FINAL REPORT
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RENDERABLE FLOCCULANTS FOR WASTEWATER TREATMENT

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Duration of Project: 12 months

Lay Summary:

Every rendering plant has a water treatment system for purification of a wide range of wastewater from rendering, poultry, meat and food processing, to recover proteins and fats to produce high quality fats and protein meals, and meet eluent standards. In many cases the use of flocculants is required to achieve optimum purification efficacy and high total solids content in the sludge. Flocculants are additives that facilitate the removal of particles or colloidal material from a liquid. Most of currently used flocculants are synthetic and their application in large scale agriculturally-related processes is becoming a growing concern for consumers, public, and regulatory entities due to lack of biodegradability and potential toxicity of accumulated contaminants. Since 2017 we have been working with Dr. Rafael Garcia on development a **novel biodegradable flocculant** prepared from blood to replace synthetic compounds. Successful development of a highly efficient biodegradable flocculant will help to address these issues and provide environmentally friendly way for treatment of wastewaters generated in rendering processes, and also reduce costs associated with use of chemicals.

The flocculants used in rendering and other wastewater treatment applications are often synthetic polymers, most commonly derivatives of polyacrylamide (PAM), a known cause of abundant problems in rendering systems, including coating of cooker surfaces and downgrading of products. While synthetic polymer flocculants are attractive due to their high effectiveness and low cost, there is growing concern regarding the environmental and health impacts of these substances. Consequently, there is currently significant academic and commercial interest in the development of bio-based alternatives to synthetic polymer flocculants. While many bio-based flocculants have been investigated, typically they must be used in at considerably higher concentrations in order to achieve equivalent results.

We teamed up with Dr. Rafael Garcia (ARS USDA) and developing a novel natural biodegradable flocculant based on hemoglobin recovered from livestock slaughter blood, that can be used as a replacement for PAM. Hemoglobin-based flocculants were found to be as efficient as PAM at similar or lower concentrations. This year we propose to use modified preparation technique to enhance flocculant efficacy, and test its performance in field conditions.

Since initiation of the project we have carried experiments with biodegradable formulations of flocculants from different blood sources including porcine, chicken and turkey. We have performed extraction and characterization of flocculants, as well as their testing in the laboratory and field conditions. We have visited a number or rendering and waste water facilities to study treatment procedures and equipment, and performed field tests.

1. **Objective (s):** <u>Preparation and characterization of biodegradable flocculant from methylated blood hemoglobin using different raw material (poultry, porcine, bovine) and optimization of preparation procedures.</u> We will work on development a scalable method of blood proteins methylation, characterize and study efficacy of flocculant prepared from different blood sources.
2. <u>Field testing of prepared flocculant formulations to achieve appropriate application rate</u>. We will travel to rendering and packing plants to test methylated blood-based flocculant and compare it to currently used synthetic flocculants.

Project Overview:

Introduction

Flocculants are substances, which destabilize colloidal suspensions and promote the clumping and settling of the suspended particles. Among the most important applications of flocculants is treatment of agricultural wastewaters, such as the wastewaters generated in livestock slaughter and processing plants, rendering plants, etc. Flocculants are also directly applied to soil to prevent erosion in agricultural and construction areas. Most of the currently used flocculants are synthetic and their application in large scale agriculturally-related processes is becoming a growing concern for consumers, public, and regulatory entities due to their lack of biodegradability and potential toxicity of accumulated contaminants. Successful development of a highly efficient biodegradable flocculant will help to address these concerns and provide an environmentally friendly way for treatment of wastewaters generated in agricultural processes. It will also lead to improved methods for reuse of wastewater because currently available methods rely primarily on the use of potentially harmful synthetic flocculants. Flocculants are widely used in agriculture for clarifying livestock slaughter and rendering waste liquids and in many other applications throughout agriculture, mining, and construction. The flocculants of choice by the livestock and rendering industries are often synthetic polymers, most commonly derivatives of polyacrylamide (PAM). While these synthetic flocculants are attractive due to their high effectiveness and low cost, there is growing concern about the environmental and health impacts of these substances. Consequently, much attention, both in industry and in academia, is currently paid to the development of bio-based alternatives to synthetic polymer flocculants.

Dr. Garcia and co-workers, from a USDA/ARS lab, recently discovered that hemoglobin can function as a powerful biodegradable flocculant suitable for wastewater recovery. The long-term goal of this work is to develop and commercialize a cost-efficient biodegradable hemoglobin-based flocculant.

Methylated hemoglobin. In a search of a more efficient formulation we attempted to prepare methylated hemoglobin. Methylation can offer several important advantages. First, it replaces negatively charged carboxyl groups to neutral methyl ester groups, thus increasing the total positive charge of the hemoglobin molecule and its flocculation efficacy. Second, it increases the isoelectric point of hemoglobin and therefore should broaden the pH range of its efficacy as a flocculant. Third, methylation can be performed in the absence of water yielding a dry product after evaporation of methanol.

This latter consideration turned out to be very important for commercialization of this product. As we found out in preliminary studies, typical application rate of our hemoglobin - zinc chloride slurry is 1000 ppm (~100 ppm by dry hemoglobin). Such dose it much higher than typical application rate of currently used synthetic polymers (10-15 ppm of dry polymers or ~100 ppm of 20 % wt. polymer solution).

Methylated hemoglobin was prepared from commercially available spray dried porcine red blood cells. Methanol was then mixed with dried red blood cells in a 2:1 wt. ratio. The mixture was left in a shaker for 24 h (some samples were removed from the shaker just after 10 min to study effect of the reaction time), followed by evaporation of methanol in a chemical hood. For flocculation experiments, methylated samples were dispersed in water at 5% wt. immediately before the start of the experiments.

We found that methylated hemoglobin was considerably more effective than regular hemoglobin. Furthemore, commercially available spray dried red blood cells themselves were not effective flocculants in a 1 g/L kaolin clay model. After methylation, same spray dried red blood cells flocculated kaolin clay suspension at the dose of 10-20 ppm (by solid Hb). We also used a diluted milk model which we felt was more relevant to rendering and animal processing wastewaters since milk contains both proteins and fats. Figure 1 shows dose-dependent flocculation of diluted milk (~0.5% solids content) by methylated hemoglobin. Coagulation starts at the dose of 300 ppm (15 ppm by methylated hemoglobin) and the precipitated easily separates from the liquid by filtration leading to almost clear water.

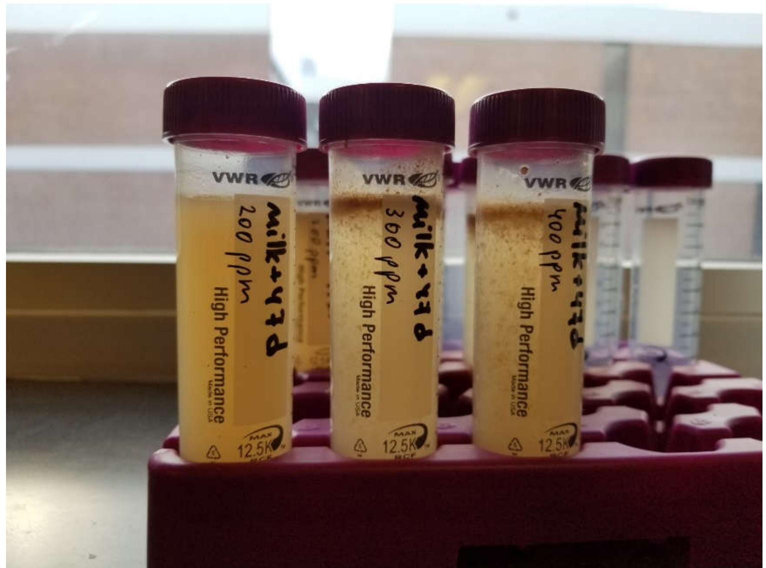

Fig. 1. Coagulation of milk by methylated hemoglobin. Coagulation starts at the dose of 300 ppm (15 ppm by solid hemoglobin)

Jar testing. We performed small scale “jar” testing of hemoglobin-based flocculants in a number of rendering and animal processing facilities in North Carolina, Georgia, Illinois, Ohio, Indiana and California.

Results of an experiment with non-methylated hemoglobin are shown in Figure 2. This challenging wastewater from a rendering plant in California has high solids content and relatively high precipitation rate on its own. Untreated wastewater forms considerable amount of

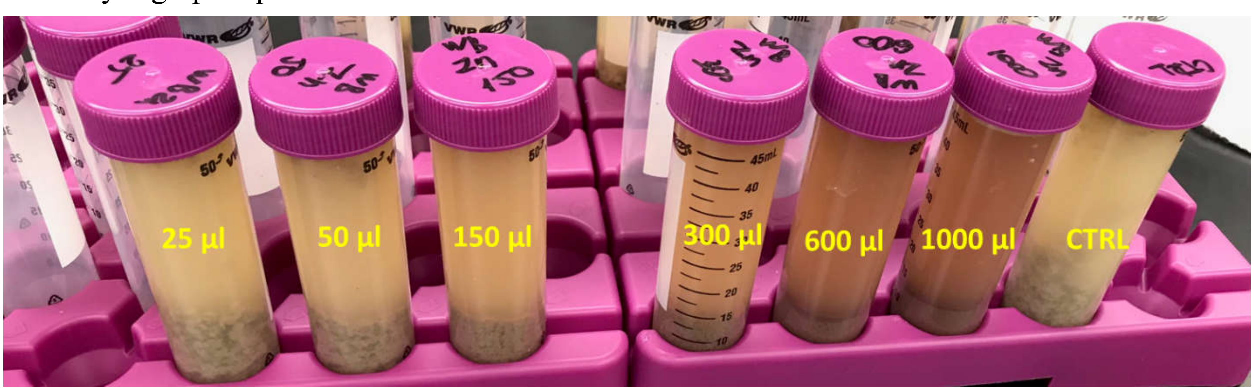

Fig. 2. Effect of non-methylated hemoglobin slurry (10% Hb, 1% $ZnCl_2$) on wastewater from a rendering plant in California. Precipitation took about 30 minutes to occur.

precipitate after standing for ~30 min (see the right most sample in Fig. 2). Adding non-methylated hemoglobin leads to the formation of a denser precipitate starting from the doses of 150-300 μL per 50 mL test tube (300-600 ppm by solid hemoglobin). Precipitation occurs slowly and does not result in good clearing of the wastewater.

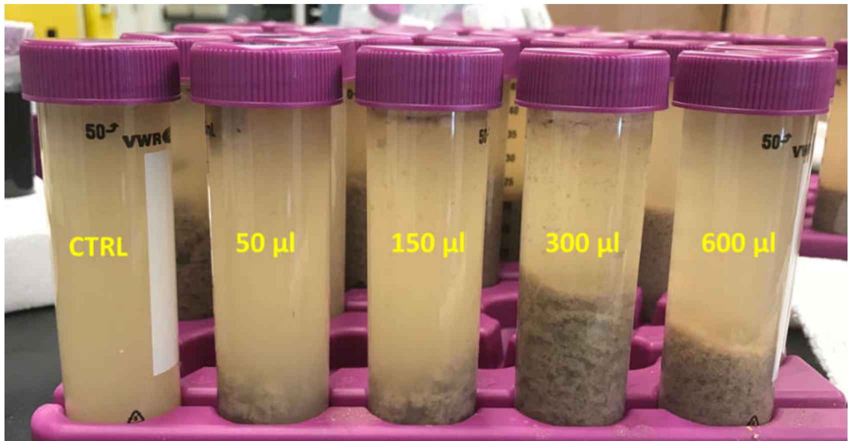

Figure 3. Effect of methylated hemoglobin (5 wt. % solid Hb) on wastewater from a rendering plant in California. Precipitation occurs immediately, and the amount of precipitate appears to increase with the amount of flocculant added.

At the same time, effect of methylated hemoglobin on the same wastewater was faster and more pronounced (Figure 3). Precipitation starts immediately, and the onset of precipitation is observed at much lower doses (50 μL per 50 mL test tube, or 50 ppm by solid methylated hemoglobin). However, good water clearance is not achieved until the dose is increased to 1000 μL, or 1000 ppm of solid methylated hemoglobin (see Fig. 4).

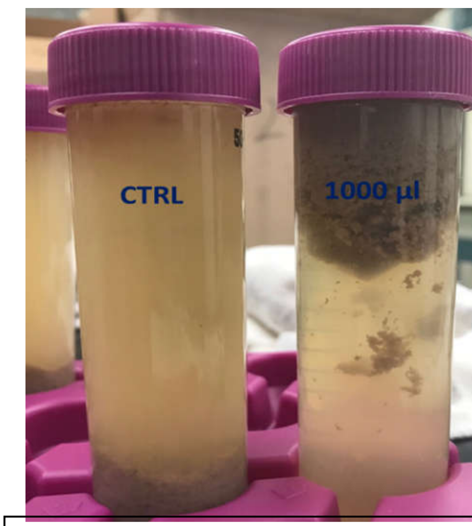

Fig. 4. High dose of 1000 ppm of methylated hemoglobin is required to achieve good water clarification.

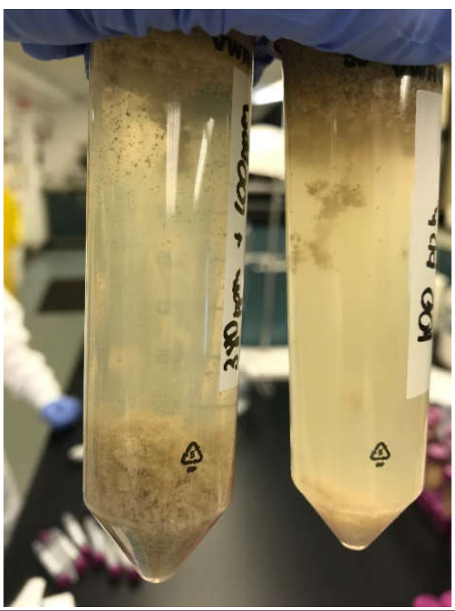

Fig. 5. Simultaneous application of methylated hemoglobin at 380 ppm and currently used inorganic coagulant at 100 ppm results in good water clarification (left). Same coagulant applied alone does not work as well (right).

The wastewater plant at this facility currently uses two precipitation aids: an inorganic coagulant and a cationic polymeric flocculant (PAM). To further improve the performance of methylated hemoglobin, it was used in conjunction with the inorganic coagulant that is currently used by this plant. As shown in Figure 5, methylated hemoglobin at the application rate of 380 ppm applied together with 100 ppm of currently used coagulant results in a desired water clarification.

Overall, similar observations were made in other locations we visited. Generally, methylated hemoglobin was more efficient than non-methylated hemoglobin (except for one location in Illinois where their performance was similar). For the facilities that use an inorganic coagulant and cationic PAM as the flocculant, methylated hemoglobin can replace the cationic polyacrylamide at the dose 300-500 ppm by solid hemoglobin. One facility in Indiana currently uses ferric chloride as the coagulant and both cationic and anionic PAM-based flocculants at their wastewater plant. In that case, combination of methylated hemoglobin and their anionic flocculant performed similarly to the currently used combination of the three reagents (Figure 6). Therefore, we would be able to replace both coagulant and cationic flocculant in this particular case. Since methylated hemoglobin is positively charged, we anticipate it will not be a very effective flocculant in the facilities that must use anionic polymers as their flocculants.

Fig. 6. Application of methylated hemoglobin in combination with currently used anionic flocculant (left) results in similar water clarification to the currently used combination of three reagents.

Impacts and Significance: Currently available biodegradable solutions are far too expensive and lack efficacy to replace synthetic flocculants such as PAM. At the same time, use of PAM becomes growingly more expensive because of additional costs associated with appropriate disposal and additional cleaning steps. Specifically, for the rendering industry, PAM accumulates in the wastewater sludge at the levels of 0.1-0.3 wt. %, leading to a number of issues. Even though wastewater sludge consists mainly from fat and protein, it cannot be returned to the rendering process because presence of PAM interferes with the final product quality and digestibility. A typical rendering facility that processes ~10^6 gallons of water per day produces about 30 tons of sludge daily. Had they been able to return this sludge back to the rendering process, it would generate additional $3,000 - 5,000 of revenue daily. Instead, renderers have to pay $1,500 - 2,000 per day for its disposal. It can be expected that use of a protein-based flocculant will help to solve this problem – and one of the goals of this project is to study whether or not the sludge generated using our flocculant can be rendered without compromise of the quality of the rendered products.

Publications: None

Outside funding: USDA SBIR Phase I and Phase II

Future Work: Since the beginning of this project we have visited multiple rendering plants and spoke with many waste plant managers, who were more worried about use of ferric in DAF systems than use of polymers. Ferric Chloride is a very efficient coagulant, which is added to waste water prior to DAF systems for destabilization of colloid solution and better extraction of sludge. Unfortunately, such sludge cannot be returned to the rendering process, due to corrosive nature of ferric compounds, and must be shipped to the municipal facilities for treatment, which involves additional charges. We have proposed to replace Ferric Chloride with biological coagulant – modified ***chitosan*** solution (NoFerric™).

NoFerric™ is a biodegradable coagulant based on chitosan. It is made of shrimp and crab shells. It can be of interest for those plants that currently use ferric chloride or alum (or any other inorganic salt) as their coagulant. It can be used in two ways:

1) Low risk approach, no need to switch from the current supplier of wastewater treatment chemicals. In this case it is used as coagulation aid along with the currently used inorganic coagulant (e.g., aluminum chloride). We found that very low dose of NoFerric™ (in the few ppm range or sometimes under 1 ppm) helps to reduce application rate of aluminum or ferric chloride by 50 to 80%, leading to the net savings on coagulants in the range of 30-40%. In addition, it leads to the formation of more dense sludge, which could be helpful if the sludge is disposed (reduces disposal costs because of lower volume of the sludge).

2) For those customers who are interested in complete elimination of inorganic coagulants and replacing with a biodegradable option, NoFerric™ can be used to replace alum or ferric chloride. NoFerric™ is about 25 times more efficient that alum and about 20 times more expensive, so in this case typical savings are in the range of 20-30%. In this case the sludge does not contain iron or aluminum, so one of our animal processing customers now sells it to a nearby rendering plant instead of paying for disposal.

At this point we are interested in jar testing to see if our solution is suitable for a particular plant. We will come and perform testing ourselves, without causing much of distraction. If it works, we will discuss the results/potential economic benefits with the plant management and see if there is further interest in a large-scale testing, which is typically performed in the low risk mode.

Acknowledgments: We would like to thank Dr. Rafael Garcia for his help with preforming the experiments and field testing.