2008 Project Abstract
For the Period Ending June 30, 2010

PROJECT TITLE: Pharmaceutical and Microbiological Pollution
PROJECT MANAGER: Timothy M. LaPara
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: ML 2007, Chap. 30, Sec. 2, Subd. 5L.

APPROPRIATION AMOUNT: $302,000

Overall Project Outcome and Results

The goal of this project was to develop technologies that eliminate antibiotic-resistant bacteria, hormones, and other pharmaceutical compounds from Minnesota's surface waters. Laboratory-scale digesters were established in which wastewater solids were treated under both aerobic and anaerobic conditions at temperatures of 72°F, 98°F, 115°F, and 130°F. Our results demonstrated that aerobic digestion had no significant effect on the destruction of these genes; in contrast, the anaerobic digesters operated at 115°F and 130°F showed a very significant ability to reduce the quantities of these genes (with 130°F performing better than 115°F). This research demonstrates that anaerobic digesters treating wastewater solids (or agricultural manure) should be operated at the highest feasible temperature to help eliminate antibiotic resistance genes, which should help slow the proliferation of these organisms. In terms of antibiotic removal, the aerobic and anaerobic digesters were effective in the removal sulfamethoxazole, trimethoprim, and tylosin, with removal generally being greater at higher temperatures. Digestion did not lead to removal of the antibacterial triclosan or the estrogens tested. Laboratory and pilot-scale photolysis experiments revealed the compounds subject to direct photolysis (triclosan, tetracycline, tylosin) are likely to be amenable to degradation in wastewater treatment stabilization ponds or treatment wetlands. Cover materials either had minimal or inhibitory effects on photolysis rates. Two compounds (sulfamethoxazole and trimethoprim) were photodegraded more rapidly in wastewater effluent than in surface water or purified water, indicating that photodegradation is more likely to occur (and perhaps should be encouraged by design) in sunlit wastewater treatment process steps than in the environment. While solar photolysis shows some promise for treatment of pharmaceuticals, no evidence for removal of antibiotic resistance genes was in the photoreactor.

Project Results Use and Dissemination

This project has been used in numerous ways. First, we have communicated the results back to the State Legislature via informal (i.e., with individual State Senators and Representatives) and formal (i.e., hearings). Second, we have communicated these results to our various partners who operate municipal wastewater treatment facilities as well as other municipalities who operate municipal wastewater treatment facilities. Finally, we have disseminated our research results as broadly as possible, including

via presentations at national and regional technical meetings as well as via publication in the peer-reviewed technical literature.

Trust Fund 2007 Work Program Final Report

Date of Report: October 15, 2010
Final Report
Date of Work program Approval: June 5, 2007
Project Completion Date: June 30, 2010

I. PROJECT TITLE: Pharmaceutical and Microbiological Pollution

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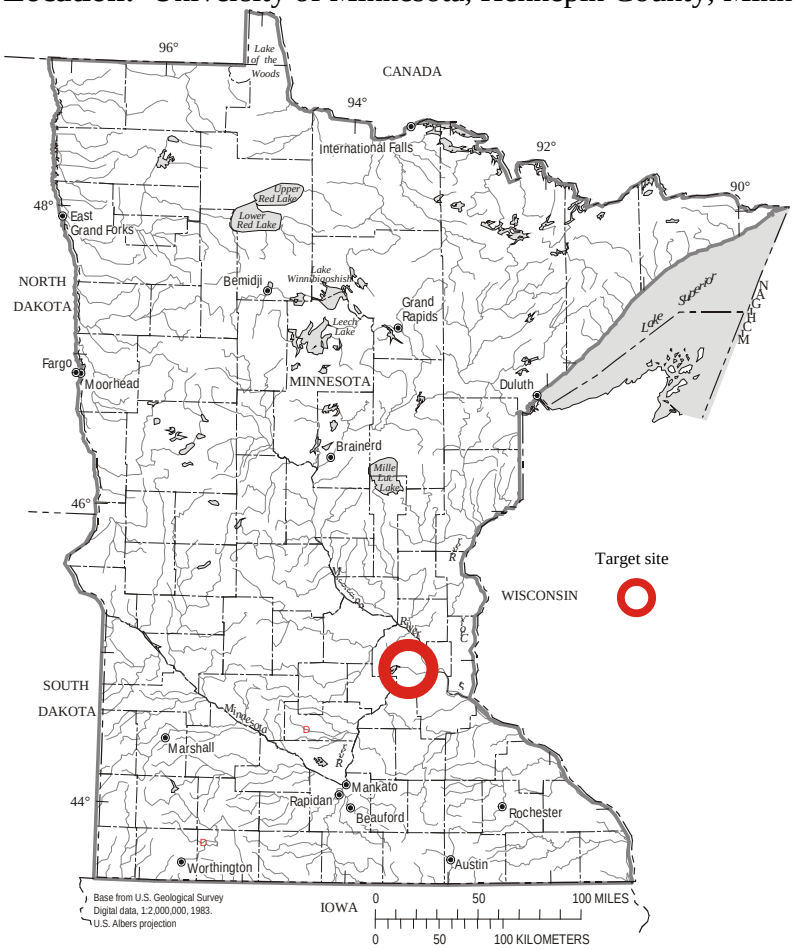

Total Trust Fund Project Budget:	**Trust Fund Appropriation:**	**$ 302,000**
	Minus Amount Spent:	**$ 302,000**
	Equal Balance:	**$ 0**

Legal Citation: ML 2007, Chap. 30, Sec. 2, Subd. 5L.

Appropriation Language: *Pharmaceutical and Microbiological Pollution Minnesota's surface waters. $302,000 is from the trust fund to the University of Minnesota to develop technologies that eliminate antibiotic-resistant bacteria, hormones, and other pharmaceutical compounds from Minnesota's surface waters.*

II. and III. FINAL PROJECT SUMMARY
Same as document 3, project abstract. Length = 300 words or less

IV. OUTLINE OF PROJECT RESULTS*:*

Result 1: Thermophilic treatment of municipal and agricultural biosolids

Description: We collected biosolids from a municipal wastewater treatment plant and used these to establish lab-scale (0.5 L) aerobic and anaerobic bioreactors operated at different temperatures (75°F, 98°F, 115°F, and 130°F; 8 total bioreactors). At the end of each batch operation, two-thirds of the reactor volume was removed and replaced with untreated biosolids. These bioreactors were operated in this semi-continuous/semi-batch-mode for at least 10 hydraulic residence times (aerobic residence time: 4 days; anaerobic residence time: 15 days) to ensure that these bioreactors are operating effectively for solids stabilization and gas production (anaerobic only) before initiating the active portion of the experiments. We monitored these digesters using typical assays to measure digester performance (*e.g.*, volatile solids, COD, etc.) as described by *Standard Methods for the Examination of Water and Wastewater*.

Once these anaerobic and aerobic digesters were established, we measured the ability of these bioreactors to inactivate tetracycline-resistant bacteria. Individual samples were collected following the initiation of an individual semi-batch cycle as well as 4-6 samples per each semi-batch cycle. We were unable to enumerate total heterotrophic bacteria as well as tetracycline-resistant bacteria by cultivation (the digester contents were a paste that was very difficult to work with). We also collected biomass samples from which we extracted total genomic DNA for quantitative PCR to characterize the inactivation of tetracycline resistant bacteria in our lab-scale digesors. Prior research has demonstrated that untreated biosolids from municipal wastewater treatment plants contains substantial quantities of tetracycline-resistant bacteria. We anticipated performing at least three replicate kinetic experiments with each digester, although became an excessive and unnecessary work load (replicate anaerobic digesters provided very reproducible results).

We used these lab-scale digesters to track the loss of antibiotics (trimethoprim, tylosin, sulfamethoxazole) and estrogens (bisphenol A, nonylphenol, estradiol) in laboratory-scale experiments We spiked a known, quantifiable concentration of these compounds at the initiation of each run and track its disappearance over time (note: we originally intended to study the antibacterial triclosan degradation in this manner, but the “background”

concentration of triclosan was higher than our experimental concentration. Thus, this background concentration was used to evaluate triclosan removal.) These experiments measured the intrinsic ability of the microbial community to degrade these compounds without explicit prior adaptation. We anticipate that the biomass will have substantial biodegradation activity given that these compounds are generally found in municipal wastewater.

Summary Budget Information for Result 1:	**Trust Fund Budget:**	**$** 151,000
	Amount Spent:	**$** 151,000
	Balance:	**$** 0

Deliverable	**Completion Date**	**Budget**	**Status**
1. Anaerobic Digestion	May 31, 2009	$76,000	Completed
2. Aerobic Digestion	March 15, 2010	$75,000	Completed
3.			

Completion Date: June 1, 2010

Final Report Summary: Substantial differences were observed in the ability of anaerobic and aerobic digesters to reduce the quantity of genes encoding tetracycline resistance and a gene encoding the integrase of Class 1 integrons. In most cases, aerobic digestion had no significant impact on gene quantities, although this could have been due to the relatively short retention time (4 days) of the experiments. In contrast, the anaerobic digesters operated at high temperatures showed significant rates of removal for most of the genes that were quantified (the exception was *tet*(L)) at temperatures of 115°F and 130°F, whereas the digesters operated at temperatures of 72°F and 98°F did cause any significant reductions in gene quantities. Of particular importance, the anaerobic digester operated at 130°C achieved a reduce in *intI1* genes from approximately 10% of the total biomass to less than 0.001% over a period of 5 days; this result is important because integrons have been implicated as a general genetic mechanism that helps the proliferation of antibiotic resistance. This research demonstrates that the operation of anaerobic digesters at high temperatures (> 125°F) offers a substantial benefit for reducing the quantity of antibiotic resistance genes in wastewater solids; with the widespread implementation of this technology, it might be possible to slow the spread of antibiotic resistance. The full details of this research is currently under review for publication in *Environmental Science and Technology*; this research was also used as data in a research proposal to the National Science Foundation that has been funded for almost $400,000 over a 3-year period.

Removal of antibiotics and estrogens from the digesters was variable depending on the compound and operating conditions. In the aerobic digesters, sulfamethoxazole was at or below the analytical detection limits within one day of spiking at all temperatures. This indicates that aerobic digestion is a viable treatment option for this antibiotic. For trimethoprim, removal varied from 40% to >95% (on a mass of compound per mass of solids basis) in the eight day monitoring period, with better removals seen at the temperatures of 115°F and 130°F (80% and >95% respectively). For tylosin, there was no removal in the 72°F digester. At 98°F and 130°F 70% removal was observed over four days. Substantially different results were observed for triclosan. Over the 8 day monitoring period, the concentration of triclosan in the solids *increased* by up to 8-fold. Concentrations are

measured on a mass of compound per mass of solids basis. Because the solids are being degraded by the digester, the denominator of this ratio decreases with time. Essentially, this result demonstrates that triclosan is not degraded in the aerobic digesters. For the three estrogens tested, nonylphenol was the only compound the produced consistent data. For all temperatures, the concentrations decreased at the same rate as that would be expected by dilution of digester caused by sampling. Because the solids mass is being reduced over time, this indicates that nonylphenol is degraded at the same rate of solids digestion. The data for bisphenol A suggests similar trends but is not conclusive. It was not possible to detect estradiol suggestion either that it degrades rapidly or that there is analytical interference. We consider the latter to be more likely. Only one temperature produced reliable for the anaerobic digesters (98°F). In this digester, removal of sulfamethoxazole (rapidly below detection limits), tylosin (80%) and trimethoprim (90%) were observed. Triclosan behavior was similar to that in the aerobic digesters, indicating no degradation of this compound.

Result 2: Solar treatment of water for pharmaceutical destruction and disinfection

Description: Laboratory Studies. Initial experiments will be performed in the laboratory. Experiments will be conducted with treated wastewater (collected prior to final chemical disinfection) and runoff collected from agricultural fields that either use treated wastewater for irrigation or manure as fertilizer. This will allow us to simulate operating conditions more closely, as the organic material (which influences light penetration and leads to indirect photolysis processes) and bacterial populations will be those expected in the field. For experiments solely focused on pharmaceutical degradation, the collected waters will be filter-sterilized (to prevent any biological degradation of the compounds), and for those focused on bacterial inactivation, no pharmaceuticals will be added (to prevent any development of resistance during the test). Experments will also be conducted on samples containing both the target pharmaceuticals and active bacteria. Pharmaceuticals will be dosed individually or in mixtures at 1-10 mg/L to facilitate detection. Our prior research suggests that substantial numbers of antibiotic resistant bacteria will be present; but if not, then we will add a known quantity of tetracycline-resistant *E. coli* harboring a *tet*(A) gene on a plasmid. The light source will be sunlight whenever possible, with the solar intensity measured via actinometry and/or using the St. Anthony Falls Laboratory weather station. When weather prevents outdoor experiments, a solar simulator will be used. For the compounds in this study, the quantum yields are known. Thus, the goal is to optimize the conditions to maximize degradation/disinfection.

In the laboratory simulation of passive systems (i.e., holding pond), the goal will be to test the light exposure times necessary to achieve a given level of compound removal and disinfection (as measured by the decrease in heterotrophic plate counts [with and without tetracycline] and quantitative real time PCR) for various depths. Experiments will be conducted in a small, open flask (~0.5-1 L capacity). Another parameter to be tested in the laboratory is important for pumped systems—the material of the cover that is used to trap the heat. Experiments will be similar to those for the passive system, except that a cover will be added to the tank. The cover enables heat to be trapped/temperature to be increased. A disadvantage is the potential alteration of the light spectrum. Microbial inactivation is optimal with UV-A wavelengths of light (320-400nm) while more energetic UV-B light (< 320nm) is often necessary for efficient organic contaminant destruction. Cover materials to be tested include quartz (completely UV transparent), Pyrex glass (blocks UV-B radiation),

and acrylics (which are more durable/lighter weight) of varying UV transparency. Temperature will also be a variable in these studies, with the reactors being heated passively (either ambiently or using a reflector to focus/increase light dosage). Control experiments (no light exposure) will be conducted in parallel to all photolysis experiments. In all experiments, aqueous samples (~ 1 mL) will be collected at selected time intervals from the reactors run in duplicate. At least seven time points will be collected.

Pilot Studies. The laboratory experiments will be used to guide the design of a pilot scale system to be set up at the Blue Lake wastewater treatment plant in Shakopee, MN. This treatment plant is particularly well suited for the study, because after undergoing activated sludge treatment, wastewater passes through a holding pond prior to final disinfection and discharge. Using data from the laboratory studies (specifically, the kinetics of bacterial inactivation and pharmaceutical destruction) the performance of the pilot system will be predicted for a given volume/depth (passive) or volume, depth, and flow rate (active system). The cover material to be used in the active system will also be based on the results of the laboratory studies. In both systems, water depth (rather than volume) is expected to be the crucial geometric parameter. The passive system will be a tank with total capacity of approximately 50-100 L. The active system will have a volume of approximately 2 L. It is expected that residence times in the active system will need to be on the order of one hour, so flow rates will vary from 0.5 to 8 L/hr.

We will focus this study on the human antibiotics (sulfamethoxazole and triclosan) and one of the estrogenic compounds (to be determined). Both passive and active systems will be studied. In the passive systems, samples will be removed to monitor pharmaceuticals, heterotrophic plate counts (with and without tetracycline), and antibiotic resistance genes as a function of time. In the active systems, influent and effluent concentrations of these parameters will be measured. The analytical methods are described below. We will also conduct a pilot-scale study of the passive system at an agricultural site using tylosin and tetracycline as the target compounds. The procedures will be the same as those described above. We anticipate that we will have to spike the waters with the target compounds for both the wastewater and the agricultural pilot tests to ensure we can routinely and easily detect the target compounds.

In both the active and passive systems, the performance of the system will be measured as a function of residence time, solar exposure (i.e., season), temperature, and water depth. With the active system, the utility of a light reflection system will also be evaluated. Temperature will be monitored using a thermocouple and a data logger. The ultimate goal is to determine if the laboratory measured parameters accurately represent pilot system performance, and if not, what correction factors are necessary to design the system to achieve the desired performance.

Summary Budget Information for Result 2:	**Trust Fund Budget:**	**$** 151,000
	Amount Spent:	**$** 151,000
	Balance:	**$** 0

Deliverable	**Completion Date**	**Budget**	**Status**
1. Laboratory Studies	January 1, 2009	$75,000	Completed
2. Pilot Studies	May 31, 2010	$76,000	Completed

Completion Date: May 1, 2010.

Final Report Summary: As expected based on previous results, all four antibiotics (sulfamethoxazole, trimethoprim, tylosin, and tetracycline) as well as the antibacterial triclosan were susceptible to direct photolysis. It was hypothesized that a cover material (either quartz, borosilicate glass, or acrylic) could be used to focus light and/or elevate temperature and enhance reaction rates. The acrylic prevented transmission of the necessary wavelengths of light and slowed photolysis by a factor of two. UV-transparent acrylic was therefore used as a substitute. None of the cover materials, however, led to enhances photolysis rates. Thus, further laboratory photolysis experiments did not use cover materials and focused on the scenario of a holding pond. Tylosin, tetracycline, and triclosan were most susceptible to photolysis both in purified water and in wastewater effluent (half-lives of 0.5-2 hours). Sulfamethoxazole and trimethoprim reacted much more slowly that the other compounds. One interesting finding of this study, however, was that the rate of photolysis of these two antibiotics was enhanced in wastewater compared to purified water (2-fold for sulfamethoxazole and 10-fold for trimethoprim). This enhanced reactive was traced to dissolved constituents of wastewater (specifically, nitrate and effluent organic matter) that produce reactive intermediates in sunlight (hydroxyl radical and triplet excited organic matter) that then react with these antibiotics. A manuscript describing these findings is currently under review for publication in the journal *Water Research.* For triclosan, tetracycline, and tylosin, this process is unimportant because the reaction caused by the direct absorption of sunlight dominated for these three compounds. Laboratory screening studies on nonylphenol, bisphenol A, and estradiol indicated that these compounds did not undergo photolysis at a sufficient rate to merit study in the pilot reactor.

Because the laboratory studies could be used to predict behavior of passive pilot systems, pilot studies focused on an active flow through system. The aqueous medium was either purified water or wastewater effluent. Field runoff was not used because experiments with river water showed no difference in reactivity to purified water (unlike wastewater effluent). The pilot reactor (3 hour residence time) demonstrated that tetracycline, triclosan, and tylosin are amenable to treatment in a flowing systems (either reactor or holding pond) that is exposed to sunlight. For the water receiving maximum solar exposure (e.g., entering the reactor around 12 noon and exiting at 3pm), up to 90% of the tetracycline, 80% of the triclosan, and 25% of the tylosin was degraded in a three hour exposure period. This was essentially unaffected by the matrix (purified water or wastewater effluent) or the presence of a cover (none or UV-transparent acrylic). Removals were lower for parcels of water receiving less intense sunlight. Photolysis will only occur to the depth to which light penetrates. Thus, large areas, shallow depths, and long retention times will be necessary in solar treatment systems if a substantial fraction of these compounds is to be removed consistently. Treatment wetlands offer such a possibility. Despite their higher photoreactivity in wastewater effluents, sulfamethoxazole and trimethoprim were not degraded in the pilot reactor. To test whether removal of triclosan occurred in a wastewater treatment holding pond, samples from the influent and effluent of the wastewater stabilization pond at the end of the treatment train at the Blue Lake Wastewater Treatment plant in Shakopee, MN was collected and analyzed. Effluent samples had triclosan levels 30-50% lower than influent samples, and biodegradation controls showed no losses, suggesting that photolysis (or another abiotic process) was responsible for the decrease.

The pilot reactor was also used to test the possibility of using sunlight for disinfection/removal of antibiotic resistance genes. Based on fecal coliform counts, disinfection efficiency was 60-95% in the 3-hour exposure period. There was no observable difference in the number of tetracycline resistant genes between the influent and effluent of the reactor. This indicates that even if the bacteria are inactivated by sunlight, the genes are not destroyed.

V. TOTAL TRUST FUND PROJECT BUDGET:

Staff or Contract Services: $222,000
Equipment: $50,000
Development: $ 0
Restoration: $ 0
Acquisition, including easements: $ 0
Other: $ 30,000. Laboratory supplies and services (e.g., analytical chemistry, microbial analyses) and travel funds to test sites and for expenses to disseminate our results to Minnesotans and to other interested individuals at local, regional, and national workshops/conferences.

TOTAL TRUST FUND PROJECT BUDGET: $302,000

Explanation of Capital Expenditures Greater Than $3,500:

A sum of $40,000 is budgeted for the purchase of a high pressure liquid chromatograph. This essential piece of equipment is required to monitor the concentrations of the target antibiotics and estrogens in the laboratory experiments as well as in the pilot-scale tests. (Trace level analysis will be performed on mass spectrometry equipment). The investigators do currently have access to an HPLC. The instrument, however, is beyond its expected lifetime, and spare parts are no longer available. If the instrument fails, the project will be unable to proceed. Thus, a new instrument is necessary to ensure the project goals are met. Given the number of samples expected to generated by the project, it is more economical to purchase an instrument rather than pay per sample fees on an instrument in another laboratory.

A sum of $10,000 is budgeted for the purchase of a real time PCR machine to quantify genes encoding resistance to tetracycline. The benefit of purchasing this instrument is that we would be able to process more samples with higher quality results (with fewer users, the instrument will be maintained better) in a shorter period of time. Additional funds will be leveraged to purchase this equipment (the LCCMR financial contribution will be $10,000 of the total instrument cost of $26,100; the remaining $16,100 will come from other research projects).

VI. OTHER FUNDS & PARTNERS:

A. Project Partners:

William A. Arnold

University of Minnesota
Department of Civil Engineering
500 Pillsbury Drive SE
Minneapolis, MN 55455

B. Other Funds Proposed to be Spent during the Project Period: $5000
An additional $5,000 towards the purchase of the HPLC will be leveraged from an unrelated grant of Dr. Arnold. Dr. Arnold is also leading a National Science Foundation sponsored project ($266,000) studying the fate of triclosan in the environment. A portion of this effort is focused on the transformation of triclosan when it is exposed to sunlight, and we will be able to leverage the results for this project.

We will also partner with the Metropolitan Council Environmental Services, Western Lake Superior Sanitary District, and anonymous farmers. Drs. LaPara and Arnold have teamed with these groups in past research efforts, and thus a good working relationship between the lead invesigators and partners already exists. The partners will provide in-kind contributions (i.e., site access, sampling assistance, staff time) at no direct cost to the proposed project.

C. Past Spending: $0

D. Time: The peer-review panel recommended a time frame of three years to complete the proposed project.

VII. DISSEMINATION ACTIVITIES

Drs. LaPara and Arnold testified at a hearing of a combined state senate/house committee on wastewater treatment on January 7, 2008. One manuscript is currently being considered for publication by *Environmental Science and Technology* and other manuscripts are currently in preparation.

VIII. REPORTING REQUIREMENTS:

Periodic work program progress reports will be submitted not later than January 2008, July 2008, January 2009, and November 2009. A final work program report and associated products will be submitted between June 30 and August 1, 2010 as requested by the LCCMR

IX. RESEARCH PROJECTS:

Research Addendum for Peer Review

Project Manager Name: Dr. Timothy LaPara

Project ID and Title: (SN-56) Pharmaceutical and Microbiological Pollution

I. Abstract

Human and veterinary antibiotics, hormones, and antibiotic resistant bacteria enter Minnesota waters via wastewater discharges, biosolids (manure), and runoff. Almost no research into approaches and technologies to control the release of pharmaceuticals and antibiotic resistant bacteria in municipal wastewater and in agricultural runoff has been performed. Practical, low cost technologies are necessary to manage the large volumes of wastewater, agricultural runoff, and biosolids generated in Minnesota. There are two treatment techniques that have potential to both destroy pharmaceuticals and to kill antibiotic resistant bacteria: thermophilic (aerobic or anaerobic) treatment of biosolids and solar treatment of water. Our goal is to determine the capabilities of both "low tech" (solar treatment) and "high tech" (thermophilic treatment) approaches with respect to the destruction of pharmaceuticals and antibiotic resistant bacteria. Although a simple technique, solar treatment is expected to be both effective and low cost and applicable to small-scale applications, such as the stereotypical family farm or a small wastewater treatment facility. Although more expensive to initially construct, thermophilic treatment should be more effective and cost-efficient for large-scale facilities, such as municipal wastewater plants and larger agricultural operations. This research will establish innovative approaches that will substantially benefit Minnesota by reducing the antibiotics, hormones, and antibiotic resistant bacteria released into our surface and ground waters.

II. Background and Hypotheses

Pharmaceuticals as contaminants

Pharmaceutical and personal care product (PPCP) contamination of surface waters was first reported in Europe, with studies conducted in Britain (Richardson and Bowron, 1985), Germany (Ternes, 1998; Hirsch et al., 1999), and other countries (Halling-Sorensen et al., 1998; Kumpel et al., 2001). More recently, American researchers have begun to take stock of the pharmaceutical contamination in US surface waters. Most prominent is the nationwide reconnaissance of organic wastewater contaminants by the US Geological Survey, in which over 95 different organic wastewater compounds were detected in US streams and rivers (Kolpin et al., 2002). The main conclusions of the European and American studies were the same: pharmaceuticals are found in the environment and can be attributed to both human and animal applications. The contamination of human origin is largely the result of incomplete removal in the wastewater treatment process. The animal-use derived contamination is more direct, coming from food animal production runoff. Recent calculations (Anderson et al., 2004) have predicted that 2 to 98 percent of specific human pharmaceuticals will pass through wastewater treatment systems and enter the environment via wastewater discharge. The percentage resistant to treatment depends on the specific compound, and the total mass of the compound released depends on usage/prescription rates. Based on the usage and removal rates provided by Anderson et al., it is predicted that 60,000 kg/year of the

antimicrobial triclosan and 36 kg/year of ethyinyl estradiol are released in to surface waters in the US. The latter number appears small, but estrogenic compounds are extremely potent, and small releases on a mass basis are of concern.

Even the fraction of the PPCPs removed via wastewater treatment can still enter the environment. A major removal pathway is association/removal with the sludge (*e.g.*,Heidler et al. 2006; Anderson et al., 2004). If this material is then processed and spread on fields as fertilizer, the pharmaceuticals may be released. This has proven to be the case, for recent work has also shown that re-use of treated wastewater and application of treated sludge as fertilizer in agricultural applications leads to pharmaceutical release into the environment (Cordy et al., 2004; Pedersen et al., 2005).

Although many PPCPs reach the environment, of specific concern is the release of antibiotic and estrogenic compounds. Both classes of compounds are known to and/or designed to have specific physiological effects, and thus these two groups of compounds have the potential to adversely affect surface waters, aquatic ecosystems, and humans. Estrogenic compounds have a demonstrated ability to interfere with the development of aquatic organisms (Stuer-Lauridsen et al., 2000; Ternes, 2001), while there is concern that the presence of antibiotics in natural waters will lead to an increase of antibiotic resistant bacteria (Hirsch et al., 1999; Kumpel et al., 2001).

Antibiotic resistant bacteria as pollutants

Although scientists have long-known that disease-causing bacteria are becoming increasingly resistant to antibiotics (Fig. 1), relatively little has been done to avoid this pending catastrophe until the last decade or so. The public health sector is now focused on limiting inappropriate antibiotic use – specifically by limiting inappropriate prescriptions (*e.g.*, for viral infections) and by educating patients to closely follow their prescription guidelines. While these changes will limit the development of newly-resistant strains, they are likely insufficient to stem the proliferation of antibiotic resistance. Also controversial is the substantial fraction of antibiotics that are used in agriculture (50-70% of total antibiotic use in the United States) for prophylaxis and growth-promotion.

A major hypothesis currently driving research in the LaPara group is that the majority of antibiotic resistant bacteria are generated in the gastrointestinal tracks of human beings and animals taking antibiotics. These resistant bacteria are then expelled from the body via defecation, suggesting that better collection and treatment of fecal material (both of human and of agricultural origin) could substantially help slow the proliferation of antibiotic resistance. Prior research by us (Firl et al., 2006; LaPara et al., 2006) and others (Auerbach et al., 2007) has demonstrated that human sewage contains substantial quantities of antibiotic resistant bacteria. Likewise,

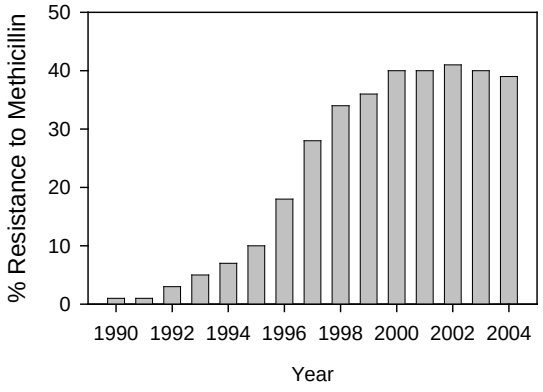

Fig. 1. Resistance to methicillin among *Staphylococcus aureus* isolated from blood cultures in England and Wales (Johnson et al., 2005).

agriculturally-generated manure is a substantial source of antibiotic resistant bacteria (Alonso et al., 2001; Onan and LaPara, 2003; Rooklidge, 2004; Ghosh and LaPara, 2006). As such, researchers now consider antibiotic resistant bacteria – as well as their genes that encode for resistance – as emerging pollutants of environmental concern (Pei et al., 2006; Pruden et al., 2006).

Goal and Hypotheses

Because of the potential adverse effects of antibiotics, estrogens, and antibiotic resistant bacteria on humans and on the environment, it is desirable to prevent the release of these pollutants. To eliminate the discharge of these contaminants, both biosolids and waters will have to be treated. The goal of this project is to evalute the utility of two technologies to achieve this goal: thermophilic digestion of biosolids and solar treatment of water.

In thermophilic digestion, microbial activity results in either the direct release of heat (aerobic) or the production of methane gas (anaerobic) that can be burned to heat the bioreactor. High reactor temperatures (> 130°F) then pasteurize the waste, which should include the destruction of the majority of resistant bacteria. The fate of antibiotics and hormones in thermophilic bioreactors is unknown. Solar treatment is simply the exposure of water to sunlight. The light destroys chemicals (pharmaceuticals, DNA) and also heats the water, which enhances these effects. The minimum requirement for this technique is passive exposure to sunlight. In an active (pumped) system, it is possible to use a solar collector to enhance the heat collected.

Based on the information presented above, we propose to test the following hypotheses in the experiments described below:

1. The elevated temperatures generated in thermophilic sludge digesters will lead to the inactivation of antibiotic resistant bacteria and the degradation of pharmaceutical compounds. This technology will be effective for large-scale facilities that can accommodate the required capital investment.

2. With sufficient dosage, storage time, and appropriate design, the combination of solar light and elevated temperature will lead to destruction of antibiotic and estrogenic compounds as well as the inactivation of antibiotic resistant bacteria in treated wastewater and agricultural runoff. The technology will be feasible for small-scale operations.

Although each is an established technology, the application of the thermophilic digestion or solar treatment targeted to the simultaneous removal of pharmaceuticals and antibiotic resistant bacteria has not been pursued. A brief background on the two technologies is provided below.

Thermophilic digestion of biosolids

There are numerous approaches to dealing with the solid residues collected at municipal wastewater treatment facilities. The most common approach, and the one that most people believe is environmentally sustainable, is to biologically stabilize the solids (i.e., reduce their organic content; a.k.a. “digestion”) and then apply these stabilized solids to farm land as both a fertilizer and a soil conditioner. The conventional technology for solids stabilization is mesophilic anaerobic digestion, which involves incubation of the solids at ~98°F in the

absence of oxygen. This technology is quite effective at stabilizing the solids, but the process is relatively ineffective at inactivating pathogens because these digesters are operated at the approximate temperature of the human body.

In an attempt to improve the stabilization process, researchers have developed two high-temperature process alternatives: (1) Thermophilic anaerobic digestion, and (2) Autothermal thermophilic aerobic digestion (ATAD). Thermophilic anaerobic digestion works similar to conventional anaerobic digestion, except that a greater fraction of the methane generated by the process is used to heat the bioreactors to much higher temperatures. ATAD processes work similar to conventional composting in which the heat released by aerobic bacterial metabolism is sufficient to "auto-heat" the reactor to high temperatures. Although both thermophilic anaerobic digestion and ATAD are used in practice, neither has achieved widespread implementation. Simply put, they are more expensive to construct and to operate, while they offer only marginal benefit for solids stabilization.

Thermophilic anaerobic digestion and ATAD processes, however, should be far superior to conventional anaerobic digestion if the inactivation of antibiotic resistant bacteria (and pathogens) becomes an explicit goal of solids digestion (i.e., in addition to stabilization). These processes operate at temperatures well above that of the human body (> 130°F), which is sufficient to inactivate virtually all bacteria that could adversely affect humans. Research is needed, however, to determine the rates at which antibiotic resistant bacteria are inactivated during thermophilic anaerobic digestion and ATAD processes. As stated above, the fate of antibiotics and hormones in the digestion process (particularly at high temperatures) is essentially unknown.

Solar treatment of water: potential for pharmaceutical destruction and disinfection
As reviewed by Boreen et al. (2003), many pharmaceuticals are subject to degradation upon direct exposure to sunlight (direct photolysis). For this reason, it is often advised to store medications in the dark and to avoid sunlight/wear sunscreen when taking specific medications. Much work in the past few years has focused on determining photolysis rates of pharmaceuticals under environmentally relevant conditions (Fasani et al., 1999; Poiger et al., 2001; Sprehe et al., 2001; Araki et al., 2002; Tixier et al., 2002; Andreozzi et al., 2003; Buerge et al., 2003; Doll and Frimmel, 2003; Valero and Costa, 2003; Latch et al., 2003a; Latch et al., 2003b; Packer et al., 2003; Boxall et al., 2004; Boreen et al., 2004; Boreen et al., 2005; Lin and Reinhard, 2005; Latch et al., 2005; Werner et al., 2005a; Werner et al. 2006). This work, including work targeting the antibiotics to be studied in this project conducted by Dr. Arnold's group, has demonstrated that direct photolysis is an important natural degradation process for pharmaceuticals that has the potential to be exploited in engineered systems.

The kinetics of direct photochemical destruction are described by the following equation (Zepp and Cline, 1977; Mill, 1999):

$$\frac{dC}{dt} = -\Phi \sum_{\lambda} \varepsilon_{\lambda} L_{\lambda} [C] = -k_p [C]$$

where ε_{λ} is the extinction coefficient at wavelength λ, L_{λ} is the averaged sunlight intensity for shallow water depth, and Φ is the quantum yield or efficiency of the process. It is important to note that a large quantum yield value (near 1) does not guarantee a rapid

reaction nor does a small value mean the direct photolysis will be slow. The key parameter is the product of □□ and the spectral overlap integral $\sum_{\lambda} \varepsilon_{\lambda} L_{\lambda}$; which quantifies the total light absorbance of the compound).

Extinction coefficients for compounds can easily be measured using a UV/visible spectrophotometer. For the compounds of interest in this study (see below), the extinction coefficients (as a function of pH) and quantum yields are known. If the solar exposure is known, the total time of solar exposure necessary to achieve a specific level of removal for a compound is readily calculated.

In addition to direct photolysis, indirect photolysis can also lead to the destruction of pharmaceuticals. For indirect processes, organic matter (which is present in both wastewater and agricultural runoff) absorbs the light and generates a variety of reactive species, including hydroxyl radicals, singlet oxygen, and triplet-state excited organic matter. Work in Dr. Arnold's laboratory has shown that indirect photolysis processes are also important in pharmaceutical degradation (Latch et al. 2003a; Werner et al., 2005a; Boreen et al., 2005).

In water, sunlight is a potent and often unexploited disinfectant. Although chemical disinfectants are effective, they have drawbacks including high operating or capital costs, toxic byproduct formation, safety issues, and the requirement of skilled operators. Recent research has shown that exposure of treated wastewater and river water to sunlight results in 99.9 to 99.99% removal of coliform bacteria (an indicator measurement of total disinfection; McLoughlin et al. 2004; Caslake et al., 2004) with 30-60 minutes of solar exposure in active/covered reactors. In fact, the process is being successfully implemented in third-world countries to reduce outbreaks of gastrointestinal illness. Some bacterial strains, however, require doses up to 5 times greater than coliform-type organisms (Gill and McLoughlin, 2007). Thus, it is important to track the disinfection rates for specific target organisms or organisms that contain a specific characteristic (*e.g.*, virulence, antibiotic resistance), and such detailed work has not yet been performed.

Solar treatment can be active or passive. A passive treatment system is as simple as a shallow holding pond that allows exposure to sunlight for a given period prior to discharge. Advantages of this system are simplicity of operation and low cost. A benefit of an active solar collector in which the water is pumped through an enclosed system (*e.g.*, clear plastic or glass tubes) is that the captured light also heats the water. In general, increasing temperature accelerates reactions and improves disinfection. The effects are relatively modest. For an increase in temperature of 10°C, photolysis rates are accelerated by less than 50% (Schwarzenbach et al., 2002). Disinfection rate has been reported to increase by ~ 3 fold if temperatures of 45°C (110°F) can be obtained (Caslake et al. 2004; Gill and McLoughlin, 2007). These increases in effectiveness need to be balanced with the capital or energy input costs. Nonetheless, such increases in rate are advantageous in that a shorter exposure time and thus reactor volume is necessary if temperature is raised. Additionally, the heat collected in the water could be used for other purposes (*e.g.*, temperature control of an onsite building).

III. Methods

In this project we will focus our efforts on bacteria that are resistant to tetracycline, which is a broad-spectrum antibiotic that serves as an excellent surrogate for the overall problem of "antibiotic resistance" (i.e., there are far too many antibiotics to study resistance to each drug). Dr. LaPara's laboratory has developed numerous cultivation-based and cultivation-independent techniques to enumerate and track the genes encode for tetracycline resistance (unpublished results). The specific antibiotics to be studied include those used in human medicine (trimethoprim, sulfamethoxazole), veterinary medicine (tylosin, tetracycline), and a household antimicrobial (triclosan). Dr. Arnold's research team has previously studied the fundamental photochemical transformation rates and mechanisms of each of these compounds (Table 1). For estrogens, bisphenol A, nonylphenol, and estradiol will be the target compounds. The basic photochemistry of these compounds has also been studied (Table 1). Each of the antibiotics and estrogens selected were detected with high frequency in U.S. surface waters (Kolpin et al., 2002) suggesting that technologies to remove these specific compounds will be particularly useful.

Table 1. Detection frequencies and quantum yields for the pharmaceuticals to be studied.

Target compound	*Surface Water Detection Frequency (Kolpin et al., 2002)*	*Quantum Yield (□)*	*Reference*
Sulfamethoxazole	19%	0.09[1]	Boreen et al., 2004
Trimethoprim	27%	0.006[2]	Werner et al., 2005b
Tylosin	13.5%	0.0014	Werner et al., 2007
Tetracycline	1.2%	0.0019[3]	Werner et al., 2006
Triclosan	58%	0.12	Latch et al., 2003a,2005
Bisphenol A	41%	-[4]	Chin et al., 2004
Nonylphenol	50%	0.003	Neamtu and Frimmel, 2006
Estradiol	10%	0.0048	Lin and Reinhard, 2005

[1] Quantum yields for sulfa drugs are pH dependent. The quantum yield reported is for the dominant protonation state expected at pH 7.
[2] Reactions are accelerated 8-fold in river water, suggesting indirect photolysis processes are also important.
[3] The quantum yields for tetracycline drugs depend upon pH and calcium ion concentration. The value reported is for pH 7.5 with $[Ca^{2+}] = 10^{-3}$ M.
[4] Direct photolysis is slow, but indirect processes in the presence of organic matter give a half-life of ~16 hours.

Thermophilic treatment of municipal and agricultural biosolids

We will collect biosolids from a municipal wastewater treatment plant and use these to establish lab-scale (0.5 L) aerobic and anaerobic bioreactors operated at different temperatures (75°F, 98°F, 115°F, and 130°F; 8 total bioreactors). At the end of each batch operation, two-thirds of the reactor volume will be removed and replaced with untreated biosolids. These bioreactors will be operated in batch-mode for at least 10 hydraulic residence times (aerobic residence time: 4 days; anaerobic residence time: 15 days) to ensure that these bioreactors are operating effectively for solids stabilization and gas production (anaerobic only) before initiating the active portion of the experiments. We will monitor these digesters using typical assays to measure digester performance (*e.g.*, volatile solids,

COD, etc.) as described by *Standard Methods* (1995). Dr. LaPara's laboratory has substantial experience in operating both thermophilic and anaerobic bioreactors (LaPara et al., 2000; Kappell et al., 2005).

Once these anaerobic and aerobic digesters are established, we will measure the ability of these bioreactors to inactivate tetracycline-resistant bacteria. Individual samples will be collected following the initiation of an individual batch run as well as 4-6 samples per each batch run. Bacterial enumerations will be used to quantify total heterotrophic bacteria as well as tetracycline-resistant bacteria. We will also collect biomass samples from which we will extract total genomic DNA so that quantitative and qualitative PCR can be used to characterize the inactivation of tetracycline resistant bacteria in our lab-scale digesors. Prior research has demonstrated that untreated biosolids from municipal wastewater treatment plants contains substantial quantities of tetracycline-resistant bacteria (LaPara et al., 2006). We anticipate performing at least three replicate kinetic experiments with each digester.

We will also use these lab-scale digesters to track the loss of antibiotics (trimethoprim, triclosan, tylosin, sulfamethoxazole, tetracycline) and estrogens (bisphenol A, nonylphenol, estradiol) in laboratory-scale experiments. We will spike a known, quantifiable concentration of these compounds at the initiation of each run and track its disappearance over time. Results will be compared to killed controls (i.e., using a portion of the digester biomass + sodium azide) operated at the same temperatures to discern the impact of biological activity on loss rates of the pharamaceutical compounds. These experiments will measure the intrinsic ability of the microbial community to degrade these compounds without explicit prior adaptation. We anticipate that the biomass will have substantial biodegradation activity given that these compounds are generally found in municipal wastewater. Depending on the results of our proposed study, future research could elucidate optimal degradation rates using explicitly acclimated anaerobic or aerobic digesters.

Solar treatment of water for pharmaceutical destruction and disinfection
Laboratory Studies. Initial experiments will be performed in the laboratory. Experiments will be conducted with treated wastewater (collected prior to final chemical disinfection) and runoff collected from agricultural fields that either use treated wastewater for irrigation or manure as fertilizer. This will allow us to simulate operating conditions more closely, as the organic material (which influences light penetration and leads to indirect photolysis processes) and bacterial populations will be those expected in the field. For experiments solely focused on pharmaceutical degradation, the collected waters will be filter-sterilized (to prevent any biological degradation of the compounds), and for those focused on bacterial inactivation, no pharmaceuticals will be added (to prevent any development of resistance during the test). Experments will also be conducted on samples containing both the target pharmaceuticals and active bacteria. Pharmaceuticals will be dosed individually or in mixtures at 1-10 mg/L to facilitate detection. Our prior research (Firl, 2006) suggests that substantial numbers of antibiotic resistant bacteria will be present; but if not, then we will add a known quantity of tetracycline-resistant *E. coli* harboring a *tet*(A) gene on a plasmid. The light source will be sunlight whenever possible, with the solar intensity measured via actinometry and/or using the St. Anthony Falls Laboratory weather station. When weather prevents outdoor experiments, a solar simulator will be used. For the compounds in this study, the quantum yields are known. Thus, the goal is to optimize the conditions to maximize degradation/disinfection.

In the laboratory simulation of passive systems (i.e., holding pond), the goal will be to test the light exposure times necessary to achieve a given level of compound removal and disinfection (as measured by the decrease in heterotrophic plate counts [with and without tetracycline] and quantitative real time PCR) for various depths. Experiments will be conducted in a small, open flask (~0.5-1 L capacity). Another parameter to be tested in the laboratory is important for pumped systems—the material of the cover that is used to trap the heat. Experiments will be similar to those for the passive system, except that a cover will be added to the tank. The cover enables heat to be trapped/temperature to be increased. A disadvantage is the potential alteration of the light spectrum. Microbial inactivation is optimal with UV-A wavelengths of light (320-400nm) while more energetic UV-B light (< 320nm) is often necessary for efficient organic contaminant destruction. Cover materials to be tested include quartz (completely UV transparent), Pyrex glass (blocks UV-B radiation), and acrylics (which are more durable/lighter weight) of varying UV transparency. Temperature will also be a variable in these studies, with the reactors being heated passively (either ambiently or using a reflector to focus/increase light dosage). Control experiments (no light exposure) will be conducted in parallel to all photolysis experiments. In all experiments, aqueous samples (~ 1 mL) will be collected at selected time intervals from the reactors run in duplicate. At least seven time points will be collected.

Pilot Studies. The laboratory experiments will be used to guide the design of a pilot scale system to be set up at the Blue Lake wastewater treatment plant in Shakopee, MN. This treatment plant is particularly well suited for the study, because after undergoing activated sludge treatment, wastewater passes through a holding pond prior to final disinfection and discharge. Using data from the laboratory studies (specifically, the kinetics of bacterial inactivation and pharmaceutical destruction) the performance of the pilot system will be predicted for a given volume/depth (passive) or volume, depth, and flow rate (active system). The cover material to be used in the active system will also be based on the results of the laboratory studies. In both systems, water depth (rather than volume) is expected to be the crucial geometric parameter. The passive system will be a tank with total capacity of approximately 50-100 L. The active system will have a volume of approximately 2 L. It is expected that residence times in the active system will need to be on the order of one hour, so flow rates will vary from 0.5 to 8 L/hr. Funds for the materials and labor for the construction of the tanks (~$2,000) and a pump (~$1,000) are included in the supply budget.

We will focus this study on the human antibiotics (sulfamethoxazole and triclosan) and one of the estrogenic compounds (to be determined). Both passive and active systems will be studied. In the passive systems, samples will be removed to monitor pharmaceuticals, heterotrophic plate counts (with and without tetracycline), and antibiotic resistance genes as a function of time. In the active systems, influent and effluent concentrations of these parameters will be measured. The analytical methods are described below. We will also conduct a pilot-scale study of the passive system at an agricultural site using tylosin and tetracycline as the target compounds. The procedures will be the same as those described above. We anticipate that we will have to spike the waters with the target compounds for both the wastewater and the agricultural pilot tests to ensure we can routinely and easily detect the target compounds.

In both the active and passive systems, the performance of the system will be measured as a function of residence time, solar exposure (i.e., season), temperature, and water depth. With the active system, the utility of a light reflection system will also be evaluated. Temperature will be monitored using a thermocouple and a data logger. The ultimate goal is to determine if the laboratory measured parameters accurately represent pilot system performance, and if not, what correction factors are necessary to design the system to achieve the desired performance.

Analytical methods and data analysis

Total heterotrophic and tetracycline-resistant heterotrophic bacteria will be enumerated by standard spread-plating techniques. LB-agar plates will be made by mixing 10 g tryptone, 5 g yeast extract, 5 g NaCl, and 15 g washed agar per liter of deionized water. This media will be sterilized by autoclaving (20 min; 121°C; 15 psig). To enumerate tetracycline-resistant bacteria, tetracycline will be added (20 mg/L final concentration) following sterilization, after allowing the media to cool to ~ 60°C. Aqueous samples will undergo a 10-fold serial dilution in phosphate-buffer saline (10 mM; pH 7) and be applied to agar plates using aseptic techniques. Bacteria will be enumerated in digester solids by adding 0.5 g of material to 9 mL of phosphate-buffer saline (10 mM; pH 7) prior to dilution. LB agar plates will be incubated at 37°C for 1 day. All assays will be performed in triplicate.

Because a substantial fraction of tetracycline-resistant bacteria might not grow on LB plates, we will also enumerate tetracycline resistant bacteria by real time quantitative PCR. Five replicate samples will be collected at each time point so that the variability of quantitative PCR can be assessed (Dionisi et al., 2003; Mumy and Finlay, 2004). Aqueous samples (about 10 mL of sample is anticipated) will be filtered through 25-mm diameter poretics filters (pore size = 0.2 μm) to concentrate the biomass. These filters will then be suspended in lysis buffer and subjected to three consecutive freeze-thaw cycles, followed by a 90 min incubation at 70°C to help lyse cells. For sludge samples, the cells will be concentrated by centrifugation and re-suspended in lysis buffer and lysed using a bead-beater approach (FastPrep FP120; Qbiogene).

Total genomic DNA will be purified from these samples using the FastDNA Spin Kit. Extracted DNA will be quantified by staining with Hoescht dye 33258 and correlating results to a standard curve calibrated with calf thymus DNA. Four different genes encoding for tetracycline resistance will be quantified by real time PCR (*tet*(A), *tet*(L), *tet*(O), and *tet*(W)) (unpublished results from the LaPara lab; Smith et al., 2004) and normalized to 16S rRNA genes, also quantified by real time PCR (as described by Pei et al., 2006). Our facilities utilize 96-well microtiter plates, and thus this technique can be very high-throughput.

We will complement these enumerations of tetracycline resistance by targeted analysis of the bacterial isolates and genes encoding tetracycline resistance. Bacterial isolates will be characterized by 16S rRNA gene sequence (i.e., for identification), multiplex PCR for 14 different genes encoding for tetracycline resistance (Ng et al., 2001), and resistance to multiple antibiotics (ciprofloxacin, ampicillin, sulfamethoxozole/trimethoprim, and erythromycin). We will also perform limited PCR-clone libraries of *tet*(A), *tet*(L), *tet*(O), and *tet*(W) to confirm our results. More detailed descriptions of these methods can be found in Firl (2006).

Pharmaceutical compound concentrations will be monitored by a variety of methods. In water samples that are dosed with higher levels of the compounds (1-10 mg/L), direct analysis of the samples with high pressure liquid chromatography with UV detection will be used. For experiments/pilot trials using lower levels, solid phase extraction will be used to concentrate the samples and analysis will be performed via either gas or liquid chromatography coupled with mass spectrometry. For biosolids samples, the material will be dried and Soxhlet extracted. The extract will then be concentrated and analyzed as described above. These methods are currently being used in Dr. Arnold's laboratory for related projects.

When a detailed temperature record is necessary, a thermocouple will be used to measure temperature and the data recorded by a personal computer (laboratory) or data logger (field). Additional water quality parameters to be measured via standard/routine methods included pH, dissolved oxygen, turbidity, total organic carbon, alkalinity, hardness (i.e., Mg^{2+} and Ca^{2+} concentrations), nitrate/ammonia concentration, and UV/visible light absorption for water samples (*Standard Methods,* 1995).

Chromatography systems will be calibrated using, at a minimum, five-point calibration curves. Precision and accuracy will be evaluated via replicate experiments and analyses. Additionally, errors (95% confidence intervals) associated with instrument calibration and detection limits will be quantified. All measured concentrations will be reported with appropriate propagated errors. Blanks, spiked samples, and replicate analyses will be used as quality assurance/quality control measures.

All data fitting and statistical analyses will be performed using commercially available software packages (*e.g., Scientist* for Windows, Microsoft *Excel*). Kinetic rate constants for pharmaceutical and baceterial removal in both laboratory and pilot studies will be regressed using averaged data from replicate experiments and 95% confidence intervals determined. Comparisions between different treatments/conditions (i.e., cover materials and temperatures in the laboratory experiments and residence time, solar exposure, temperature, and water depth in the pilot studies) will be made at the 95% confidence interval to determine if the removal rates are statistically different from each other and from zero.

IV. Results and Products

It is expected that both thermophilic treatment of biosolids and solar treatment of water will effectively remove both the target pharmaceuticals and antibiotic resistant bacteria. Specifically, both types of thermophilic digestion will be excellent at inactivating antibiotic resistant bacteria, but that there will be substantial differences between anaerobic and aerobic thermophilic digestion to biodegrade antibiotics and pharmaceutical compounds (*e.g.,* some compounds will be rapidly biodegraded under aerobic conditions but not under anaerobic conditions). The deliverable product is evaluation of which thermophilic treatment method (aerobic or anaerobic) provides the best removal efficiencies. This research will extend the suitable applications of these established technologies, fulfilling a technology need that will benefit Minnesota. This research will also demonstrate the further importance of thermophilic digestion, which is currently employed within Minnesota at the Western Lake

Superior Sanitary District. The implementation of thermophilic digestion is also under consideration at least one other Minnesota municipality (Knoff, 2007).

For the solar treatment of water, we expect the active/covered systems to be more effective for disinfection. For the removal of pharmaceuticals, the active/covered system will have positive and negative effects. The increased temperature and the possibility to focus the light (using a reflective surface) will enhance removal, but the covering materials will partially attenuate the UV-B radiation that generally leads to pharmaceutical destruction. Thus, we expect an open system with shallow depth will give most effective removal of the pharmaceuticals. The outcomes of this result are (1) determination of the feasibiltiy of solar treatment to simultaneously destroy pharmaceuticals and bacteria and (2) quantification of necessary light doses/exposure times as a function of season, and (3) required capacity and reactor depth to treat a given volume (passive) or flow rate (active) of water.

V. Timetable

The research tasks outlined above will be accomplished according to the following schedule. Shaded regions are continuous efforts and X's mark discreet events.

	Year 1				Year 2				Year 3			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Result 1: Thermophilic treatment of biosolids												
Biosolid collection	X				X							
Establish reactors												
-anaerobic												
-aerobic												
Reactor operation												
-anaerobic												
-aerobic												
Data analysis												
Product delivery							X					X
Result 2: Solar treatment of water												
Laboratory studies												
-Water collection	X	X	X									
-Optimize depth, time, and temperature												
-Evaluate cover materials												
Pilot-scale reactor operation												
Data analysis												
Product delivery							X					X

VI. Deliverable products correlated to the timetable

We estimate that after eighteen months, we will be able to report on the performance of the anaerobic digesters. At this point, we will also have determined the necessary times to destroy the individual pharmaceuticals and will have determined the best cover material for active solar treatment systems.

VII. Budget requirements

The requested funds from LCCMR total $302,000. The total cost breakdown is as follows.

Staff or Contract Services. Over the three year project, Drs. LaPara and Arnold will devote 8% (1 month per year) of time to the project (total salary and fringe benefits of $81,052). The responsibilities of the principal investigators includes experimental design, product coordination, data analysis, student guidance, and report/product preparation. Two graduate student researchers pursuing M.S. or Ph.D. degrees will conduct the day-to-day experiments described above. A 50% appointment is considered full time for students (and includes tuition payment and health insurance), and funds for two 40% appointments is budgeted ($146,530). The remaining 10% appointment will be supplied by teaching assistantships (TAs) or funding from other research grants. Support for the environmental engineering laboratory manager/technician is also requested (5%; $9,932). Responsibilities of the technician include aiding the students in sample preparation/analysis, equipment maintenance, and enforcing laboratory safety protocols.

Equipment. A sum of $40,000 is budgeted for the purchase of a high pressure liquid chromatograph. This essential piece of equipment is required to monitor the concentrations of the target antibiotics and estrogens in the laboratory experiments as well as in the pilot-scale tests. (Trace level analysis will be performed on mass spectrometry equipment, see below). The investigators do currently have access to an HPLC. The instrument, however, is beyond its expected lifetime, and spare parts are no longer available. If the instrument fails, the project will be unable to proceed. Thus, a new instrument is necessary to ensure the project goals are met. Given the number of samples expected to generated by the project, it is more economical to purchase an instrument rather than pay per sample fees on an instrument in another laboratory.

Other. Additional funds totaling $24,486 dollars are requested for travel (mileage charges for trips to partner wastewater facilities/farms; $1,000), laboratory supplies (glassware, chemicals, analytical reagents/consumables, gloves, data storage media, materials for reactor construction); $12,486), analytical services (microbial analyses, gene sequencing, instrument time on liquid chromatograph-mass spectrometers for trace chemical analysis; $10,000), and publication/dissemination costs ($1,000).

Leveraged funds. An additional $5,000 towards the purchase of the HPLC will be leveraged from an unrelated grant of Dr. Arnold. Dr. Arnold is also leading a National Science Foundation sponsored project ($266,000) studying the fate of triclosan in the environment. A portion of this effort is focused on the transformation of triclosan when it is exposed to sunlight, and we will be able to leverage the results for this project.

We will also partner with the Metropolitan Council Environmental Services, Western Lake Superior Sanitary District, and anonymous farmers. Drs. LaPara and Arnold have teamed with these groups in past research efforts, and thus a good working relationship between the lead invesigators and partners already exists. The partners will provide in-kind contributions (i.e., site access, sampling assistance, staff time) at no direct cost to the proposed project.

The proposed project is a result of past research and collaborations of Drs. Arnold and LaPara. Previous funds totaling $340,000 have been spent on studies on the proliferation on antibiotic resistant bacteria in agricultural soils, the fate of pharmaceutical and personal care products in surface waters, and the release of antibiotic resistant bacteria from the Metropolitan Wastewater Treatment plant (St. Paul). The knowledge and techniques from these past projects will be used in the proposed LCCMR project.

VIII. Investigators' qualifications

Drs. LaPara and Arnold are nationally-known researchers with expertise on antibiotic resistance/thermophilic digestion and the environmental chemistry of pharmaceutical compounds, respectively. Dr. LaPara will be responsible for studying the inactivation/disinfection of antibiotic resistant bacteria in the targeted treatment systems and design/operation of the thermophilic digestion experiments. Dr. Arnold will focus on the degradation of the pharamceuticals and have responsibility for the design/operation of the solar treatment studies.

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PROFESSIONAL EXPERIENCE

2006- **Associate Professor**, Department of Civil Engineering, University of Minnesota, Minneapolis, MN and member of the graduate faculty in Water Resources Sciences

2000-2006 **Assistant Professor**, Department of Civil Engineering, University of Minnesota, Minneapolis, MN and member of the graduate faculty in Water Resources Sciences

EDUCATION

1999 **Ph.D.** Purdue University, West Lafayette, IN, School of Civil Engineering, Geography and Environmental Engineering.

1995 **B.S.C.E.** University of Notre Dame, Notre Dame, IN, Civil Engineering

RESEARCH INTERESTS

Biological wastewater treatment, wastewater microbiology, environmental microbiology, structure-function relationships in mixed microbial communities, antibiotic resistant bacteria, microbial ecology, and microbial evolution

RELEVENT CURRENT AND PAST RESEARCH FUNDING

"Antibiotics in the Environment: Linking Environmental Fate to the Proliferation of Antibiotic Resistant Bacteria", PI, US Department of Agriculture, $240,000, 9/1/03-8/31/07.

"The current role of municipal wastewater treatment facilities in the proliferation of antibiotic resistant bacteria", PI, Center for Urban and Regional Affairs, $45,054, July 1, 2004 – December 31, 2005

SELECTED HONORS AND AWARDS

2005 *Bonestroo, Rosene, Anderlink & Assoc. Undergraduate Teaching Award*, Univ. of Minnesota

1999 *Donald E. Bloodgood Memorial Award*, Purdue University

1995 *Walter L. Shilts Award***,** University of Notre Dame

PROFESSIONAL AFFILIATIONS

American Society for Microbiology, Water Environment Federation, International Water Association, International Society for Microbial Ecology, Association of Environmental Engineering and Science Professors

RELEVANT PEER REVIEWED PUBLICATIONS

1. Wammer KH, **TM LaPara**, K McNeill, WA Arnold, and DL Swackhamer. 2006. Changes in antibacterial activity of triclosan and sulfa drugs due to photochemical transformations. *Environmental Toxicology and Chemistry* **25**(6):1480-1486.
2. Kappell AS, MJ Semmens, PJ Novak and **TM LaPara**. 2005. A novel application of oxygen-transferring membranes to improve anaerobic wastewater treatment. *Biotechnology and Bioengineering* **89**(4):373-380.
3. Onan LJ and **TM LaPara**. 2003. Tylosin-resistant bacteria cultivated from agricultural soil. *FEMS Microbiology Letters* **220**(1):15-20.
4. **LaPara TM**, CH Nakatsu, LM Pantea and JE Alleman. 2001. Aerobic biological treatment of a pharmaceutical wastewater: effect of temperature on COD removal and bacterial community development. *Water Research* **35**(18):4417-4425.
5. **LaPara TM**, A Konopka, CH Nakatsu and JE Alleman. 2001. Thermophilic aerobic biological wastewater treatment of a synthetic wastewater in a membrane-coupled bioreactor. *Journal of Industrial Microbiology and Biotechnology* **26**(4):203-209.
6. **LaPara TM**, CH Nakatsu, LM Pantea and JE Alleman. 2000. Phylogenetic analysis of bacterial communities in mesophilic and thermophilic bioreactors treating pharmaceutical wastewater. *Applied and Environmental Microbiology* **66**(9):3951-3959.
7. **LaPara TM**, A Konopka, CH Nakatsu and JE Alleman. 2000. Thermophilic aerobic wastewater treatment in continuous-flow bioreactors. *Journal of Environmental Engineering* **126**(8):739-744.
8. **LaPara TM**, A Konopka, CH Nakatsu and JE Alleman. 2000. Effects of elevated temperature on bacterial community structure and function in bioreactors treating a synthetic wastewater. *Journal of Industrial Microbiology and Biotechnology* **24**(2):140-145.
9. Konopka A, T Zakharova and **TM LaPara**. 1999. Bacterial function and community structure in reactors treating biopolymers and surfactants at mesophilic and thermophilic temperatures. *Journal of Industrial Microbiology and Biotechnology* **23**(2): 127-132.
10. **LaPara TM** and JE Alleman. 1999. Thermophilic aerobic biological wastewater treatment. *Water Research* **33**(4):895-908.

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PROFESSIONAL EXPERIENCE

2006-2007 and **Visiting Researcher,** Eawag, The Swiss Federal Institute of Aquatic Science Technology, Dübendorf, Switzerland

2005- **Associate Professor**, Department of Civil Engineering, University of Minnesota, Minneapolis, MN and member of the graduate faculty in Water Resources Sciences and in Stream Restoration Science and Engineering

1999-2005 **Assistant Professor**, Department of Civil Engineering, University of Minnesota, Minneapolis, MN and member of the graduate faculty in Water Resources Sciences

EDUCATION

1999 **Ph.D.** The Johns Hopkins University, Baltimore, MD, Department of Geography and Environmental Engineering.

1995 **M.S.** Yale University, New Haven, CT, Department of Chemical Engineering.

1994 **S.B.** Massachusetts Institute of Technology, Cambridge, MA, Chemical Engineering, Minor in Chemistry.

RESEARCH INTERESTS

Fate and abiotic transformations of anthropogenic chemicals in natural and engineered aquatic systems, including

- Redox reactions at metal and mineral surfaces
- Photochemistry in surface waters and for water treatment
- Disinfection by-product degradation and formation
- Reactive membrane systems

RELEVENT CURRENT AND PAST RESEARCH FUNDING

"Collaborative Research: Formation of Polyhalogenated Dioxins and Furans from Triclosan and PBDEs in Rivers", PI, National Science Foundation, $266,000, 6/1/06-5/31/08.

"Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency", co- PI, National Institutes for Water Resources, $134,070, 9/1/02-2/28/06.

"Antibiotics in the Environment: Linking Environmental Fate to the Proliferation of Antibiotic Resistant Bacteria", co-PI, US Department of Agriculture, $240,000, 9/1/03-8/31/07.

"Environmental Estrogens and Antibacterials in Aquatic Systems: Occurrence, Persistence, and Fate", co-PI, Dreyfus Foundation, $96,000, 8/1/03-7/31/05.

"Photodegradation of Pharmaceutical Compounds Discharged and Detected in Natural Waters", PI, National Institutes for Water Resources, $102,656, 9/1/01-8/31/03.

SELECTED HONORS AND AWARDS

2005 Excellence in review award from *Environmental Science and Technology*

2003 1st Place Montgomery-Watson-Harza Consulting Engineers/AEESP Master's Thesis Award for Jennifer L. Packer's Thesis

2003 Bonestroo, Rosene, Anderlik and Associates Undergraduate Faculty Award (for excellence in teaching, advising, and mentoring of students)
2003 MFES Minnesota Young Engineer/Science & Technology Professional of the Year
2003 ASCE Minnesota Section Young Engineer of the Year

PROFESSIONAL AFFILIATIONS

American Chemical Society (Environmental Chemistry Division), American Geophysical Union (Hydrology Section)**,** American Society of Civil Engineers, Association of Environmental Engineering and Science Professors, Licensed Professional Engineer (Minnesota)

RELEVANT PEER-REVIEWED PUBLICATIONS

1. Werner, J.J.; **Arnold, W.A.**; McNeill, K., 2006. Water hardness as a photochemical parameter: tetracycline photolysis as a function of calcium concentration, magnesium concentration, and pH, *Environmental Science and Technology*, v. 40, 7236-7241.
2. Edhlund, B.L.; **Arnold**, **W. A.;** McNeill, K. 2006. Aquatic photochemistry of nitrofuran antibiotics, *Environmental Science and Technology*, v. 40, 5422-5427.
3. Wammer, K. H.; LaPara, T. M.; McNeill, K.; **Arnold**, **W. A.;** Swackhamer, D. L. 2006. Changes in antibacterial activity of triclosan and sulfa drugs due to photochemical transformations, *Environmental Toxicology and Chemistry*, v. 25, 1480-1486.
4. Boreen, A.L.; **Arnold, W.A.**; McNeill, K., 2005. Triplet-sensitized photodegradation of sulfa drugs containing six-memebered heterocyclic groups: identification of an SO_2 extrusion photoproduct, *Environmental Science and Technology*, v. 39, 3630-3638.
5. Werner, J. J.; McNeill, K.; **Arnold, W.A.**, 2005. Environmental photodegradation of mefenamic acid, *Chemosphere*, v. 58, 1339-1346.
6. Latch, D. E.; Packer, J. L.; Stender, B. L.; VanOverbeke, J; **Arnold, W.A.**, McNeill, K., 2005. Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-*p*-dioxin and oligomerization products, *Environmental Toxicology and Chemistry*, v. 24, 517-525.
7. Boreen, A.L.; **Arnold, W.A.**; McNeill, K., 2004. Photochemical Fate of Sulfa Drugs in the Aquatic Environment: Sulfa Drugs Containing Five-Membered Heterocyclic Groups. *Environmental Science and Technology*, v. 38, 3933-3940.
8. Boreen, A.L.; **Arnold, W.A.**, McNeill, K., 2003. Photodegradation of pharmaceuticals in the aquatic environment: a review. *Aquatic Sciences*, v. 65, 320-341.
9. Packer, J.L.; Werner, J.J.; Latch, D.E.; McNeill, K.; **Arnold, W.A.**, 2003. Photochemical fate of pharmaceuticals in the environment: naproxen, diclofenac, clofibric acid, and ibuprofen. *Aquatic Sciences*, v. 65, 342-351.
10. Latch, D.E.; Stender, B.L.; Packer, J.L.; **Arnold, W.A.** ; McNeill, K., 2003. Photochemical fate of pharmaceuticals in the environment: cimetidine and ranitidine. *Environmental Science and Technology*, v. 37, 3342-3350.
11. Latch, D.E.; Packer, J.L.; **Arnold, W.A.**; McNeill, K., 2003. Photochemical conversion of triclosan to 2,8-dichlorodibenzo-*p*-dioxin in aqueous solution. *Journal of Photochemistry and Photobiology A: Chemistry*, v. 158, 63-66.

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Attachment A: Budget Detail for 2007 Projects - Summary and a Budget page for each partner (if applicable)

Project Title: *Pharmaceutical and Microbiological Pollution Minnesota's surface waters; 5(L)*

Project Manager Name: *Timothy M. LaPara*

Trust Fund Appropriation: $302,000

1) See list of non-eligible expenses, do not include any of these items in your budget sheet

2) Remove any budget item lines not applicable

2007 Trust Fund Budget	**Result 1 Budget:**	**Result 1 Revised Budget:**	**Amount Spent 6/30/10**	**Balance 6/30/10**	**Result 2 Budget:**	**Amount Spent 6/30/10**	**Balance 6/30/10**	**TOTAL BUDGET**
	Thermophilic treatment of municipal and agricultural biosolids	*Thermophilic treatment of municipal and agricultural biosolids*			*Solar treatment of water for pharmaceutical destruction and disinfection*			
BUDGET ITEM								
PERSONNEL: wages and benefits	~~118,684~~	103,684	102,284	16,400	118,684	112,563	2,613	~~237,368~~
Other direct operating costs *(laboratory supplies, travel for pilot studies and wastewater collection, publication costs, and analytical services such as DNA sequencing and real-time PCR)*	~~11,316~~	16,316	18,038	-6,722	11,316	18,383	-2,559	~~22,632~~
Equipment / Tools *(High Pressure Liquid Chromatograph; cost = $40,000; real time thermal cycler, trust fund cost = $10,000 out of $26,100 total -- additional funds will come from other research grants)*	~~20,000~~	30,000	30,000	-10,000	20,000	20,000	0	~~40,000~~
Printing	0	0	0	0	500			500
Travel expenses in Minnesota	0	0	0	0	500			500
Other *(ST Rents & Leases)*	1,000	1,000	678	322	0	54	-54	1,000
COLUMN TOTAL	**$151,000**	**$151,000**	**$151,000**	**$0**	**$151,000**	**$151,000**	**$0**	**$302,000**