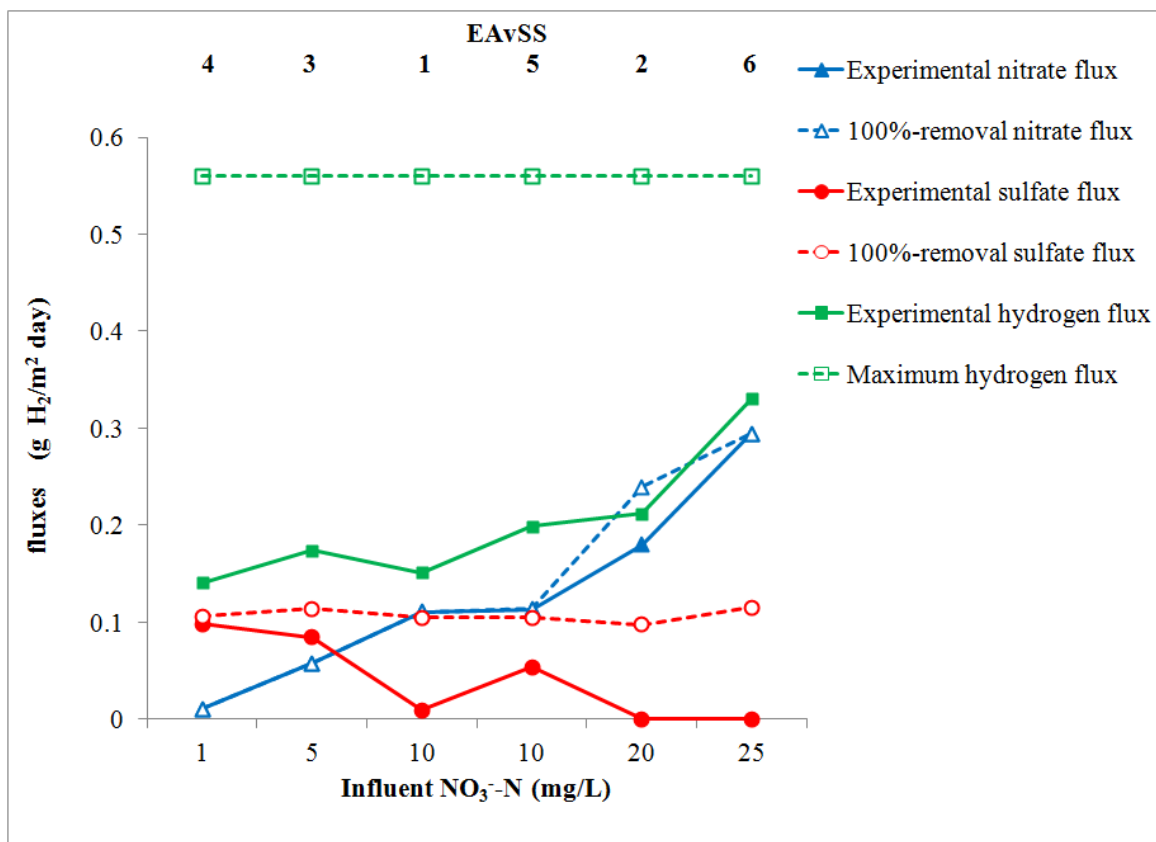
Ontiveros-Valencia, A., M. Ziv-El., H.-P. Zhao, L. Feng, B. E. Rittmann, and R. Krajmalnik-Brown (2012). Interactions between nitrate-reducing and sulfate-reducing bacteria coexisting in a hydrogen-fed biofilm. *Environ. Sci. Technol.* **46**: 11289-11298.

To explore the relationships between DB and SRB in H<sub>2</sub>-fed biofilms, we operated two H<sub>2</sub>-based MBfRs (bench-scale) with or without restrictions on H<sub>2</sub> availability. H<sub>2</sub> availability was gauged from the H<sub>2</sub> pressure and the H<sub>2</sub> permeabilities reported by Tang et al. (2012) (previous section) for the polypropylene fibers. Since DB and SRB compete for H<sub>2</sub> and space in the biofilm, SO<sub>4</sub><sup>2-</sup> reduction should be out-competed by NO<sub>3</sub><sup>-</sup> reduction when H<sub>2</sub> is limiting inside the biofilm, as SRB are slower growing than DB. We know that DB and PRB can co-exist, but the SRB appear to have a negative impact on PRB. Therefore, having DB out-compete SRB should have a profound positive impact on the PRB.

As shown in Figure 18 for experimental set EDvSS (standing for Electron-Donor-variable Steady States), NO<sub>3</sub><sup>-</sup> reduction was proportional to the H<sub>2</sub> pressure when the supply of H<sub>2</sub> was restricted (at the lower H<sub>2</sub> pressures). Denitrification was complete at a H<sub>2</sub> pressure of 3 atm, and SO<sub>4</sub><sup>2-</sup> reduction began at H<sub>2</sub> ≥ 3.4 atm. The H<sub>2</sub> flux for complete denitrification and no SO<sub>4</sub><sup>2-</sup> reduction was ~0.4 g H<sub>2</sub>/m<sup>2</sup>-day (corresponding to a NO<sub>3</sub><sup>-</sup> removal flux of about 1.1 gN/m<sup>2</sup>-day). Figure 19 shows the results from experimental set EAvSS (standing for Electron-Acceptor-variable Steady States), in which the H<sub>2</sub> availability always was sufficient. NO<sub>3</sub><sup>-</sup> was the preferred electron acceptor, and SO<sub>4</sub><sup>2-</sup> was reduced only when the NO<sub>3</sub><sup>-</sup> surface loading was ≤ ~0.2 g H<sub>2</sub>/m<sup>2</sup>-day, which corresponds to a NO<sub>3</sub><sup>-</sup> flux of ~0.5 g N/m<sup>2</sup>-day.



**Figure 18.** Removal fluxes of  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  for the 6 steady states of EDvSS (noted at the top of the graph), 100%-reduction fluxes for these acceptors, the total  $\text{H}_2$  removal flux for all acceptors, and the maximum-possible  $\text{H}_2$  flux deliverable by the experimental  $\text{H}_2$  pressure (in the range of 1.7 to 3.7 atm). All fluxes are expressed as  $\text{H}_2$  equivalents. The  $\text{H}_2$  flux due to  $\text{O}_2$  reduction of  $0.12 \text{ g H}_2/\text{m}^2\text{-day}$  for each EDvSS is included in the total  $\text{H}_2$  flux. The EDvSS numbers indicate the chronological order of the experiments. The results are presented here in ascending order of ascending  $\text{H}_2$  pressure.

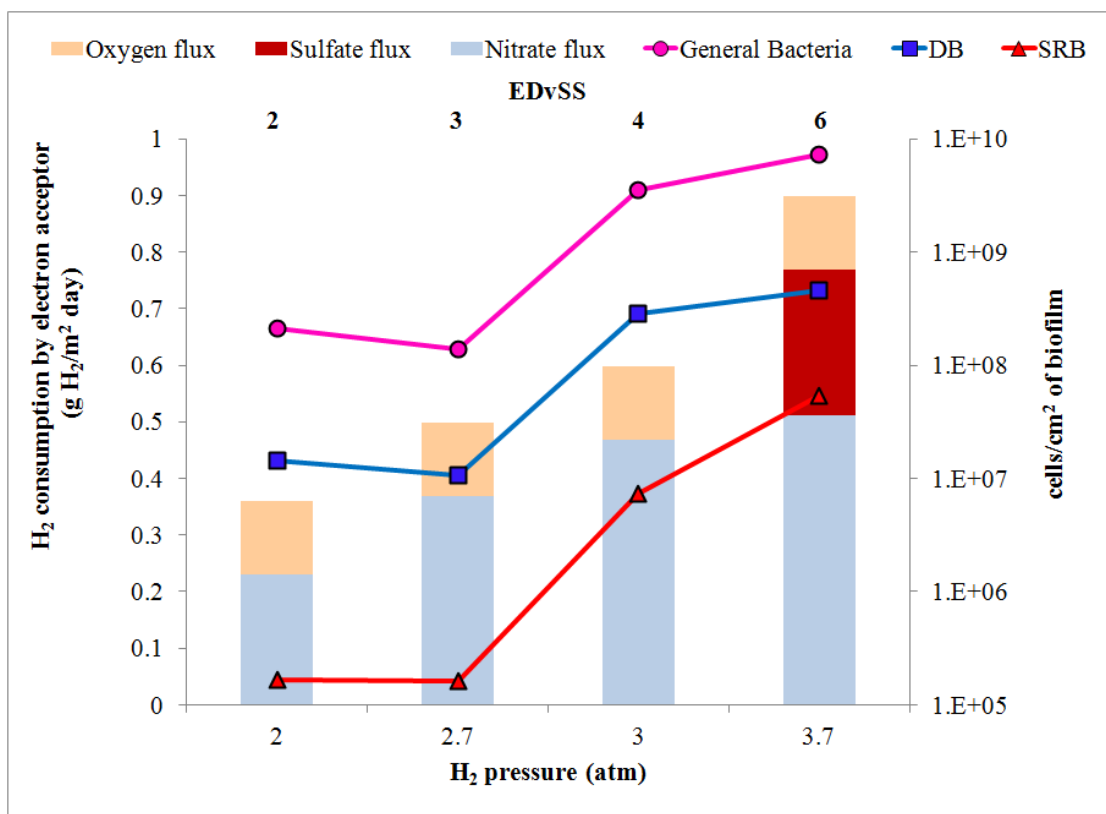


**Figure 19.** Removal fluxes of NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup> for the 6 steady states of EAvSS (indicated across the top of the graph), 100%-removal fluxes for these acceptors, the total H<sub>2</sub> removal flux for all acceptors, and the maximum-possible H<sub>2</sub> flux deliverable with a H<sub>2</sub> pressure of 25 psig (2.7 atm). All fluxes are expressed as H<sub>2</sub> equivalents. The H<sub>2</sub> flux due to O<sub>2</sub> reduction of 0.03 g H<sub>2</sub>/m<sup>2</sup>-day for each EAvSS is included in the total H<sub>2</sub> flux. The EAvSS numbers indicate the chronological order of the experiments. The results are presented here in ascending order of influent NO<sub>3</sub><sup>-</sup> concentration, and two experiments has an influent concentration of 10 mgN/L.

For biofilm samples, we assayed DB and SRB by the quantitative Polymerase Chain Reaction (qPCR) targeting the nitrite reductases and dissimilatory sulfite reductase, respectively. Figures 20 and 21 present the qPCR results. While DB and SRB increased with higher H<sub>2</sub> pressures when H<sub>2</sub> availability was limiting (Figure 20), SRB did not decline with higher NO<sub>3</sub><sup>-</sup> removal flux when H<sub>2</sub> availability was not limiting (Figure 21), even when SO<sub>4</sub><sup>2-</sup> reduction was absent. The SRB trend reflects that the SRB's metabolic diversity allowed them to remain in the biofilm whether or not they were reducing SO<sub>4</sub><sup>2-</sup>. In all scenarios tested, the SRB were able to initiate strong SO<sub>4</sub><sup>2-</sup> reduction only when competition for H<sub>2</sub> inside the biofilm was relieved by nearly complete removal of NO<sub>3</sub><sup>-</sup>. Therefore, the onset of SO<sub>4</sub><sup>2-</sup> reduction was suppressed by the presence of sufficient denitrification to out-compete SRB for H<sub>2</sub> and space in the biofilm.

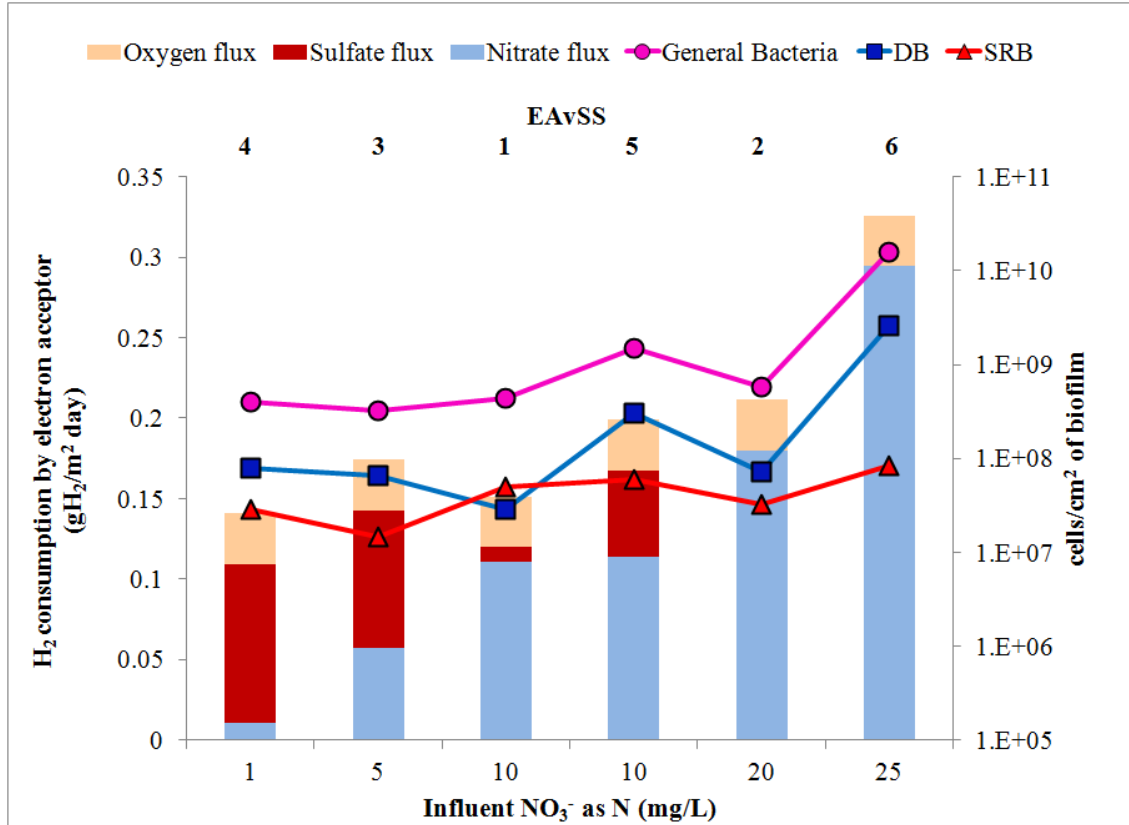
In summary, denitrification out-competed  $\text{SO}_4^{2-}$  reduction when the  $\text{H}_2$  flux for denitrification was  $\sim 0.4 \text{ g H}_2/\text{m}^2\text{-day}$  and  $\text{H}_2$  delivery was limiting. In this situation, SRB increased significantly when the  $\text{NO}_3^-$  removal flux was low enough to allow  $\text{SO}_4^{2-}$  reduction to begin. When  $\text{H}_2$  delivery was not limiting, SRB were significantly present in all biofilms, and the  $\text{H}_2$  flux due to denitrification had to be  $\geq 0.2 \text{ g H}_2/\text{m}^2\text{-day}$  to suppress  $\text{SO}_4^{2-}$  reduction.

Similar trends were observed in the pilot MBfRs. With  $\text{H}_2$  limitation, a  $\text{NO}_3^-$  flux of  $0.3 \text{ g H}_2/\text{m}^2\text{-day}$  suppressed  $\text{SO}_4^{2-}$  reduction, while  $0.17 \text{ g H}_2/\text{m}^2\text{-day}$  slowed  $\text{SO}_4^{2-}$  reduction when  $\text{H}_2$  delivery was not limiting. Likewise, SRB were present in all cases, but stimulated when conditions allowed  $\text{SO}_4^{2-}$  reduction in the lag MBfR.



**Figure 20.** Abundances (in cells/cm<sup>2</sup>) of DB (sum of *nirS* and *nirK* genes), SRB, and general bacteria for four biofilm samples from EDvSS, along with the  $\text{H}_2$  consumption rate by each electron acceptor.





**Figure 21.** Abundances (in cells/cm<sup>2</sup>) of DB (sum of *nirS* and *nirK* genes), SRB, and general bacteria for six biofilm samples from EAvSS, along with the H<sub>2</sub> consumption rate by each electron acceptor. The EAvSS numbers indicate the chronological order of the experiments. The results are presented here in ascending order of influent NO<sub>3</sub><sup>-</sup> concentration.

### 6.3 Phylogenetic analysis of DB and SRB in hydrogen-fed biofilms

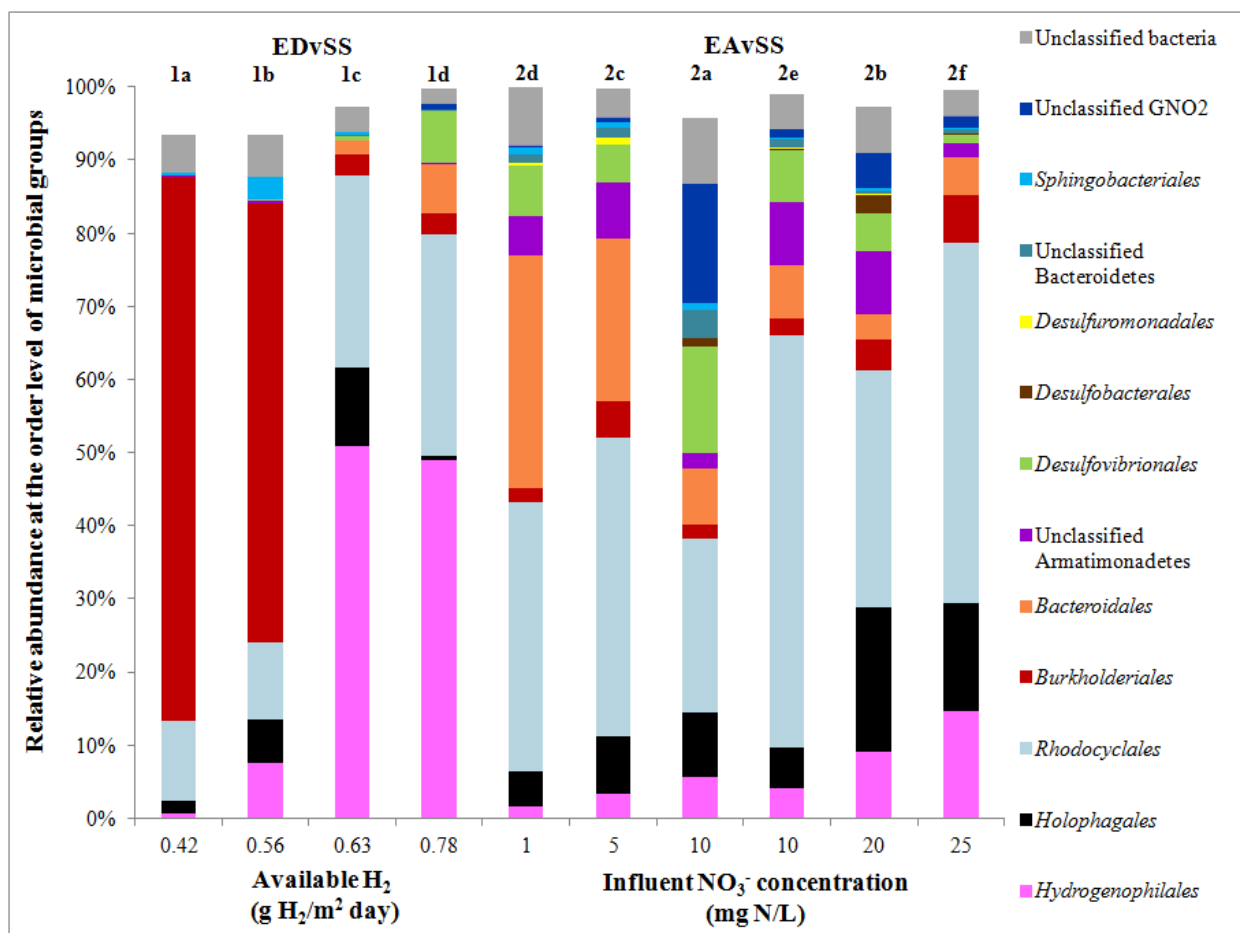
Ontiveros-Valencia A, Ilhan ZE, Kang DW, Rittmann BE, Krajmalnik-Brown R (2013a) Phylogenetic analysis of nitrate- and sulfate-reducing bacteria in a hydrogen-fed biofilm *FEMS Microbial Ecology*, 85: 158-167.

Using pyrosequencing analysis, we studied the ecological interactions between DB and SRB in the MBfRs from the study in the previous section (Ontiveros-Valencia et al., 2012). We achieved a comprehensive understanding of the microbial community structure and function by employing two MBfR: one with changes to electron donor availability (called EDvSS) and the other with different electron acceptor (NO<sub>3</sub><sup>-</sup>) surface loadings (called EAvSS).

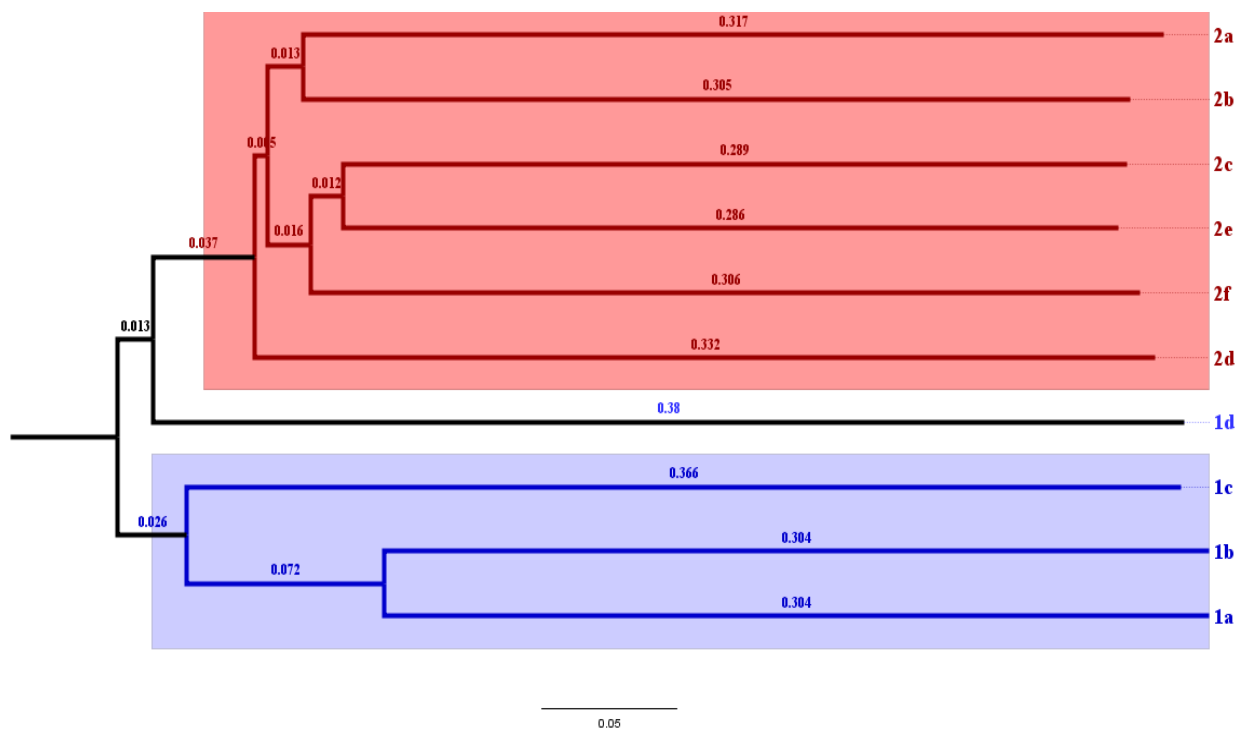
In EDvSS and with severe electron-donor limitation (samples 1a and 1b in Figure 22), the biofilm community was dominated by heterotrophic DB (i.e., *Burkholderiales*); when the limitation on H<sub>2</sub> decreased, the biofilm became more autotrophic, with DB-phylotypes closely related to *Rhodocyclales* and *Hydrogenophilales* dominating the biofilm community (samples 1c and 1d in Figure 20). In EAvSS and without donor limitation, the biofilm community was dominated by autotrophic DB; however, a decrease of NO<sub>3</sub><sup>-</sup> loading allowed SRB (mostly *Desulfovibrionales*) to compete better for space in the biofilm. *Bacteroidales* were associated

with strong  $\text{SO}_4^{2-}$  reduction activity (samples 1d for EDvSS, and 2c, 2d for EAvSS) as they likely consumed soluble microbial products released by SRB.

Principal coordinate analysis (PCoA, not shown) and UniFrac analysis (Figure 23) pointed out that electron-donor availability and electron-acceptor surface loading framed the microbial community, with restricted electron-donor availability significantly decreasing the microbial diversity of the biofilm. The onset of  $\text{SO}_4^{2-}$  reduction led the biofilm communities of EDvSS and EAvSS to cluster together (sample 1d for EDvSS and all samples from EAvSS) (Figure 23), showing the importance of  $\text{SO}_4^{2-}$  reduction in determining community structure.



**Figure 22.** Relative abundances of the most abundant microbial phylotypes at the order level for EDvSS and EAvSS. The EDvSS and EAvSS letter and number codes show the chronological order of samples. Samples for EAvSS are shown according to increasing  $\text{NO}_3^-$  concentration. The sum does not add up to 100% in all cases because minor phylotypes are not shown.



**Figure 23.** Clustering based on the unweighted UniFrac analysis. The branch length represents the distance between biofilm samples in UniFrac units, as indicated by the scale bar. 1a-1d correspond to EDvSS, with 1a = 0.42 g H<sub>2</sub>/m<sup>2</sup> day, 1b = 0.56 g H<sub>2</sub>/m<sup>2</sup> day, 1c = 0.63 g H<sub>2</sub>/m<sup>2</sup> day, and 1d = 0.78 g H<sub>2</sub>/m<sup>2</sup> day. 2a-2f correspond to EAvSS, with 2a = 10 mg N/L, 2b = 20 mg N/L, 2c = 5 mg N/L, 2d = 1 mg N/L, 2e = 10 mg N/L, and 2f = 25 mg N/L.

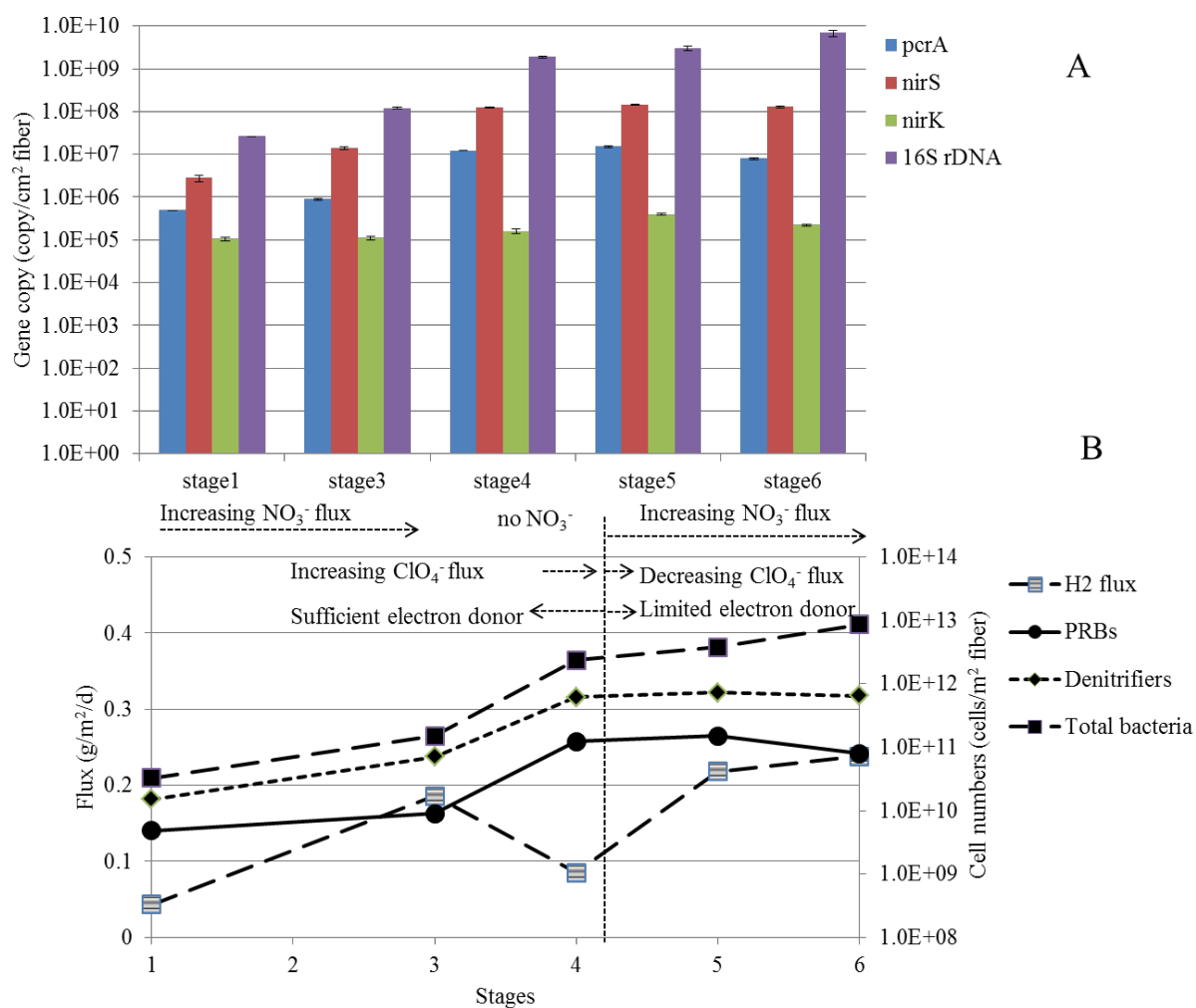
#### 6.4 Interactions Between DB and PRB in a H<sub>2</sub>-Based Biofilm

Zhao, H. P., Van Ginkel, S., Tang, Y., Kang, D.-W., Rittmann, B. E., and Krajmalnik-Brown, R. (2011) Interactions between perchlorate and nitrate reductions in the biofilm of a hydrogen-based membrane biofilm reactor. *Environ. Sci. Technol.* 45: 10155-10162.

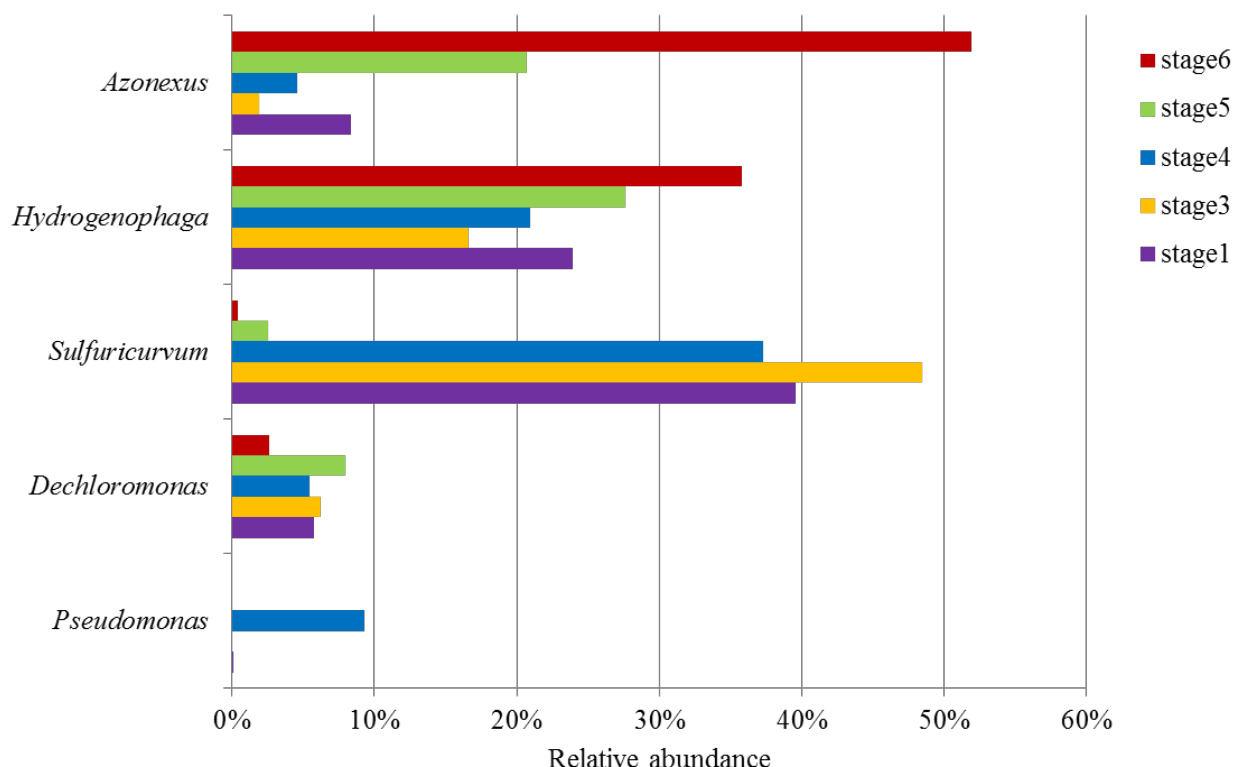
We studied the direct functional and structural interactions between NO<sub>3</sub><sup>-</sup> and ClO<sub>4</sub><sup>-</sup> reductions in the H<sub>2</sub>-based MBfR. We utilized qPCR directed toward the nitrate and perchlorate reductase genes, as well a high-throughput pyrosequencing. Figure 24 presents the H<sub>2</sub> fluxes and qPCR results according to the electron acceptor. Figure 25 summarizes the predominant genera according to high-throughput pyrosequencing.

When H<sub>2</sub> delivery was not limiting (especially Steady States 1 and 4, based on H<sub>2</sub> permeability of the polypropylene fiber (Tang et al., 2012)), ClO<sub>4</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> reductions were complete (not shown), and the MBfR's biofilm was composed mainly of bacteria from the  $\gamma$  and  $\beta$ -*proteobacteria* classes, with autotrophic genera *Sulfuricurvum*, *Hydrogenophaga*, and *Dechloromonas* dominating the biofilm. Based on functional-gene and pyrosequencing assays, *Dechloromonas* played the most important role in ClO<sub>4</sub><sup>-</sup> reduction, while *Sulfuricurvum* (an autotrophic denitrifier) and *Hydrogenophaga* were responsible for NO<sub>3</sub><sup>-</sup> reduction.

When H<sub>2</sub> delivery was insufficient to completely reduce both electron acceptors (especially Stages 5 and 6, when the H<sub>2</sub> flux was  $\geq 0.22$  g H<sub>2</sub>/m<sup>2</sup>-day), NO<sub>3</sub><sup>-</sup> reduction out-competed ClO<sub>4</sub><sup>-</sup> reduction for electrons from H<sub>2</sub>, and mixotrophs (i.e., bacteria that can use H<sub>2</sub> or organics as the electron donor) became important in the MBfR biofilm. Under H<sub>2</sub> limiting conditions in steady state 5 and 6,  $\beta$ -*proteobacteria* became the dominant class: *Azonexus* and *Hydrogenophaga* (mixotrophs) replaced *Sulfuricurvum* (an autotrophic denitrifier) as a main genus.



**Figure 24.** (A) *pcrA*, *nirK*, *nirS*, and 16S rRNA gene copy numbers per cm<sup>2</sup> fiber for, respectively, PRB, DB, DB, and general bacteria. (B) Estimated cells densities associated with the total flux of H<sub>2</sub>. The vertical dashed line distinguishes no limitation from H<sub>2</sub> delivery (stages 1 – 4) from limitation from H<sub>2</sub> delivery in stages 5 and 6.



**Figure 25.** Predominant bacterial genera by relative abundance (%) in the MBfR biofilms.

The community differences suggest that mixotrophic  $\text{NO}_3^-$ -reducing bacteria had advantages over strict autotrophs when  $\text{H}_2$  was limiting, because organic microbial products became important electron donors when  $\text{H}_2$  was severely limiting. In this case, DB had an advantage over PRB (such as *Dechloromonas*), and  $\text{ClO}_4^-$  reduction was suppressed. This result underscores the value of identifying specific PRB, as well as the general presence of PRB using qPCR. While *Dechloromonas* is a prominent and apparently effective PRB, other PRB may be present instead of or along with *Dechloromonas*, especially when  $\text{H}_2$  delivery is limited. Further investigations into the characteristics of the different PRB seem justified.

### 6.5 Competition Among Perchlorate, Nitrate, and Sulfate Reductions

Zhao, H. P., Z.-E. Ilhan, A. Ontiveros-Valencia, B. E. Rittmann, and R. Krajmalnik-Brown (2013b). Interactions among multiple electron acceptors and their effects on the microbial ecology in a hydrogen-based biofilm. *Environ. Sci. Technol.*, 47: 7396-4703.

To investigate interactions among multiple electron acceptors in a  $\text{H}_2$ -fed biofilm, we operated an MBfR with  $\text{H}_2$ -delivery capacity sufficient to reduce all acceptors. The research goal was to study the interactions among the reductions of the multiple electron acceptors when the practical goal is to obtain a very low effluent concentration of  $\text{ClO}_4^-$ . In particular, we

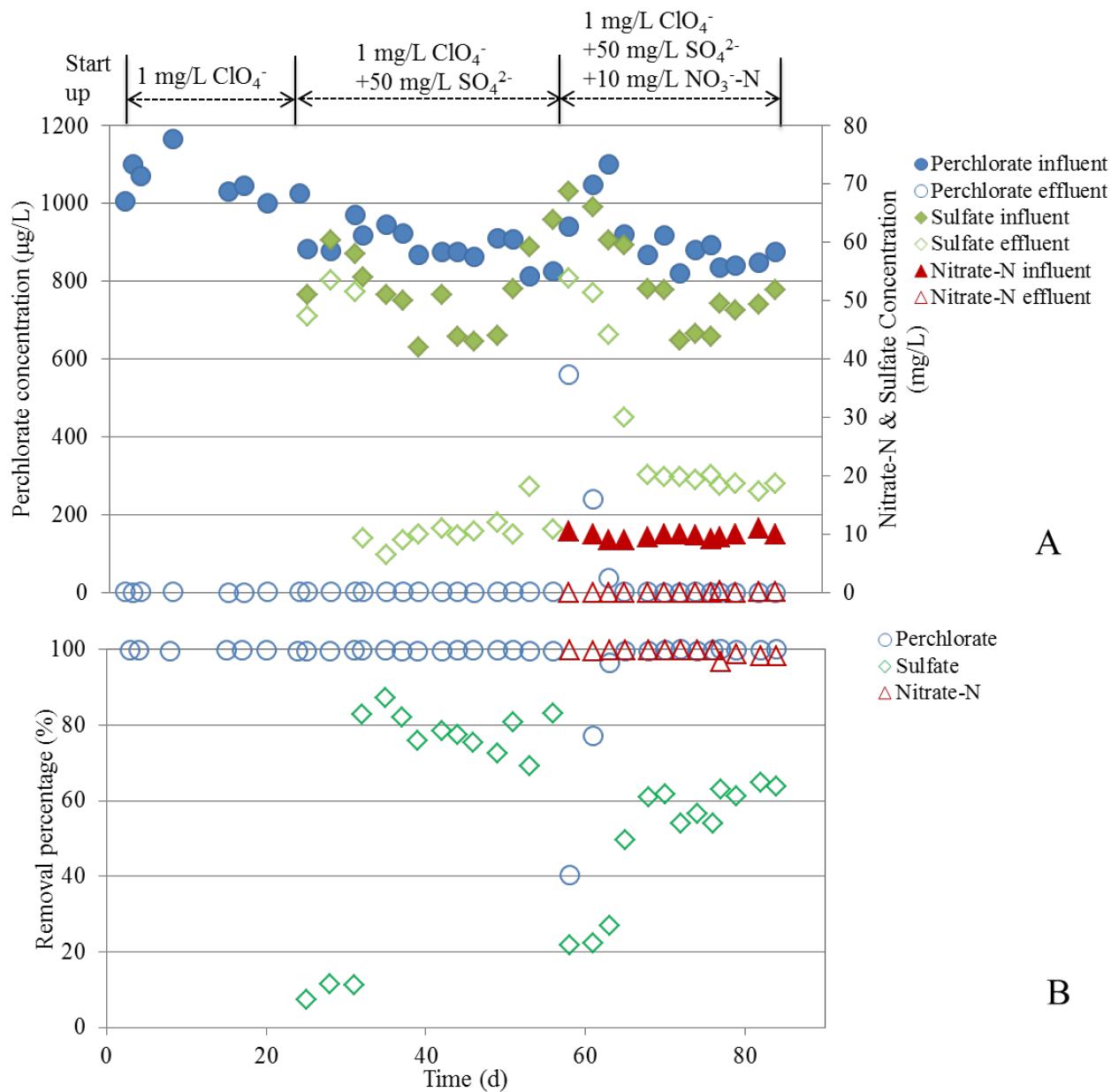
sought to understand if any fundamental ecological or kinetic phenomena were preventing full  $\text{ClO}_4^-$  reduction in the presence of the other acceptors. Based on  $\text{H}_2$  permeabilities with the composite fibers (Tang et al., 2012), we carried out all experiments with sufficient  $\text{H}_2$ -delivery capacity.  $\text{ClO}_4^-$  and  $\text{O}_2$  were input electron acceptors in all stages at surface loadings of  $0.08 \pm 0.006 \text{ g/m}^2\text{-d}$  ( $10 \pm 0.7 \text{ e}^- \text{ meq/m}^2\text{-d}$ ) for  $\text{ClO}_4^-$  and  $0.51 \text{ g/m}^2\text{-d}$  ( $76 \text{ e}^- \text{ meq/m}^2\text{-d}$ ) for  $\text{O}_2$ .  $\text{SO}_4^{2-}$  was added in Stage 2 at  $3.77 \pm 0.39 \text{ g/m}^2\text{-d}$  ( $331 \pm 34 \text{ e}^- \text{ meq/m}^2\text{-d}$ ), and  $\text{NO}_3^-$  was further added in Stage 3 at  $0.72 \pm 0.03 \text{ g N/m}^2\text{-d}$  ( $312 \pm 13 \text{ e}^- \text{ meq/m}^2\text{-d}$ ).

As summarized in Figure 26,  $\text{ClO}_4^-$ ,  $\text{O}_2$ , and  $\text{NO}_3^-$  (when present in the influent) were completely reduced at steady state for each stage; measured  $\text{SO}_4^{2-}$  reduction decreased from  $78 \pm 4\%$  in Stage 2 to  $59 \pm 4\%$  in Stage 3, when  $\text{NO}_3^-$  was present. Thus, we found no intrinsic barrier to full  $\text{ClO}_4^-$  reduction when  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  were being reduced in parallel. Based on qPCR targeting the reductase genes (not shown), PRB remained stable throughout, although SRB became dominant when a high concentration of  $\text{SO}_4^{2-}$  was added.

While perchlorate-reducing bacteria (PRB), assayed by qPCR targeting the *pcrA* gene (not shown), remained stable throughout, sulfate-reducing bacteria (SRB), assayed by the *dsrA* gene, became dominant (35% of total bacteria, TB) when a high concentration of  $\text{SO}_4^{2-}$  was added. Denitrifying bacteria (DB), assayed by the *nirK* and *nirS* genes, became dominant (54% of TB) in Stage 3, but SRB remained important (13% of TB).

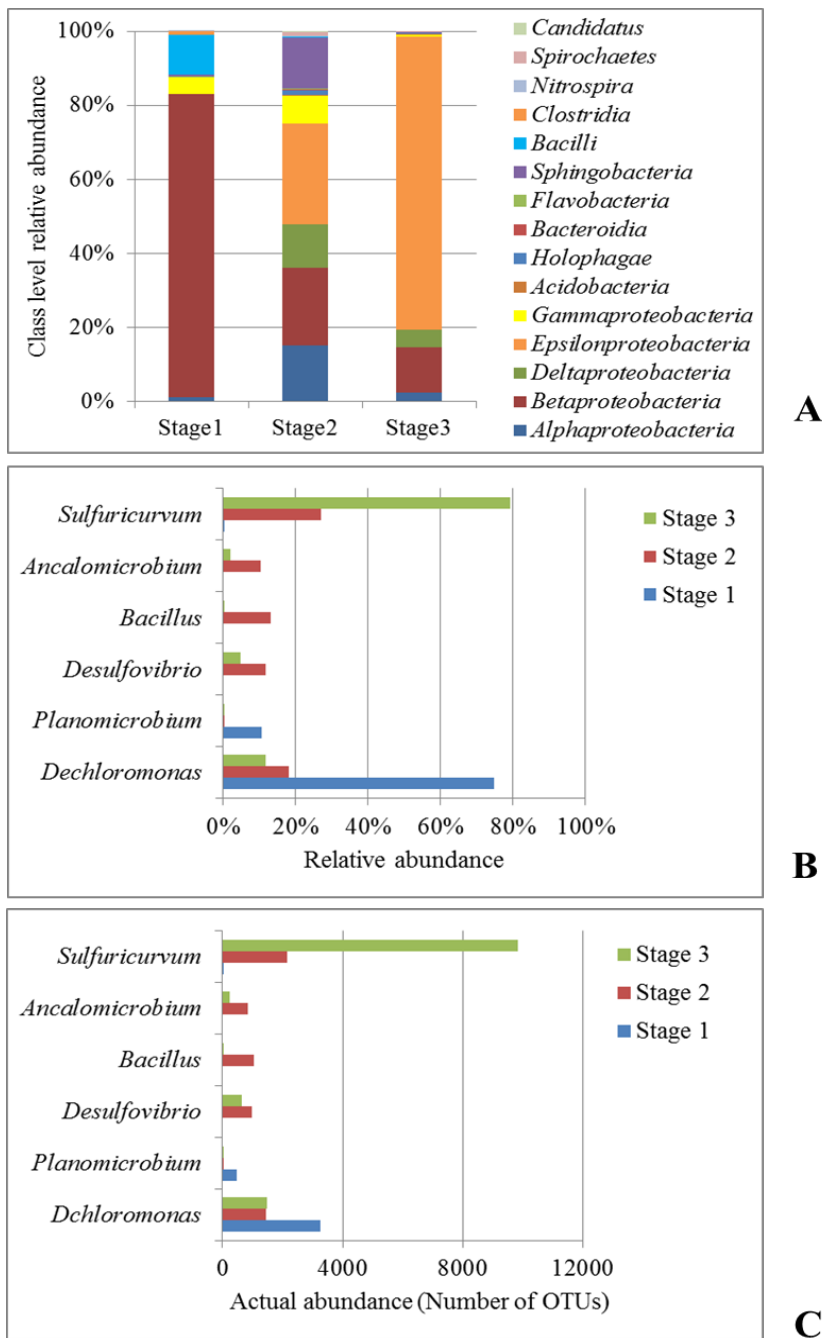
Figure 27 shows pyrosequencing results of the communities.  *$\beta$ -Proteobacteria* dominated in Stage 1, but  *$\epsilon$ -Proteobacteria* became more important in Stages 2 and 3, when the input of multiple electron acceptors favored genera with broader electron-accepting capabilities. *Sulfuricurvum* (a sulfur oxidizer and  $\text{NO}_3^-$  reducer) and *Desulfovibrio* (a  $\text{SO}_4^{2-}$  reducer) become dominant in Stage 3, suggesting redox cycling of sulfur in the biofilm. Redox cycling probably involved  $\text{SO}_4^{2-}$  reduction to elemental sulfur ( $\text{S}^0$ ) and sulfide ( $\text{S}^{2-}$ ) by SRB and  $\text{S}^0$  oxidation by *Sulfuricurvum*.

In summary, we demonstrated that it was possible to achieve  $\mu\text{g/L}$  concentrations of  $\text{ClO}_4^-$  in parallel to reductions of  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ . The  $\text{NO}_3^-$  flux was  $0.25 \text{ g H}_2/\text{m}^2\text{-day}$ , the  $\text{NO}_3^- + \text{O}_2 + \text{ClO}_4^-$  flux was  $0.34 \text{ g H}_2/\text{m}^2\text{-day}$ , the  $\text{SO}_4^{2-}$  flux was as high as  $0.25 \text{ g H}_2/\text{m}^2\text{-day}$ , and the total  $\text{H}_2$  flux (up to  $0.59 \text{ g H}_2/\text{m}^2\text{-day}$ ) was less than the  $\text{H}_2$ -delivery capacity of the composite fibers. *Dechloromonas* appeared to be the main PRB, and having multiple electron donors present favored the genera with broader electron-accepting capabilities (e.g., *Sulfuricurvum* and *Dechloromonas*) in Stage 3.



**Figure 26.** (A) Perchlorate, sulfate, and nitrate concentrations in the MBfR. (B) Perchlorate, sulfate and nitrate removal percentages.





**Figure 27.** (A) Class-level abundances based on pyrosequencing from the MBfR biofilms (X stands for unclassified by class). (B) Relative abundance (%) of the predominant bacterial genera in the MBfR biofilms. (C) Actual abundance (Number of OTUs) of the predominant bacterial genera in the MBfR biofilms. (Sequences that are 5% or less divergent to each other were combined as one OTU.)

## 6.6 Two-Stage MBfR to Achieve Full Perchlorate Reduction with Rialto's Groundwater

Zhao, H.-P., A. Ontiveros-Valencia, Y. Tang, B.-O. Kim, Z.-E. Ilhan, R. Krajmalnik-Brown, and B. E. Rittmann (2013a). Using a two-stage hydrogen-based membrane biofilm reactor (MBfR) to achieve complete perchlorate reduction in the presence of nitrate and sulfate. *Environ. Sci. Technol.* **47**: 1565 – 1572.

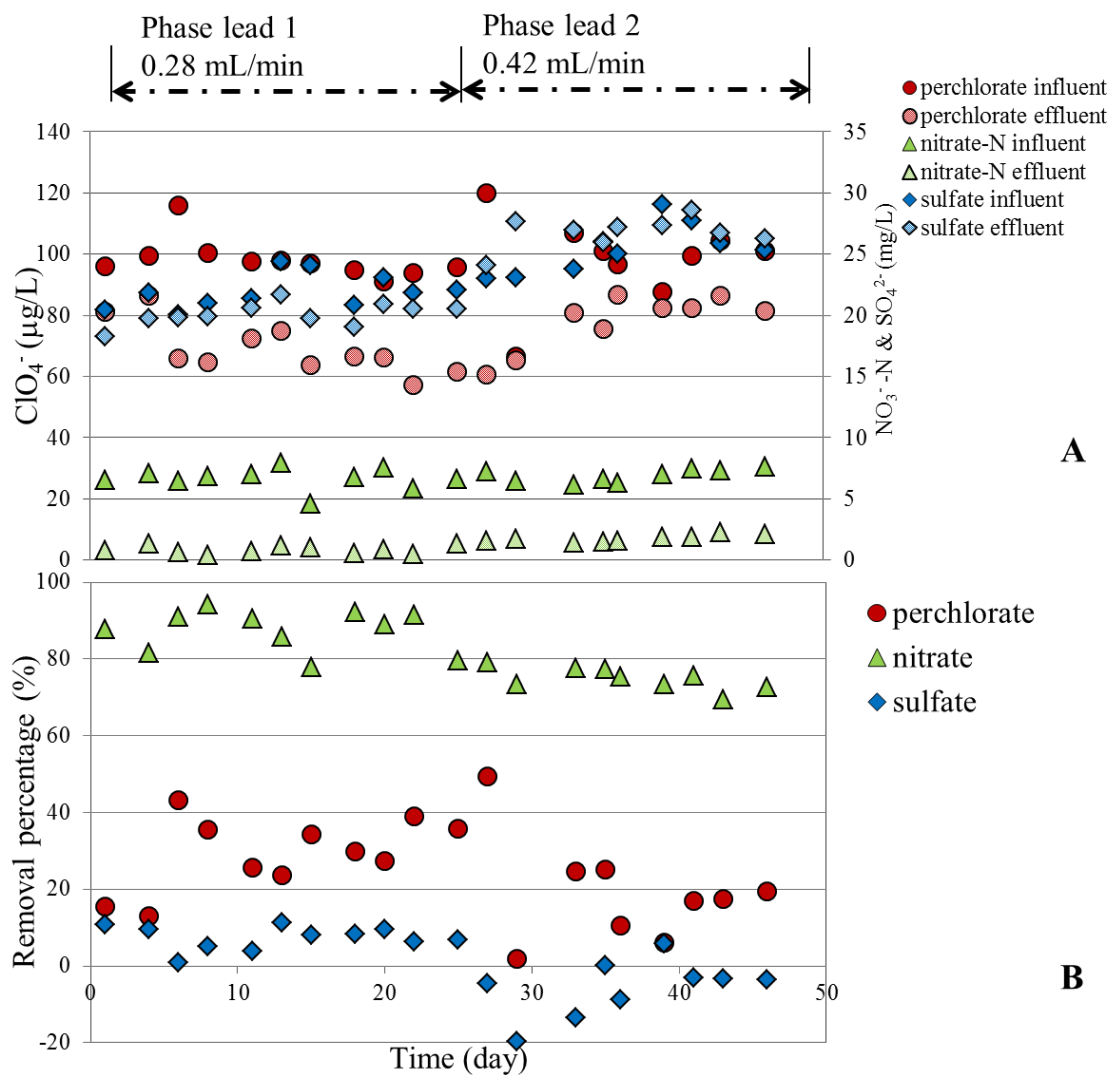
This work evaluated a two-stage strategy for achieving complete reduction of  $\text{ClO}_4^-$  without  $\text{SO}_4^{2-}$  reduction in a  $\text{H}_2$ -based MBfR treating the Rialto groundwater, in which  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  are present in much higher concentrations than  $\text{ClO}_4^-$ . This study was set up specifically to help understand why the field results did not show full  $\text{ClO}_4^-$  reduction and to find ways to overcome the roadblock to full  $\text{ClO}_4^-$  reduction. Furthermore, our preliminary results with one stage-treatment did not lead to full  $\text{ClO}_4^-$  reduction in a bench-scale MBfR treating a groundwater having 10 mg/L  $\text{ClO}_4^-$  (Ontiveros-Valencia et al., 2013b).

As shown in Figures 28 and 29, we achieved full  $\text{ClO}_4^-$  reduction in the Rialto groundwater (i.e., in the presence of much higher concentrations of  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$ ) by using a two-stage MBfR with controlled  $\text{NO}_3^- + \text{O}_2$  surface loadings and polypropylene fibers. With a  $\text{NO}_3^- + \text{O}_2$  surface loading larger than 0.6 g N/m<sup>2</sup>-d (0.21 g  $\text{H}_2$ /m<sup>2</sup>-day), the lead MBfR removed about 80% of  $\text{NO}_3^-$  and 30% of  $\text{ClO}_4^-$ . This decreased the surface loading of  $\text{NO}_3^- + \text{O}_2$  to 0.3–0.5 g N/m<sup>2</sup>-d (0.11 – 0.18 g  $\text{H}_2$ /m<sup>2</sup>-day) for the lag MBfR, in which  $\text{ClO}_4^-$  was reduced to non-detectable concentrations.  $\text{SO}_4^{2-}$  reduction also was eliminated without compromising full  $\text{ClO}_4^-$  reduction using a higher flow rate that gave a  $\text{NO}_3^- + \text{O}_2$  surface loading of 0.94 g N/m<sup>2</sup>-d (0.34 g  $\text{H}_2$ /m<sup>2</sup>-day) in the lead MBfR and 0.53 g N/m<sup>2</sup>-d (0.18 g  $\text{H}_2$ /m<sup>2</sup>-day) in the lag MBfR.

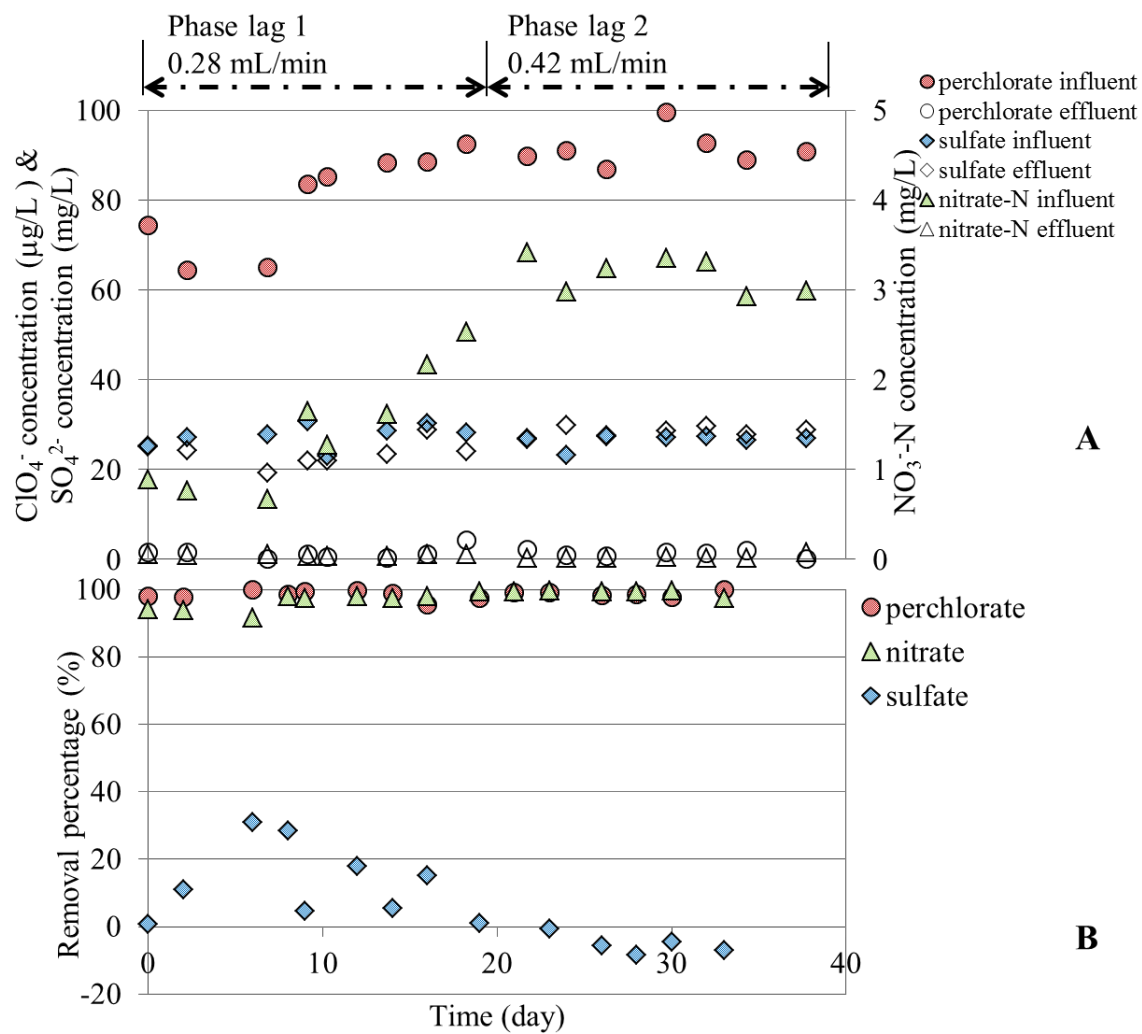
Results from qPCR (not shown) and pyrosequencing (Figure 30) show that the lead and lag MBfRs had distinctly different microbial communities. DB, quantified using the *nirS* and *nirK* genes, dominated the biofilm in the lead MBfR, but PRB, quantified using the *pcrA* gene, became more important in the lag MBfR. The facultative anaerobic bacteria *Dechloromonas*, *Rubrivivax*, and *Enterobacter* were dominant genera in the lead MBfR, where their main function was to reduce  $\text{NO}_3^-$ . With a modest  $\text{NO}_3^-$  surface loading and full  $\text{ClO}_4^-$  reduction, the dominant genera shifted to *Sphaerotilus*, *Rhodocyclaceae*, and *Rhodobacter* in the lag MBfR. *Dechloromonas* was present in the lag MBfR, but not dominant. The difference in community structures probably was related to the fact that the lead and lag positions were not switched. For the pilot MBfRs, the microbial communities were similar for the lead and lag MBfRs, probably due to regular switching. Another difference is that the influent to the lag MBfR in our study was oxygenated.

These results show that it was possible to obtain essentially 100% reduction of  $\text{ClO}_4^-$  treating the Rialto groundwater when the electron-acceptor surface loadings were properly managed: lag-MBfR's  $\text{NO}_3^- + \text{O}_2$  loading  $\leq \sim 0.5$  g N/m<sup>2</sup>-day, or 0.18 g  $\text{H}_2$ /m<sup>2</sup>-day. Furthermore, it was possible to attain this good result without incurring  $\text{SO}_4^{2-}$  reduction with the  $\text{NO}_3^- + \text{O}_2$  loading  $\geq 0.18$  g  $\text{H}_2$ /m<sup>2</sup>-day to the lag MBfR. Finally, it was not necessary to have the

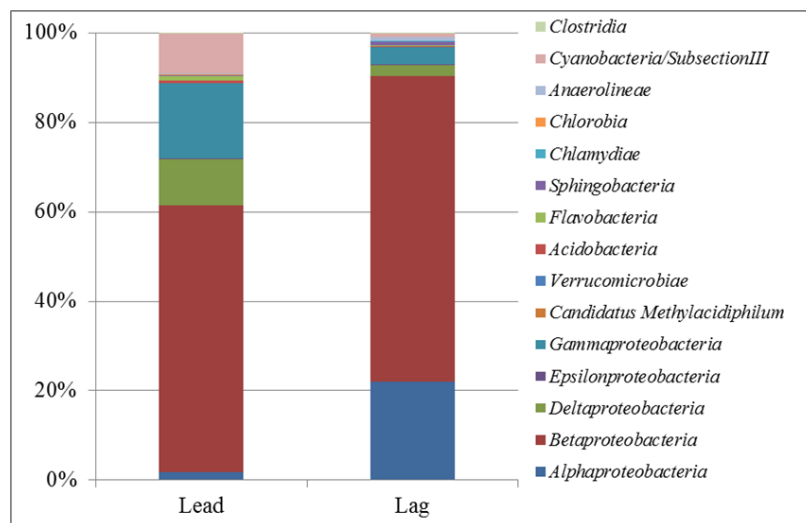
biofilm dominated by *Dechloromonas* to achieve complete  $\text{ClO}_4^-$  reduction in the lag MBfR. These findings suggest that an “ideal”  $\text{NO}_3^- + \text{O}_2$  loading to a lag MBfR is  $\sim 0.18 \text{ g H}_2/\text{m}^2\text{-day}$  to suppress  $\text{SO}_4^{2-}$  reduction while encouraging  $\text{ClO}_4^-$  reduction. In this study, the  $\text{NO}_3^- + \text{O}_2$  surface loading was controlled by  $\text{O}_2$ , since the lead MBfR removed most of the  $\text{NO}_3^-$ . This illustrates the value of using  $\text{O}_2$  addition to the second stage, a practice not utilized for the field study.



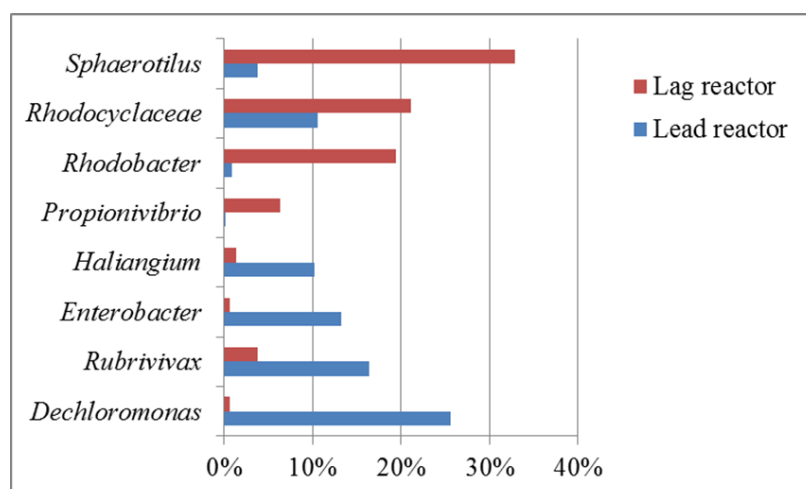
**Figure 28.** Concentrations and removals of  $\text{NO}_3^-$ ,  $\text{ClO}_4^-$ , and  $\text{SO}_4^{2-}$  in the lead MBfR.



**Figure 29.** Concentrations and removals of  $\text{NO}_3^-$ ,  $\text{ClO}_4^-$ , and  $\text{SO}_4^{2-}$  in the lag MBfR.



A



B

**Figure 30.** Microbial community structure in the MBfRs for steady state at the flow rate of 0.28 mL/min. The top panel is the distribution of bacteria at the class level. The bottom panel is the distribution of the most common genera.

## Chapter 7. Exploring Mechanisms and Performance with Mechanistic Modeling

In parallel to the bench-scale experiments, the ASU team developed and applied a series of mechanistic mathematical models. The primary objective of the mathematical modeling was to integrate and quantify the many ecological, kinetic, and transport mechanisms occurring when  $H_2$  is delivered to a biofilm of bacteria exposed to multiple electron acceptors. We developed two models having different combinations of electron acceptors, but a common framework. One model described  $NO_3^-$  and  $ClO_4^-$  reductions occurring in a  $H_2$ -based biofilm. The second model represented  $NO_3^-$  and  $SO_4^{2-}$  reductions. The models were published in three manuscripts that are summarized in this section.

### 7.1 Developing the Multi-Species Model for a $H_2$ -Based Biofilm Able to Do $NO_3^-$ and $ClO_4^-$ Reductions

Tang, Y. H. P., Zhao, A. K. Marcus, and B. E. Rittmann (2012c). A steady-state biofilm model for simultaneous reduction of nitrate and perchlorate -- Part 1: model development and numerical solution. *Environ. Sci. Technol.* **46**: 1598 – 1607.

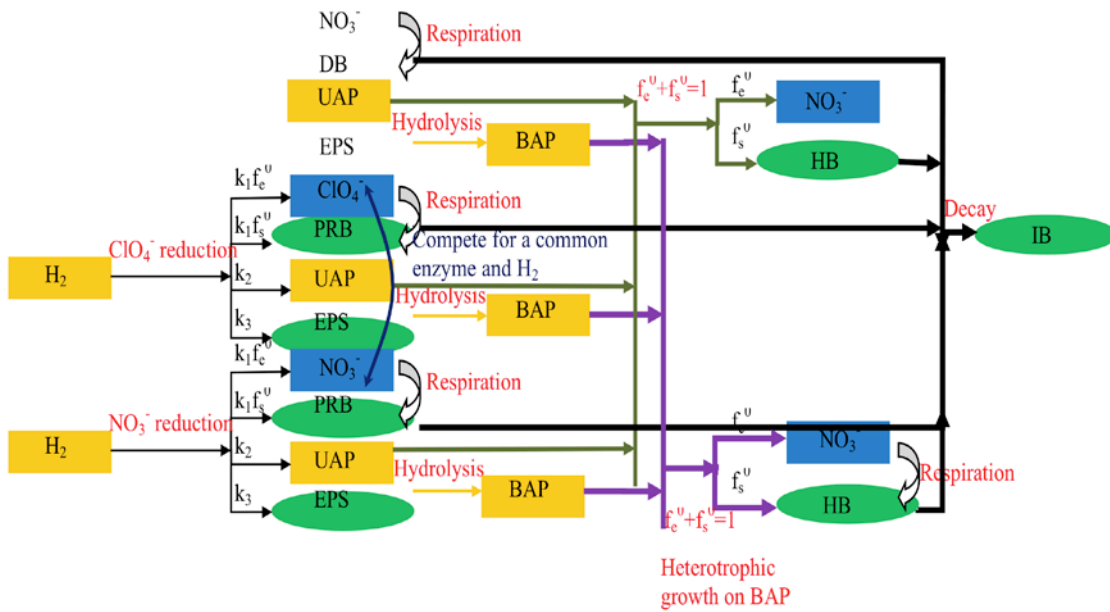
We developed a multispecies biofilm model for simultaneous reduction of  $NO_3^-$  and  $ClO_4^-$  in the biofilm of a  $H_2$ -based MBfR. The one-dimension model includes dual-substrate Monod kinetics for a steady-state biofilm with five solid and five dissolved components. The solid components are autotrophic DB, autotrophic PRB, heterotrophic bacteria (HB), inert biomass (IB), and EPS. The dissolved components are  $NO_3^-$ ,  $ClO_4^-$ ,  $H_2$ , substrate-utilization-associated products (UAP), and biomass-associated products (BAP). The structure of the model is illustrated in Figure 31.

The model explicitly considers four mechanisms involved in how three controllable operating conditions ( $H_2$  pressure,  $NO_3^-$  loading, and  $ClO_4^-$  loading) affect  $NO_3^-$  and  $ClO_4^-$  removals: 1) competition for  $H_2$ , 2) promotion of PRB (such as *Dechloromonas*) due to having two electron acceptors ( $NO_3^-$  and  $ClO_4^-$ ), 3) competition between  $NO_3^-$  and  $ClO_4^-$  reductions for the same resources in the biofilm (electrons and enzymes), and 4) competition for space in the biofilm. Two other special features of the model are having  $H_2$  delivered from the membrane substratum and solving directly for steady state using a novel three-step approach: finite-difference for approximating partial differential and/or integral equations, Newton-Raphson for solving non-linear equations, and an iterative scheme to obtain the steady-state biofilm thickness.

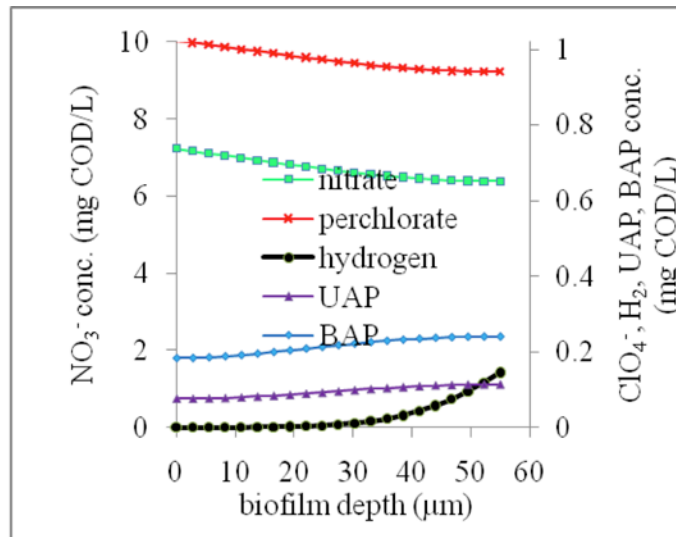
An example result illustrates the model's features. Figure 32 presents profiles of the five soluble components and distributions of the five solid components in the biofilm, which has a thickness of 55  $\mu m$  at steady state for this example. The  $NO_3^-$  and  $ClO_4^-$  concentrations are higher on the liquid side, while the  $H_2$  concentration is higher on the membrane side, but essentially zero on the liquid side. The latter demonstrates that  $H_2$  delivery is limiting inside the biofilm. Because UAP and BAP are produced in the biofilm and diffuse into the liquid,

their concentrations are higher on the membrane side. Competition for space and  $H_2$  results in non-uniform biomass distributions inside the biofilm. The fraction of DB and PRB are higher on the membrane side, because  $H_2$  is limiting and depleted before the outside of the biofilm. In this example, the fraction of PRB is much higher than that of DB; this is due to relatively low nitrate and perchlorate concentrations in the biofilm. Low  $NO_3^-$  and  $ClO_4^-$  concentrations mean weak competition for a common enzyme and for electrons from  $H_2$  in PRB; thus, PRB can grow faster than DB, since PRB can use nitrate and perchlorate for growth. This promotion effect allows PRB to outcompete DB for  $H_2$  and space in the biofilm.

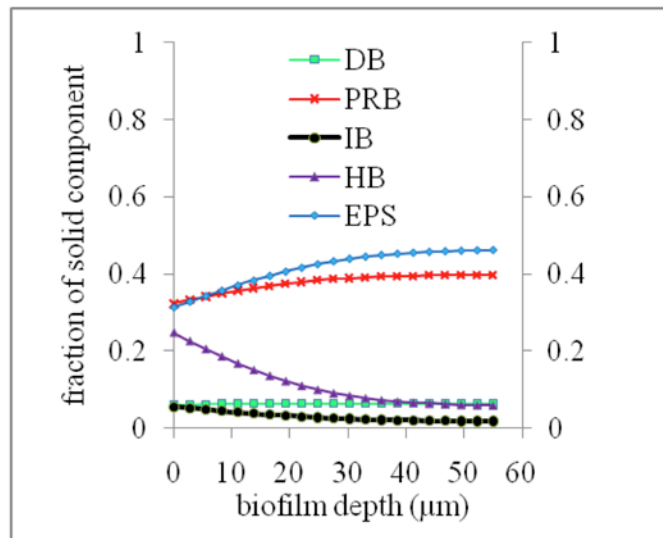
Consistent with our measurements from the side reactors of the field pilots, EPS comprised the largest fraction of the biomass, up to 50% near the membrane. According to the model, EPS are higher on the membrane side, since most EPS are produced by DB and PRB. HB also are significant (up to 24%), and they are higher on the liquid side of the biofilm, because competition from DB and PRB is lessened due to depleted  $H_2$ . IB is a low fraction (< 7%), but is denser near the outer surface, where  $H_2$  is depleted so that competition from DB and PRB is lessened.



**Figure 31.** Schematic describing how the dissolved components (rectangles) and solid components (ellipses) interact.



a.



b.

**Figure 32.** Substrate profiles and biomass distributions in the example. The liquid is to the left, and the membrane is to the right. The thickness of the biofilm is shown by the extent of the symbols to the right.

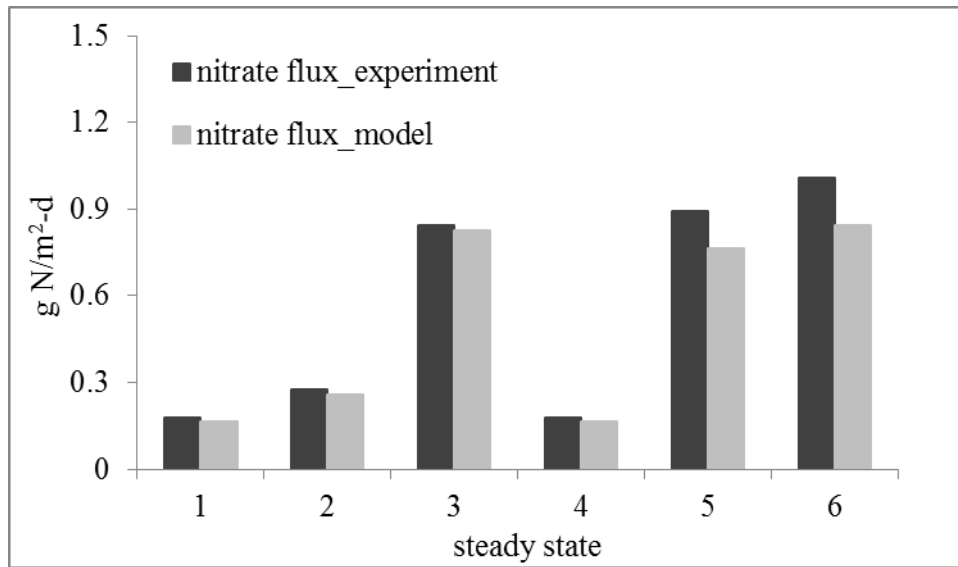


## 7.2 Application of the $\text{NO}_3^-$ and $\text{ClO}_4^-$ Model to MBfR Performance

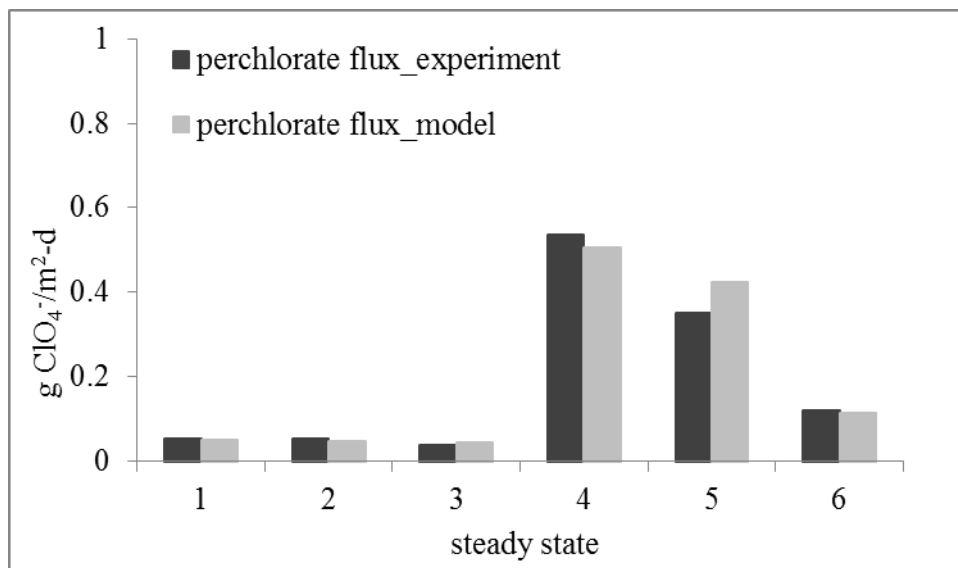
Tang, Y., H. P., Zhao, A. K. Marcus, R. Krajmalnik-Brown, and B. E. Rittmann (2012d). A steady-state biofilm model for simultaneous reduction of nitrate and perchlorate -- Part 2: parameter optimization and results and discussion. *Environ. Sci. Technol.* **46**: 1608 – 1615.

Part 1 developed a steady-state, multispecies biofilm model for simultaneous reductions of  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  in the  $\text{H}_2$ -based MBfR and presented a novel method to solve it. In Part 2, we optimize the half-maximum-rate concentrations and inhibition coefficients of  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  by fitting data from experiments with different combinations of influent  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  concentrations. Figure 33 compares the model-generated and experimental values (from the bench-scale studies of Zhao et al. (2011) (reported above)) for  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  fluxes, along with the optimized parameter values. The model accurately captured the experimental trends for steady states that had high versus low fluxes of  $\text{NO}_3^-$  and  $\text{ClO}_4^-$ .

We next used the model with optimized parameters to quantitatively and systematically explain how three important operating conditions ( $\text{NO}_3^-$  loading,  $\text{ClO}_4^-$  loading, and  $\text{H}_2$  pressure) affect  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  reductions and biomass distributions in the biofilm.  $\text{ClO}_4^-$  reduction and accumulation of PRB in the biofilm are affected by four promotion or inhibition mechanisms: (1) simultaneous use of  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  by PRB, and competition for (2)  $\text{H}_2$ , (3) the same enzymes in PRB, and (4) space in a biofilm. Table 4 summarizes the patterns identified by the model. For the  $\text{H}_2$  pressure evaluated experimentally, a low  $\text{NO}_3^-$  loading ( $< 0.1 \text{ g N/m}^2\text{-day}$ , or  $< 0.036 \text{ g H}_2/\text{m}^2\text{-day}$ ) slightly promotes  $\text{ClO}_4^-$  removal, because of the beneficial effect from PRB using both acceptors. However, a  $\text{NO}_3^-$  loading  $> 0.6 \text{ g N/m}^2\text{-day}$  ( $0.21 \text{ g H}_2/\text{m}^2\text{-day}$ ) begins to inhibit  $\text{ClO}_4^-$  removal, as the competition effects become dominant. Therefore, the  $\text{NO}_3^-$  loading needs to be held below about  $0.2 \text{ g H}_2/\text{m}^2\text{-day}$  to make excellent  $\text{ClO}_4^-$  reduction feasible. This result is coincident with the two-stage bench-scale results of Zhao et al. (presented above). The field pilot had fluxes of  $0.02 - 0.18 \text{ g H}_2/\text{m}^2\text{-day}$  in the lag MBfR, but it differed from the bench-scale study in that the lead and lag MBfRs were switched in the field study, the lag MBfR had  $\text{SO}_4^{2-}$  reduction, and dissolved oxygen was introduced to the lag reactor in the laboratory study.



a.



b.

**Figure 33.** Comparison of fluxes of  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  from the experiments and from the model with optimized parameters ( $K_1 = K_{21} = 0.2 \text{ mg N/L}$  and  $K_{22} = 0.8 \text{ mg ClO}_4^-/\text{L}$ ).

**Table 4** The Mechanistic Effects of Nitrate Loading on PRB and Perchlorate Removal

| Nitrate-Loading Situation                                                                       | Promotion Mechanism                                                   | Inhibition Mechanisms                              |                                   |                            | Net Effect        |
|-------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|----------------------------------------------------|-----------------------------------|----------------------------|-------------------|
|                                                                                                 | 2<br>Using $\text{NO}_3^-$ and $\text{ClO}_4^-$ as electron acceptors | 3<br>Competition for the same resources within PRB | 1<br>Competition for $\text{H}_2$ | 4<br>Competition for space |                   |
| <b>1</b><br>< 0.1 g N/m <sup>2</sup> -day<br>(< 0.036 g H <sub>2</sub> /m <sup>2</sup> -day)    | +                                                                     | +                                                  | No                                | +                          | promotion         |
| <b>2</b><br>0.1-0.6 g N/m <sup>2</sup> -d<br>(0.036-0.22 g H <sub>2</sub> /m <sup>2</sup> -day) | ++                                                                    | ++                                                 | No                                | ++                         | no effect         |
| <b>3</b><br>0.6-1.0 g N/m <sup>2</sup> -d<br>(0.22-0.36 g H <sub>2</sub> /m <sup>2</sup> -day)  | +++                                                                   | +++                                                | No                                | +++                        | inhibition        |
| <b>4</b><br>>1.0 g N/m <sup>2</sup> -d<br>(>0.36 g H <sub>2</sub> /m <sup>2</sup> -day)         | ++++                                                                  | ++++                                               | Yes                               | ++++                       | strong inhibition |

Notes: +: very weak effect; ++: weak effect; +++: strong effect; ++++: very strong effect. The effects are qualitative.

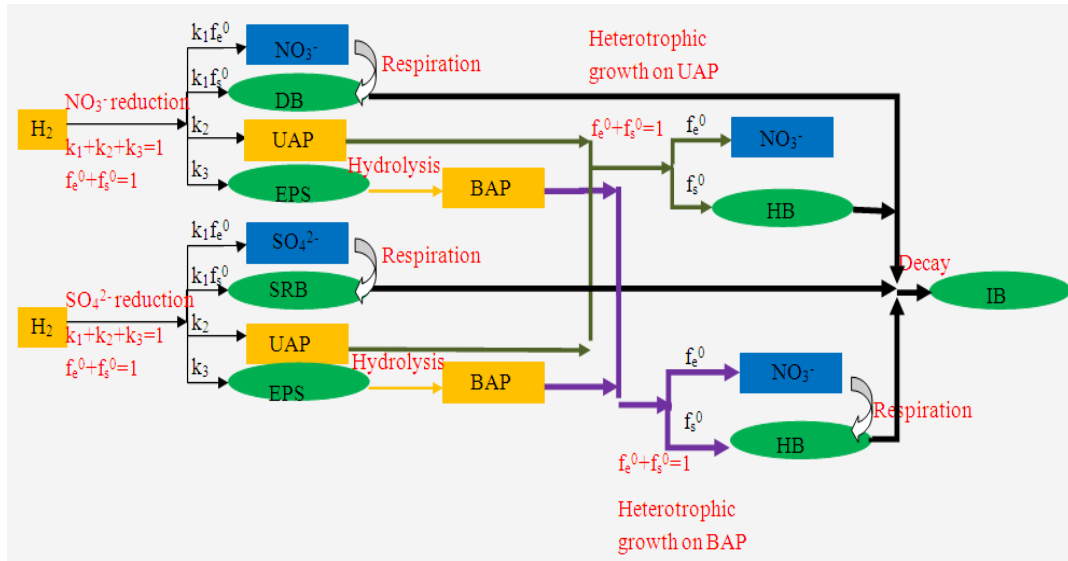
### 7.3 A Model for $\text{NO}_3^-$ and $\text{SO}_4^{2-}$ Reductions in the MBfR Biofilm

Tang, Y., A. Ontiveros-Valencia, L. Feng, C. Zhou, R. Krajmalnik-Brown, and B. E. Rittmann (2012a). A biofilm model to understand the onset of sulfate reduction in denitrifying membrane biofilm reactors. *Biotechnol. Bioengr.* 110: 763 – 772.

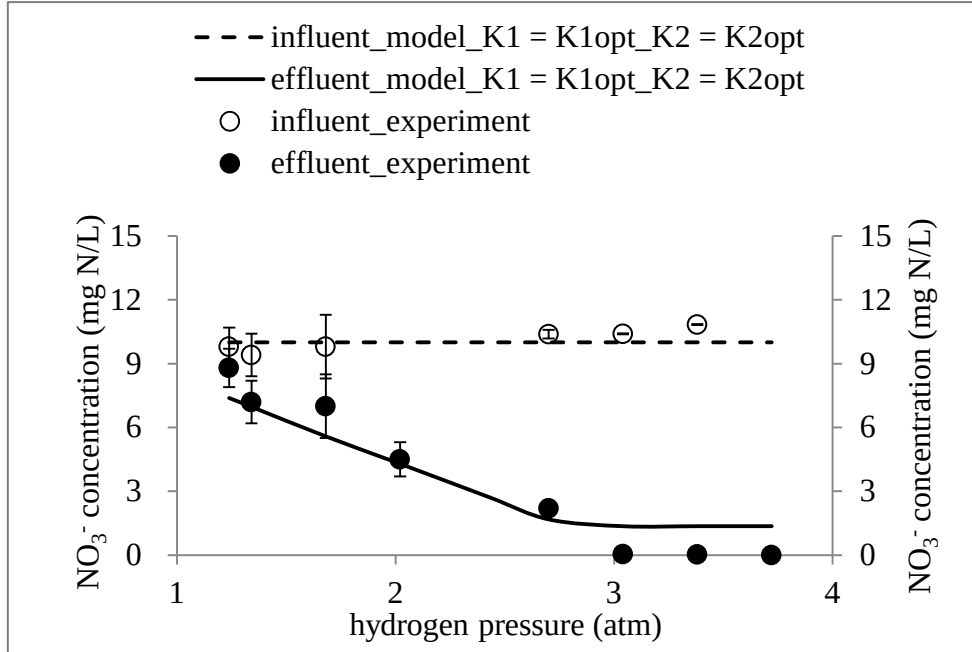
This work developed a multispecies biofilm model that describes the co-existence of DB and SRB in the  $\text{H}_2$ -based MBfR. The model is based upon the same framework as the model for  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  reductions (described in the previous two sections), but considers the unique metabolic and physiological characteristics of autotrophic SRB that use  $\text{H}_2$  as their electron donor. The framework is illustrated in Figure 34. A critical feature is that the SRB do not reduce  $\text{NO}_3^-$ , while the DB do not reduce  $\text{SO}_4^{2-}$ . This differs from the case with PRB, which are able to reduce  $\text{NO}_3^-$  and  $\text{ClO}_4^-$ .

To evaluate the model, we simulated effluent  $\text{H}_2$ , UAP, and BAP concentrations, along with biomass distributions in the biofilm. We compared all of these results to chemical analyses and qPCR data from the experiments on Ontiveros-Valencia et al. (2012) (reported above). Figure 35 shows that model outputs and experimental results match for all major trends and explain when  $\text{SO}_4^{2-}$  reduction does or does not occur in parallel with denitrification. Figure 35b indicates that  $\text{SO}_4^{2-}$  reduction is suppressed when all of the  $\text{H}_2$  is consumed inside the biofilm (Fig. 34). In accordance with the experimental results of Ontiveros-Valencia et al. (2012),  $\text{SO}_4^{2-}$  reduction could be suppressed with a  $\text{NO}_3^-$  surface loading that is  $0.4 \text{ g H}_2/\text{m}^2\text{-day}$  when  $\text{H}_2$  delivery is limiting. Mechanistically, the model tells us that the onset of  $\text{SO}_4^{2-}$  reduction occurs only when the  $\text{NO}_3^-$  concentration at the fiber's outer surface is low enough so that the growth rate of the denitrifying bacteria is equal to that of the SRB. The model also predicts that effluent UAP and BAP increase noticeably when  $\text{SO}_4^{2-}$  reduction begins (Fig. 36). The increase of UAP and BAP translates into a rise of the DOC concentration, a factor observed in the field pilot study, when  $\text{SO}_4^{2-}$  reduction was significant (Evans et al., 2013).

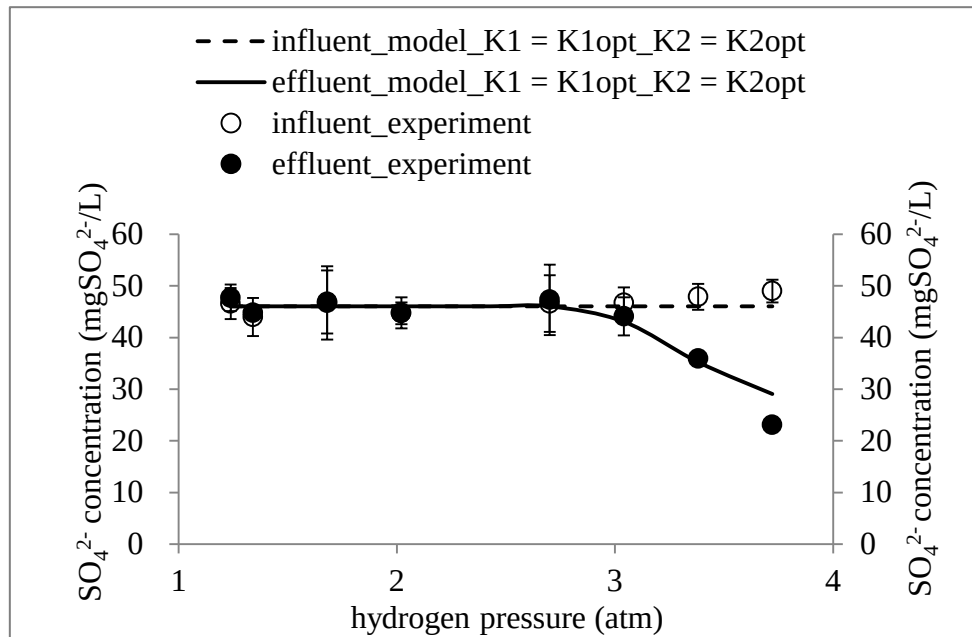
An example shows how to use the model to design an MBfR that achieves satisfactory  $\text{NO}_3^-$  reduction, but suppresses  $\text{SO}_4^{2-}$  reduction. In Figure 37, good  $\text{NO}_3^-$  reduction is possible without  $\text{SO}_4^{2-}$  reduction when the flow rate is around  $10 \text{ m}^3/\text{day}$ , which corresponds to  $\text{NO}_3^-$  surface loadings of approximately  $0.94 \text{ g N}/\text{m}^2\text{-day}$  or  $0.33 \text{ g H}_2/\text{m}^2\text{-day}$ . The effluent  $\text{NO}_3^-$  concentration increases for higher flow rates and  $\text{NO}_3^-$  surface loadings, reaching the 10-mg N/L MCL at around  $1.5 \text{ g N}/\text{m}^2\text{-day}$  ( $\text{NO}_3^-$  surface flux of about  $0.5 \text{ g H}_2/\text{m}^2\text{-day}$ ).



**Figure 34.** Schematic describing how the dissolved components (rectangles) and solid components (ellipses) interact for the biofilm model with  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  reductions.

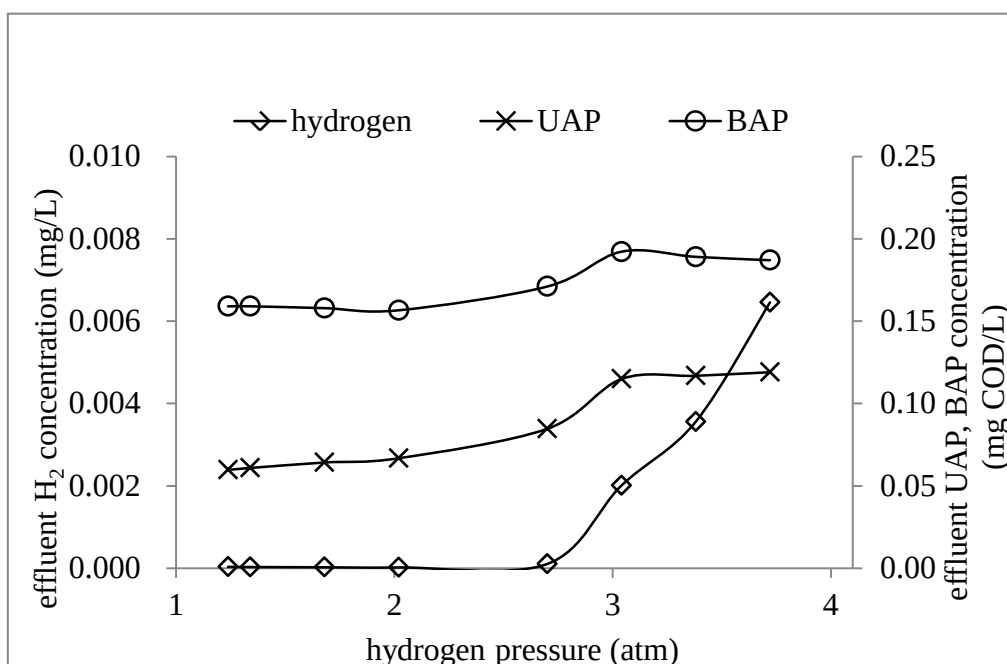


a.

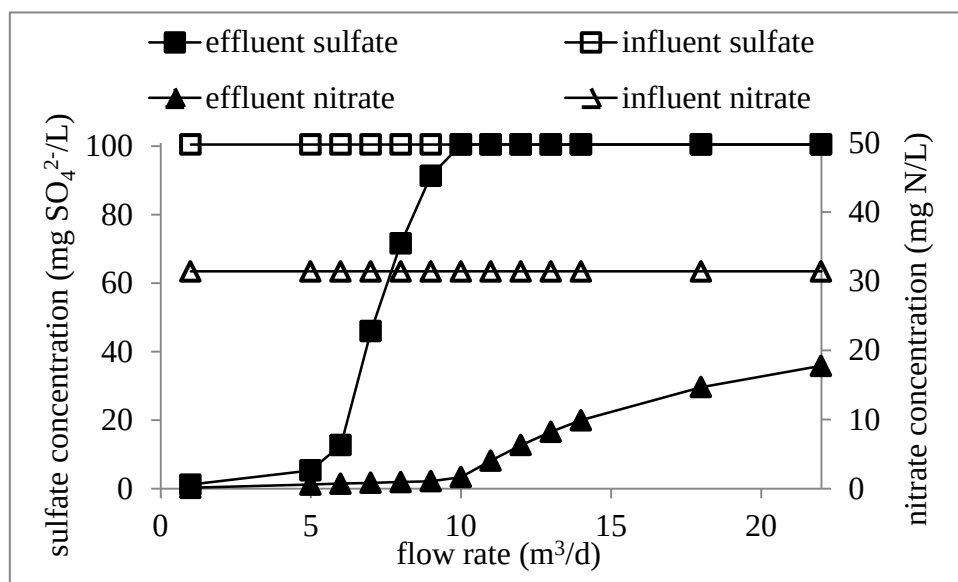


b.

**Figure 35.** Comparison of effluent  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  concentrations from the experiment (symbols) and from the model (lines) with optimized parameters ( $K_1 = 0.2$  mg N/L and  $K_2$  equals 1.6 mg  $\text{SO}_4^{2-}$ /L).



**Figure 36.** Model-simulated concentrations of H<sub>2</sub>, UAP, and BAP in the effluent.



**Figure 37.** Model-simulated effluent NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup> concentrations at flow rates of 1 to 22 m<sup>3</sup>/d in a module having 320 m<sup>2</sup> of fiber surface area.

## Chapter 8. Special Modeling Evaluation of the Pilot MBfRs

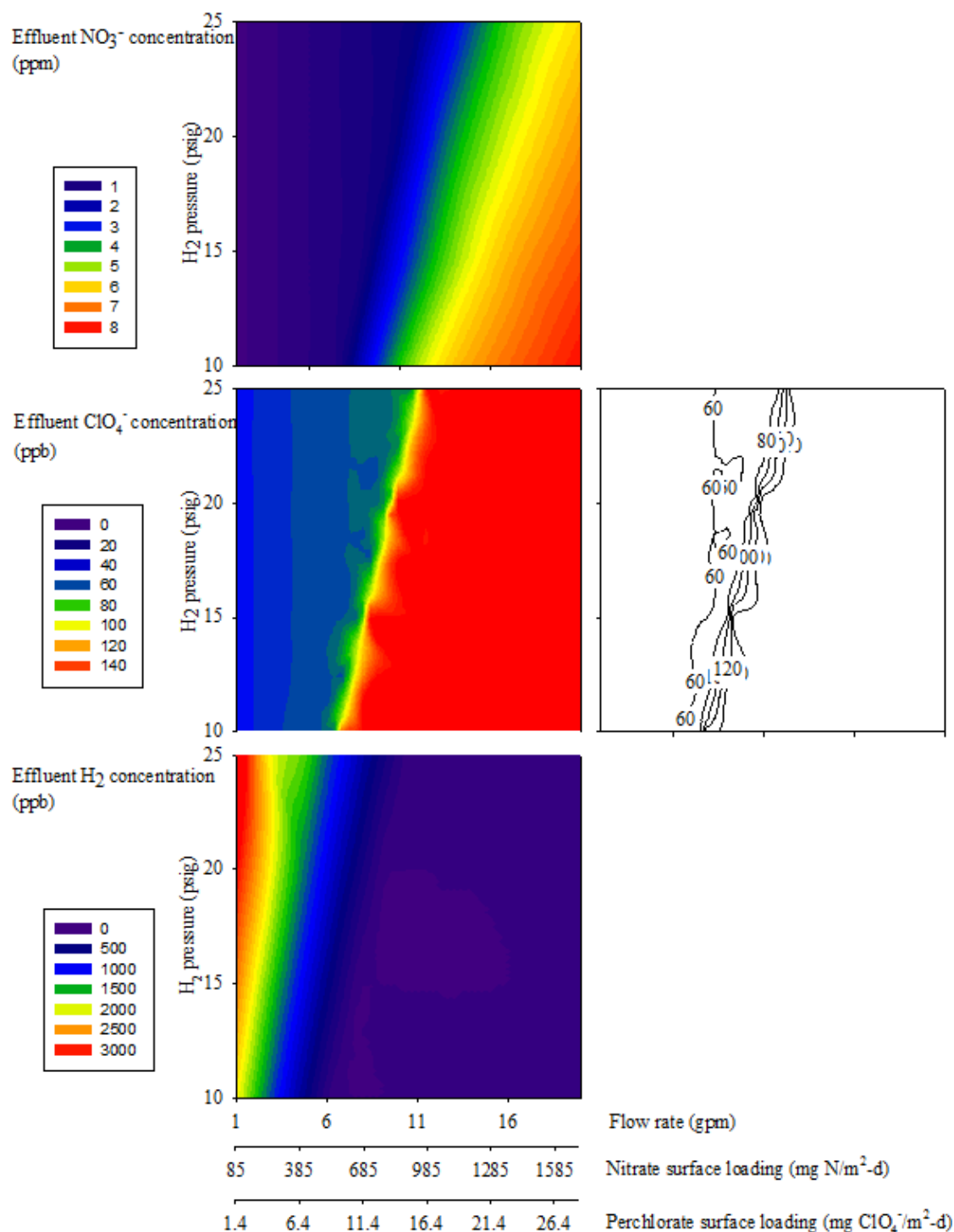
We applied the  $\text{NO}_3^- + \text{ClO}_4^-$  model of Tang et al. (2012c,d) (reviewed previously) to the conditions of the Rialto pilot MBfRs. Here, we summarize the main modeling results in ways that help explain why pilot-MBfR performance changed with different conditions. In particular, we investigate why the  $\text{ClO}_4^-$  concentration could not be reduced consistently to below 6  $\mu\text{g/L}$  in the pilot-scale MBfR.

Figures 38 and 39 plot simulated effluent  $\text{NO}_3^-$ ,  $\text{ClO}_4^-$ , and  $\text{H}_2$  concentrations relevant to the pilot-scale lead and lag MBfRs, respectively. Operating conditions are identified in the figure captions. The simulated operating conditions cover the range used in the pilot test. Extreme operating conditions are not simulated, as they are not relevant in practice or to the pilot experiments.

Important features to see while comparing the modeling results for the lead MBfR (Figure 38) to experimental results are:

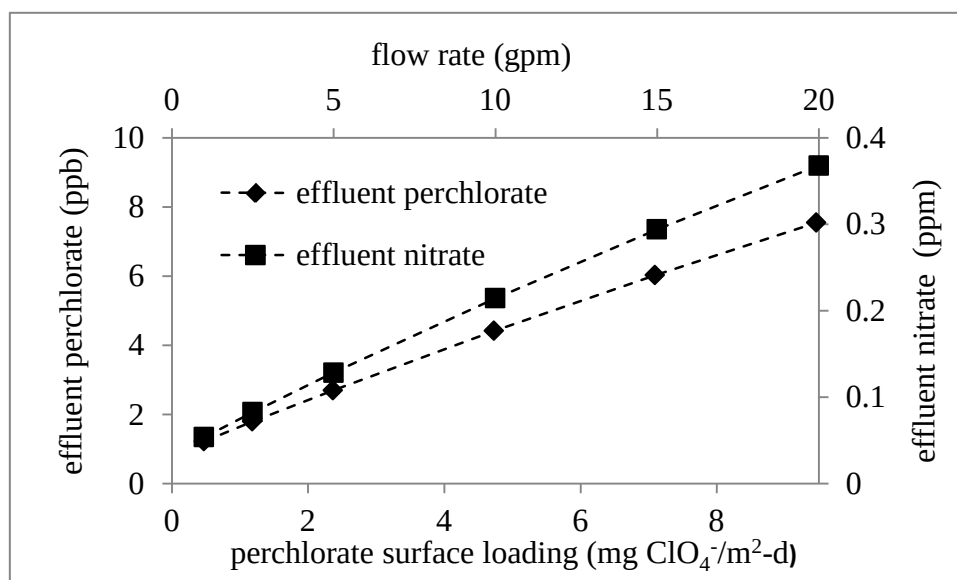
- 1) The  $\text{ClO}_4^-$  panel can be divided into three areas:
  - a) Blue area, in which, the effluent  $\text{ClO}_4^-$  is stable at  $\sim 50 \mu\text{g/L}$ . For the simulated conditions, the lead MBfR never takes the  $\text{ClO}_4^-$  concentration below about 50  $\mu\text{g/L}$ , because the half-maximum-rate concentration for perchlorate is 780  $\mu\text{g/L}$  in the model simulation, and this gives an  $S_{\min}$  value of 56  $\mu\text{g/L}$ . Since the flow rate is used to control the  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  surface loadings, they are proportional to each other. We see that stable low  $\text{ClO}_4^-$  concentration begins when the effluent  $\text{NO}_3^-$  concentration is less than  $\sim 1 \text{ mg N/L}$  and when the  $\text{H}_2$  concentration is more than  $\sim 500 \mu\text{g/L}$ . Smaller  $\text{NO}_3^-$  and larger  $\text{H}_2$  concentrations (due to lower flow rates) do not enhance  $\text{ClO}_4^-$  removal further. The border for entering the blue region is a  $\text{NO}_3^-$  surface loading of 0.5 to 0.7  $\text{g N/m}^2\text{-day}$  (0.18 to 0.25  $\text{g H}_2/\text{m}^2\text{-day}$ ), depending on the  $\text{H}_2$  pressure.
  - b) Yellow area, in which the effluent perchlorate is very sensitive to  $\text{H}_2$  pressure and flow rate. If a reactor is operated at this area, effluent  $\text{ClO}_4^-$  can change significantly for small changes in flow rate or  $\text{NO}_3^-$  loading.
  - c) Red area, in which  $\text{ClO}_4^-$  reduction does not occur due to competition from  $\text{NO}_3^-$  reduction. We expect no  $\text{ClO}_4^-$  removal for  $\text{NO}_3^-$  surface loading above 0.65 to 0.9  $\text{g N/m}^2\text{-day}$  (0.24 to 0.32  $\text{g H}_2/\text{m}^2\text{-day}$ ), depending on the  $\text{H}_2$  pressure.
- 2) The pilot-scale lead MBfR was operated in either the blue area or the yellow area, depending on the hydrogen pressure and flow rate.
- 3) The effluent  $\text{H}_2$  pressure represents the potential for  $\text{SO}_4^{2-}$  reduction. Seeing non-trivial  $\text{H}_2$  signifies that  $\text{SO}_4^{2-}$  reduction is likely, because  $\text{H}_2$  remains in the biofilm and can allow SRB growth and accumulation near the substratum. In particular, the green, yellow, and red areas to the upper left of the  $\text{H}_2$  panel show where  $\text{SO}_4^{2-}$  reduction is likely in the lead MBfR; this is where the effluent  $\text{ClO}_4^-$  concentration is about 50  $\mu\text{g/L}$ .





**Figure 38.** Simulated effluent  $\text{NO}_3^-$  (top),  $\text{ClO}_4^-$  (middle left and right), and  $\text{H}_2$  (bottom) concentrations for the pilot-scale lead MBfR. The operating conditions are: fiber surface area =  $576 \text{ m}^2$ , influent DO =  $8 \text{ mg/L}$ , influent nitrate =  $9 \text{ mg N/L}$ , influent perchlorate =  $150 \text{ } \mu\text{g/L}$ ,  $\text{H}_2$  pressures =  $10\text{--}25 \text{ psig}$ , and flow rates =  $1\text{--}20 \text{ gpm}$ .

Figure 39 summarizes the model-simulated performance of a lag MBfR. The input conditions, noted in the caption, are typical of what is in the effluent of a lead MBfR and are consistent with the right edge of the blue area in the  $\text{ClO}_4^-$  plot of Figure 36. Figure 39 says that the lag MBfR ought to be able to achieve an effluent  $\text{ClO}_4^-$  concentration well below 6  $\mu\text{g/L}$  for the range of conditions evaluated, since competition with DB is much lower than for the lead MBfR. Also, the relatively low  $\text{NO}_3^-$  concentration fed to the lag reactor promotes the growth of PRB, which help reduce  $\text{ClO}_4^-$  to an even lower level (Tang et al., 2012c,d). Since the flow rate is used to control the loadings of  $\text{ClO}_4^-$  and  $\text{NO}_3^-$ , the loadings and effluent concentrations of both electron acceptors vary together with flow rate. The  $\text{NO}_3^-$  concentration remains less than 0.4 mg N/L for all flow rates, which give a  $\text{NO}_3^-$  surface loading of 0.1 to 2 g  $\text{N/m}^2\text{-day}$ , or up to 0.7 g  $\text{H}_2/\text{m}^2\text{-day}$ . The effluent  $\text{ClO}_4^-$  concentration increases from  $\leq 2 \mu\text{g/L}$  to about 6  $\mu\text{g/L}$  as the  $\text{ClO}_4^-$  loading increased from 0.5 to 7.5 mg  $\text{ClO}_4^-/\text{m}^2\text{-day}$ . Thus, the lag MBfR seems capable of producing an effluent concentration less than 6  $\mu\text{g/L}$  if sulfate reduction is minimized. This is consistent with the bench-scale results of Zhao et al. (2013a), reviewed earlier in the report.



**Figure 39.** Simulated effluent  $\text{NO}_3^-$  and  $\text{ClO}_4^-$  concentrations for the pilot-scale lag MBfR. The operating conditions are: fiber surface area = 576  $\text{m}^2$ , influent DO = 0 mg/L, influent nitrate = 2 mg N/L, influent perchlorate = 50  $\mu\text{g/L}$ ,  $\text{H}_2$  pressures = 15 psig, and flow rates = 1-20 gpm. Since  $\text{H}_2$  pressure was always not limiting in the lag reactor, only one  $\text{H}_2$  pressure is modeled. The effluent  $\text{H}_2$  concentration was > 80  $\mu\text{g/L}$ .

Because the pilot results did not achieve  $\text{ClO}_4^-$  concentrations less than 6  $\mu\text{g/L}$ , we used the model to simulate the effluent  $\text{ClO}_4^-$  concentrations for scenarios that correspond to one or more of the following possible causes of poorer  $\text{ClO}_4^-$  removal in the lag MBfR compared to model-simulated expectations:

- 1) No  $O_2$  was available in the lag MBfR's influent. Dissolved  $O_2$  is an electron acceptor for PRB, and its reduction can increase the PRB biomass.
- 2) The PRB in the pilot MBfRs have a higher half-maximum-rate concentration (K) for  $ClO_4^-$  than used in the model, which was able to simulate bench-scale results well. A higher K value slows the kinetics for  $ClO_4^-$  reduction, particularly for the desired low concentrations.
- 3) The mass-transport resistance of  $ClO_4^-$  to the biofilm is increased due to the spacers and inert biomass building up in and on the spacers. This effect is simulated by increasing the thickness of the liquid diffusion layer (L).

These conditions are outlined in Table 5, which also summarizes the results. On the one hand, scenario 1 (adding  $O_2$  to the lag-MBfR influent) brings about a significant increase in the biofilm mass, but this results in only a small increase in  $ClO_4^-$  removal. On the other hand, scenarios 2 (higher K for  $ClO_4^-$ , probably due to different microbial populations), 3 (thicker L due to spacers or the flow path), and the combination of 2 and 3 show significant negative impacts on the effluent  $ClO_4^-$  concentration. Taken alone, scenario 2 or 3 increases the simulated  $ClO_4^-$  concentration from 2.7  $\mu\text{g/L}$  to 7.0 or 7.5  $\mu\text{g/L}$ , respectively. Together, they lead to an effluent concentration of 11.4  $\mu\text{g/L}$ .

The results in Table 5 suggest that physical details of the pilot MBfRs may have led to accumulation of biomass that increased mass-transport resistance and also may have selected for somewhat less-efficient PRB. When coupled with the field data, the modeling analysis suggests that improvements to the internal configuration of the MBfR may be the most efficient strategy for improving performance when the goal is to achieve a very low effluent concentration. In particular, experiments varying the recirculation ratio in the field pilot showed that the reduction kinetics for all acceptors were sensitive to the recirculation rate (Evans et al., 2013). This is consistent with the findings in Table 5 that external mass-transport resistance could have been a key factor for why the  $ClO_4^-$  concentration could not be driven below 6  $\mu\text{g/L}$ . Likewise, the pilot MBfRs were operated with excess  $H_2$  delivery capacity and had significant  $SO_4^{2-}$  reduction, which slows  $ClO_4^-$  reduction kinetics, as shown in bench scale research by Ontiveros-Valencia et al., (2013b).

**Table 5.** Simulated scenarios to investigate the difference in perchlorate removal in a typical lag MBfR

| Simulated scenarios                                                                                                                                                                                                                                                                    | Parameters change                                                                     | Simulated effluent perchlorate ( $\mu\text{g/L}$ ) | Simulated effluent nitrate ( $\text{mg/L}$ ) | Simulated effluent hydrogen ( $\text{mg/L}$ ) | Simulated biofilm thickness ( $\mu\text{m}$ ) |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|----------------------------------------------------|----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| <b>Standard condition<sup>a</sup></b>                                                                                                                                                                                                                                                  | none                                                                                  | 2.7                                                | 0.13                                         | 2.06                                          | 13.5                                          |
| <b>1. Oxygen addition (S)</b>                                                                                                                                                                                                                                                          | S increases from 0 to 8 $\text{mg/L}$                                                 | 2.2                                                | 0.26                                         | 1.71                                          | 24.3                                          |
| <b>2. Higher half-maximum-rate concentration (K)</b>                                                                                                                                                                                                                                   | K increases from 0.8 to 4.0 $\text{mg/L}$                                             | 7.0                                                | 0.13                                         | 2.06                                          | 13.5                                          |
| <b>3. Thicker diffusion layer (L)</b>                                                                                                                                                                                                                                                  | L (liquid diffusion layer) increases from 100 to 500 $\mu\text{m}$                    | 7.5                                                | 0.37                                         | 2.03                                          | 12.4                                          |
| <b>4. Combined scenarios 2 and 3</b>                                                                                                                                                                                                                                                   | K increases from 0.8 to 4.0 $\text{mg/L}$ ; L increases from 100 to 500 $\mu\text{m}$ | 11.4                                               | 0.37                                         | 2.03                                          | 12.3                                          |
| a. The standard condition in the lag reactor of the pilot-scale MBfR: fiber surface area = 576 $\text{m}^2$ , influent DO = 0 $\text{mg/L}$ , influent nitrate = 2 $\text{mg N/L}$ , influent perchlorate = 50 $\mu\text{g/L}$ , $\text{H}_2$ pressures = 15 psig, flow rates = 5 gpm. |                                                                                       |                                                    |                                              |                                               |                                               |

## Chapter 9. Global Synthesis

The field pilot study at Rialto, CA showed that, although the two-stage MBfR system was effective for reducing  $\text{NO}_3^-$  and  $\text{ClO}_4^-$ , it did not achieve one of the goals: an effluent  $\text{ClO}_4^-$  concentration less than 6  $\mu\text{g/L}$ . Attempts to reduce the loading rates of the electron acceptors by lowering the flow rate did not lead to the desired outcome of  $\text{ClO}_4^- < 6 \mu\text{g/L}$  for sustained operation, but led to  $\text{SO}_4^{2-}$  reduction, an undesired outcome. Likewise, regularly switching the lead and lag positions did not bring about the desired result.

The ASU Team carried out a wide range of studies to uncover why the two-stage MBfR system did not achieve the 6- $\mu\text{g/L}$  goal, even though it performed well in other ways. The team carried out extensive analyses of the acceptor and donor ( $\text{H}_2$ ) fluxes during the pilot study and correlated them to a range of analyses of biofilm samples sent to ASU from Rialto. In parallel, the ASU team carried out bench-scale MBfR experiments and developed mechanistic mathematical models to identify and quantify the kinetic and ecological mechanisms underpinning the performance of the pilot and bench-scale MBfRs. The preceding sections of this report summarize all of those results.

This section synthesizes all of the results to provide a comprehensive understanding of what controlled the performance of the pilot MBfRs and what changes hold promise for improving MBfR performance when the goal is achieving a very low  $\text{ClO}_4^-$  concentration when a range of other electron acceptors is present.

The biofilms in the reactor modules in the MBfR vessels and side-reactors had consistent characteristics that reflect the operating conditions at the pilot site. While the biofilm thickness varied in the pilot MBfRs, it typically was about 200  $\mu\text{m}$ . The biofilm was only about 10% inorganic, which means that precipitation was prevented well by the pH-control system. While the biofilm contained around 40 – 50% EPS, the cells were predominantly live, particularly near the membrane substratum. For all MBfRs, PRB always made up the smallest fraction of the active bacteria, SRB always were present, and SRB became more important when  $\text{SO}_4^{2-}$  reduction was the a major sink for  $\text{H}_2$ . In the bench-scale studies done at ASU, *Dechloromonas* was an important DB and PRB when  $\text{ClO}_4^-$  reduction was successful, but it was not always the main PRB.

Pilot, bench-scale, and modeling results were consistent about the need to manage the fluxes of  $\text{NO}_3^-$ ,  $\text{O}_2$ , and  $\text{SO}_4^{2-}$  to allow for complete  $\text{ClO}_4^-$  reduction. In order to compare all fluxes, we express all of them in terms of their  $\text{H}_2$  consumption flux, or in  $\text{g H}_2/\text{m}^2\text{-day}$ .

1. According to modeling results and bench-scale experiments, a moderate flux of  $\text{NO}_3^- + \text{O}_2$  is one key to helping promote PRB and  $\text{ClO}_4^-$  reduction by giving the PRB three electron acceptors ( $\text{ClO}_4^-$ ,  $\text{NO}_3^-$ , and  $\text{O}_2$ ) without causing too much competition for  $\text{H}_2$  and space.
  - a. Modeling says that a  $\text{NO}_3^- + \text{O}_2$  flux of 0.036 to 0.21  $\text{g H}_2/\text{m}^2\text{-day}$  promotes  $\text{ClO}_4^-$  reduction, while a flux  $\geq 0.36 \text{ g H}_2/\text{m}^2\text{-day}$  begins to cause serious inhibition to  $\text{ClO}_4^-$  reduction.
  - b. With synthetic medium in a bench-scale MBfR, full  $\text{ClO}_4^-$  was possible at a  $\text{NO}_3^- + \text{O}_2$  flux up to 0.33  $\text{g H}_2/\text{m}^2\text{-day}$  when the  $\text{SO}_4^{2-}$  flux was as high as 0.25  $\text{g H}_2/\text{m}^2\text{-day}$ . It is possible the S cycling between  $\text{SO}_4^{2-}$  and  $\text{S}^0$  played a role in enhancing  $\text{ClO}_4^-$  reduction despite significant  $\text{SO}_4^{2-}$  reduction by lowering the  $\text{H}_2$  demand for  $\text{SO}_4^{2-}$  reduction.

- c. When using Rialto groundwater at the bench scale, a  $\text{NO}_3^- + \text{O}_2$  flux  $\leq 0.18 \text{ g H}_2/\text{m}^2\text{-day}$  in a lag MBfR (with polypropylene fibers) allowed full  $\text{ClO}_4^-$  reduction. A  $\text{NO}_3^- + \text{O}_2$  flux  $\geq 0.21 \text{ g H}_2/\text{m}^2\text{-day}$  allowed partial ( $\sim 30\%$ )  $\text{ClO}_4^-$  reduction in the same Rialto groundwater in the lead MBfR.
  - d. For comparison, the pilot MBfRs gave the highest  $\text{ClO}_4^-$  flux in the lag MBfR when the  $\text{NO}_3^- + \text{O}_2$  flux was  $\sim 0.17 \text{ g H}_2/\text{m}^2\text{-day}$ , which is in the range that modeling and bench-scale studies suggest should give good  $\text{ClO}_4^-$  reduction.
2. Modeling and bench-scale results consistently point to keeping the  $\text{NO}_3^- + \text{O}_2$  flux in a moderate range to suppress  $\text{SO}_4^{2-}$  reduction.
    - a. A flux of  $0.2 - 0.4 \text{ g H}_2/\text{m}^2\text{-day}$  from  $\text{NO}_3^- + \text{O}_2$  definitely prevented  $\text{SO}_4^{2-}$  reduction, with a typical target flux of  $\sim 0.3 \text{ g H}_2/\text{m}^2\text{-day}$ .
    - b. In a two-stage MBfR with polypropylene fibers and fed Rialto groundwater, a  $\text{NO}_3^- + \text{O}_2$  flux of  $0.18 \text{ g H}_2/\text{m}^2\text{-day}$  could stop  $\text{SO}_4^{2-}$  reduction, even though SRB were present.
    - c. For comparison, a  $\text{NO}_3^- + \text{O}_2$  fluxes  $\geq 0.1 \text{ g H}_2/\text{m}^2\text{-day}$  slowed  $\text{SO}_4^{2-}$  reduction in the pilot lag MBfR, while  $> 0.3 \text{ g H}_2/\text{m}^2\text{-day}$  stopped  $\text{SO}_4^{2-}$  reduction in the pilot lead MBfR.
  3. Bench-scale results suggest that  $\text{SO}_4^{2-}$  reduction by itself did not necessarily slow  $\text{ClO}_4^-$  reduction, although the pilot results gave the best  $\text{ClO}_4^-$  flux with the smallest  $\text{SO}_4^{2-}$  flux, which was associated with a  $\text{NO}_3^- + \text{O}_2$  flux of  $\geq 0.17 \text{ g H}_2/\text{m}^2\text{-day}$ . Whether or not  $\text{SO}_4^{2-}$  reduction slows  $\text{ClO}_4^-$  reduction, it should be suppressed to avoid other water-quality problems, as were observed in the pilot MBfRs (Evans et al., 2013).

Factors relating to the operation of the pilot MBfRs may have affected performance for  $\text{ClO}_4^-$  reduction. Special modeling runs for the pilot conditions suggested that external mass-transport resistance may have been greater in the pilot MBfRs than in the bench-scale MBfRs. This interpretation may be supported by our observation that a large amount of biomass accumulated between the spacers. The special modeling runs also point to the possibility that the pilot biofilms selected for different and less-efficient PRB.

Another difference between operation of the pilot two-stage MBfRs and the bench-scale two-stage MBfRs is that the lead and lag positions for the pilots were switched regularly, while they were not switched for the bench-scale MBfRs. Consistent with these operating differences is that the biofilm communities were similar between lead and lag MBfR for the pilot, but distinctly different for the bench-scale MBfRs.

Another difference is that the influent of the lag MBfRs was oxygenated in the bench-scale experiments. Oxygenation made it possible to control the  $\text{NO}_3^- + \text{O}_2$  surface loading to the lag MBfR, keeping it in the optimal range even though most of the  $\text{NO}_3^-$  was removed in the lead MBfR. This seems like a good option to build into MBfR systems designed for reducing  $\text{ClO}_4^-$  and  $\text{NO}_3^-$ .

One more difference is that the lead and lag MBfRs were switched regularly in the field pilot, while they were maintained as lead or lag for the bench-scale studies and special modeling analysis. The switching definitely caused the biofilm communities to be similar between lead and lag MBfRs. It also may have played a role in accentuating accumulation of SRB by making the biofilm thicker in the lag MBfR.

In summary, the modeling and bench-scale results show no intrinsic roadblock for achieving a very low  $\text{ClO}_4^-$  concentration when the influent water contains much higher

concentrations of  $\text{NO}_3^-$ ,  $\text{O}_2$ , and  $\text{SO}_4^{2-}$ . One clear key is managing the  $\text{NO}_3^- + \text{O}_2$  loading so that it promotes PRB while simultaneously suppressing  $\text{SO}_4^{2-}$  reduction. The two-stage strategy is well tuned for this goal, and particular attention has to be paid to the  $\text{NO}_3^- + \text{O}_2$  loading to the lag MBfR. While the results do not give an exact target value, they suggest that the lag MBfR should have a  $\text{NO}_3^- + \text{O}_2$  flux of around  $0.18 \text{ g H}_2/\text{m}^2\text{-day}$  to meet the  $\text{ClO}_4^-$  and  $\text{SO}_4^{2-}$  goals together.

Besides  $\text{NO}_3^- + \text{O}_2$  surface loadings, liquid velocities past the membranes and their corresponding mass-transfer resistances and biofilm-detachment rates probably were different between the bench-scale and pilot-scale setups. Larger external mass-transport resistance in the pilot MBfR could have slowed  $\text{ClO}_4^-$  removal in the pilot setup (Evans et al., 2013). Less biofilm detachment in the pilot-scale MBfRs may have led to more accumulation of SRB. The impact of detachment is worth mentioning, because other operational conditions that promoted SRB activity (e.g., lower total-acceptor loading and delivery of excess  $\text{H}_2$ ) allowed SRB to outnumber the normally faster-growing DB and PRB (Ontiveros-Valencia et al., 2013b).

## Chapter 10. References

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