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Treatment of Wastewater Containing Phenol and Heavy Metals Using Natural Zeolite and Bioaugmentation

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TREATMENT OF WASTEWATER CONTAINING PHENOL AND HEAVY METALS USING NATURAL ZEOLITE AND BIOAUGMENTATION

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Submitted in Partial Fulfillment of Requirements for the Degree of MASTER OF SCIENCE IN ENVIRONMENTAL ENGINEERING CLEVELAND STATE UNIVERSITY December, 2007

This thesis has been approved for the Department of Civil and Environmental Engineering and the College of Graduate Studies by:

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PATRICK JAMESON

ABSTRACT

Preliminary experiments and 9 trials were conducted to evaluate a bench scale wastewater treatment experiment. Wastewater consisted of 3 heavy metals including cadmium (Cd), copper (Cu), and lead (Pb). An organic pollutant, phenol, was added at 4 concentrations ranging from 0 to 250 mg/L. Organic substrate was used at 3 levels of strength corresponding to high, medium, and low. Treatment consisted of 2 phases, a physical-chemical removal of metal with an ion exchange mineral known as zeolite and biological oxidation through bioaugmentation. Zeolite was added at doses of high, medium, low, and a reference of zero. Bioaugmentation consisted of 4 types of Live Liquid MicroOrganisms (LLMO) including strains E1, S1, G1, and N1. Pb was effectively removed with up to 100% removal while Cd and Cu were not as effectively removed. Success of bioaugmentation was measured using a total organic carbon analyzer and results were mixed. LLMO improved biological oxidation at the lowest organic substrate levels after ion exchange. Pb wastewaters with a high dose of zeolite, phenol concentrations of low or zero, and low organic substrate showed the best total organic carbon removal performance with the addition of LLMO. LLMO strains of E1, S1, and G1 showed slightly better performance over N1.

Keywords: Zeolite, LLMO, Bioaugmentation, Ion Exchange, Heavy Metals, Phenol

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CHAPTER I

INTRODUCTION

Wastewaters containing heavy metals and organic chemicals are common in the iron and chemical industries (1). Metals such as cadmium, copper, and lead are quite toxic and inhibit biological oxidation of organic wastes such as phenol. Treatment of wastewater containing organic chemicals and heavy metals requires the removal of soluble and solid metals before bio-oxidation can be effectively used.

Heavy metal removal via ion exchange has existed for decades. One of the first materials or media used was zeolite. Zeolite is a large family of minerals, including natural and synthetic species, which have a wide variety of uses in industry.

Biological treatment of certain organic chemical wastes, such as municipal wastewater and to a limited extent, phenol, is an effective and inexpensive technology provided optimum conditions are matched to the correct population (type and number) of microorganisms. For example, concentrated organic waste can be too toxic for biodegradation inhibiting bio-oxidation or killing the organism. However, the organic waste is treatable at dilute concentrations and long treatment time.

The microorganism community is responsible for biological oxidation and the establishment of microorganism populations in biological treatment is critical. One way

to get the correct microbiological population is to use seed sludge or pre-mixed inoculums such as Live Liquid Micro-Organisms (LLMO). Various LLMO types exist which are formulated for a myriad of pollutant types, treatments, and environments.

CHAPTER II

OBJECTIVES

The research for this thesis was conducted in bench scale reactors simulating Activated Sludge (AS) treatment systems. The objectives of this study consist of the following:

- 1. Determine inhibiting concentrations of phenol, cadmium, copper, and lead upon LLMO.
- 2. Evaluate the removal via ion exchange of soluble cadmium, copper, and lead using 3 doses of zeolite and a control.
- 3. Evaluate total organic carbon removal of wastewaters containing 3 concentrations of organic substrate, and 3 concentrations of phenol with 1 control in the presence of a heavy metal using zeolite to remove the metal and LLMO to provide the microorganisms.
- 4. Evaluate 4 types of LLMO to determine treatment efficiency.

CHAPTER III

LITERATURE REVIEW

3.1. Overview of Treatment Processes

 Domestic or municipal wastewater (WW) is relatively innocuous and easy to treat. The same can not be said for industrial wastewaters because they can be excessively toxic to microorganisms. Often industrial wastewater is pre-treated on site before release to the sewer or wastewater treatment system. Essentially, microorganisms utilize soluble, and to a lesser degree insoluble, organic pollutants and metabolize them into waste gases, heat, and biomass.

 Wastewater treatment systems, in developed, urban areas consist of sewers for collection and concentration; and a wastewater treatment plant (WWTP) for processing. Two conventional methods of wastewater treatment are trickle filters (TF) and activated sludge (AS). These two methods can exist with or without the use of return sludge. The essential difference is TFs utilize media, typically as structured plastic sheets in various geometries, to provide a surface for microorganism adhesion. The attached microorganisms remove soluble waste which trickles through via gravity (2, 3). AS systems are deemed more efficient as pollutant removal centers on the aeration tank (AT) and following settling tank. Optimum performance in the AT is dependant upon sludge age and the use of return sludge.

Figure 1. Typical (General) Process Flow Chart of Biological Wastewater Treatment Plants.

AS and TF systems vary from plant to plant as well as regionally, but many components of the process remain the same. A typical process can be seen in Figure 1. Wastewater influent contains objects and abrasive particulates which can damage equipment. The first stage in treatment to remove large objects such as toilet paper, diapers, and plastics from the waste stream via a bar screen. Following the bar screen comes abrasive particulate removal in a grit chamber. Typically, after grit removal WW continues to primary treatment in a primary settling tank (PST) or Dissolved Air Flotation (DAF) unit. In a PST, solids, typically organic, and grease are removed. Grease is skimmed off the surface and processed separately. The organic solids, known as primary sludge settles to the bottom and is removed for solids treatment via pumping or gravity. At this point the wastewater is relatively solid free and mostly soluble pollutants exist. Wastewater proceeds to either a TF or AT. A TF is straight-forward and WW is fed from the top of the filter and trickles down to a collection unit at the bottom. After the TF, a second settling tank is found to settle sludge which sloughs off trickle

filter media. Water which has passed biological treatment and settling proceeds to a tertiary stage for physical-chemical treatment.

 AS treatment differs from trickle filters as WW enters an AT as opposed to a TF. In the AT, WW enters and is typically mixed with return sludge and air. Oxygen is necessary for aerobic microorganisms to metabolize soluble organic pollutants. Following the AT, WW and sludge enters a final settling tank (FST) where biomass in the form of solids is removed. A portion of the biomass is returned to the AT and the rest is processed with solids from the PST or DAF. At this point biological treatment is completed and tertiary treatment removes low levels of heavy metals or recalcitrant organic pollutants via physical-chemical means and the effluent is disinfected. In theory, AS is straightforward and simple, in practice it is not, as mixing the correct amounts of WW with return sludge and air must take many variables into account. Some of these include sludge age, WW characteristics, temperature, and microbiological composition of sludge, flow rates, and others. Arguably, the most important factor for determining treatment efficiency is sludge age or mean cell residence time (3). As a general rule of thumb, the older the sludge, the more efficient the treatment.

3.2 Organic and Inorganic Pollutants

 Industrial wastes incorporate a vast array of substances and chemicals. In the narrow scope of this thesis, only water soluble chemicals will be considered. Soluble waste is also a broad category and this thesis concentrates on a single organic waste, phenol and water soluble heavy metals. Phenol is a common organic pollutant composed of carbon, hydrogen, and oxygen. The formula is C_6H_5OH and consists of a benzene ring

and a single hydroxyl group (4). Phenol is soluble in water at 80,000 mg/L and used as a germicidal agent (4). Heavy metals such as lead (Pb), cadmium (Cd), and copper (Cu) are included under the large umbrella classification of inorganic molecules. These metals, when found in water commonly exist as positive ions with a charge of $+2$, although ions of other charges do exist.

 Treating mixed wastewaters is a typical problem posed to industry. Phenol and heavy metals are no exception and are found in iron and petro-chemical industries (1). Phenol is toxic to many microorganisms at concentrations as low as 25 mg/L but some are able to metabolize phenol at concentrations exceeding 500 mg/L (4, 5, 6, 7, 8).

While some microorganisms may be able to oxidize phenol at moderate concentrations, the addition of soluble heavy metals may inhibit oxidation or outright kill them (9). However, toxicity of heavy metal depends upon metal type, concentration, and microorganism. *Pseudomonas sp*. is noted as a robust genus of bacteria which can resist inhibition and death caused by heavy metals and phenol. Meanwhile, nitrification bacteria such as *Nitrosomonas sp*. and *Nitrobacter sp*. are extremely sensitive to heavy metals. Lee et al. found nitrification was inhibited at a copper (II) concentration of 5 mg/L (10). Cu^{2+} concentrations between 5.52-11.35 mg/L also inhibited phytoplankton and bacteria Northeast of Singapore (11). Cadmium was observed to be less toxic than $Cu²⁺$ in an upflow anaerobic sludge blanket reactor at concentrations greater than 100 mg/L (12). However, in a study using *Pseudomonas putida*, Malakul et al. did not witness inhibition until Cd^{2+} concentrations were greater than 80 mg/L (13). In a toluene remediation study, Burggraf et al. found bacteria respiration inhibited at lead (II) concentrations above 15 mg/L (14). The toxicity order from greatest to weakest appears

to be $Cu^{2+} > Cd^{2+} > Pb^{2+}$. Soluble heavy metals are not biodegradable and with the exception of acclimatization, stabilization, or removal, their toxicity can not be reduced. A typical solution for WW containing moderately toxic organic waste and very toxic metallic waste involves physical or chemical separation of soluble metals from organic pollutants. Physical/chemical separation of metallic constituents from WW is most practical for industrial waste. The presence of soluble metals waste and strong organic waste presents a very serious problem for WWTPs using biological treatment. Environmental policies and regulations require industry to pre-treat wastes before release into the domestic sewer. In many cases, pre-treatment is not good enough and toxic organic and metallic wastes are not allowed into the sewerage system. A high concentration of toxic organic or metallic waste is known as a shock load and can make biological treatment completely ineffective. Indeed, once soluble metal waste enters the collection system little can be done until after secondary treatment. After a shock load is purged from a WWTP re-establishing a robust microbiological community can take weeks or months (15, 16).

 Physical separation of mixed wastewaters borrows heavily from chemical engineering principles. Geankoplis writes about these processes and the theories behind them, in his highly regarded textbook, Transport Processes and Separation Process Principles (17). A typical method for mixed waste such as phenol and metals is to use adsorption, ion exchange, and other phenomena to remove the heavy metals from solution. This leaves microorganisms an environment more conducive for biological oxidation of phenol. Selection of the most cost effective as well as best performing

adsorbent or ion exchange medium is a significant challenge and continues to drive research.

3.3 Zeolite and its Application

 Zeolite is defined as an alumina-silicate mineral and has a broad range of applications. Natural zeolite is generally found in tufa rock outcrops near active or extinct volcanoes (18). The mineral is formed as alkaline seas cover volcanic deposits and as of 1997, there were 86 different species or types of zeolite (19, 20). Natural zeolites have been augmented in laboratories resulting in minerals of slightly differing chemical composition, structure, and properties. Applications of zeolites range from ion exchange for natural zeolite to hydrogen storage for hydrogen fuel cells in synthesized zeolite to feed supplements for livestock.

 In wastewater treatment, zeolite is commonly applied as an adsorbent, ion exchanger, or molecular sieve medium. Physical adsorption occurs through phenomena such as Van der Waals forces between solute and adsorbent surface (4, 17). Ion exchange and molecular sieving are different mechanisms, but the end results are similar for heavy metal cations and zeolite. Zeolites tend to be dominant in a certain metallic ion, the most common being calcium (Ca^{2+}) , sodium (Na^{+}) , and potassium (K^{+}) . This dominant cation is known as the counter-ion and can have significant effects upon kinetics, affinity, and sorption capacity governing ion exchange. For example, El-Bishtawi and Al-Haj Ali noticed lead ion exchange with a $Na⁺$ dominated zeolite resulted in the release of $Na⁺ ions$, which in turn raised the pH. Raising of the pH actually improved the affinity of lead towards zeolite and driving faster removal rates (21). In

comparison to synthetic or laboratory-augmented zeolites, a given quantity of natural zeolite varies widely in composition and chemical properties. In addition, the characteristics of an "identical" natural zeolite will vary from one geologic formation to the next (18). The theory of ion exchange is the metal or cation in solution, such as ammonium (NH₄⁺) or lead (Pb²⁺) will exchange places with the zeolite counter-ion of it is energetically favorable (22, 23). The most common, and therefore cheap, species of natural zeolite is clinoptilolite. Clinoptilolite has been documented as being able to selectively remove Pb²⁺, zinc (Zn^{2+}) , nickel (Ni^{2+}) , cadmium (Cd^{2+}) , iron (Fe^{2+}) , copper (Cu^{2+}) , and manganese (Mn^{2+}) (18, 24).

 Due to its abundance and inexpensive cost, a great deal of research has been performed upon this species of natural zeolite known as clinoptilolite. Ammonium removal in anaerobic conditions has been a focus for ion exchange applications of clinoptilolite (23, 25, 26, 27). Research in heavy metal removal has also shown much promise. A good, as well as bad, property of clinoptilolite as an ion exchanger is its selectiveness. Zeolites are known to be "species specific" for metal immobilization and, likewise, this is where cation removal research tends to focus. Typical studies select several types of soluble heavy metals, as well as ammonium, and attempt to remove them from a wastewater or polluted water (9, 18, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34).

 Zeolite has also been investigated for its ability to remove other chemical components and even bacteria. The removal of phenol using clinoptilolite as an adsorbent is generally considered ineffective without surfactant-augmentation of the mineral (35, 36). Hrenovic et al. asserts zeolite adsorbs phosphate and provides a surface

for bacterial colonization (37). Milan et al modified clinoptilolite with heavy metals to reduce bacterial populations in treated wastewaters (38).

 Contaminant removal with clinoptilolite is dependent, in part, upon crystalline structure, ratios of aluminum (Al) to silicon (Si), and the dominant cation. As previously stated, while there is natural variation and varying levels of trace elements in a geological formation, the most common cations of clinoptilolite are potassium, sodium, and calcium. Erdem et al. reported a mol: mol ratio of Si: Al of 4.66 and a $(Na + K)$: Ca ratio of 1.85 from a sample in Western Turkey (18).

 The process of ion exchange substitutes the original cation, or counter-ion, in the crystalline framework, with a soluble cation in the wastewater. Clinoptilolite has a net negative charge and is relatively unstable. Ion exchange with heavy metals and ammonium allow the mineral to achieve a more energetically favorable and therefore more stable state (22). Consistent results via ion exchange, generally requires a homoionic zeolite. A homo-ionic refers to a zeolite with a homogenous counter-ion population. A homo-ionic zeolite can be achieved by simply washing the particulate zeolite (usually in granular form) in a salt solution of the desired cation (19).

 Chemical and physical adsorption, ion exchange, and molecular sieving depend upon the porosity of the zeolite as well as charge balance dictated by Al: Si ratios (39). The nature of porosity, or the size of the pores, allows molecules of certain sizes to pass through the crystal lattice of the mineral. Naturally, smaller molecules have an easier time passing through. Erdem et al. states cations with smaller hydrated radii become immobilized faster and more completely than ions of larger diameters (18). Yapar and Yilmaz (35) report an average pore diameter of 38.6 angstroms (\hat{A}) and Cabrera et al.

Cabrera et al. found a pore diameter of 101\AA (28). The difference in pore size can be explained, in part, by the fact the zeolites came from different geologic formations located in different continents. Relatively small pore size, partially helps to explain why ammonium and metallic cations are suitable for ion exchange while large organic molecules, such as proteins are not favored for removal.

 Previous studies have found a wide range of results highlighting zeolite quantities for metal removal. General trends of metal removal from solution remain similar. Cabrera et al. observed a zeolite doseage of 20 g/L removed 85% of soluble Cu (28). However, it is important to note the concentration was very low at 0.13 mg/L. Erdem et al. noticed 66.1% of a Cu solution containing 50 mg/L was removed at a doseage of 5 g/L (18). The ion exchange trial length lasted 5.5 hours. Some studies expressed results as quantity removed per quantity zeolite added. For example, at a zeolite doseage of 20 g/L , Mozgawa and Bajda found 22.6 mg of Pb are removed for each gram of zeolite added. They found 10.4 mg of Cd are removed for each gram of zeolite added (22). In a similar study using a zeolite doseage of 5 g/L, Ouki and Kavannagh noticed 6 mg of Pb are removed per gram of zeolite. Cu removal was 3.8 mg per gram of zeolite and Cd was 3.7 mg per gram zeolite (24).

Another trend or phenomenon observed in these studies was the effect of pH. pH plays an important part in ion exchange of clinoptilolite but the mechanism is not well understood (24). Literature shows metal cations are removed from solutions at higher pHs such as 5-7. A pH range from 6-8 is preferred as many microorganisms do not perform well outside of this range. Another point of importance is the physical size of the medium or clinoptilolite. Smaller particle sizes provide greater surface area for
penetration and sorption of metals. However, if particle size is too small, solid settling rates may be very low. This is a problem of concern as a major component of WW treatment is the removal of total suspended solids (TSS).

 Research on ion exchange using clinoptilolite pays close attention to specific selectivity with respect to heavy metal removal. Clinoptilolite removes Pb greater than any other metal (22, 24). The performance of ion exchange with other metals is much less and there appears to be controversy of how the trend should proceed. Presumably, the selectivity trend after Pb is in dispute because consistent evidence does not exist for the development of a conclusive selectivity trend. However, for the purpose of this thesis the results of Ouki and Kavannagh show clinoptilolite removes Cu better than Cd (24). Another important point is the initial heavy metal concentration. Soluble metal concentrations are very low and the amount of zeolite required for removal is relatively high at 5 to 20 g/L .

Solid surfaces are used in nature and some WW treatment applications to form biofilms for microbial communities. Zeolites are no exception and the immobilization of microorganisms could increase microbial detention time, or sludge age, in a bioreactor as well as providing habitat for diverse microbiological communities. In turn, a diverse microbial community can degrade a wider range of pollutants to a greater degree than a homogenous population of organisms. This phenomenon of biosorption for bacteria removal has potential due to negative charges surrounding bacteria and positive counterions in clinoptilolite (37, 40, 41, 42).

3.4 LLMO and Bioaugmentation

Biological treatment is the most common and cost effective method to treat wastewaters of low toxicity. The correct microbiological community is critical for insuring optimum treatment. The variety of microorganisms and their corresponding physical/chemical requirements as well as treatment applications is vast, requiring constant monitoring and maintenance of optimal conditions. During WWTP start-up, or after a shock load, microorganisms have to be re-introduced in a sufficient quantity (2). Live Liquid Micro-Organisms (LLMO) cultures were developed by General Environmental Science of Cleveland, Ohio to satisfy this need. The addition of LLMO or other microbiological cultures changes the microbial community and is known as bioaugmentation. The process includes the addition of many species of microorganisms mixed together as different species of bacteria favor different substrates and environments (43, 44, 45, 46, 47, 48, 49, 50, 51).

The exact contents of LLMO are proprietary but the organism blend is given and consists of the following:

- *Bacillus subtilis*
- *Bacillus amyloliquefaciens*
- *Bacillus licheniformis*
- *Cellulomonas sp.*
- *Cellulomonas biazotea*
- *Pseudomonas stutzeri*
- *Pseudomonas denitrificans*
- *Rhodopseudomonas palustris*

The correct blending of these organisms makes the LLMO mixture effective and each organism has unique qualities. *Bacillus* bacteria produce exoenzymes for degradation of substrates outside the bacteria. This initial degradation breaks down nonsoluble substrates for easier transport into the organism. *Cellumonas* bacteria degrade cellulose, a very difficult sugar to biologically oxidize (46). *Pseudomonas sp*. has been shown to degrade phenol in biofilm reactors (8). *Pseudomonas* has also shown the uncommon tolerance of heavy metals (52). *Rhodopseudomonas* is known to oxidize organic chemicals and produces a red pigment which is helpful for determining if the treatment is working (48).

3.5 Phenol Biodegradation and LLMO

As stated earlier, phenol is a common, toxic organic pollutant produced in many industrial processes and can be detoxified using chemical and biological means. Phenol's chemical formula is C_6H_5OH and its structure consists of a benzene ring and a single hydroxyl group. While phenol is toxic, microorganisms can be acclimatized to a point where they can utilize it as a secondary substrate and in some cases a primary substrate (1). Co-metabolism is another biological mechanism of bio-oxidation used by bacteria upon phenol based pollutants (2, 53, 54). The addition of a primary carbon source can also be of help for the breakdown of phenol. In general, phenol is relatively non-toxic when compared to its derivatives such as chlorinated phenols.

3.6 Biosorption

In addition to adsorption and ion exchange of pollutants, biosorption to known to occur upon clinoptilolite and to organic materials. Adsorption of heavy metals to particulate, organic material is well known and is a method used to remove heavy metals from polluted waters (55). Generally, it can be assumed if organic particulates exist in a metallic wastewater, then heavy metals will adsorb to the material. It makes little difference if the material is bacterial biomass or powdered milk.

Biosorption is thought to occur because of bacteria containing negative charges are attracted to the counter-ions of zeolites. Hrenovic et al. showed this to be the case using the bacteria *Acinetobacter calcoaceticus* at clinoptilolite concentrations of 5 and 15 g/L of wastewater (37). The author notes *A. calcoaceticus* as an organism with a large number of phosphate groups on its cell wall which are attracted to clinoptilolite.

The presence of biofilm can be very helpful for the development of a diverse and effective microbial community. It is thought the thickness of biofilm protects bacteria when shock loads become lethal (8). Research has been performed on using biofilms to oxidize phenol but the microbial parameters become more complex with the addition of heavy metals.

If a two stage treatment is used, then heavy metals will be sorbed to the *clinoptilolite* before the addition of LLMO. Will the sorbed heavy metal have a negative effect upon biofilm formation? Milan et al. showed that 2 mg Cu/g zeolite has a deleterious effect upon fecal coliform bacteria (38). This corroborates Nakamura and Sawada who studied phenol (100 mg/L) degradation in the presence of Cu ions (0.05 to 0.5 mg/L) in solution (1). Nakamura and Sawada found Cu partially inhibits bio-

oxidation of phenol by *Acinetobacter calcoaceticus*. However, the effect of free Zn ions was not as detrimental. It's not known how the copper reduced the bacterial population. In addition, if sorbed Cu had a negative effect, would Pb and Cd have the same inhibitory effect?

CHAPTER IV

MATERIALS AND METHODS

4.1 Materials

4.1.1 Metal Wastewaters

 Wastewaters used in this experiment consisted of heavy metals, phenol, and an organic substrate provided by dissolved, dehydrated milk. The procedure produced synthesized a WW consisting of heavy metals; later this was added to known quantities of organic material including phenol and dehydrated milk. The heavy metals used consisted of cadmium, copper, and lead. The desired concentration of heavy metal in WW was 100 mg/L. In preliminary experiments, metal salts were dissolved in deionized water (DI water).

4.1.1.1 Lead

 The metal salt used to produce a wastewater of soluble lead was lead nitrate $(Pb(NO₃)₂)$ from Fisher Scientific. Lead nitrate has a molecular weight (MW) of 331.2 g/mol and has a solubility of 52 $g/100$ ml of water (56). The molecular weight of Pb is 207.2 g/mol. To achieve a concentration in DI water of 100 mg/L the percentage lead by

weight was determined and assuming complete dissolution the following equations are applied:

$$
Pb(NO_3)_2(s) \rightarrow Pb^{2+}(aq) + 2NO_3(aq)
$$

% Pb²⁺ =
$$
\frac{Pb2 +}{Pb(NO3)2}
$$
 = 100 × $\frac{207.2(g/mol)}{331.2(g/mol)}$ = 62.6 % Pb²⁺

mg $Pb(NO₃)₂/L =$ 62.6 % Pb2 100 mg Pb2 + $+$ = 159.7 mg Pb(NO₃)₂/L \approx 160 mg Pb(NO₃)₂/L

 However, in practice, DI water is too simple to replicate a wastewater. Therefore, the dissolving medium was degassed tap water. The tap water was degassed to remove the disinfectant, gaseous chlorine and residuals. Tap water contains sodium chloride (NaCl), other salts, and particles which pass through a 2 µm filter; these species are referred holistically as total dissolved solids (TDS). Typical TDS concentrations range from 20 mg/L to 1000 mg/L and 300 mg/L is a common value (Sawyer et al, 2003). The chloride ion in NaCl reacts with the soluble lead ion to form PbCl2 in the following equation:

 $Pb(aq) + 2Cl(aq) \rightarrow PbCl2(s)$

 Lead chloride has a solubility of 10 g/L but generally precipitates out of solution at room temperature (20° C) (57). The presence of chloride ions results in the formation of other molecules and complexes including $PbCl⁺$, $PbCl₃$, and $PbCl₄²$ (Sawyer et al., 2003). The precipitation of the listed salts and complexes takes time as does the settling of precipitate. In order to get a high concentration of soluble lead excess $Pb(NO₃)₂$ had to be added to react with available chloride ions. After several experiments of trial and error it was decided to add 475 mg $Pb(NO_3)/L$ of degassed tap water. This represents

nearly 3 times the amount needed in DI water. Synthesized lead WW was mixed the day before the experiment to allow precipitated lead species to settle.

4.1.1.2 Copper

 Copper was added in the form of Copper (II) sulfate pentahydrate crystals otherwise known as cupric sulfate pentahydrate with a formula of $CuSO₄·5H₂O$ from J.T. Baker Chemical Co. CuSO₄:5H₂O has a molecular weight of 249.7 g/mol and a solubility of 31.7 g/100 ml of water (58). Copper has a MW of 63.5 g/mol. To achieve a concentration of 100 mg/L of copper cations (Cu^{2+}) the following equations were used:

$$
CuSO_4:5H_2O(s) \to Cu^{2+}(aq) + SO_4^{2-}(aq) + 5 H_2O(l)
$$

$$
\% \text{ Cu}^{2+} = \frac{\text{Cu2} + \text{CuSO4} + 5 \text{H2O}}{\text{CuSO4} + 5 \text{H2O}} = 100 \times \frac{63.5(g/mol)}{249.7(g/mol)} = 25.4 \% \text{ Cu}^{2+}
$$

mg CuSO₄·5H₂O/L =
$$
\frac{100 \text{ mg Cu2} + 25.4\% \text{Cu2} + 294 \text{ mg CuSO}_4 \cdot 5\text{H}_2\text{O/L}}
$$

 Preliminary experiments utilized dissolution in DI water and later experiments used degassed tap water. Again, the presence of TDS and chloride ions affected the concentration of Cu^{2+} through the formation of $CuCl₂$ and other unidentified products. However, the amount of CuSO₄:5H₂O added to the simulated wastewater was not augmented.

4.1.1.3 Cadmium

Cadmium was added in the form of anhydrous cadmium chloride $(CdCl₂)$ from Fisher Scientific. CdCl₂ has a MW of 183.3 g/mol and is freely soluble in water (59). The ion cadmium (Cd^{2+}) has a MW of 112.4 g/mol. To determine the amount of $CdCl₂$ necessary for a concentration of 100 mg/L Cd^{2+} , the following equations were used:

$$
CdCl_2(s) \to Cd^{2+}(aq) + 2Cl_2(aq)
$$

\n%
$$
Cd^{2+} = \frac{Cd^2 +}{CdCl_2} = 100 \times \frac{112.4(g/mol)}{183.3(g/mol)} = 61.3 \% Cd^{2+}
$$

\nmg
$$
CdCl_2/L = \frac{100 \text{ mg } Cd^{2+}}{61.3\% Cd^{2+}} = 163 \text{ mg } CdCl_2/L
$$

As CdCl₂ freely dissolves in DI water the presence of TDS was not expected to affect solubility and no variance from theoretical concentrations were made.

4.1.2 Phenol and Milk

Phenol (C_6H_5OH) and dehydrated milk were added at the same time by adding pre-weighed amounts to 500 ml of simulated, heavy metal wastewater. Phenol (C_6H_5OH) was added to the wastewater as loose crystals from Mallinckrodt. The concentrations of phenol used were 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L. Pre-weighed amounts of 0 mg, 25 mg, 75 mg, and 125 mg were individually kept in sealed containers to prevent volatile loss and contamination.

 Dehydrated milk (Food Club® Instant Non-Fat Dry Milk) was used to simulate non-toxic organic pollutants, or primary substrate, found in WW. Milk was used as it contains nitrogen (N), phosphorus (P), and trace elements in sufficient quantities for microorganisms to thrive. Dehydrated milk was crushed and added at concentrations of

100 mg Total Organic Carbon (Total Organic Carbon)/L, 200 mg Total Organic Carbon/L, and 300 mg Total Organic Carbon/L in Cu and Cd trials. Dehydrated milk was added at concentrations of 50 mg Total Organic Carbon/L, 100 mg Total Organic Carbon/L, and 200 mg Total Organic Carbon/L for Pb trials. This corresponds to dry weights of 13 mg, 25 mg, 50 mg, and 75 mg for 50 mg Total Organic Carbon/L, 100 mg Total Organic Carbon/L, 200 mg Total Organic Carbon/L, and 300 mg Total Organic Carbon/L, respectively. The pre-weighed amounts of milk were added to containers holding previously weighed amounts of phenol. The milk/phenol dry mix was reconstituted with 500 ml of heavy metal wastewater to achieve desired concentration.

4.1.3 Zeolite

 The zeolite used in this series of experiments was provided by Dr. Pete Bunger of ZEO,Inc. in McKinney, Texas, USA. The product used is known as ZeoSand® Natural Zeolite Filter Media. This product is principally designed for filtration in swimming pools use can also be applied to water treatment, wastewater treatment, aquariums, aquaculture, and zoos. ZeoSand $\mathcal D$ is potassium dominated clinoptilolite (K⁺clinoptilolite) and has the important, industrial characteristic of removing ammonium ions from water. ZeoSand® has the empirical formula of (Na^+, K^+, Ca^{++}) , Mg^{++} ⁺⁶[(AlO₂)₆(SiO₂)₃₀]⁻⁶·24H₂O. Other characteristics include the following as provided by ZEO, Inc.:

Table I. Typical Properties of ZeoSand® Natural Zeolite Filter Media from ZEO,

The chemical analysis of ZeoSand® consists of the following:

Table II. Chemical Analysis of ZeoSand® Natural Zeolite Filter Media from ZEO,

Inc (2007)

 Commercially, ZeoSand® is not specifically used for heavy metal removal. The principal cation targeted for removal is ammonium. However, it is still useful for polishing waters which may contain slight concentrations of heavy metals. The ion exchange experiments of this research consisted of weighing amounts of zeolite and adding them to wastewater containing heavy metal concentrations. The K^+ -clinoptilolite was added at doses of 0, low, medium, and high. The correct dosage was determined during preliminary ion exchange experiments. Final dosages varied with the heavy metal WW. Cd and Cu had dosages of 0 g/L, 5 g/L, 10 g/L, and 20 g/L. Pb had treatment

doses of 0 g/L, 2.5 g/L, 5 g/L, and 10 g/L. These doses were pre-weighed and added to WW containing the heavy metal, phenol, and milk.

4.1.4 LLMO

 Live Liquid Micro-Organisms were added after ion exchange to remove organic contaminants from the wastewater. LLMO was acquired from General Environmental Science of Cleveland, Ohio. LLMO strains added consisted of E1, S1, G1, and N1. LLMO is a blend of bacteria which is commercially used for small scale treatments of heavily loaded (organic) ponds and wastewater treatment units. The above strains have different biological components or bacteria depending on the desired treatment. However, all strains have some common components including: $NH₄Cl$, $K₂HPO₄$, MgSO4, Sodium Acetate, Yeast Extract, Viable Bacteria Mass, and Water. The listed components include trace minerals and energy substrates which allow for an extended shelf life of up to 3 years.

 The bacteria compositions of LLMO used in these experiments can be separated into 2 groups of E1, S1, G1, and N1 as a separate group. E1, S1, and G1 have similar objectives of consuming waste while N1 is used from ammonia removal. The consuming group of E1, S1, and G1 contains the following bacteria:

- *Bacillus subtilis*
- *Bacillus amyloliquefaciens*
- *Bacillus licheniformis*
- *Cellulomonas sp.*
- *Cellulomonas biazotea*
- *Pseudomonas stutzeri*
- *Pseudomonas denitrificans*
- *Rhodopseudomonas palustris*

The N1 strain of LLMO consists of the following organisms:

- *Bacillus subtilis*
- *Bacillus amyloliquefaciens*
- *Bacillus licheniformis*
- *Nitrosomonas sp.*
- *Nitrobacter sp.*

Bacillus sp. produce exo-enzymes, enzymes which are secreted outside the cell wall for the purpose degrading complex organic substrates to a point where they can be transported across the cell membrane and cell wall for complete metabolization. *Cellulomonas sp.* are known for their ability to metabolize cellulose. Cellulose is the rigid molecule which makes up the "skeleton," and the bulk of the mass of plants (and makes up the cell walls of bacteria too). Again, the enzymes necessary for such degradation need to be secreted outside the bacterium as cellulose is a very large, complex organic molecule. *Pseudomonas sp*. represent bacteria resistant to a myriad of unfavorable conditions. Previous studies have noted their ability to live in conditions with high concentrations of phenol and heavy metals (5, 6, 8, 40). *Rhodopseudomonas* represents bacteria with similar qualities of *Pseudomonas*. The difference is *Rhodopseudomonas* has a red color under certain conditions; therefore significant growth is quite obvious (51). This is beneficial as treatment status can actually be seen.

Nitrosomonas sp. convert ammonia to nitrite which *Nitrobacter sp*. utilize and convert to nitrate in the denitrification process with the N1 strain.

 While E1, S1, and G1 contain the same microorganisms, the blends vary in quantity of specific bacteria. The exact composition of the blends in terms of relative population is a trade secret. The different blends are marketed towards different treatments.

 E1 is known as a start-up, shock, and efficiency strain. Applications involve use in toxic wastewaters as well as wastewaters with a high nutrient loading. It is also used for start up situations in WWTPs. The blend also helps to increase metabolic efficiency in wastewater treatment and remediation. It would be expected to have high populations of *Pseudomonas sp.* and *Bacillus sp*.

 S1 is referred to as the sludge reduction strain. It is used to aerobically oxidize particulate, organic matter. Waters choked with sludge or vegetable matter are ideal for S1. *Cellulomonas sp*. and *Bacillus sp*. would be expected to dominate the microbial blend.

 G1 is a blend to oxidize grease and fats. Grease and fats are not easily biodegraded and take a comparatively long time to treat in comparison to most soluble, organics. For this reason, most WWTPs have skimmers to remove grease for treatment or combustion.

 N1 is used for nitrification/denitrification. Its primary use is to treat waters with high concentrations of ammonia. Ammonia is oxidized forming nitrite and oxidized again forming nitrate. Nitrification is a slow process requiring high sludge concentrations, long sludge age, and copious amounts of oxygen.

4.2 Equipment

A. Atomic Absorption Spectrometer

Perkin-Elmer 3100 Atomic Absorption Spectrometer was used to measure concentrations of soluble cadmium, copper, and lead. Acetylene was used as fuel and air was used as the carrier gas.

B. Batch Reactors

1. Batch Reactor –Ion Exchange

Ion exchange trials were conducted in a collection of large beakers and flasks. Due to limited availability, a beaker set of uniform size and shape was not possible. Beakers and flasks capacities ranged from 800 ml to 2000 ml were used.

2. Batch Reactor –Bioaugmentation

After ion exchange, samples were poured into individual batch reactors and LLMO was added. Bioaugmentation batch reactors consisted of 120 ml (4 oz) pee cups with lids from VWR®. A hole of approximately $\frac{3}{4}$ inch diameter was drilled through the top of the lid. A piece of surgical tubing or nylon reinforced tubing of approximately 1.5 to 2 inches in length and $\frac{3}{4}$ inch outer diameter was inserted into the hole. The hole with tube was designed to allow the exchange of gases and prevent liquid spillage.

C. Balance

A Mettler Balance PL303, Mettler-Toledo, Inc. was used to measure small quantities of dehydrated milk, phenol, cadmium chloride, copper sulfate pentahydrate, lead nitrate, and zeolite between 13 mg to 10 g.

D. Glass Filters

Glass Microfibre Filters from Whatman type 934-AH were used to filter samples. They have a diameter of 24 mm and fit inside a gooch crucible. They have a pore size of 1.5 μ m and are designed for filtering liquids containing microorganisms.

E. Gooch Crucible

Gooch crucibles with glass filters were used in conjunction with a vacuum pump to filter samples prior to testing for total organic carbon and heavy metal concentration.

F. Oven

A Fisher Scientific model 175, Illinois was used to dry zeolite after rinsing.

G. pH Meter

A Fisher Scientific Accumet model 10 pH meter was used to take pH readings before and after ion exchange.

H. Platform Shaker

A New Brunswick Scientific Innova 2300 Platform Shaker was used to agitate batch reactors for ion exchange and bioaugmentation. This platform shaker utilizes a counter-clockwise rotation and speeds of 130 and 200 rpm were used. A cage was made of wood and drilled to the platform to prevent batch reactors from leaving the platform and spilling on the floor. In bioaugmentation trials,

additional slats of wood were drilled into wooden frames with 1.5 inch coarse wood screws, preventing the batch reactors from moving at high rotational speeds.

I. Stir Plates and Stir Bars

2 Corning Stirrers, model PC-353 with 2 inch stir bars were used to mix ion exchange batch reactors for the first ten minutes of the trial.

J. Total Organic Carbon Analyzer

A Shimadzu Total Organic Carbon Analyzer -5050A was used to analyze samples for Total Organic Carbon. Ultra Zero Air from AirGas was used as the carrier gas.

K. Vacuum Pump

A Fisher Scientific, IL, USA Vacuum Pump with Flask/Filter Apparatus was used to filter samples prior to testing for total organic carbon or heavy metal concentration.

4.3 Methods

4.3.1 Procedures-Overview

 Bench scale treatment of wastewaters was conducted in batch reactors. Prior to the main experiment, preliminary experiments needed to be conducted to determine the following:

- Ion exchange capacity and treatment dose for Cd, Cu, and Pb.
- Time duration of ion exchange trials.
- Inhibition concentrations of Cd, Cu, Pb, and phenol.

Bench scale treatment was also used in the trials of the main experiment as can be seen in Figure 2. 500 ml of metallic wastewater was added to a beaker and pre-weighed amounts of dehydrated milk, phenol, and zeolite were added. The mixture was heavily mixed for 10 minutes on a stir plate with 2 inch stir bar. After 10 minutes the mixture was placed on a platform shaker for an additional 4 hours at a speed of 130 rpms. After 4 hrs, the WW with zeolite was allowed to settle for 30 minutes. 100 ml of treated water was slowly poured into 4 pee cup bioreactors. 1 ml of LLMO was added, the lids were closed, put on the shaker and locked down for 48 hours at a speed of 200 rpms. The remaining 100 ml was filtered, place in a separate pee cup, and tested for pH and heavy metal concentration. All sample analysis was performed as described by standard methods (60).

Figure 2. Flow Chart of Experiment

At the end of the trial period (48 hrs) the samples were allowed to sit for 30 minutes. Then they were filtered and placed in pyrex test tubes between 20 ml and 50 ml. 4 drops of concentrated sulfuric acid (H_2SO_4) was added to stop further bio-oxidation.

 The generic run protocol for trials in the main experiment can be seen in Table III. A total of 144 ion exchange trials were performed and followed with 576 biological trials. Each run consisted of one metal type, a total organic carbon concentration, a phenol concentration, a zeolite doseage, and an LLMO aliquot. Some examples are also given in the table below for a lead trial and a Cd trial. Exact run protocols will be discussed further.

Run	Metal	Total Organic	Phenol	Zeolite	LLMO
		Carbon	Concentration	Doseage	Type
		Concentration			
576	C _d	High	High	High	E1
Biological	Cu	Medium	Medium	Medium	S ₁
Trials $\&$	Pb	Low	Low	Low	G1
144 Ion			Reference	Reference	N ₁
Exchange					
Trials					
Ex. 1	Pb	Low	Low	Medium	E1
Ex. 2	C _d	High	High	Low	N1

Table III. Run Protocol Determination and Example

4.3.2 Preliminary Experiments

4.3.2.1 Inhibition Concentration

 Inhibition concentrations of Cd, Cu, Pb, and phenol upon E1 strain of LLMO were conducted in bioaugmentation batch reactors. Stock solutions of Cd, Cu, Pb, and phenol were made as seen in Table IV. A pre-weighed amount of solute was added to 1 L of DI Water.

Solute	Mass Solute Added (mg)	Concentration
Cadmium	163	100
Copper	394	100
Lead	160	100
Phenol	1000	1000

Table IV. Stock Solution Preparation

 Milk was analyzed for percentage total organic carbon via trial and error. It was determined that dehydrated milk is approximately 40% total organic carbon. Likewise, it was added to 100 ml of waste at a concentration of 50 mg/100 ml (200 mg Total Organic Carbon/L). Appropriate amounts of stock solution were added to the milk as seen in Table V. DI water was added, if necessary, for a total volume of 100 ml.

Pollutant	Amount of Pollutant Added	Pollutant Concentration	
	(ml)	(mg/L)	
Control (200 mg Total	θ	θ	
Organic Carbon/L)			
Phenol	25	250	
Phenol	50	500	
Phenol	75	750	
Phenol	100	1000	
Cadmium	1	1	
Cadmium	2.5	2.5	
Cadmium	5	5	
Cadmium	7.5	7.5	
Cadmium	10	10	
Copper	1	1	
Copper	2.5	2.5	
Copper	5	5	
Copper	7.5	7.5	
Copper	10	10	
Lead	1		
Lead	2.5	2.5	
Lead	5	5	
Lead	7.5	7.5	
Lead	10	10	

Table V. Preparation of Inhibition Experiment Concentrations

2 types of LLMO, E1 and S1, were added (1 ml) for a total of 40 bioreactors. A portion of each sample was filtered for total organic carbon analysis at t=0 and at t=48 (hours).

4.3.2.2 Ion Exchange Capacity and Exchange Duration

 Preliminary ion exchange experiments were conducted with Pb, Cu, and Cd to identify approximate amounts of ZeoSand® needed and time length of ion exchange trials. The bulk of the work for kinetics experiments was performed using Pb. Literature showed Pb is most effectively removed from solution. All samples were filtered and analyzed on a Perkin-Elmer 3100 AAS. The first experiment was conducted with Pb

(100 mg/L) in DI water which was agitated using stir plate and stir bar. Samples were filtered before zeolite addition (t=0), t=1, t=2, t=3, t=4, and t=24 hours.

 A second kinetics experiment with lead was run using 100 mg/L of soluble lead in the presence of dehydrated milk as listed in the following table. Samples were taken over a period of 4 hours at times of 0, 0.25 hrs. , 0.5 hrs., 0.75 hrs., 1 hr., 1.25 hrs, 1.5 hr, 1.75 hrs., 2 hrs., 3 hrs., and 4 hrs.

A third kinetics experiment was performed using 100 mg Pb/L in the presence of milk but zeolite dose was changed as seen in Table VII. Samples were filtered before the addition of zeolite and 4 hours after the addition of zeolite.

Mixture Description	Zeolite Addition (g/L)
100 mg Pb/L	0.1
100 mg Pb/L	
100 mg Pb/L	3
100 mg Pb/L	5
100 mg Pb/L	10
$(100 \text{ mg Pb} + 100 \text{ mg Total Organic C/L})$	0.1
$(100 \text{ mg Pb} + 100 \text{ mg Total Organic C/L})$	1
$(100 \text{ mg Pb} + 100 \text{ mg Total Organic C/L})$	3
$(100 \text{ mg Pb} + 100 \text{ mg Total Organic C/L})$	5
$(100 \text{ mg Pb} + 100 \text{ mg Total Organic C/L})$	10
$(100 \text{ mg Pb} + 200 \text{ mg Total Organic C/L})$	0.1
$(100 \text{ mg Pb} + 200 \text{ mg Total Organic C/L})$	1
$(100 \text{ mg Pb} + 200 \text{ mg Total Organic C/L})$	3
$(100 \text{ mg Pb} + 200 \text{ mg Total Organic C/L})$	5
$(100 \text{ mg Pb} + 200 \text{ mg Total Organic C/L})$	10
$(100 \text{ mg Pb} + 300 \text{ mg Total Organic C/L})$	0.1
$(100 \text{ mg Pb} + 300 \text{ mg Total Organic C/L})$	1
$(100 \text{ mg Pb} + 300 \text{ mg Total Organic C/L})$	$\overline{3}$
$(100 \text{ mg Pb} + 300 \text{ mg Total Organic C/L})$	5
$(100 \text{ mg Pb} + 300 \text{ mg Total Organic C/L})$	10

Table VII. Set-up for Varied Zeolite Doses of Pb Wastewater

Similar experiments were done with solutions of 100 mg/L of Cu and Cd to determine the amount of zeolite necessary for adequate treatment.

4.3.3 Treatment of Cadmium Wastewaters

 A general overview of wastewater treatment was given previously and can be seen in Figure 2. A soluble Cd concentration with a target of 100 mg/L was mixed using 163 mg CdCl2 added to degassed tap water. Pre-weighed amounts of dehydrated milk and phenol were added to a beaker and filled to a volume of 500 ml with Cd laden wastewater. Next, pre-weighed amounts of zeolite were added. Experiments were conducted with milk total organic carbon concentrations of 100 mg/L, 200 mg/L, and 300 mg/L. Phenol concentrations used consisted of 0 mg/L, 50 mg/L, 150 mg/L, and 250

mg/L. Zeolite doses consisted of 0 g/L, 5 g/L, 10 g/L, and 20 g/L. Tables X through XII in Appendix A list specific run scenarios.

4.3.4 Treatment of Copper Wastewaters

 The treatment of copper wastewaters was nearly identical to cadmium treatment. A soluble Cu concentration with a target of 100 mg/L was mixed using 394 mg CuSO4·5H2O added to degassed tap water. Pre-weighed amounts of dehydrated milk and phenol were added to a beaker and filled to a volume of 500 ml with Cu laden wastewater. Next, pre-weighed amounts of zeolite were added. Experiments were conducted with milk total organic carbon concentrations of 100 mg/L, 200 mg/L, and 300 mg/L. Phenol concentrations used consisted of 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L. Zeolite doses consisted of 0 g/L, 5 g/L, 10 g/L, and 20 g/L. Tables XIII through XV in Appendix A list specific run scenarios.

4.3.5 Treatment of Lead Wastewaters

The treatment of lead wastewaters was similar to cadmium and copper. A soluble Pb concentration with a target of 100 mg/L was mixed using 475 mg $Pb(NO₃)₂$ added to degassed tap water. Theoretically, and with DI water, the amount added is nearly 3 times the amount needed and the wastewater should have a concentration of 300 mg/L. Preweighed amounts of dehydrated milk and phenol were added to a beaker and filled to a volume of 500 ml with Pb laden wastewater. Next, pre-weighed amounts of zeolite were added. Experiments were conducted with milk total organic carbon concentrations of 50 mg/L, 100 mg/L, and 200 mg/L. Phenol concentrations used consisted of 0 mg/L, 50

mg/L, 150 mg/L, and 250 mg/L. Zeolite doses consisted of 0 g/L, 2.5 g/L, 5 g/L, and 10

g/L. Tables XVI through XVIII in Appendix A list specific run scenarios.

CHAPTER V

RESULTS AND DISCUSSION

5.1 Overview

 The results in the following chapter are divided into 10 sections. The first section describes results of preliminary experiments. Preliminary results are divided into 3 subsections describing inhibition concentrations, ion exchange kinetics, and metal removal due to ion exchange. Trials 1-9 describe results in terms of metal removal and total organic carbon removal. Trials 1-3 describe results in cadmium wastewater with varying levels of total organic carbon. Trials 4-6 describe results in copper wastewater with varying levels of total organic carbon. Trials 7-9 describe results in lead wastewater with varying levels of total organic carbon.

 Results in this section were tabulated and presented as tables in Appendix A and figures in Appendix B. Important figures and tables are placed in the results summary section of results.

 Experiments were conducted in as consistent a manner as possible. Naturally, problems due to equipment and inclement weather occurred which may have affected results. These irregularities are noted where appropriate.

5.2 Preliminary Results

5.2.1 Inhibition Concentrations

 Inhibition concentrations for the main experiment were conducted using known quantities of phenol, cadmium, copper, and lead in the presence of 200 mg Total Organic Carbon/L and LLMO over a period of 48 hours. The specifics of the set-up can be seen in Table V of Material and Methods. The purpose of this preliminary experiment was to determine performance of 2 types of LLMO in concentrations of pollutants. Phenol inhibition data was then used to determine maximum concentrations of phenol allowable in treatment. Likewise, a desired range for metal concentration was observed.

 Results for these series of experiments can be seen in Appendices A and B. Tables XIX and XX of Appendix A; and Figures $11 - 18$ of Appendix B list and illustrate total organic carbon removal by LLMO. The positive control was simply 1 ml of LLMO in a solution of 200 mg Total Organic Carbon/L and removal rates were 57.73% for E1 and 55.43% for S1.

 Phenol was tested at relatively high concentrations and observed to inhibit total organic carbon reduction at 250 mg/L. At this concentration E1 removed 22.71% and S1 removed 19.85% of total organic carbon. At higher concentrations of 500, 750, and 1000 mg/L; total organic carbon removal was negative. Total organic carbon was not actually gained in the bio-reactors. The increase in total organic carbon is probably due to increased dissolution of dehydrated milk over a 48 hour period of vigorous shaking. At this point, it was decided the maximum concentration of phenol to use in the main experiment would be 250 mg/L. Therefore, phenol concentrations decided upon for the main trials were 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L.

 Copper concentrations tested for inhibition were 1 mg/L, 2.5 mg/L, 5 mg/L, 7.5 mg/L, and 10 mg/L. LLMO strain E1 showed significant inhibition with respect to total organic carbon removal at only 1 mg/L (Total organic carbon removal = 36.9% compared to 57.73% removal in control). However, S1 did not show inhibition at 1 mg/L (60.47% removal). At 2.5 mg/L, both strains showed significant inhibition with removal rates of 2.31% and 16.21% for E1 and S1 respectively.

Lead concentrations tested for inhibition were the same as copper at 1 mg/L , 2.5 mg/L, 5 mg/L, 7.5 mg/L, and 10 mg/L. Lead concentrations of up to 10 mg/L appeared to have a uniform, slight inhibitory effect upon the E1 strain. Total organic carbon removal for E1 strain hovered around 50% for all concentrations of lead. The S1 strain showed sensitivity to Pb with total organic carbon removal rates of 23.78% and 28.01% for lead concentrations of 2.5 mg/L and 5 mg/L respectively.

Cadmium concentrations tested for inhibition were the same as copper and lead at 1 mg/L, 2.5 mg/L, 5 mg/L, 7.5 mg/L, and 10 mg/L. E1 showed sensitivity to Cd at a concentration of 2.5 mg/L where total organic carbon removal was 17.9%. S1 did not show significant inhibition until a Cd concentration 5 mg/L was tested. At 5 mg/L total organic carbon removal was 0.37%.

5.2.2 Ion Exchange Kinetics

Ion exchange trials focusing on time were conducted with lead, copper, and cadmium to determine ion exchange trial times. Literature showed the time needed for ion exchange with zeolite is quite short as can be seen in Table I. Experiments with lead were meticulously conducted and recorded. Results from Pb ion exchange time trials

were compared to Cu and Cd. Results for Cu and Cd were not recorded as a time for ion exchange was already decided after the lead experiment. The time used in the lead experiment was simply applied to Cd and Cu and checked. Pb removal was conducted in wastewater composed of varying levels of organic substrate and 100 mg/L of soluble lead in DI water as seen in Table VI.

Results can be seen in Tables XXI and XXII. A sample was collected before zeolite was added and another immediately after zeolite was added at a concentration of 10 g/L. 3 trends can be drawn from these tables. First, dehydrated milk concentration has an effect upon the concentration of soluble lead in wastewater. Higher organic substrate concentrations resulted in a lower soluble Pb concentration. All samples had a theoretical concentration of 100 mg/L. Initial concentrations ranged from 95.4 mg/L for Pb in DI water to 77 mg/L in the presence of 200 mg Total Organic Carbon/L. The lead concentration in the presence of 300 mg Total Organic Carbon/L was similar to 200 mg Total Organic Carbon/L with a soluble Pb concentration of 78 mg/L.

The second trend is the speed of ion exchange. In lead ion exchange is very fast with the bulk of removal occurring in the first fifteen minutes. Indeed, removal rates within 2 minutes of zeolite addition are between 30 and 80%. The degree of variation can possibly be explained due to the time needed to filter the wastewater from the zeolite treatment. After 15 minutes removal rates ranged from 99.58% for Pb in DI water to 90.51% in 300 mg Total Organic Carbon /L. After 4 hours final removal rates were 100%, 98.79%, 98.31%, and 96.79% for organic loads of 0 mg Total Organic Carbon /L, 100 Total Organic Carbon /L, 200 Total Organic Carbon L, and 300 Total Organic Carbon /L respectively.

The third observation or trend was that high organic loads of milk retarded ion exchange rates. Lead removal in the absence of organic substrate resulted in nearly complete removal in 15 minutes. However, in the presence of milk, a maximum removal of 98.79% took 4 hours. The highest concentration of milk 300 mg Total Organic Carbon /L reached maximum Pb removal of 96.79% after 4 hours. At this point, it was decided 4 hours of ion exchange would be used in the main trials of the experiment.

5.3 Trial 1 – Cd, Total Organic Carbon = 100 mg/L

 Trial 1 was conducted with cadmium mixed with wastewater with an organic substrate concentration of 100 mg Total Organic Carbon/L. The ion exchange portion of the experiment consisted of 16 variations as seen in Table VIII of Materials and Methods. After ion exchange and 30 minutes of settling 100 ml of the 16 runs was poured into 120 ml batch reactors. 100 ml was left over which was filtered and analyzed for pH and Cd concentration. 1 ml of LLMO was added to each reactor for a total of 64 reactors. After incubation they were tested for total organic carbon concentration.

5.3.1 Cadmium Removal

 Cadmium was tested for removal using 4 concentrations of zeolite including 0 g/L, 5 g/L, 10 g/L, and 20 g/L at 4 levels of phenol including 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L. Higher zeolite doses may have yielded better Cd removal but the zeolite supply was starting to get low. The reduction in Cd can be seen in Table XXIII and Figure 19. While 100 mg/L was the theoretical Cd concentration, actual initial concentrations of Cd ranged from 36 mg/L to 84 mg/L. The Cd concentration of the

wastewater containing 250 mg/L phenol was significantly lower than other initial measurements. It is unclear why this was the case. However, all initial concentrations were well above inhibiting concentrations as found in preliminary experiments. For that matter so were all samples treated with zeolite.

 The percentage of Cd removed was calculated by comparing concentrations treated with zeolite against the control which lacked zeolite. As can be seen in Table XXIII, the percentage of Cd removed ranged from 77.78% when phenol concentration was 0 mg/L and zeolite concentration was 20 g/L to 41.67% when phenol concentration was 250 mg/L and zeolite concentration was 20 g/L. Regardless, of the percentage removed, the final Cd concentration did not reach a threshold which allowed LLMO to flourish.

5.3.2 Effect on pH

 pH was monitored in all trials as a simple observation. A pH measurement was taken before zeolite was added as well as after ion exchange. pH was taken due to reactions in the wastewaters between TDS and soluble metal cations. Such observations can be helpful when unexpected results are attained. The effect of ion exchange upon pH in Cd wastewater of 100 mg Total Organic Carbon/L can be seen in Table XXXII. pH ranged from 6.8 to 7 before ion exchange and 7 to 7.3 afterwards. As seen in the table pH changed relatively little and ion exchange had no effect on pH.

5.3.3 Total Organic Carbon Removal

 Removal of total organic carbon for the cadmium and 100 mg Total Organic Carbon/L can be seen in Tables XXXXI through XXXXIV and Tables LXXVII through LXXX. Tables XXXXI through XXXXIV compares total organic carbon removal within each LLMO strain and Tables LXXVII through LXXX compare total organic carbon removal as a function of LLMO strain.

 Some of the results for this trial show an actual increase in total organic carbon concentration after 48 hours. This was not expected and was a trend observed in other trials. In Tables XXXXI through XXXXIV the best removal of total organic carbon occurred when phenol was not present. In each table a trend appears where total organic carbon removal became worse as phenol concentrations increased. Zeolite concentration didn't appear to have a positive effect upon total organic carbon removal. As zeolite concentration increased, Cd concentration decreased and total organic carbon removal was expected to increase.

 Tables LXXVII through LXXX compare performance of LLMO strains against each other using parameters of phenol concentration, zeolite concentration and associated Cd concentration. No conclusive results can be made in terms of total organic carbon removal in these tables. The two conclusions which can be drawn is the Cd concentrations were too high inhibiting microbial growth and the 48 hour shaking period eventually dissolved particulate milk.

5.4 Trial 2 – Cd, Total Organic Carbon = 200 mg/L

5.4.1 Cadmium Removal

 Cadmium was tested for removal using 4 concentrations of zeolite including 0 g/L, 5 g/L, 10 g/L, and 20 g/L at 4 levels of phenol including 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L. The reduction in Cd can be seen in Table XXIV and Figure 20. While 100 mg/L was the theoretical Cd concentration, actual initial concentrations of Cd ranged from 83 mg/L to 72 mg/L. The variability in these initial concentrations was more acceptable than the trial at 100 mg Total Organic Carbon/L. Again, all initial concentrations were well above inhibiting concentrations as found in preliminary experiments as were all samples treated with zeolite.

 The percentage of Cd removed was calculated by comparing concentrations treated with zeolite against the control which lacked zeolite. As can be seen in Table XXIV, %Cd removed ranged from 60.81% when phenol concentration was 250 mg/L and zeolite concentration was 20 g/L to 46.67% when phenol concentration was 50 mg/L and zeolite concentration was 20 g/L. However, the zeolite dose was not high enough to remove an adequate amount of Cd to allow microbial growth.

5.4.2 Effect on pH

 The effect of ion exchange upon pH in Cd wastewater of 200 mg Total Organic Carbon/L can be seen in Table XXXIII. pH ranged from 6.8 to 7 before ion exchange and 6.3 to 7.6 afterwards. The trend observed here is pH decreases when zeolite is not added but milk total organic carbon is. Zeolite had the effect of raising pH in most samples. There was one exception, sample 12, which had pH decrease by 0.1.

5.4.3 Total Organic Carbon Removal

 Removal of total organic carbon for the cadmium and 200 mg Total Organic Carbon/L can be seen in Tables XXXXV through XXXXVIII and Tables LXXXI through LXXXIV. Tables XXXXV through XXXXVIII compares total organic carbon removal within each LLMO strain and Tables LXXXI through LXXXIV compare total organic carbon removal as a function of LLMO strain.

 Results in trial 2 were more palatable as total organic carbon removal was positive for all 16 phenol and zeolite combinations and their 4 subsequent treatments with LLMO. Tables XXXXV through XXXXVIII show total organic carbon removal decreased as phenol concentration increased. Table XXXXV shows total organic carbon removal with LLMO strain E1 worked best with a zeolite concentration of 20 g/L and a phenol concentration of 50 mg/L (removal of 39.53%). This result is slightly better than treatment of the wastewater containing 0 mg/L of phenol (removal of 38.11%). It appears that a concentration of 50 mg/L of phenol had no inhibitory effect upon E1. Table XXXXVI shows total organic carbon removal with LLMO strain S1. The results in this table are very similar to results achieved by E1 except total organic carbon removal is slightly better. The best removal rates of 41.77% and 39.15% occurred with a zeolite concentration of 20 g/L and phenol concentrations of 50 mg/L and 0 mg/L respectively. Table XXXXVII lists total organic carbon removal with the G1 strain developed for grease. Total organic carbon removal (41.35%) was best with a zeolite concentration of 20 g/L and a phenol concentration of 0 mg/L. An increase of phenol concentration to 50 mg/L had little effect while higher phenol concentrations decreased removal percentages. Treatment with the N1 strain is listed in Table XXXXVIII. Results

here were unexpected as performance was similar to other strains of LLMO. Best total organic carbon removal of 40% occurred with zeolite concentrations of 20 g/L for phenol concentrations of 0 and 50 mg/L.

 Tables LXXXI through LXXXIV compares LLMO strain performance against each other when phenol zero. As can be seen, the strains of LLMO acted similarly and did not show significant differences. An explanation for this is Cd concentrations were high enough regardless of zeolite dose to inhibit bio-oxidation by LLMO. In fact, most of the total organic carbon removal in all trials probably occurred due to sedimentation.

5.5 Trial 3 – Cd, Total Organic Carbon = 300 mg/L

5.5.1 Cadmium Removal

 Cadmium was tested for removal using 4 concentrations of zeolite including 0 g/L, 5 g/L, 10 g/L, and 20 g/L at 4 levels of phenol including 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L. The reduction in Cd can be seen in Table XXV and Figure 21. Again, 100 mg/L was the theoretical Cd concentration, but actual initial concentrations of Cd ranged from 64 mg/L to 69 mg/L. Again, all initial concentrations were well above inhibiting concentrations as found in preliminary experiments as were all samples treated with zeolite. Final concentrations of Cd treated with 20 g/L zeolite were 32 mg/L, 18 mg/L, 18 mg/L, and 24 mg/L for 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L of phenol respectively.

 The percentage of Cd removed was calculated by comparing concentrations treated with zeolite against the control which lacked zeolite. As can be seen in Table XXV, %Cd removed ranged from 73.91% when phenol concentration was 50 mg/L and

zeolite concentration was 20 g/L to 52.24% when phenol concentration was 0 mg/L and zeolite concentration was 20 g/L. Again, the zeolite dose was not high enough to remove an adequate amount of Cd to allow microbial growth.

5.5.2 Effect on pH

 The effect of ion exchange upon pH in Cd wastewater of 300 mg Total Organic Carbon/L can be seen in Table XXXIV. pH ranged from 6.8 to 7 before ion exchange and 7.1 to 7.4 afterwards. The trend observed here is pH increased slightly regardless of whether zeolite was added or not. The logical conclusion is zeolite had no effect on pH in this trial.

5.5.3 Total Organic Carbon Removal

 Removal of total organic carbon for the cadmium and 300 mg Total Organic Carbon/L can be seen in Tables IL through LII and Tables LXXXV through LXXXVIII. Tables IL through LII compares total organic carbon removal within each LLMO strain and Tables LXXXV through LXXXVIII compare total organic carbon removal as a function of LLMO strain.

 Tables IL through LII show the best total organic carbon removal rates occurred with phenol concentrations of 0 mg/L. The E1 strain showed a best removal rate of 39.01% when phenol was zero and the zeolite concentration was 5 g/L . S1, G1, and N1 strains show similar removal rates at the same parameters.

 Tables LXXXV through LXXXVIII compare LLMO strain performance when all other parameters were the same. Table LXXXV shows total organic carbon removal
rates were about the same regardless of LLMO when phenol concentration was zero. The best performance occurred with E1 with a removal rate of 39.01% and a zeolite dose of 5 g/L. The worst performance was by S1 with a removal rate of 20.91% at a zeolite dose of 10 g/L. Tables LXXXV through LXXXVIII, again, show LLMO strains behaving approximately the same as high zeolite doses appear to result in less total organic carbon removal. The one group which had unexpected results is in Table LXXXVIII where the phenol concentration was 250 mg/L and the zeolite dose was 10 g/L.

5.6 Trial 4 – Cu, Total Organic Carbon = 100 mg/L

5.6.1 Copper Removal

 Copper was tested for removal using 4 concentrations of zeolite including 0 g/L, 5 g/L, 10 g/L, and 20 g/L at 4 levels of phenol including 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L. The reduction in Cu due to ion exchange can be seen in Table XXVI and Figure 22. 100 mg/L of Cu was the theoretical concentration, but actual initial concentrations of Cu were significantly lower. The solubility of $CuSO₄·5H₂O$ is 31.7 $g/100$ ml and is relatively low in comparison to CdCl₂. The presence of TDS and various ions may have decreased the solubility of $CuSO_4:5H_2O$. Also, other unknown reactions may have occurred with dissolved ions and dehydrated milk components causing insoluble copper salts and complexes to precipitate. The important point is all initial concentrations were well above inhibiting concentrations as found in preliminary experiments.

Initial concentrations of Cu or Cu in the presence of 100 mg Total Organic Carbon/L without zeolite were 44.5 mg/L, 37 mg/L, 35 mg/L, and 38 mg/L for phenol

concentrations of 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L respectively. Final concentrations of Cu treated with 20 g/L zeolite were 7 mg/L, 7 mg/L, 4 mg/L, and 5 mg/L for 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L respectively. The decrease in copper due to ion exchange is significant but as inhibition experiments show, these final concentrations are enough to inhibit microbial activity.

 The percentage of Cu removed was calculated by comparing concentrations treated with zeolite against the control which lacked zeolite. As can be seen in Table XXVI, %Cu removed ranged from 81.08% when phenol concentration was 50 mg/L and zeolite concentration was 20 g/L to 88.57% when phenol concentration was 150 mg/L and zeolite concentration was 20 g/L.

5.6.2 Effect on pH

 The effect of ion exchange upon pH in Cu wastewater of 100 mg Total Organic Carbon/L can be seen in Table XXXV. pH ranged from 5.8 to 5.9 before ion exchange and 5.9 to 6.2 afterwards. A couple observations can be made here. One is that cupric sulfate pentahydrate, when dissolved in degassed tap water has the effect of lowering pH. The second point is that zeolite has the effect of slightly raising pH . A low pH of ≤ 6 can have inhibiting consequences on many microorganisms making successful wastewater treatment difficult.

5.6.3 Total Organic Carbon Removal

 Removal of total organic carbon for the copper and 100 mg Total Organic Carbon/L can be seen in Tables LIII through LVI and Tables LXXXIX through

LXXXXIV. Tables LIII through LVI compares total organic carbon removal within each LLMO strain and Tables LXXXIX through LXXXXIV compare total organic carbon removal as a function of LLMO strain.

 Results in tables LIII through LVI are not consistent with wide fluctuations in total organic carbon values and removal rates. Other tables such as LXXXIX through LXXXXIV comparing total organic carbon removal by LLMO strain were not reliable either. The only runs which provide reliable data are the samples tested with a phenol concentration of 0 mg/L. This is best observed in Table LXXXIX. In this table, total organic carbon removal varied from $\leq 10\%$ to greater than 60%. Treatment with 5 g/L of zeolite resulted in consistent total organic carbon % removals of around 60%. Treatment with 20 g/L zeolite with the N1 strain was also wildly unrealistic at 73.29%. However, if these samples are disregarded a slight trend can be seen where total organic carbon removal with no zeolite results in removal rates of 8.01 to 17.76% and total organic carbon removal increases to approximately 20% with zeolite doses of 10 and 20 g/L. These total organic carbon removal rates are logical as some removal occurred due to sedimentation but more removal occurred as Cu concentrations decreased to 16 and 7 mg/L. However, these concentrations of Cu are still high enough to cause significant inhibition of LLMO.

5.7 Trial 5 – Cu, Total Organic Carbon = 200 mg/L

5.7.1 Copper Removal

 Copper was tested for removal using 4 concentrations of zeolite including 0 g/L, 5 g/L, 10 g/L, and 20 g/L at 4 levels of phenol including 0 mg/L, 50 mg/L, 150 mg/L, and

250 mg/L. The reduction in Cu due to ion exchange can be seen in Table XXVII and Figure 23. Again, 100 mg/L of Cu was the theoretical concentration, but actual initial concentrations of Cu were significantly lower ranging from 35 mg/L to 38 mg/L. Still, the initial concentrations were well above inhibiting concentrations as found in preliminary experiments.

Initial concentrations of Cu or Cu in the presence of 200 mg Total Organic Carbon/L without zeolite were 38 mg/L, 35 mg/L, 36 mg/L, and 36 mg/L for phenol concentrations of 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L of phenol respectively. Final concentrations of Cu treated with 20 g/L zeolite were 14.5 mg/L, 9 mg/L, 8.5 mg/L, and 8 mg/L for 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L respectively. The decrease in copper due to ion exchange is significant but as inhibition experiments show, these final concentrations are enough to inhibit microbial activity.

 The percentage of Cu removed was calculated by comparing concentrations treated with zeolite against the control which lacked zeolite. As can be seen in Table XXVII, %Cu removed ranged from 61.84% when phenol concentration was 0 mg/L and zeolite concentration was 20 g/L to 77.78% when phenol concentration was 250 mg/L and zeolite concentration was 20 g/L. The concentration of Cu treated with a phenol concentration of 0 mg/L treated with 20 g/L zeolite was significantly higher than other wastewaters treated with 20 g/L. In fact, it was only 0.5 mg/L lower than zeolite treatment using 10 g/L.

5.7.2 Effect on pH

The effect of ion exchange upon pH in Cu wastewater of 200 mg Total Organic Carbon/L can be seen in Table XXXVI. pH ranged from 5.8 to 5.9 before ion exchange and 5.8 to 6.3 afterwards. Again, it appears Cu ion exchange with zeolite may slightly raise pH.

5.7.3 Total Organic Carbon Removal

 Removal of total organic carbon for the copper and 200 mg Total Organic Carbon/L can be seen in Tables LVII through LX and Tables LXXXXII through LXXXXV. Tables LVII through LX compares total organic carbon removal within each LLMO strain and Tables LXXXXII through LXXXXV compare total organic carbon removal as a function of LLMO strain.

 Tables LVII through LX show total organic carbon removal rates decrease as phenol concentration increased. Table LVII compares results with strain E1 which had total organic carbon removal percentages of 21.74 to 34.07% when the phenol concentration was zero to removal rates of 7.62 to 25.68% with a phenol concentration of 250 mg/L. Similar results occurred with S1, G1, and N1.

 Comparison of removal rates via strain can be observed in Tables LXXXXII through LXXXXV. Table LXXXXII shows the strains E1, S1, and G1 remove total organic carbon slightly better than N1 in treatments of 10 and 20 g/L with a phenol concentration of zero. This trend was repeated in Table LXXXXIV when the phenol concentration was 150 mg/L and the zeolite dose was 20 g/L. Table LXXXXV also shows this trend when the phenol concentration was 250 mg/L and the zeolite concentration was 10 g/L.

5.8 Trial 6 – Cu, Total Organic Carbon = 300 mg/L

5.8.1 Copper Removal

Copper was tested for removal using 4 concentrations of zeolite including $0 \frac{g}{L}$, 5 g/L, 10 g/L, and 20 g/L at 4 levels of phenol including 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L. The reduction in Cu due to ion exchange can be seen in Table XXVIII and Figure 24. Again, 100 mg/L of Cu was the theoretical concentration, but actual initial concentrations of Cu were significantly lower. However, the initial concentrations were above inhibiting concentrations as found in preliminary experiments.

Initial concentrations of Cu or Cu in the presence of 300 mg Total Organic Carbon/L without zeolite were 36.5 mg/L, 34.5 mg/L, 35 mg/L, and 32 mg/L for phenol concentrations of 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L respectively. Final concentrations of Cu treated with 20 g/L zeolite were 7 mg/L, 6 mg/L, 6 mg/L, and 5 mg/L for 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L respectively. Again, the decrease in copper due to ion exchange is significant but as inhibition experiments show, these final concentrations are enough to inhibit microbial activity.

 The percentage of Cu removed was calculated by comparing concentrations treated with zeolite against the control which lacked zeolite. As can be seen in Table XXVIII, percentage of Cu removed ranged from 80.82% when phenol concentration was 0 mg/L and zeolite concentration was 20 g/L to 84.38% when phenol concentration was 250 mg/L and zeolite concentration was 20 g/L. There appeared to be a trend with Cu removal as final concentration decreased (percentage Cu removed increased) as phenol concentrations became stronger. However, more replicates and more testing are necessary to statistically show a significant trend.

5.8.2 Effect on pH

 The effect of ion exchange upon pH in Cu wastewater of 300 mg Total Organic Carbon/L can be seen in Table XXXVII. pH ranged from 5.8 to 5.9 before ion exchange and 5.9 to 6.2 afterwards. Again, it appears Cu ion exchange with zeolite may slightly raise pH.

5.8.3 Total Organic Carbon Removal

 Removal of total organic carbon for the copper and 300 mg Total Organic Carbon/L can be seen in Tables LXI through LXIV and Tables LXXXXVI through IC. Tables LXI through LXIV compares total organic carbon removal within each LLMO strain and Tables LXXXXVI through IC compare total organic carbon removal as a function of LLMO strain.

 Total organic carbon removal rates in Tables LXI through LXIV which grouped LLMO strains together showed inconsistent results. Expected trends of increasing rates of total organic carbon removal with increased doses of zeolite were not observed. In fact, the opposite was true. However, tables LXXXXVI through IC compare performance of LLMO strains directly against each other show limited evidence that E1, S1, G1 perform better than N1. For example, in Table LXXXXVI where the phenol concentration was 0 mg/L, the trend is seen in zeolite doses of 0 g/L, 5 g/L, and 20 g/L. The best evidence of this trend exists when the zeolite concentration was 20 g/L and the final Cu concentration was 7 mg/L. In this comparison, E1 removed nearly 35%, G1 removed nearly 31%, S1 removed about 27%, and N1 removed 24.5% of initial total organic carbon. Table LXXXXVII shows a similar trend where the phenol concentration was 50 mg/L and zeolite concentration was 20 g/L. The associated Cu concentration was 6 mg/L. Total organic carbon removal rates were 18.92%, 16.55%, 16.52%, and 11.39% for LLMO strains E1, S1, G1, and N1 respectively.

5.9 Trial 7 – Pb, Total Organic Carbon = 50 mg/L

5.9.1 Lead Removal

Lead was tested for removal using 4 concentrations of zeolite including $0 g/L$, 2.5 g/L , 5 g/L, and 10 g/L at 4 levels of phenol including 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L. The reduction in Pb due to ion exchange at an organic substrate concentration of 50 mg Total Organic Carbon/L can be seen in Table XXIX and Figure 25. Approximately 300 mg/L of Pb was the theoretical concentration, but actual initial concentrations of Pb were around 90 mg/L. As mentioned in Materials and Methods this probably due to the presence of chloride ions in the water. Soluble lead reacts with chloride ions to form $PbCl₂$ and related complexes which precipitate out of solution. Therefore, excess $Pb(NO₃)₂$ was added via trial and error to overcome the loss due to reactions. Otherwise, the initial concentrations would not be high enough to inhibit microbial growth. In addition, a relatively high concentration of soluble Pb was needed as zeolite removes these cations from solution so effectively.

Initial concentrations of Pb or Pb in the presence of 50 mg Total Organic Carbon/L without zeolite were 89 mg/L, 83.5 mg/L, 88.25 mg/L, and 91 mg/L for phenol concentrations of 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L respectively. Final concentrations of Pb treated with 10 g/L zeolite were 0 mg/L, 0.1 mg/L, 0.05 mg/L, and 0.3 mg/L for 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L respectively.

 The percentage of Pb removed was calculated by comparing concentrations treated with zeolite against the control which lacked zeolite. As can be seen in Table XXIX, %Pb removed was essentially 100% for all concentrations of phenol when wastewater was treated with 10 g zeolite/L. It is quite obvious that lead is very efficiently removed when compared with copper and cadmium species.

5.9.2 Effect on pH

 The effect of ion exchange upon pH in Pb wastewater of 50 mg Total Organic Carbon/L can be seen in Table XXXVIII. pH ranged from 5.7 to 5.8 before ion exchange and 5.7 to 6.5 afterwards. High rates of ion exchange appear to substantially increase the pH of wastewater. The greater the removal of Pb from solution the greater the pH increased. This had the benefit of creating an environment more hospitable for bacterial growth.

5.9.3 Total Organic Carbon Removal

 Removal of total organic carbon for the lead and 50 mg Total Organic Carbon/L can be seen in Tables LXV through LXVIII and Tables C through CIII. Tables LXV through LXVIII compares total organic carbon removal within each LLMO strain and Tables C through CIII compare total organic carbon removal as a function of LLMO strain.

 Table LXV lists total organic carbon removal rates for the E1 strain. No definite trends were observed for phenol concentrations of 150 mg/L and 250 mg/L as total organic carbon removal rates were between 10 to 15%. However, phenol concentrations

of 0 mg/L and 50 mg/L show increased total organic carbon removal with zeolite treatment. When phenol and zeolite concentrations were zero the Pb concentration was 89 mg/L and the total organic carbon removal rate was 31.09% . The addition of 2.5 g/L of zeolite reduced the Pb concentration to 11.05 mg/L and total organic carbon removal rate increased to 40.69%. The addition of 5 g/L and 10 g/L of zeolite reduced the Pb concentration to less than 1 mg/L. Consequently, total organic carbon removal rates were very close at 45.16% and 45.76% for zeolite doses of 5 g/L and 10 g/L respectively. The same trend existed when the phenol concentration was 50 mg/L except total organic carbon removal rates were lower and treatment with 5 g/L of zeolite resulted in a total organic carbon removal rate of only 15.87%. The S1 strain on Table LXVI did not show the trends of increasing total organic carbon removal rates as zeolite dose increased. However, the G1 strain did in Table LXVII with phenol concentrations of 0 mg/L and 50 mg/L.

 Tables C through CIII which compared total organic carbon removal via LLMO strain had mixed results. In these tables the N1 strain performed the same or better than the other strains of LLMO. This was unexpected as N1 is blended for the purpose of nitrification/denitrification and shouldn't oxidize organic compounds faster than the other strains.

5.10 Trial 8 – Pb, Total Organic Carbon = 100 mg/L

5.10.1 Lead Removal

 Lead was tested for removal using 4 concentrations of zeolite including 0 g/L, 2.5 g/L, 5 g/L, and 10 g/L at 4 levels of phenol including 0 mg/L, 50 mg/L, 150 mg/L, and

250 mg/L. The reduction in Pb due to ion exchange at an organic substrate concentration of 100 mg Total Organic Carbon/L can be seen in Table XXX and Figure 19. Again, 300 mg/L of Pb was the theoretical concentration, but actual initial concentrations of Pb ranged from 69 to 79 mg/L.

Initial concentrations of Pb or Pb in the presence of 100 mg Total Organic Carbon/L without zeolite were 69 mg/L, 79.5 mg/L, 79 mg/L, and 77.5 mg/L for phenol concentrations of 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L respectively. Final concentrations of Pb treated with 10 g/L zeolite were 0.15 mg/L, 0.6 mg/L, 0.65 mg/L, and 0.35 mg/L for 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L respectively.

 The percentage of Pb removed was calculated by comparing concentrations treated with zeolite against the control which lacked zeolite. As can be seen in Table XXX, %Pb removed was greater than 99% for all concentrations of phenol when wastewater was treated with 10 g zeolite/L.

5.10.2 Effect on pH

 The effect of ion exchange upon pH in Pb wastewater of 100 mg Total Organic Carbon/L can be seen in Table XXXIX. pH ranged from 5.7 to 5.8 before ion exchange and 5.7 to 6.3 afterwards. Again, in most cases pH increased but only slightly. It is important to note that initial Pb concentrations in this trial were not as high as the previous trial with Pb concentrations around 90 mg/L. Initial Pb concentrations in this trial ranged from 69 to 79 mg/L. The decrease in Pb concentration, in turn, means less Pb was available for ion exchange. Therefore, fewer ions were available to buffer or raise the pH.

5.10.3 Total Organic Carbon Removal

 Removal of total organic carbon for the lead and 100 mg Total Organic Carbon/L can be seen in Tables LXIX through LXXII and Tables CIV through CVII. Tables LXIX through LXXII compares total organic carbon removal within each LLMO strain and Tables CIV through CVII compare total organic carbon removal as a function of LLMO strain.

 Results in Tables LXIX through LXXII vary wildly with very high total organic carbon removal rates when lead concentrations were at their highest. Comparing treatment by dose did not yield consistent results. However, comparing performance by strain in Tables CIV through CVII showed some evidence that E1, S1, and G1 performed better than N1. In Table CIV where the phenol concentration was zero this trend was observed with zeolite treatments of 0 and 5 g/L. The N1 run with a zeolite dose of 10 g/L had a total organic carbon removal rate of 98.74%. This result may be best explained as the drops of H2SO4 may not have been added resulting in continued bio-oxidation.

5.11 Trial 9 – Pb, Total Organic Carbon = 200 mg/L

5.11.1 Lead Removal

 Lead was tested for removal using 4 concentrations of zeolite including 0 g/L, 2.5 g/L , 5 g/L, and 10 g/L at 4 levels of phenol including 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L. The reduction in Pb due to ion exchange at an organic substrate concentration of 100 mg Total Organic Carbon/L can be seen in Table XXXI and Figure 27. Again, 300 mg/L of Pb was the theoretical concentration, but actual initial concentrations of Pb

ranged from 56.25 mg/L to 66.5 mg/L. This marks an interesting trend as the increase in total organic carbon noticeably decreases available lead in solution.

Initial concentrations of Pb or Pb in the presence of 200 mg Total Organic Carbon/L without zeolite were 60.75 mg/L, 57.25 mg/L, 56.25 mg/L, and 66.5 mg/L for phenol concentrations of 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L respectively. Final concentrations of Pb treated with 10 g/L zeolite were 0.05 mg/L, 0.65 mg/L, 0.3 mg/L, and 0.85 mg/L for phenol concentrations of 0 mg/L, 50 mg/L, 150 mg/L, and 250 mg/L respectively.

 The percentage of Pb removed was calculated by comparing concentrations treated with zeolite against the control which lacked zeolite. As can be seen in Table XXXI, %Pb removed ranged from 98.72% to 99.92% when wastewater was treated with 10 g zeolite/L.

5.11.2 Effect on pH

 The effect of ion exchange upon pH in Pb wastewater of 200 mg Total Organic Carbon/L can be seen in Table XXXX. pH ranged from 5.7 to 5.8 before ion exchange and varied from 5.4 to 6.2 afterwards. Again, in most cases pH increased but only slightly, and there were quite a few examples where pH decreased. It is believed these low pHs were exceptions and could possibly be explained by differences in temperature between measurements. Again, initial Pb concentrations in this trial were not as high as the previous trial (50 mg Total Organic Carbon/L) with Pb concentrations around 90 mg/L. Initial Pb concentrations in this trial ranged from 56 to 66 mg/L. Less Pb exchange with zeolite meant fewer potassium ions to raise the pH.

5.11.3 Total Organic Carbon Removal

 Removal of total organic carbon for the lead and 200 mg Total Organic Carbon/L can be seen in Tables LXXIII through LXXVI and Tables CVIII through CXI. Tables LXXIII through LXXVI compares total organic carbon removal within each LLMO strain and Tables CVIII through CXI compare total organic carbon removal as a function of LLMO strain.

 Table LXXIII shows increases in total organic carbon removal rates as zeolite dose increased for the E1 strain. However, zeolite concentrations of 2.5 g/L appeared more effective than a zeolite dose of 5 g/L at phenol concentrations of 0 mg/L, 50 mg/L, and 250 mg/L. S1 showed similar trends in total organic carbon removal rates in Table LXXIV, especially for the runs conducted at a phenol concentration of 50 mg/L. The total organic carbon removal rate increased from 29.19% without zeolite to 41.29% with 10 g/L zeolite. The G1 strain in Table LXXV showed a similar trend to E1 and S1, although the increase was not as great. Finally, total organic carbon removal rates with the N1 strain were unexpectedly high at zeolite doses of 10 g/L. Removal rates with treatment of zeolite at 10 g/L ranged from 36.5% to 97.12%.

 Tables CVIII through CXI showed mixed results. Table CVIII where the phenol concentration was zero showed a slight trend of increased total organic carbon removal performance of E1, S1, and G1 over N1 at zeolite doses of 0 and 2.5 g/L . However, this trend was not seen at higher zeolite doses of 5 and 10 g/L. When the phenol concentration was increased to 50 mg/L in Table CIX; E1, S1, and G1 performed better than N1 at zeolite doses of 2.5 g/L and 5 g/L. However, treatment with 10 g/L did not show this as the removal rate with N1 was again, unexpectedly high at 79.84%. Finally,

total organic carbon removal rates were inconsistent, indicative of inhibition, in Tables CX and CXI where phenol concentrations were 150 mg/L and 250 mg/L.

5.12 Summary of Performance in Presence of Cadmium

 Results for the cadmium trials fall into two categories of physical-chemical and biological processes. In short, Cd removal through ion exchange worked while biological oxidation of organic wastewater was inconclusive. The parameters measured in the 3 trials of differing organic substrate (milk) concentrations of 100, 200, and 300 mg Total Organic Carbon/L were Cd concentration, total organic carbon concentration, and pH.

 Soluble Cd was removed effectively from the wastewater mixture. The differing concentrations of organic substrate did not appear to have an effect upon Cd concentration which ranged from 36 mg/L (36 mg/L was quite unexpected and abnormally low) to 84 mg/L in all total organic carbon concentrations. The best Cd removal occurred with the largest dose of zeolite at 20 g/L. Final concentrations of Cd with the highest zeolite dose range 14 mg/L to 40 mg/L. Unfortunately, these Cd concentrations are still very high and preliminary experiments showed inhibition of LLMO at Cd concentrations below 10 mg/L.

 pH is an indicator of environmental conditions affecting bacterial growth and was noted as slightly increasing as zeolite doses increased. In the Cd trials pH was not very important as the range was very close to neutral. pH range without addition of zeolite was 6.3 to 7.3 which is fine for bio-oxidation of organic compounds. The pH range after ion exchange was 6.8 to 7.4.

 Total organic carbon removal in all trials was inconclusive. In runs where total organic carbon concentration was 100 mg/L there were some cases of total organic carbon removal but more than half the samples showed an increase in total organic carbon. Total organic carbon removal was more acceptable with initial concentrations of 200 and 300 mg Total Organic Carbon/L. In these trials total organic carbon removal rates decreased as phenol concentrations increased. Also, total organic carbon removal rates were not greatly affected by Cd removal with zeolite. Total organic carbon was removed regardless of zeolite dose. Preliminary experiments showed the final concentrations of Cd were more than adequate to inhibit bacterial oxidation. Thus, the best explanation for total organic carbon removal is through sedimentation of organic solids in the milk wastewater.

5.13 Summary of Performance in Presence of Copper

 Copper results were similar to cadmium results as the physical-chemical experiments worked well but biological oxidation was inconclusive. The wastewaters had a theoretical Cu concentration of 100 mg/L and organic substrate concentrations of 100, 200, and 300 mg Total Organic Carbon/L. Results were measured as soluble Cu concentration, pH, and total organic carbon concentration.

 Cu was removed from wastewater to a final lower concentration lower than Cd trials. Concentrations of soluble copper were much lower than expected. Dissolution of $CuSO₄·5H₂O$ was very good in DI water but not very good in degassed tap water. The increase in organic carbon and the presence of phenol also appeared to have the effect of slightly reducing Cu concentrations as seen in Tables XXVI through XXVIII. When total

organic carbon concentration was 100 mg/L the Cu concentration without zeolite or phenol was 44.5 mg/L but decreased as phenol was added to 35 and 38 mg/L for phenol concentrations of 150 and 250 mg/L respectively. Likewise, Cu concentration decreased as organic substrate concentration increased. For example, 44.5 mg Cu/L in 100 mg Total Organic Carbon/L decreased to 38 mg/L at 200 mg Total Organic Carbon/L, and decreased further to 36.5 mg/L at 300 mg Total Organic Carbon/L. Thus, the lowest initial concentration of Cu was 32 mg/L at 300 mg Total Organic Carbon/L and 250 mg phenol/L.

 The best removal of Cu occurred with a zeolite dose of 20 g/L. Final Cu concentrations with this treatment varied from 4 to 14.5 mg/L with most concentrations around 7 mg/L. Unfortunately, Cu concentrations this high still had an inhibiting effect upon LLMO, especially when phenol was present. Literature as seen in Table 1 shows evidence where zeolite removes Cu slightly better than Cd (24). Research in these experiments suggests Cd is removed better than Cu. However, the difference is moot as the Cu concentration was still high enough to inhibit bio-oxidation.

 As stated previously, pH affects bio-oxidation and was low in Cu trials in comparison to cadmium trials. pH was not effected by differing concentrations of organic substrate and ranged from 5.8 to 5.9 before addition of zeolite. After 4 hours of ion exchange pH remained the same or slightly increased in most runs. pH after treatment ranged from 5.8 to 6.2. The low pH may have affected LLMO metabolism slightly but to what degree is unknown. At any rate, Cu and phenol concentrations inhibit growth more than pH of this range does.

 total organic carbon reduction was inconclusive in Cu trials. When theoretical total organic carbon concentration was 100 mg/L, total organic carbon concentration actually increased after 48 hours for some runs. In total organic carbon concentrations of 200 mg/L and 300 mg/L zeolite treatment did not consistently improve total organic carbon removal due to bioaugmentation. This can be observed in Tables LVII through LXIV as zeolite appeared to make a difference in some cases while zeolite appeared to increased total organic carbon in other cases. The greatest factor influencing total organic carbon removal appears to be phenol concentration. As phenol concentration increased, total organic carbon removal decreased while the potential for inhibition increased. However, Cu concentrations were still adequate, regardless of zeolite dose, to inhibit bioaugmentation.

Comparisons of LLMO performance did not yield conclusive results either. The expected trend was that E1, S1, and G1 would perform better than N1. Sometimes this did occur but many times it did not. The N1 strain is blended for nitrificationdenitrification and should not remove organic carbon as well as the other strains. It appears most of total organic carbon removal that occurred in these trials was due to sedimentation.

5.14 Summary of Performance in Presence of Lead

 Lead results were similar to cadmium and copper results as the physical-chemical experiments worked very well but biological oxidation was inconclusive. The wastewaters had a theoretical Pb concentration of 300 mg/L but after precipitation from PbCl₂ as well as other complex formations the actual concentration of Pb was less than

100 mg/L. Organic substrate concentrations were reduced to 50, 100, and 200 mg Total Organic Carbon/L. Organic substrate concentrations were reduced as this was the last set of experiments and it was hoped better evidence of bioaugmentation would be seen at lower substrate concentrations. Results were measured as soluble Pb concentration, pH, and total organic carbon concentration.

 Initial lead concentrations decreased significantly with increased organic substrate concentration. This can be seen in Tables XXIX through XXXI. Initial concentrations at 50 mg Total Organic Carbon/L were between 83.5 mg/L and 91 mg/L. These concentrations dropped to a range of 69 to 79.5 mg/L and 56.25 to 66.5 mg/L for total organic carbon concentrations of 100 mg/L and 200 mg/L respectively. The decrease in Pb concentration was probably due to adsorption and precipitation reactions with components of the dehydrated milk. The presence of chloride ions would certainly have caused more lead to precipitate, albeit not many chloride ions would be expected. The adsorption of Pb onto organic material probably accounted for more Pb removal than precipitation due to milk addition.

 Physical-chemical treatment with zeolite was very effective. As Tables XXIX through XXXI show a mere zeolite dose of 2.5 g/L reduced soluble Pb to concentrations of 15.6 mg/L or less. 5 g/L dropped Pb concentrations to 3.35 mg/L and 10 g/L dropped Pb concentrations to 0.85 mg/L or less. Organic substrate and phenol concentrations did not appear to have a significant effect upon Pb removal. As literature asserted, zeolite is very selective towards Pb. Preliminary experiments showed that these zeolite treatments got the Pb concentration low enough to where inhibition of LLMO should have been significantly reduced.

Similar to CuSO₄·5H₂O, the addition of Pb($NO₃$)₂ to degassed tap water dropped the pH. Before ion exchange pH ranged 5.7 to 5.8 as seen in Tables XXXVIII through XXXX. Afterwards, pH usually increased to above 6. The increase in pH was not large but may have large enough to provide a better environment for LLMO.

 total organic carbon removal in Pb trials as seen in Tables LXV through LXXVI and Tables C through CXI was disappointing and inconclusive. As stated previously, there appear to be some trends of increased total organic carbon removal as zeolite doses increased and Pb concentrations decreased. These trends occur at phenol concentrations of 0 and 50 mg/L. However, the trends are slight and not conclusive. LLMO strain did not have a significant effect upon total organic carbon removal either. In short, total organic carbon removal was unpredictable.

5.15 Summary of Best Total Organic Carbon Removal Performance

The summary of performance is limited to the lowest total organic carbon concentrations in each metal. This means 100 mg total organic carbon for Cu and Cd and 50 mg/L for Pb. If total organic carbon removal due to bio-oxidation does exist, it will be best observed at the lowest organic carbon concentrations. 1 ml of LLMO was added to each bio-reactor regardless of total organic carbon concentration. Therefore, a total organic carbon concentration of 100 mg/L would have more bacteria metabolizing the organic carbon as opposed to a total organic carbon concentration of 200 mg/L.

 One way to compare total organic carbon removal is to present graphs of total organic carbon removal compared against metal species and zeolite dose for each LLMO type. These results can be seen in Figures 3 through 6 where Figure 3 represents results

using E1, Figure 4 represents results using S1, Figure 5 represents results using G1, and Figure 6 represents results using N1. The results compare no zeolite added to a maximum amount of zeolite added. The maximum amount added to Cd and Cu was 20 g/L and the maximum amount added to Pb was 10 g/L . Parameters of importance, including metal concentration, for the following figures can be seen in Table XVII. The purpose of not including results from phenol concentrations greater than 0 mg/L is identify any trends due to LLMO type and maximum zeolite dose. The presence of phenol complicates results by reducing total organic carbon removal rates because phenol is not removed in appreciable amounts by such a short period of biological oxidation, and phenol inhibits bio-oxidation.

Metal	Total Organic Carbon Conc. (Theoretical)	Phenol Conc. (mg/L)	Zeolite Conc. (g/L)	Metal Conc. Post - Exchange (mg/L)
Cd $Zeo = 0$	100	$\mathbf 0$	0	63
Cd $Zeo = 20$	100	$\mathbf 0$	20	14
Cu $Zeo = 0$	100	0	0	44.50
Cu $Zeo = 20$	100	$\mathbf 0$	20	7.00
Pb $Zeo = 0$	50	0	0	89
Pb $Zeo = 10$	50	0	10	0

Table VIII. Parameters Affecting Total Organic Carbon Removal in Figures 3-6

Figure 3. Total Organic Carbon Removal % in Absence of Phenol Using E1 as a Function of Metal Type and Zeolite Dose

Figure 4. Total Organic Carbon Removal % in Absence of Phenol Using S1 as a Function of Metal Type and Zeolite Dose

Figure 5. Total Organic Carbon Removal % in Absence of Phenol Using G1 as a Function of Metal Type and Zeolite Dose

Figure 6. Total Organic Carbon Removal % in Absence of Phenol Using N1 as a Function of Metal Type and Zeolite Dose

 Figures 3 through 6 illustrate the effects of LLMO type, metal concentration, and zeolite dose upon total organic carbon removal. In the Cd trials, total organic carbon removal rates actually decrease with the addition of zeolite. This probably occurred because the Cd concentration was 14 mg/L and still too high for biological oxidation to occur. In addition, particulate milk remaining in the wastewater after settling had 48 hours to dissolve at a high shaking speed. total organic carbon removal with Cu removal increased as expected by about 5% in E1, S1, and G1 LLMO types. This occurred even as the Cu concentration after ion exchange was 7 mg/L, a level shown in preliminary experiments as inhibiting biological oxidation. The N1 experiment showed a large increase in total organic carbon removal and it is not clear why this occurred. Nitrification bacteria are autotrophic and should increase total organic carbon concentration in wastewater instead of remove it. total organic carbon removal rates varied as lead was removed from the wastewater. E1 showed an increase in removal of about 15%, G1 showed an increase of about 11%, S1 remained unchanged, and N1 slightly decreased.

 The increases and decreases of Table XVII and Figures 3 through 6 are show illustrate total organic carbon removal due to bioaugmentation and zeolite treatment better than any other trials in this period of research as phenol was absent and total organic carbon concentrations were low. Bioaugmentation should have worked best with LLMO addition to Pb wastewater with 10 g/L of zeolite. The addition of 10 g/L of zeolite resulted in a Pb concentration of zero. Metal concentrations after the addition of 20 g/L zeolite to Cu and Cd were still very high and inhibited total organic carbon removal through bioaugmentation. Even with complete removal of soluble Pb

bioaugmentation increased total organic carbon removal by a maximum of 15% over the 48 hour growth period.

 The addition of 50 mg/L of phenol had the effect of reducing total organic carbon removal as seen in Figures 7 through 10. Cd and Cu concentrations, as seen in Table XVIII were still too high after the addition of 20 g/L of zeolite and inhibited bioaugmentation. total organic carbon removal rates in the Cd trial were negative regardless of LLMO strain. It is not clear why the data is negative as organic carbon was removed through settling, not added. A slight decrease in removal rates was expected with a maximum of dissolution over 48 hours and inhibition of LLMO was expected. However, an increase in total organic carbon of up to 30% was certainly not expected. The obviously incorrect results could be due to operator error and re-calibration of the Total Organic Carbon Analyzer.

Metal	Total Organic Carbon Conc. (Theoretical)	Phenol Conc. (mg/L)	Zeolite Conc. (g/L)	Metal Conc. Post - Exchange (mg/L)
Cd $Zeo = 0$	100	50	0	84
Cd $Zeo = 20$	100	50	20	32
Cu $Zeo = 0$	100	50	0	37.00
Cu $Zeo = 20$	100	50	20	7.00
Pb $Zeo = 0$	50	50	0	83.5
Pb $Zeo = 10$	50	50	10	0.1

Table IX. Parameters Affecting Total Organic Carbon Removal in Figures 7-10

Total organic carbon removal in the Cu trial was slightly negative without the addition of zeolite was slightly negative meaning the organic carbon concentrations increased slightly due to dissolution over 48 hours. However, the addition of 20 g/L of clinoptilolite increased total organic carbon removal rates up to nearly 50% for LLMO strains E1 and S1 in Figures 7 and 8; and a modest 5-7% as seen in Figures 9 and 10. It is unclear why a vast difference occurred between strains E1/S1 and G1/N1. Bioaugmentation effectively removed some organic carbon but the Cu concentration was still 7 mg/L and high enough to inhibit bacterial growth, especially in the presence of phenol.

Figure 7. Total Organic Carbon Removal % in Presence of 50 mg/L Phenol Using E1 with Respect to Metal Type and Zeolite Dose

Figure 8. Total Organic Carbon Removal % in Presence of 50 mg/L Phenol Using S1 with Respect to Metal Type and Zeolite Dose

Figure 9. Total Organic Carbon Removal % in Presence of 50 mg/L Phenol Using G1 with Respect to Metal Type and Zeolite Dose

Figure 10. Total Organic Carbon Removal % in Presence of 50 mg/L Phenol Using N1 with Respect to Metal Type and Zeolite Dose

 The best or most consistent performance was expected to occur with the lead trials. Again, soluble Pb was effectively removed from the wastewater to a concentration of 0.1 mg/L with a zeolite dose of 10 g/L. Without zeolite addition the total organic carbon removal rate ranged from 7 to 17%. After Pb removal, total organic carbon removal increased to 38% for E1, 25% for S1, 24% for G1, and 28% for N1. The listed total organic carbon removals are consistent and show LLMO addition removed total organic carbon. However, these removal rates are not as good as when phenol was absent where removal rates were above 40%. The lower removal rates can be explained by 2 reasons. One is the general total organic carbon concentration is higher with phenol and phenol was not oxidized by LLMO. Therefore, a certain amount of total organic carbon, i.e. 24 mg/L in for Pb and 50 mg total organic carbon of milk/L was unable to be

removed. This automatically diluted the total organic carbon removal rate. The second reason is 50 mg/L of phenol slightly inhibited biological oxidation.

Phenol concentrations of 150 mg/L and 250 mg/L in combination with heavy metal presence inhibited bioaugmentation. Even with the optimum metal removal treatment of Pb with 10 g/L of zeolite a decrease in total organic carbon removal was observed. It is probable that some heavy metals adsorbed to the milk and was always present in the wastewater regardless of soluble concentration after ion exchange. In combination with higher phenol concentrations, this may have been enough to inhibit biological oxidation to a degree not seen with phenol concentrations of 0 and 50 mg/L.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- 1. Pollutants used in these experiments inhibited LLMO inoculations at various concentrations including 20% inhibition with the following pollutants and concentrations: phenol concentration of 250 mg/L, copper and cadmium concentrations of 2.5 mg/L. Lead inhibited LLMO bio-oxidation by 10% at a concentration of 10 mg/L.
- 2. Zeolite effectively removed soluble, heavy metals from the simulated wastewater. Phenol did not effect ion exchange removal of heavy metals. The zeolite used in these experiments removed lead effectively with an optimum removal rate of 100%. Removal of copper and cadmium was very close in performance. Best removal rates of copper and cadmium were 88.6% and 77.8% respectfully. However, final concentrations of copper and cadmium were still above inhibiting concentrations determined in preliminary experiments and probably inhibited total organic carbon removal using LLMO.
- 3. Total Organic Carbon removal was due to sedimentation and biological oxidation. However, total organic carbon removal was influenced by metal type, zeolite doseage, phenol concentration, and initial total organic carbon concentration. Phenol had the effect of raising total organic carbon concentration Sedimentation removed over 50% total organic carbon in some cases when phenol concentrations were low or zero. Phenol had the effect of raising total organic carbon concentration but was not removed by sedimentation or biological oxidation. Therefore, not only had inhibitory effects upon oxidation it also lowered the percentage of total organic carbon which could be removed from the wastewater. LLMO was observed to improve total organic carbon removal by 10- 15% for lead and copper trials of maximum zeolite dose, low total organic carbon and low phenol concentrations. The combination of heavy metals and phenol had a greater inhibiting effect upon bio-oxidation than single exposure to a pollutant. Biological treatment worked best in the following order of Pb>Cu>Cd corresponding to the metal removal selectivity order by zeolite at highest doseage.
- 4. LLMO improved total organic carbon removal rates but each strain behaved differently. E1 performed better than G1, G1 performed better than S1, which performed better than N1. In optimum conditions of lead trials E1 improved efficiency by 15%, G1 by about 10%, S1 slightly improved by less than 1%, and N1 showed no improvement.

6.2 Recommendations for Future Research

Future research evaluating treatment of wastewaters containing phenol, heavy metals, and organic substrate should consider the following recommendations:

- 1. Spend more time and experiments characterizing the wastewaters. Pollutant concentrations observed using DI water will probably not be the same as pollutant concentrations in degassed tap water. This is especially true for heavy metals such as copper, cadmium, and lead.
- 2. Investigate synergistic-antagonistic effects upon LLMO of heavy metals and phenol at very dilute concentrations before the main experiment trials.
- 3. Use relatively low concentrations of organic substrate. Initial total organic carbon concentrations should not exceed 100 mg/L. total organic carbon removal performance using microorganisms is easier to test for at lower substrate concentrations. At a decreased F/M ratio, microorganisms will remove organic substrates faster and to a larger degree as compared to high total organic carbon concentrations.
- 4. Organic substrates must be selected carefully. While dehydrated milk represents the organic components of wastewater very well it is difficult to disperse and dissolve resulting in substantial amounts lost to sedimentation. Future research may want to use another carbon source such as glucose. Unfortunately, this means additional compounds are necessary for nitrogen, phosphorous, and trace elements essential for a growing, healthy microbiological population.
- 5. Amount of LLMO added is very important. This experiment used 1 ml of LLMO per reactor. An increase to 5 ml should increase total organic carbon removal.

6. Future research may want to acclimatize LLMO to pollutants. Microorganisms perform better in wastewater experiments after an exposure period to dilute concentrations of pollutants such as phenol and heavy metals.

6.3 Engineering Applications

 Large slug flows or shock loads of pollutants are rarely encountered anymore in publicly owned treatment plants of the USA anymore but certainly occur in other parts of the world. Shock loads can inhibit or kill microorganisms essential for biological treatment. This research would be best applied to individual industrial plants as a pre-treatment before release to municipal sewerage. The point to be made is the toxic substances have to present on a constant basis. A shock load of once a month will kill or inhibit microorganisms. However, if acclimatization periods at low pollutant concentrations are gradually increased to desired treatment levels the bacteria can survive heavy metals while oxidizing phenol and other organic substrates.

 Design for such a treatment system would be similar to current technologies. Wastewater characteristics would need to analyzed and to a large degree would depend upon the concentration or organic carbon which could be used as a substrate for biological oxidation. If the organic substrate was lacking it would be best to treat the WW strictly by physical-chemical methods. The treatment scheme would start with removal of course and damaging solids. Next, primary solids would be removed by DAF or settling. After this, the primary treated water would need extensive dilution with recirculated, treated water. Recirculation ratios would depend upon heavy metal concentration in the water as well as the known concentration which bacteria can still effectively oxidize pollutants. After biological oxidation, secondary sludge removal water would pass through a sand filter which would use zeolite instead of sand. Zeolite could be used to remove waters of mixed metals provided the metal species have an affinity for zeolite and exist at quantities which can be removed. An additional filter of

activated carbon would be helpful to remove phenol as little phenol would be removed in biological operations. Treated water would then be recirculated to dilute influent as necessary or released to the sewer. There would be a significant challenge with how to handle the sludge. The sludge would contain high concentrations of heavy metals and would have to be disposed of properly at a high cost.

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APPENDIX A

Table X. Run Protocol for Trial 1 – Cadmium with 100 mg Total Organic Carbon/L

Table XI. Run Protocol for Trial 2 – Cadmium with 200 mg Total Organic Carbon/L

Table XII. Run Protocol for Trial 3 – Cadmium with 300 mg Total Organic Carbon/L

Table XIII. Run Protocol for Trial 4 - Copper with 100 mg Total Organic Carbon/L

Table XIV. Run Protocol for Trial 5 – Copper with 200 mg Total Organic Carbon/L

Table XV. Run Protocol for Trial 6 – Copper with 300 mg Total Organic Carbon/L

Table XVI. Run Protocol for Trial 7 - Lead with 50 mg Total Organic Carbon/L Run # Pb (mg/L) Total Organic | Zeolite (g/L) | Phenol (mg/L) LLMO Type

Table XVII. Run Protocol for Trial 8 – Lead with 100 mg Total Organic Carbon/L

Run#	Cd (mg/L)	Total	Zeolite (g/L)	Phenol	LLMO
		Organic		(mg/L)	Type
		Carbon			
		(mg/L)			
$\mathbf{1}$	100	200	$\boldsymbol{0}$	$\boldsymbol{0}$	E1
$\overline{2}$	100	200	$\boldsymbol{0}$	$\boldsymbol{0}$	S1
3	100	200	$\boldsymbol{0}$	$\boldsymbol{0}$	G ₁
$\overline{4}$	100	200	$\boldsymbol{0}$	$\boldsymbol{0}$	N1
$\overline{5}$	100	200	$\boldsymbol{0}$	50	E1
6	100	200	$\boldsymbol{0}$	50	S1
$\overline{7}$	100	200	$\boldsymbol{0}$	50	G1
$\,8\,$	100	200	$\boldsymbol{0}$	50	N1
9	100	200	$\boldsymbol{0}$	150	E1
10	100	200	$\boldsymbol{0}$	150	S1
11	100	200	$\boldsymbol{0}$	150	G1
12	100	200	$\boldsymbol{0}$	150	N1
13	100	200	$\boldsymbol{0}$	250	E1
14	100	200	$\boldsymbol{0}$	250	S1
15	100	200	$\boldsymbol{0}$	250	G ₁
16	100	200	$\boldsymbol{0}$	250	N1
17	100	200	2.5	$\boldsymbol{0}$	E1
18	100	200	2.5	$\boldsymbol{0}$	S1
19	100	200	2.5	$\boldsymbol{0}$	G ₁
20	100	200	$2.\overline{5}$	$\boldsymbol{0}$	N1
21	100	200	2.5	50	E1
22	100	200	$\overline{2.5}$	50	S ₁
23	100	200	$\overline{2.5}$	50	G1
24	100	200	2.5	50	N1
$\overline{25}$	100	200	$\overline{2.5}$	150	E1
26	100	200	2.5	150	S ₁
27	100	200	2.5	150	G1
28	100	200	$\overline{2.5}$	150	N1
29	100	200	2.5	250	E1
30	100	200	2.5	250	S ₁
31	100	200	2.5	250	G ₁
32	100	200	2.5	250	N ₁
33	100	200	5	$\boldsymbol{0}$	E1
34	100	200	$\overline{5}$	$\boldsymbol{0}$	S ₁
35	100	200	$\overline{5}$	$\boldsymbol{0}$	G1
36	100	200	$\overline{5}$	$\boldsymbol{0}$	N ₁
37	100	200	$\overline{5}$	50	E1
38	100	200	$\overline{5}$	50	S ₁
39	100	200	$\overline{5}$	50	G ₁

Table XVIII. Run Protocol for Trial 9 – Lead with 200 mg Total Organic Carbon/L

			Total	Total	% Total
		Contaminant	Organic	Organic	Organic
Sample	LLMO	Concentration	Carbon	Carbon	Carbon
#	Type	(mg/L)	$t=0$ hr	$t=48$ hr	Removal
1	S ₁	Reference	171.8	76.57	55.43
$\overline{\mathbf{c}}$	S ₁	Phenol = 250	366.7	293.9	19.85
$\overline{3}$	\overline{S}	Phenol = 500	560.8	605.4	-7.95
4	S ₁	Phenol = 750	746	811.9	-8.83
$\overline{5}$	S ₁	Phenol = 1000	978.8	1057	-7.99
6	S ₁	$Cu = 1$	204.3	80.76	60.47
7	S ₁	$Cu = 2.5$	194.3	162.8	16.21
8	$\overline{S1}$	$Cu = 5$	128	150.7	-17.73
9	S ₁	$Cu = 7.5$	183.9	200.2	-8.86
10	S ₁	$Cu = 10$	173.9	202.2	-16.27
11	S ₁	$Pb = 1$	193.9	87.52	54.86
12	S ₁	$Pb = 2.5$	193	147.1	23.78
13	S ₁	$Pb = 5$	187.4	134.9	28.01
14	S ₁	$Pb = 7.5$	187.6	206.9	-10.29
15	S ₁	$Pb = 10$	178.3	75.61	57.59
16	S ₁	$Cd = 1$	190.6	113.2	40.61
17	S ₁	$Cd = 2.5$	197.2	94.81	51.92
18	S ₁	$Cd = 5$	190.2	189.5	0.37
19	S ₁	$Cd = 7.5$	196.1	203.5	-3.77
20	S ₁	$Cd = 10$	188.5	193.2	-2.49

Table XX. Inhibition of S1: Pollutant Concentration vs. Total Organic Carbon Removal

Table XXI. Preliminary Experiment: Pb Concentration vs. Time

Table XXIII. Cadmium Concentration at 4 Hours with 100 mg Total Organic Carbon/L

Table XXIV. Cadmium Concentration at 4 Hours with 200 mg Total Organic Carbon/L

Sample #	Total Organic Carbon (mg/L)	Phenol Conc. (mg/L)	Zeolite Conc. (g/L)	C _d Conc. (mg/L)	% Cd Removed
1	300	0	0	67	0.00
$\overline{2}$	300	0	5	48	28.36
3	300	0	10	42	37.31
$\overline{\mathbf{4}}$	300	0	20	32	52.24
5	300	50	$\mathbf 0$	69	0.00
6	300	50	5	44	36.23
$\overline{7}$	300	50	10	36	47.83
8	300	50	20	18	73.91
9	300	150	0	64	0.00
10	300	150	5	49	23.44
11	300	150	10	38	40.63
12	300	150	20	18	71.88
13	300	250	0	64	0.00
14	300	250	5	57	10.94
15	300	250	10	40	37.50
16	300	250	20	24	62.50

Table XXV. Cadmium Concentration at 4 Hours with 300 mg Total Organic Carbon/L

Table XXVI. Copper Concentration at 4 Hours with 100 mg Total Organic Carbon/L

Table XXVII. Copper Concentration at 4 Hours with 200 mg Total Organic Carbon/L

Table XXVIII. Copper Concentration at 4 Hours with 300 mg Total Organic Carbon/L
Sample #	Total Organic C Conc. (mg/L)	Phenol Conc. <u>(mg/L)</u>	Zeolite Conc. (g/L)	Pb Conc. (mg/L)	%Pb Removed
1	50	0	0	89	0.00
\overline{c}	50	0	2.5	11.05	87.58
3	50	0	5	0.9	98.99
$\overline{\mathbf{4}}$	50	0	10	0	100.00
5	50	50	0	83.5	0.00
6	50	50	2.5	11.6	86.11
$\overline{7}$	50	50	5	3.3	96.05
8	50	50	10	0.1	99.88
9	50	150	0	88.25	0.00
10	50	150	2.5	6.3	92.86
11	50	150	5	2.15	97.56
12	50	150	10	0.05	99.94
13	50	250	0	91	0.00
14	50	250	2.5	7.65	91.59
15	50	250	5	2.9	96.81
16	50	250	10	0.3	99.67

Table XXIX. Lead Concentration at 4 Hours with 50 mg Total Organic Carbon/L

Table XXX. Lead Concentration at 4 Hours with 100 mg Total Organic Carbon/L

Table XXXI. Lead Concentration at 4 Hours with 200 mg Total Organic Carbon/L

Table XXXII. Effect of Ion Exchange on pH in $Cd + 100$ mg Total Organic Carbon/L with Respect to Zeolite Dose

Table XXXIII. Effect of Ion Exchange on pH in $Cd + 200$ mg Total Organic Carbon/L with Respect to Zeolite Dose

Table XXXIV. Effect of Ion Exchange on pH in $Cd + 300$ mg Total Organic Carbon/L with Respect to Zeolite Dose

Table XXXV. Effect of Ion Exchange on pH in $Cu + 100$ mg Total Organic Carbon/L with Respect to Zeolite Dose

Table XXXVI. Effect of Ion Exchange on pH in $Cu + 200$ mg Total Organic Carbon/L with Respect to Zeolite Dose

Table XXXVII. Effect of Ion Exchange on pH in $Cu + 300$ mg Total Organic Carbon/L with Respect to Zeolite Dose

Table XXXVIII. Effect of Ion Exchange on pH in $Pb + 50$ mg Total Organic Carbon/L with Respect to Zeolite Dose

Table XXXIX. Effect of Ion Exchange on pH in $Pb + 100$ mg Total Organic Carbon/L with Respect to Zeolite Dose

Table XXXX. Effect of Ion Exchange on pH in Pb + 200 mg Total Organic Carbon/L with Respect to Zeolite Dose

Table XXXXI. Total Organic Carbon Removal in Cd Wastewater: Milk Conc. $= 100$ mg/L, E1

Table XXXXII. Total Organic Carbon Removal in Cd Wastewater: Milk Conc. = 100 mg/L, S1

Table XXXXIII. Total Organic Carbon Removal in Cd Wastewater: Milk Conc. = 100 mg/L, G1

Table XXXXIV. Total Organic Carbon Removal in Cd Wastewater: Milk Conc. = 100 mg/L, N1

Table XXXXV. Total Organic Carbon Removal in Cd Wastewater: Milk Conc. = 200 mg/L, E1

Table XXXXVI. Total Organic Carbon Removal in Cd Wastewater: Milk Conc. = 200 mg/L, S1

Table XXXXVII. Total Organic Carbon Removal in Cd Wastewater: Milk Conc. = 200 mg/L, G1

Table XXXXVIII. Total Organic Carbon Removal in Cd Wastewater: Milk Conc. = 200 mg/L, N1

Sample #	Phenol Conc. (mg/L)	Zeolite Conc. (g/L)	C _d Conc. Post Exch. (mg/L)	рH Post Exch.	LLMO Type	Total Org. $C = 0$ hrs (mg/L)	Total Org. $C = 4$ hrs (mg/L)	Total Org. C $t = 48$ hrs (mg/L)	Total Org. C% Removal
1	Ω	0	67	7.1	E ₁	264.05	167.05	184.55	30.11
$\overline{2}$	0	5	48	7.1	E1	264.05	160.40	161.05	39.01
3	0	10	42	7.2	E ₁	264.05	162.40	166.50	36.94
4	Ω	20	32	7.1	E1	264.05	159.80	181.55	31.24
5	50	0	69	7.3	E1	276.10	200.35	194.25	29.65
6	50	5	44	7.2	E1	276.10	201.15	211.20	23.51
$\overline{7}$	50	10	36	7.2	E1	276.10	198.50	241.60	12.50
8	50	20	18	7.2	E1	276.10	201.10	252.05	8.71
9	150	0	64	7.3	E1	361.25	270.35	271.80	24.76
10	150	5	49	7.3	E1	361.25	277.15	279.80	22.55
11	150	10	38	7.4	E ₁	361.25	286.45	303.65	15.94
12	150	20	18	7.3	E1	361.25	281.55	306.00	15.29
13	250	0	64	7.3	E ₁	427.80	342.65	333.15	22.12
14	250	5	57	7.4	E1	427.80	355.95	347.10	18.86
15	250	10	40	7.4	E1	427.80	359.55	444.60	-3.93
16	250	20	24	7.2	E1	427.80	367.50	382.00	10.71

Table IL. Total Organic Carbon Removal in Cd Wastewater: Milk Conc. = $300 \text{ mg/L}, E1$

Sample #	Phenol Conc. (mg/L)	Zeolite Conc. (g/L)	Cd Conc. Post Exch. (mg/L)	рH Post Exch.	LLMO Type	Total Org. $C = 0$ hrs (mg/L)	Total Org. $C = 4$ hrs (mg/L)	Total Org. C $t = 48$ hrs (mg/L)	Total Org. C% Removal
1	0	0	67	7.1	S ₁	264.05	167.05	187.05	29.16
$\overline{2}$	0	5	48	7.1	S ₁	264.05	160.40	163.15	38.21
3	0	10	42	7.2	S ₁	264.05	162.40	208.85	20.91
4	0	20	32	7.1	S ₁	264.05	159.80	179.05	32.19
5	50	Ω	69	7.3	S ₁	276.10	200.35	192.85	30.15
6	50	5	44	7.2	S ₁	276.10	201.15	215.20	22.06
$\overline{7}$	50	10	36	7.2	S ₁	276.10	198.50	237.60	13.94
8	50	20	18	7.2	S ₁	276.10	201.10	249.90	9.49
9	150	Ω	64	7.3	S ₁	361.25	270.35	271.00	24.98
10	150	5	49	7.3	S ₁	361.25	277.15	280.60	22.33
11	150	10	38	7.4	S ₁	361.25	286.45	303.05	16.11
12	150	20	18	7.3	S ₁	361.25	281.55	316.20	12.47
13	250	Ω	64	7.3	S ₁	427.80	342.65	333.15	22.12
14	250	5	57	7.4	S ₁	427.80	355.95	342.70	19.89
15	250	10	40	7.4	S ₁	427.80	359.55	443.05	-3.56
16	250	20	24	7.2	S ₁	427.80	367.50	387.10	9.51

Table L. Total Organic Carbon Removal in Cd Wastewater: Milk Conc. = $300 \text{ mg/L}, S1$

Sample #	Phenol Conc. (mg/L)	Zeolite Conc. (g/L)	Cd Conc. Post Exch. (mg/L)	рH Post Exch.	LLMO Type	Total Org. C $t=0$ hrs (mg/L)	Total Org. C $t = 4$ hrs (mg/L)	Total Org. C $t = 48$ hrs (mg/L)	Total Org. C % Removal
1	Ω	0	67	7.1	G ₁	264.05	167.05	183.30	30.58
$\overline{2}$	$\mathbf 0$	5	48	7.1	G ₁	264.05	160.40	161.45	38.86
3	Ω	10	42	7.2	G ₁	264.05	162.40	167.10	36.72
4	$\mathbf 0$	20	32	7.1	G ₁	264.05	159.80	182.70	30.81
5	50	$\mathbf 0$	69	7.3	G ₁	276.10	200.35	193.65	29.86
6	50	5	44	7.2	G ₁	276.10	201.15	217.45	21.24
$\overline{7}$	50	10	36	7.2	G ₁	276.10	198.50	242.75	12.08
8	50	20	18	7.2	G ₁	276.10	201.10	259.15	6.14
9	150	0	64	7.3	G ₁	361.25	270.35	266.15	26.33
10	150	5	49	7.3	G1	361.25	277.15	284.40	21.27
11	150	10	38	7.4	G ₁	361.25	286.45	305.00	15.57
12	150	20	18	7.3	G ₁	361.25	281.55	305.80	15.35
13	250	0	64	7.3	G ₁	427.80	342.65	326.70	23.63
14	250	5	57	7.4	G1	427.80	355.95	342.90	19.85
15	250	10	40	7.4	G ₁	427.80	359.55	442.90	-3.53
16	250	20	24	7.2	G ₁	427.80	367.50	391.05	8.59

Table LI. Total Organic Carbon Removal in Cd Wastewater: Milk Conc. = $300 \text{ mg/L}, G1$

Table LII. Total Organic Carbon Removal in Cd Wastewater: Milk Conc. $=$ 300 mg/L, N1

Table LIII. Total Organic Carbon Removal in Cu Wastewater: Milk Conc. $= 100$ mg/L, E1

Sample #	Phenol Conc. (mg/L)	Zeolite Conc. (g/L)	Cu Conc. Post Exch. (mg/L)	рH Post Exch.	LLMO Type	Total Org. C $t=0$ hrs (mg/L)	Total Org. C $t = 4$ hrs (mg/L)	Total Org. C $t = 48$ hrs. (mg/L)	Total Org. C % Removal
1	Ω	Ω	44.50	6.1	S1	88.68	83.17	81.58	8.01
$\overline{2}$	0	5	24.00	6.2	S ₁	88.68	39.13	34.52	61.07
3	$\overline{0}$	10	16.00	6.1	S ₁	88.68	67.11	68.33	22.95
$\overline{4}$	$\overline{0}$	20	7.00	6.2	S ₁	88.68	69.59	69.21	21.96
5	50	Ω	37.00	6	S ₁	114.05	113.40	118.90	-4.25
6	50	5	24.00	6.1	S ₁	114.05	111.35	116.45	-2.10
$\overline{7}$	50	10	16.50	6.1	S ₁	114.05	117.00	111.00	2.67
8	50	20	7.00	6.2	S ₁	114.05	62.77	57.33	49.73
9	150	Ω	35.00	6	S ₁	176.75	214.05	217.70	-23.17
10	150	5	21.50	6	S ₁	176.75	199.55	205.10	-16.04
11	150	10	17.00	6.1	S ₁	176.75	178.00	191.45	-8.32
12	150	20	4.00	6.1	S ₁	176.75	227.40	123.25	30.27
13	250	0	38.00	5.9	S ₁	267.55	276.55	290.35	-8.52
14	250	5	23.00	5.9	S ₁	267.55	305.45	306.50	-14.56
15	250	10	15.00	6	S ₁	267.55	314.75	320.40	-19.75
16	250	20	5.00	6	S ₁	267.55	159.05	166.75	37.68

Table LIV. Total Organic Carbon Removal in Cu Wastewater: Milk Conc. $= 100$ mg/L, S1

Table LV. Total Organic Carbon Removal in Cu Wastewater: Milk Conc. $= 100$ mg/L, G1

Table LVI. Total Organic Carbon Removal in Cu Wastewater: Milk Conc. $= 100$ mg/L, N1

Table LVII. Total Organic Carbon Removal in Cu Wastewater: Milk Conc. $= 200$ mg/L, E1

Table LVIII. Total Organic Carbon Removal in Cu Wastewater: Milk Conc. $= 200$ mg/L, S1

Table LIX. Total Organic Carbon Removal in Cu Wastewater: Milk Conc. $= 200$ mg/L, G1

Table LX. Total Organic Carbon Removal in Cu Wastewater: Milk Conc. $= 200$ mg/L, N1

Table LXI. Total Organic Carbon Removal in Cu Wastewater: Milk Conc. $=$ 300 mg/L, E1

Table LXII. Total Organic Carbon Removal in Cu Wastewater: Milk Conc. $=$ 300 mg/L, S1

Table LXIII. Total Organic Carbon Removal in Cu Wastewater: Milk Conc. $=$ 300 mg/L, G1

Table LXIV. Total Organic Carbon Removal in Cu Wastewater: Milk Conc. $=$ 300 mg/L, N1

Table LXV. Total Organic Carbon Removal in Pb Wastewater: Milk Conc. $=$ 50 mg/L, E1

Table LXVI. Total Organic Carbon Removal in Pb Wastewater: Milk Conc. $=$ 50 mg/L, S1

Table LXVII. Total Organic Carbon Removal in Pb Wastewater: Milk Conc. $=$ 50 mg/L, G1

Table LXVIII. Total Organic Carbon Removal in Pb Wastewater: Milk Conc. $=$ 50 mg/L, N1

Table LXIX. Total Organic Carbon Removal in Pb Wastewater: Milk Conc. $= 100$ mg/L, E1

Table LXX. Total Organic Carbon Removal in Pb Wastewater: Milk Conc. $= 100$ mg/L, S1

Table LXXI. Total Organic Carbon Removal in Pb Wastewater: Milk Conc. $= 100$ mg/L, G1

Table LXXII. Total Organic Carbon Removal in Pb Wastewater: Milk Conc. $= 100$ mg/L, N1

Table LXXIII. Total Organic Carbon Removal in Pb Wastewater: Milk Conc. $= 200$ mg/L, E1

Table LXXIV. Total Organic Carbon Removal in Pb Wastewater: Milk Conc. $= 200$ mg/L, S1

Sample #	Phenol Conc. (mg/L)	Zeolite Conc. (g/L)	Pb Conc. Post Exch. (mg/L)	рH Post Exch.	LLMO Type	Total Org. C $t=0$ hrs (mg/L)	Total Org. C $t=4$ hrs (mg/L)	Total Org. $C = 48$ hrs (mq/L)	Total Org. C % Removal
1	0	0	60.75	5.7	G ₁	149.95	111.65	108.60	27.58
$\overline{2}$	Ω	2.5	8.55	6	G1	149.95	82.84	111.40	25.71
3	0	5	1.55	6	G1	149.95	11.54	107.15	28.54
4	Ω	10	0.05	5.9	G ₁	149.95	56.26	99.84	33.42
5	50	Ω	57.25	5.6	G ₁	196.8	136.85	139.70	29.01
6	50	2.5	3.4	5.8	G ₁	196.8	120.70	136.55	30.61
$\overline{7}$	50	5	1.9	5.7	G ₁	196.8	135.10	140.05	28.84
8	50	10	0.65	5.6	G ₁	196.8	80.51	137.35	30.21
9	150	Ω	56.25	5.6	G ₁	269.95	217.05	214.85	20.41
10	150	2.5	7.55	5.7	G1	269.95	200.70	216.15	19.93
11	150	5	1.05	5.8	G1	269.95	208.50	208.65	22.71
12	150	10	0.3	5.8	G ₁	269.95	138.00	213.95	20.74
13	250	Ω	66.5	5.3	G ₁	347.8	296.85	285.55	17.90
14	250	2.5	5.35	5.4	G ₁	347.8	279.45	280.40	19.38
15	250	5	1.9	5.7	G1	347.8	279.50	285.15	18.01
16	250	10	0.85	6.2	G ₁	347.8	284.20	277.15	20.31

Table LXXV. Total Organic Carbon Removal in Pb Wastewater: Milk Conc. $= 200$ mg/L, G1

Table LXXVI. Total Organic Carbon Removal in Pb Wastewater: Milk Conc. $= 200$ mg/L, N1

Table LXXVII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cd Wastewater: Total Organic Carbon = 100 mg/L , Phenol = 0 mg/L

Table LXXVIII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cd Wastewater: Total Organic Carbon = 100 mg/L , Phenol = 50 mg/L

Table LXXIX. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cd Wastewater: Total Organic Carbon = 100 mg/L , Phenol = 150 mg/L

Table LXXX. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cd Wastewater: Total Organic Carbon = 100 mg/L , Phenol = 250 mg/L

Table LXXXI. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cd Wastewater: Total Organic Carbon = 200 mg/L , Phenol = 0 mg/L

Table LXXXII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cd Wastewater: Total Organic Carbon = 200 mg/L , Phenol = 50 mg/L

Table LXXXIII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cd Wastewater: Total Organic Carbon = 200 mg/L , Phenol = 150 mg/L

Table LXXXIV. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cd Wastewater: Total Organic Carbon = 200 mg/L , Phenol = 250 mg/L

Table LXXXV. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cd Wastewater: Total Organic Carbon = 300 mg/L , Phenol = 0 mg/L

Table LXXXVI. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cd Wastewater: Total Organic Carbon = 300 mg/L , Phenol = 50 mg/L

Table LXXXVII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cd Wastewater: Total Organic Carbon = 300 mg/L , Phenol = 150 mg/L

Table LXXXVIII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cd Wastewater: Total Organic Carbon = 300 mg/L , Phenol = 250 mg/L

Table LXXXIX. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cu Wastewater: Total Organic Carbon = 100 mg/L , Phenol = 0 mg/L

Table LXXXX. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cu Wastewater: Total Organic Carbon = 100 mg/L , Phenol = 50 mg/L

Table LXXXXI. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cu Wastewater: Total Organic Carbon = 100 mg/L , Phenol = 150 mg/L

Table LXXXXII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cu Wastewater: Total Organic Carbon = 100 mg/L , Phenol = 250 mg/L

Table LXXXXIII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cu Wastewater: Total Organic Carbon = 200 mg/L , Phenol = 0 mg/L

Table LXXXXIV. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cu Wastewater: Total Organic Carbon = 200 mg/L , Phenol = 50 mg/L

Table LXXXXV. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cu Wastewater: Total Organic Carbon = 200 mg/L , Phenol = 150 mg/L

Table LXXXXVI. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cu Wastewater: Total Organic Carbon = 200 mg/L , Phenol = 250 mg/L

Table LXXXXVII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cu Wastewater: Total Organic Carbon = 300 mg/L , Phenol = 0 mg/L

Table LXXXXVIII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cu Wastewater: Total Organic Carbon = 300 mg/L , Phenol = 50 mg/L

Table IC. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cu Wastewater: Total Organic Carbon = 300 mg/L , Phenol = 150 mg/L

Table C. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Cu Wastewater: Total Organic Carbon = 300 mg/L , Phenol = 250 mg/L

Table CI. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Pb Wastewater: Total Organic Carbon = 50 mg/L, Phenol = 0 mg/L

Table CII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Pb Wastewater: Total Organic Carbon = 50 mg/L, Phenol = 50 mg/L

Table CIII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Pb Wastewater: Total Organic Carbon = 50 mg/L, Phenol = 150 mg/L

Table CIV. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Pb Wastewater: Total Organic Carbon = 50 mg/L, Phenol = 250 mg/L

Table CV. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Pb Wastewater: Total Organic Carbon = 100 mg/L , Phenol = 0 mg/L

Table CVI. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Pb Wastewater: Total Organic Carbon = 100 mg/L , Phenol = 50 mg/L

Table CVII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Pb Wastewater: Total Organic Carbon = 100 mg/L , Phenol = 150 mg/L

Table CVIII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Pb Wastewater: Total Organic Carbon = 100 mg/L , Phenol = 250 mg/L

Table CIX. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Pb Wastewater: Total Organic Carbon = 200 mg/L , Phenol = 0 mg/L

Table CX. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Pb Wastewater: Total Organic Carbon = 200 mg/L , Phenol = 50 mg/L

Table CXI. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Pb Wastewater: Total Organic Carbon = 200 mg/L , Phenol = 150 mg/L

Table CXII. Comparison of LLMO Strains Illustrated by Total Organic Carbon Removal in Pb Wastewater: Total Organic Carbon = 200 mg/L , Phenol = 250 mg/L

APPENDIX B

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