

FINAL REPORT
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Solvent Extraction of Oils from DAF Sludge and Reuse of Recovered Flocculant

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Lay Summary: The current industry standard for wastewater treatment in the rendering industry employs dissolved air flotation (DAF) to remove oils, protein, and other suspended particulate matter from wastewater before disposal or further treatment. The DAF process uses flocculants to aid in the coagulation and removal of the suspended solids. A result of the DAF process is a large volume of DAF sludge material that must be dealt with. Current practices are to dispose of the sludge, which is costly, or to put the sludge back into the front end of the rendering process, which introduces polyacrylamide flocculant into the rendered meal products. Polyacrylamide is commonly used in DAF processes and there is rising concerns with regard to the presence of the polyacrylamide in the meals used for agricultural and pet foods. The focus of this research is to investigate novel methods of isolating valuable oil components from the DAF sludge and the potential for reuse of recovered DAF flocculant.

Objective (s):

- Perform lab-scale progressive solvent soxhlet extractions of DAF sludge obtained from rendering facilities. Solvents of initial investigation will include hexane, acetone, ethanol, and ethyl acetate. Other generally recognized as safe (GRAS) FDA solvents will also be investigated.
- Characterize the oil extracted from DAF sludge to determine purity, composition, and quality.
- Determine the composition of the residual matrix after extraction and evaluate the potential for reuse in the DAF process.

Project Overview: The goal of this work is to investigate the use of solvent extraction for the treatment of DAF sludge with the goal of recovering the oil component and potential reuse of the remaining flocculant in the DAF process. If successful, this process could provide alternative means for the treatment of DAF sludge where other methods have failed. Once the DAF sludge has been dewatered, it can contain 60 to 80 % oil content, which makes it very difficult to process by alternative means. In addition, the lack of bone in the sludge hinders the ability to remove the fat by pressing techniques, commonly used in the industry. Hexane extraction is the most commonly used method of oil extraction worldwide and currently accounts for 95% of the world market for seed oils; primarily soybean and canola. Benefits of hexane include the high solubility of oil compounds and the low boiling point for ease of recovery by distillation. Drawbacks of hexane are the high flammability and toxicity, regardless hexane extraction is commonly used on extremely large scales and for the production of edible oils.

In this work, we have demonstrated the improved extraction ability of alternative solvents to hexane, specifically methanol, ethanol and acetone. Experimentally, 20 g of DAF sludge was measured and dried in a vacuum oven at 40°C for 2 hrs to remove residual water. The average water content in the DAF sludge was 3.7±0.2%. The DAF was then placed in a soxhlet extraction unit and extracted for 5, 10, 15, and 20 hours, measuring the weight percent extracted at each time point. Figure 1 displays the rate of oil extraction using the four solvents, demonstrating that methanol and acetone have the greatest extraction rate, while hexane has the slowest. Furthermore, methanol extracted the greatest percentage of lipids (67%) from the DAF sludge compared to the other solvents (63%). The increased extraction efficiency of methanol is likely due to the increased polarity of the solvent and the higher propensity of free fatty acids

over conventional bio-oils. Methanol would also have an advantage in extraction when the sample contains an appreciable fraction of water.

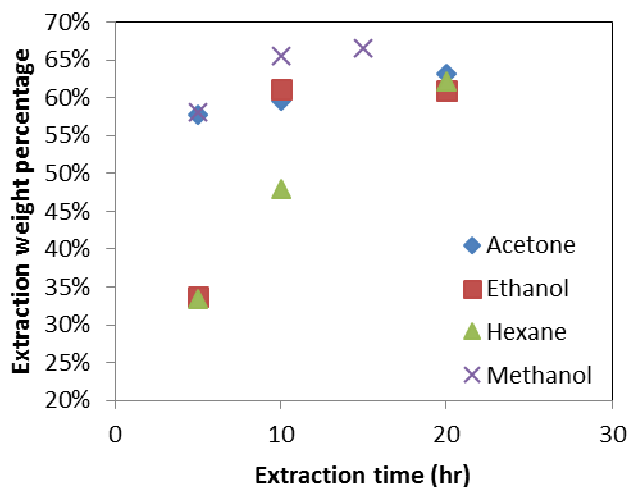


Figure 1. Extracted oil weight percentage vs extraction time for different solvents

After extraction, the DAF became a fine powder and the color changed from a dark brown to a pale tan color consistent with an extracted protein material. The extraction solvent became a brown color. For hexane, precipitation was observed when the extract cooled to room temperature (25 °C). This indicates the presence of lower solubility lipid components such as polar lipids or free fatty acids.

Thin layer chromatography (TLC) was used to qualitatively determine the composition of the lipid extracts for each solvent at each time point. With TLC, different class of lipids can be identified by the relative distance of travel up a TLC plates (Figure 2). In Figure 2, two reference standards were used to determine the different fractions identified by TLC; triacylglycerol (TG), cholesterol ester (CE), free fatty acid (FA), diacylglycerol (DG), cholesterol (C), and monoacylglycerol (MG). Other polar lipids (phospholipids, etc.) were also identified in the extracted samples but at lower levels. The lipids extracted by the solvents overall (Figure 2) and as a function of time (Figure 3) showed some variation. Both the neutral and polar lipids were extracted from the DAF by each solvent at each time point. Acetone and Ethanol seem to have higher extraction selectivity for relatively polar lipids (FA, DG, C and MG) than neutral ones (TG and CE). However, this is difficult to quantify due to the qualitative nature of the TLC method. Regardless, the ability to selectively extract different classes of lipids from the DAF using different solvents and extraction time was not achieved.

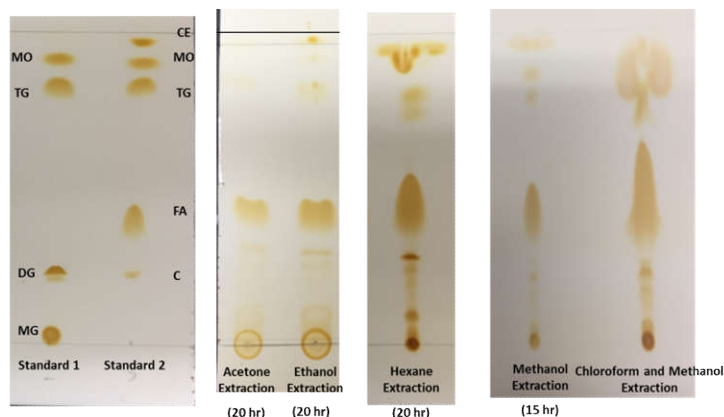


Figure 2. Visual verification of lipids with TLC in different solvent extractions. In the standard 1 and 2 chromatography (from NU-CHEKCPREP, INC. GLC Reference Standard), TG: Triacylglycerol (Triolein in standard sample); DG: Diacylglycerol (Diolein in standard sample); MG: Monoacylglycerol (Monoolein in standard sample); C: Cholesterol; CE: Cholesterol ester (Cholesterol Oleate in standard sample); FA: Free fatty acid (Oleic Acid in standard sample). During TLC process, solvent flows from bottom to top.

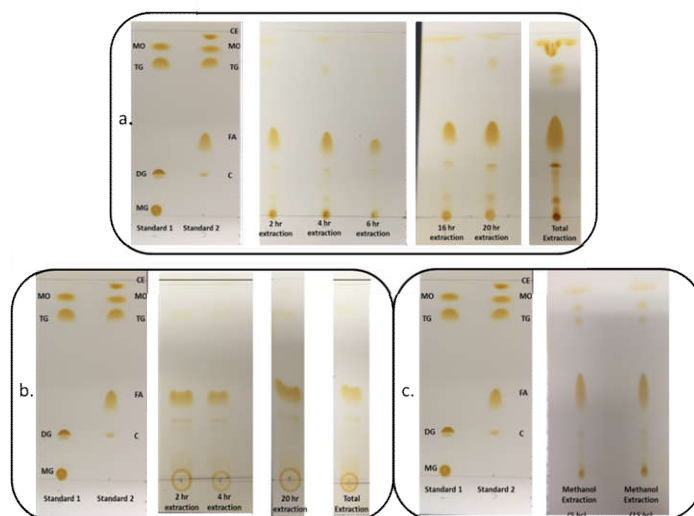


Figure 3. Visual verification of lipids with TLC in hexane (a), acetone(b) and methanol(c) extraction over time. During TLC process, solvent flows from bottom to top. The standards are the same as in Figure 2.

To further explore the potential for selective extraction, a sequential extraction was investigated where been applied, here. With carefully controlled lipid deposition on each TLC plate (100 μg), no obvious difference was observed in the extracts (Figure 4). Despite hexane having higher solubility for non-polar lipid fractions and acetone and alcohols having a higher solubility of polar lipids, the other components still have limited solubilities and mutual solubilities are also appreciable. Thus proving difficult to selectively extract one lipid class over another.

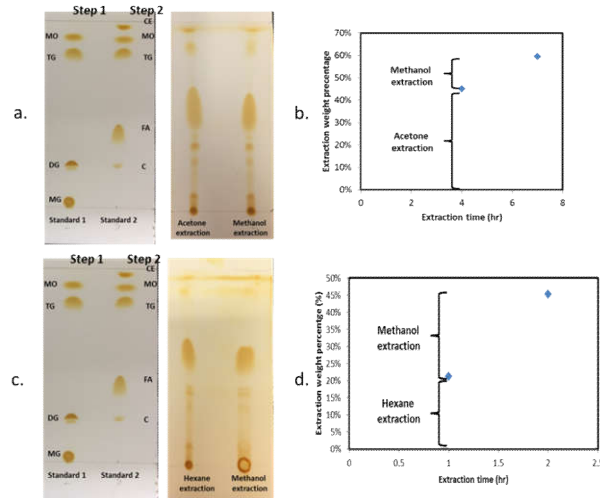


Figure 4. TLC characterization of extracted lipids with sequential solvent extractions. a,b) DAF sludge first extracted by acetone for 4 hours, then by methanol for 3 hours; c,d) DAF sludge first extracted by acetone for 4 hours, then by methanol for 3 hours. a and c are the TLC images; b and d are the extraction weight percentage change in each step.

Considering that methanol and hexane are two immiscible solvents and that precipitation of lipid fractions was observed with hexane cooling, an alternative method of lipid separation was explored. In short, methanol extraction was performed, followed by hexane liquid-liquid extraction. The TLC results in Figure 5 show that the non-polar lipids (CE, MO, TG) are extracted into the hexane phase, while the polar lipids and FA remain in the methanol phase. Thus, it may be possible to take advantage of this competitive liquid-liquid extraction, combined with transesterification treatment to produce esters from the extracted lipid.

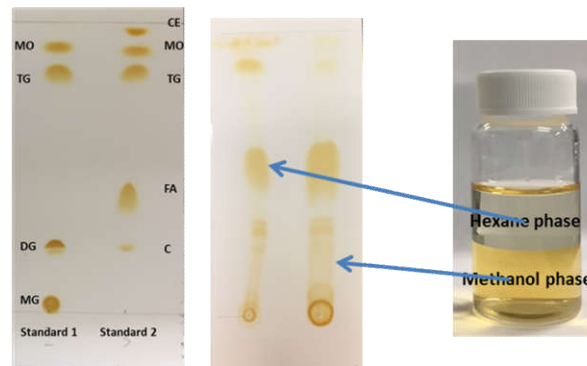


Figure 5. TLC visual verification of lipids from liquid-liquid extraction.

Analysis of Extracted Solids:

Fourier transform infrared (FTIR) spectroscopy was used to examine the composition of the remaining solids after DAF sludge extraction. Figure 6 shows the FTIR spectra after methanol and hexane extraction, yielding typical protein spectrum with NH stretching band at 3277 and 2926 cm^{-1} , C=O stretching band at 1626 cm^{-1} and CN stretching band at 1524 cm^{-1} .⁷

Furthermore, there are little differences between the spectra for the solids remaining after methanol and hexane extraction. Because the protein and polyacrylamide have similar chemical

functional groups, it is difficult to identify the presence of the polyacrylamide. Furthermore, the relatively low concentrations of flocculant used make it difficult to recover, however, there may be potential to add the extracted protein solids back into the DAF process.

Figure 7 is the FTIR spectra of the dried lipids obtained from the methanol and hexane extractions. Peaks at 3000-2800, 1800-1700 and 1500-1000 cm^{-1} are indicative of OH, C=O and CO stretching of fatty acids and tri, di and monoglycerides. This confirms the basic lipid types extracted from the DAF sludge.^{8,9} Spectral subtraction of the methanol extract from hexane extract suggests potential existence of an amide group at $\sim 1600 \text{ cm}^{-1}$ and CN stretching band at $\sim 1500 \text{ cm}^{-1}$. This suggests that methanol extracts an additional amide containing compound, however this is indistinguishable between polyacrylamide or protein.

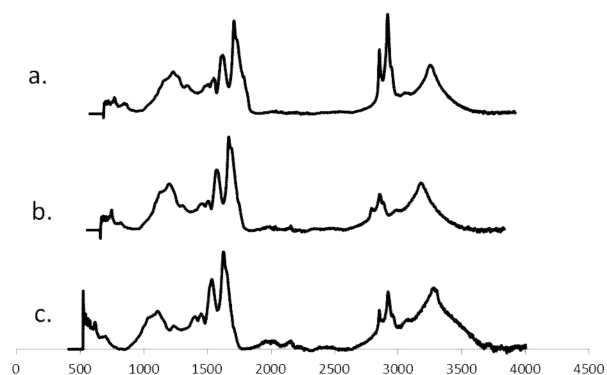


Figure 6. FTIR of the 4000-600 cm^{-1} region of DAF sludge extracted powder after a) 5 hrs methanol extraction; b) 15 hrs methanol extraction; and c) 20 hrs hexane extraction.

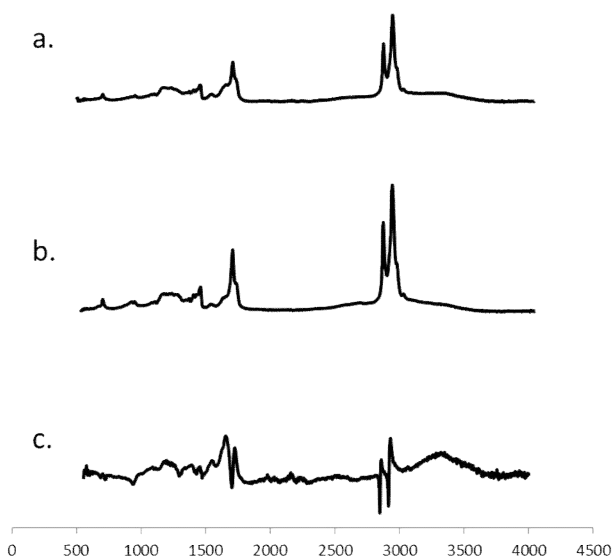


Figure 7. FTIR spectrum of the 4000-600 cm^{-1} region of extracted lipid after solvent drying: a, spectrum of dried methanol extract residue; b, spectrum of dried hexane extract residue; c, differential spectrum of a and b (subtraction factor:0.58)

Conclusions:

Methanol was found to be a much more effective solvent for the extraction of lipid components from DAF sludge as compared to the traditional hexane. The extracted lipids were found to consist of polar and nonpolar components, but the presence of phospholipids was less than expected. Despite having preferential solubility of different lipid compounds, selective extraction with different solvents was not achieved. Furthermore, identification of the residual polyacrylamide was not achieved due to the spectral overlap with the proteins. Some results that were successful and hold promise are related to the liquid-liquid extraction of non-polar fractions from the methanol extract. Methanol is a reactant in lipid trans-esterification reactions to produce methyl esters, which have value in the commodity chemicals market. This suggests potential for a reactive extraction scenario where methanol is used for extraction, followed by reactive conversion of selected lipid fractions to yield a more consistent and uniform extract product that contains value. Preliminary results along this line are presented in the future works section.

Impacts and Significance: The significance of this work is to create a value added option for DAF sludge materials. Currently DAF sludge is a waste byproduct that must be accommodated. If successful, our methods could provide a solution that can obtain value added materials from this waste product. Furthermore, these methods could also be applied to other DAF sludge streams from municipal waste water treatment.

Publications: None to date for this work.

Outside funding:

- **USDA - \$500k to investigate the mechanisms of nutrient release from rendered materials as an alternative organic-based fertilizers.**
- **Private Funding - \$3k to investigate bio-derived asphalt release agents.**

Future Work:

Based on the success in using methanol to effectively extract lipids from DAF sludge, we decided to investigate the potential of reactive modification of the lipids to produce a more valuable product. We first explored direct alkaline transesterification of the extracted lipids with sodium hydroxide because alkali-catalyzed transesterification is the most economical process requiring low temperatures and pressures to achieve a high ester yield. However, the yields from different reaction conditions all showed relatively low yields, less than 32%. Furthermore, TLC result indicated the presence of free fatty acids and other polar lipids in the product solution. Alkaline transesterification is very sensitive to both water and free fatty acids content, which can lower conversion and activate side reactions that effectively cease the primary reaction. An alternative solution involves introducing an acid pretreatment for fatty acid esterification. Acid catalyzed transesterification reactions have slower kinetics than the base catalyzed alternative but are less sensitive to the presence of polar lipids. Here are the developed methods.

Acid Pretreatment ^{1,2}

The extracted lipid mixture was combined with methanol and H₂SO₄ (lipid to sulfuric acid ratio 1:0.05 w/w; lipid to methanol ratio 1:10 v/v) and was heated to 65°C with mechanical stirring at 1000 rpm for 2 hr. The reaction product was then mixed with equal amount water to enhance the

isolation of the lipid product phase. The final lipid product was taken for TLC characterization and alkaline transesterification.

Alkaline Transesterification⁴

The pretreated or non-pretreated lipids were transesterified to fatty acid methyl esters by addition of certain amount of sodium hydroxide-methanol solution in 5 ml of lipid methanol extract and stirred at 60 °C for 3 hrs. Samples were then neutralized by addition of HCl. Fatty acid methyl esters (FAMES) were extracted by addition of 10 ml hexane and vigorous mixing. The upper hexane phase has been taken as the production solution. Without acid pretreatment, the transesterification reaction had a maximum yield of 32%. With acid pretreatment, a yield of 62% was achieved. Future work will further investigate the feasibility of this reactive conversion coupled with separation to yield a higher value product.

Acknowledgments: Mingzhe Jiang, PhD student.

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