Self-Organizing Wetland Bioreactors (SOWBs) application to mmining reclamation: Direct and indirect bioremediation as a design tool for Mine Influenced Water (MIW) benefaction

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Abstract

Self-organizing wetland bioreactors (SOWBS) provide a designer of mine influenced water (MIW) treatment systems with a tool to harness ubiquitous microbiological processes in methodical and novel layouts for a variety of MIW load reclamation and beneficiation. SOWBs efficacy comes from the ability to grow and maintain very high masses of attached biofilms that are self selecting dependent on the MIW load entering any portion of a SOWB treatment system. As the MIW load is sequestered or remediated, the net water biochemistry changes, developing new and ever changing selective pressures on the predominant, but ever shifting, biofilm metabolisms found throughout the treatment train. Depending on the MIW's load, influences such as iron, aluminum and manganese are either directly or indirectly remediated by the self organized biofilms. Direct bioremediation (DBR) is when a microbe uses the influence in its metabolic triplet to respire, grow and reproduce. Indirect bioremediation, or IBR, are processes such as biofilms "stickiness" and the tendency to colonize and coat all surfaces in the SOWB, including precipitating matter, that leads to capture and sequestration in the SOWB of influences that are not part of a metabolic triplet. This paper provides designers with a rudimentary framework to apply self organization by elucidating mine influence specific DBR and IBR. This knowledge can be applied to other waste streams beyond mining. The experiences herein are not learned through classical experimental design using controls and variables, but on iterative trial and error, so this study is, by definition, long and involved.

Keywords: Self-Organizing Wetland Bioreactors (SOWBs), acid mine drainage (AMD), direct bioremediation (DBR), indirect bioremediation (IDB), mine influenced water (MIW)

Introduction

Self-Organizing Wetland Bioreactors (SOWBs), or manufactured wetlands, were developed in 2012 to sequester dissolved manganese from a closed mine site to meet permitted total maximum daily loads.

SOWBs operate on classic principles elucidated by Sergei Winogradsky, a preeminent Kyiv born Ukrainian microbiologist working in the 1880s to 1920s, who was one of the first to notice and explain the effects of vertical stratification in an open top glass column full of pond muck that developed from a homogeneous substrate of mud. His work identifying microbes that regulate nitrogen fixation are foundational. Today, these classroom mesocosms are known as Winogradsky columns and demonstrate in colorful profusion the key principle that allows SOWBs to horizontally self organize and stratify influences. In this manner, a Winogradsky column with no flow or added load is by definition a batch reactor, much like a wine vat. A column which has flow through and is open to the general environment, providing constant influx of substrate and wild microbes, is a wetland. The ability to self organize and stratify is the key characteristic and difference between a wine vat and a wetland.

Influences are self-sorted into a metabolic reduction/oxidation ladder which is represented by the number of adenosine triphosphate (ATP) produced by the triplet (Schlesigner 2013). For instance, the atmospheric diatomic oxygen (O_2) you breathe ultimately provides 32 molecules of ATP per molecule of glucose, which is the ultimate fate of your food. This highly efficient system of metabolism allows your body to be a huge multicellular beauty of evolution. On the other side of the spectrum are tiny carbon dioxide reducing microbes that produce methane, such as those that colonize cattles' many stomachs. These microbes only produce 4 ATP per molecule of glucose, making them very inefficient scavengers of whatever is left over in their localized environment. With these ratios in mind, designers can now predict where and when the influences will directly or indirectly transition from phase to phase throughout a wetland or system's ever adapting treatment train.

SOWBs remediate mining related influences in two main ways: direct and indirect bioremediation. Direct bioremediation (DBR) sequesters a variety of influences by fostering the growth of biofilms that use contaminants in their biological reduction/oxidation triplet to grow and reproduce, referenced here as their metabolic pathway. A living cell's metabolic triplet consists of a terminal electron donor (TED) or the energy source, a terminal electron acceptor (TEA) as the exhaust gas, and a carbon source. For instance, as one reads this, the reader is breathing oxygen (TEA) and the body is breaking down lunch to be an energy and carbon source. This makes the reader a chemoheterotroph wherein their body uses chemical energy harvested through secondary productivity. The sun, which provides energy for almost all living things on earth powers all plants (photoautotrophs) which gain energy from photosynthetically active radiation (PAR), carbon from the atmosphere, and O2 as the off gassing electron acceptor.

Examples of DBR in mining reclamation are low pH iron oxidation and selenate/sulfate reduction to gas or further reduction to elemental (Schlesinger 2013). In each case of DBR, the biofilm's microbes reduce or oxidize the mining influence to fuel cellular metabolism, growth, and reproduction. DBR waste products are off-gassed, sequestered as a solid, or pass through the SOWBs if the influence has a miscibility the likes of classic salts such as sodium, chloride, or nutrient salts like phosphorus or potassium. If there is more energy provided by the contaminant, the microbe's growth and reproductive advantage increases in an open environment. The influence is used up by the biofilm as the load passes through the treatment train to the point where the influences' scarcity halts the competitive advantage of one triplet and the next most energy dense triplet now provides a growth advantage to another triplet that provides less energy.

The authors then classify indirect bioremediation (IBR) to include all other effects related to the biofilm's presence and shifting population as influences are reduced or oxidized and change phases. These effects are not truly abiotic, as life is still the prime catalyst where IBR is a secondary or tertiary result. For example, the biofilm's "stickiness" functions as a living flocculant that readily sequesters aluminum floc. The production of in-situ biomass that functions as biological oxygen demand (BOD) when reducing environments are desired is another example of IDB. The biomass may not be a direct carbon source for DBR of influences but it can use up all of the dissolved O2 in a load, indirectly leading to the redox ladder and the selective pressure that allows the next most energetic couplet to outcompete the one below it on the redox ladder.

Another major potential benefit of sulfate reduction and outgassing, if done safely, is beneficially increasing pH without needing exogenous alkalinity sources like limestone or caustic soda. These biologically rendered waste products, such as oxide or sulfide solids, are periodically flushed to a dewatering bag for sale or a dewatering basin for long term storage.

Dangerous gasses such as ammonia or hydrogen sulfide are filtered through steel wool or activated carbon air filters. Also, if the treatment system is powered, it may be possible to capture these cases in solution by bubbling the offgassing products through distilled water or other solution for capture and storage, removing them safely from the environment. In this way hydrogen sulfide may be transformed to sulfuric acid, a highly useful

Generally, SOWBs are installed at strategic positions, with considerations to the redox ladder, on reclamation sites where total maximum daily load (TMDL) compliance requires a treatment boost or a “paydirt” has been identified.

Methods

The primary investigator has been building, installing, and maintaining self organizing wetland bioreactors since 2012. SOWBs generally have four primary components, 1) a vessel through which water flows, 2) a load of water that is influences in some form, 3) at least two to three chambers or large bore pipes inside the vessel which deliver and channelize the load through a generally non-reactive loose fiber organic matrix of high lignocellulosic content (e.g. hemp, coconut coir, straw), 4) and a sludge vault that captures suspended solids from upstream while supporting and screening the matrix in the vault above it so that, when an individual SOWB is flushed, the matrix is maintained and the accumulated sludges are broken free from the matrix and drained in a slurry through a flush manifold. SOWBs may be run in a series or parallel, like batteries, in order to adapt to intentional and successional changes in the load.

The PI has deployed 50 to 60 SOWBs on 10 or so sites between 2012 and 2024, with at least 30 or 40 modifications from the original units. These modifications work to make the matrix volume larger as a ratio to the total volume, flow rates smoother, treatment rates higher, atmospheric seals stronger, sludge flushing easier, and overall maintenance time as low as possible.

SOWBs have been made from epoxy and plywood, polytotes, azek (sheet PVC) and most recently welded high density poly-ethylene (HDPE) sheet and pipe. Some of those changes and designs proved to be less than useful and nearly ended the whole venture, and others were immediately adopted and have informed the experiences that follow. The PI has settled on HDPE as the superior material for workability, longevity, and because it is good enough for food grade applications. It is also highly resistant to a range of pH, which is critical for MIW remediation and benefaction. The expected

Figure 1. In-Situ Alkalinity Production Driving Direct and Indirect Bioremediation of Mine Impacted Acidic Water Using Self Organizing Wetland Bioreactors (sowbs)

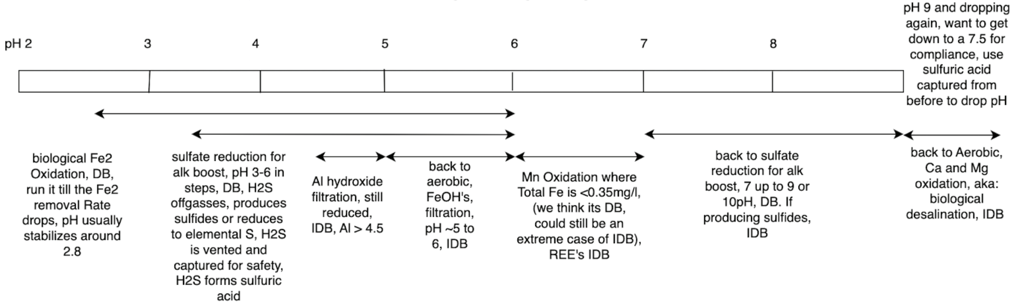

***Figure 1** is a hypothetical direct and indirect bioremediation (DBR/ IDB) process diagram for producing alkalinity in order to remove oxide and sulfide solids from mine influenced waters by vacillating between aerobic and anaerobic environments. Metals usually produced as a heterogeneous sludge are separated as discrete sulfides and oxides captured in stabilized niches through the treatment train. Generally, in Pennsylvania and West Virginia mine influenced waters, sulfate is > 1000 mg/L. The PI proposes to produce a balance of hydroxyl and hydronium ions (pH) so that influences are less soluble. A system need only produce enough alkalinity to equal the acidity (pH 7), not drive off all the sulfate which is a relatively inefficient metabolic pathway*

lifespan of wood/epoxy is 5 years, while polytotes will last for about 10 years before their cage rots out, and azek units have been seen to last at least 8 years without cracking a chemical weld. Some of these early azek units are still commercially in service, with 20 years likely to be a good lifespan. HDPE, on the other hand, if buried in the ground, could theoretically last a century. The gaskets on the flush valves will need to be replaced every 10–15 years, so SOWBs are always installed such buried valves and their protective collars can be readily exposed for servicing.

Results

During the PI's 14 years of work to date, many pathways and techniques have been observed to support and advance the general techniques laid out herein. For instance, a designer can encourage iron (Fe^{2+}) oxidation as the terminal electron donor (TED) for low pH iron oxidizing biofilm. Using SOWBs, DBR of reduced iron will continue if the primary metabolites are available down to an as yet undefined concentration. The primary metabolites are Fe^{2+} as the energy source, O_2 in solution as the oxidant, and CO_2 or carbon rich substrate as the carbon source. The carbon source could be both organic and CO_2, or one then the other, providing another interesting avenue of research as it implies multiple species of low pH iron oxidizers in paired consortium, making better use of the total organic and inorganic carbon (TOIC).

From observation and followed up with lab data, the pH will be its lowest between 2.6 to 2.8 no matter how much "energy", or Fe^{2+} remains in solution. If this were an abiotic effect, one might expect the net reaction to "runaway" and plunge deeper into acidity, but there appears to be a modulation of the acid load to maintain that biofilm's "comfort zone", or its selective advantage. A human analogue is homeostasis, where an internal environment is maintained to keep a body alive. The PI hypothesizes that biofilm self regulation slows metabolism (reducing Fe^{2+} oxidation kinetics) to maintain maximum growth downstream until the TED is depleted. To date, the amount of metabolite to be oxidized to reach the "exhaustion state" of that metabolic pathway is not known by the PI, but it is possibly site and load specific and future studies should look into this value.

The author has also witnessed a site where an anoxic lime drain (ALD) discharges mining influenced waters that are well buffered by the ALD but still produce the classic terrigenous iron formation (TIF) with the paired acidogenesis. Yet, this production only occurs when the discharged waters are aerated upon discharge. Circumneutral pH iron oxidation in well pre-buffered waters discharging from and ALD also appears to produce terracing classically related to low pH iron oxidation, which was quite a surprise to the PI. In abiotic terms this would seem unlikely, but it is possible when considered in terms of DBR.

Once the useful low pH Fe^{2+} oxidation rate decreases a designer needs alkalinity to buffer the partially acidified load (considering total net acidogenesis) and can promote reducing environments using SOWBs sealed from Earth's O2 rich atmosphere to produce alkalinity from the reduction of sulfate in the load. Increasing alkalinity through sulfate reduction and hydrogen sulfide offgassing is a critical tool to achieve as much DBR/IDB as can be expected with the lowest net electroconductivity (EC).

Low pH iron oxidation, and any reaction where the TEA is dissolved O_2, is much more energetically efficient than sulfate reduction in the production of ATP, or about 32:8 / O_2 to SO_4. To adapt to these changing metabolisms, larger, more, or parallel series of SOWBs are required to return the influenced load to anaerobic conditions without restricting the load and causing overflows at a ratio of about 4:1, or four times the SOWB volume for SO_4^{2-} to O_2's base volume (n). For example, carbon dioxide (CO_2) reduction to methane (CH4) is about 32:4, requiring 8 times the SOWB volume to achieve the same throughput.

Because O_2 is such a good TEA, it is used up first to fuel aerobic metabolism. One method of measuring the total oxygen consumption requirements of a load is through biological oxygen demand measured over five days (BOD5). The chemical and biological oxygen demand (CBOD) combined make up the load, so that if no atmospheric oxygen is added to the load, the load and internal environments drift to reduced states. Typically, substrate such as straw, spent mushroom compost, or ethanol are added to a system to force reduction, but if a designer is able to cut off the introduction of fresh atmospheric O_2 through the use of SOWBs designed for that purpose, then a designer can eliminate another exogenous amendment that needs replenishment at cost to operations and maintenance (O&M).

Returning to the treatment train walkthrough, residual low pH iron oxides are captured alongside aluminum hydroxides in the now reducing SOWBs as they precipitate, even though there is no O_2 in solution. Low pH iron oxidation has halted due to lack of O_2 but $Al(OH)_3$ oxidation and precipitation is just beginning. In-situ aluminum oxidation, floc coagulation, and sequestration that normally occurs in ponds or other typical treatment embodiments is achieved in a sulfate reducing SOWB without hampering alkalinity production.

Generally, aluminum floc inhibits an ALD's alkalinity production because the limestone is coated by the aluminum floc and decreases alkalinity availability (Hedin 2002), but SOWBs use lignocellulosic fibers as attachment surface growth matrix.

The alkalinity production from sulfate reducers is throughout the embodiment and not predicated on limestone surface area and purity dynamics. The mass of sulfate reducers in attached biofilms is then a factor of surface area, dissolved O_2 consumption upstream, and sulfate availability. Here, though, one must also consider latent carbon availability: TEAs, TEDs, and carbon sources. A surplus of each must be available to a niche in any treatment train to stabilize and be useful towards treatment. The carbon amendments these researchers saved upstream to achieve reduction may not be enough to maintain it. This is why the PI suggests a mix of coconut coir and straw as the matrix, where the straw is consumed as BOD but the lignin heavy coir is not likely to decay in a reducing environment and will last for decades. Due to this, the active biofilm is not lost every time a SOWB is flushed of its accumulated $Al(OH)_3$. Straw is replaced and mixed back into the coir to replace what is consumed by way of the SOWB's sealed hatches after full and careful degassing is complete.

Flushing and stirring the SOWBs during cleanouts deflates and inflates the SOWBs loose fiber matrix, breaking free biofilm coated aluminum and iron of all varieties and biologies for removal. The PI has extensive experience with SOWBs and aerobic aluminum hydroxide capture. Aluminum typically achieves regulated loadings at this stage in a SOWB treatment train and non-detect by the end of the system.

Aluminum, unlike iron, manganese and sulfate, is not a part of any relevant metabolic triplet, so the capture of aluminum by the matrix and sticky biofilm that grows upon it is IDB for the purposes of this work. Once the aluminum has been mostly oxidized and captured in the reducing SOWBs, the designer again turns to aerobic conditions. Great care must be taken after any reducing step as residual hydrogen sulfide (H_2S) is offgassing and this poses a real hazard to maintenance workers unless the H_2S is captured in air filters filled with iron oxides and/or carbon filters. Iron filings are used to capture residual H_2S from methane gas streams, reducing the filings and chemically capturing the hydrogen sulfide. The quantity of H_2S produced from the proposed system is much greater than for methane production so this must be taken into account in the early acid balance and design steps. Again, only a fraction of the overall sulfate load needs to be reduced to drive off enough hydrogen sulfide or produce enough elemental sulfur if the sulfur is fully reduced and its out electron shell is filled.

The iron oxides produced through low pH iron oxidation have a very high surface area to mass ratio and may be the perfect reactant to this highly reactive and dangerous gas, which is another benefit of the system. Care must again be taken to ensure the liquid gathered at this filtration step, for there will be water vapor issuing in tandem, is kept from reintroduction to the treatment train, as it is likely to be a highly molar sulfuric acid.

The target pH range at this step (post 1st sulfate reduction step), and conversely how much sulfate needs to be reduced to have enough alkalinity to buffer the net acidogenesis, should reach pH 4–5 in the reducing SOWBs and before atmospheric aeration.

The next step in the process is abiotic iron oxidation (iron hydroxide floc) and requires more "stored up" alkalinity by alkagenesis to buffer the next steps of acidogenesis of iron and manganese oxidation. Classically, iron oxidation begins around pH 5.5 and biotic manganese oxidation appears to begin around 6.5 pH. This classic iron sludge is again captured by the SOWBs through IDB in the same manner as aluminum floc to very low or non-detect far upstream in the treatment train from final discharge.

Once all the remaining total iron is oxidized and captured (total or dissolved to less than 0.35mg/L) the manganese, from multiple replications on different sites over several years, begins to "spill out". There is evidence collected over the years strongly suggesting that Mn oxidation is also DB, wherein the Mn^{2+} is the TED just like in low pH iron oxidation. However, the PI is still awaiting direct evidence that the manganese oxides produced are in fact byproducts of cellular metabolism. This evidence could come through further metagenomics study, though initial anecdotal evidence indicates the high rates of removal might be a IBR mineralization upon the matrix, and the Mn oxidation is Abiotic, just occurring at rates and pH ranges far from the observed norms. Whether the oxidation of Mn is achieved through DB or IDB, the Mn is oxidized and captured as discrete mineral forms in the SOWBs at a range of ~6 to 8 pH.

Mn oxides are known as wonderful ion scavengers due to their high surface area, cation exchange capacity, and available oxidation states. In reference to rare earth elements (REEs), which are least soluble in this similar pH range (Ziemkiewicz 2022), the manganese oxide and REEs produced, after all aluminum and Fe^{2+} are removed upstream, are of high quality and concentration.

Conclusion

This paper attempts to provide context and methods for the application of self organization to MIWs by defining the biogeochemical characteristics of MIWs and strategically using this knowledge to design systems that use MIWs, which are also metabolites, to clean the water. In essence, the SOWBs, using layouts explained herein, use the load's biogeochemical potential energies to remediate the selfsame load while also beneficially separating the individual components of the load.

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