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Article

Fourier-Transform Infrared (FTIR) spectroscopy analysis of seven wisconsin biosolids

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SUMMARY

Our research explores the use of Fourier-transform infrared (FTIR) spectroscopy to better understand the phosphorus (P) characteristics of biosolids and how these characteristics can affect agricultural production. Our question was whether FTIR spectra can differentiate between the organic and inorganic species of phosphorus in biosolids by showing shifts in the peaks that indicate species of phosphorus. We obtained biosolids from five Wisconsin wastewater treatment plants (Madison, Delafield, East Troy, Mukwonago, and Fort Atkinson). From the Madison location, we obtained several samples from different parts of the treatment process — cake, final liquid (labeled as Metrogro), and composted biosolids. We compared the FTIR spectra from these samples to P standards, aiming to identify the dominant P species in each biosolid. We employed several FTIR methods: attenuated total reflectance (ATR), sodium chloride (NaCl) salt plates with mineral oil, and pressed potassium bromide (KBr) pellets. We found that the FTIR method that best shows P species differences in the biosolids was the pressed KBr pellet method. Additionally, the P standards indicated two critical wavenumber regions in which the inorganic species' FTIR peaks are shifted to a higher wavenumber than those of the organic species. This work indicated that we can use KBr pellet FTIR as a simple and rapid method to start characterizing P species in biosolids. These research findings could provide farmers useful information about P availability to crops and risk of P loss in runoff when applying biosolids to their fields.

INTRODUCTION

Phosphorus is both a key structural component and a catalyst for numerous biochemical reactions in plants. Not only is it a vital component of nucleic acids including DNA and RNA, the carriers of genetic information, phosphorus is also a part of ATP, the carrier of energy. Thus, phosphorus plays an important role in plants' growth and reproduction as it stimulates root development, increases stalk and stem strength, and improves flower formation and seed production (1). Food production through modern agriculture is highly reliant on the application of fertilizers containing phosphorus (2).

With increasing population and higher food demand, global phosphorus reserves are depleted (2). One possible solution of this problem is to recover phosphorus from water and wastewater (2). Concentrated phosphorus can potentially be recovered in the agriculturally reusable form of biosolids—beneficial and economical resources that contain essential plant nutrients (3). In general, 10% of the phosphorus in wastewater is insoluble and can be removed by primary settling. Removal of the remaining phosphorus can be achieved by chemical, biological, and physical methods, resulting in different forms of phosphorus in biosolids. Chemical precipitation and adsorption can be used to recover inorganic phosphorus compounds, producing co-precipitation with iron or aluminum salts. Biological treatment methods are based on stressing the microorganisms, so that they will take up excess phosphorus resulting in organic phosphorus compounds (4). Organic phosphorus compounds consist of a phosphate molecule associated with a carbon-based molecule, such as phytic acid (P storage compound) or nucleic acids (DNA/RNA) in plant or animal tissue. Inorganic phosphorus is phosphate (PO_4^{3-}) and can be precipitated in a mineral form such as Ca, Fe, or Al phosphate). In the environment, organic and inorganic P compounds behave differently in their chemistry, transport, uptake, etc. (5).

Although phosphorus is necessary for agriculture, excess application of phosphorus-containing chemicals to lawns and farm fields can cause eutrophication in lakes, rivers, and other bodies of water, which would adversely impact aquatic life and potability of water in the affected areas (6). Nutrient pollutants stemming from untreated sewage, industrial wastewater, and agricultural runoff can cause massive algae blooms, depleting oxygen in the contaminated water and causing numerous aquatic organisms to die. Secondary metabolites produced by algae blooms can also be deadly to both aquatic life and humans (6).

Therefore, appropriate management of biosolids and other soil amendments is important because it can reduce the loss of phosphorus-containing compounds and prevent excessive eutrophication. In order to protect the quality of surface water, regulations in many places limit biosolid application to only the quantities that provide nitrogen and/or phosphorus needed by crops (6).

Differences in biosolid phosphorus availability resulting from varying processes used at wastewater treatment plants are not well understood, and biosolid variations are not incorporated into the Wisconsin Phosphorus Index (P Index).

The P Index is a nutrient management tool for farmers to help predict phosphorus loss from fields, including factors such as soil test phosphorus, proximity to surface water, and soil amendments (such as manure, fertilizer, or biosolids). However, the equations for the P Index only include the water-solubility factors for different types of manure. All biosolids are treated as though they have the same P solubility factor, and the risk index for phosphorus losses from different biosolids cannot be accurately predicted (7).

To better understand the differences among biosolids, we used Fourier-transform infrared (FTIR) spectroscopy to analyze seven biosolids in Wisconsin. FTIR spectrometers collect the entire infrared absorption spectrum of solid compounds at once and then reconstruct it using Fourier-transform mathematics (8). Infrared spectroscopy has been used to analyze organic phosphorus compounds since around 1959 (9). FTIR peaks are relatively narrow and, in many cases, can be associated with the vibration of a particular chemical bond in the molecule (10). We compared three FTIR techniques: the attenuated total reflectance (ATR) method, the NaCl salt plates with mineral oil method, and the pressed potassium bromide (KBr) pellet method. In FTIR-ATR method, we placed the sample on the top of an ATR crystal and pressed down using a small metal cylinder, and we used a Mattson (4020 Galaxy FTIR) to conduct the analysis. We used the Bruker Tensor 27 (**Figure 1**) for the salt plates method and the pressed potassium bromide (KBr) pellet method. In the salt plates method, we placed each sample between two NaCl salt plates with a thin layer of mineral oil. In the pressed KBr pellet method, we mixed KBr and the sample together and transferred to a metal press. By tightening the bolt tips together, we made a translucent pellet (**Figure 2**) and placed in the Bruker FTIR (**Figure 1**).

Figure 1. Bruker Tensor 27 FTIR used for the second (NaCl salt plate with mineral oil) and third (KBr pellet) methods of our experiment.

We hypothesized that FTIR spectra can differentiate between the organic and inorganic species of phosphorus in biosolids by showing shifts in the peaks that indicate species of phosphorus. If found, these shifts and their relative ratio of abundance will indicate phosphorus availability in the biosolids and provide farmers with information to better manage biosolids application. Among seven biosolids, we predicted that the spectra of biologically-treated biosolids would be similar to those of pure organic phosphorus compounds, whereas the spectra of biosolids with metal treatments would be similar to those of pure inorganic phosphorus compounds. Of the three FTIR methods investigated, the KBr pellet method provided the clearest spectra and greatest amount of information. In the spectra of inorganic compounds, there appeared to be a slight shift to the left for phosphorus vibrational peaks; whereas in those of organic compounds, there appears to be a slight shift to the right. The findings from this study can help to predict the risk of phosphorus runoff and inform land application rates of biosolids, which contain both inorganic and organic phosphorus compounds.

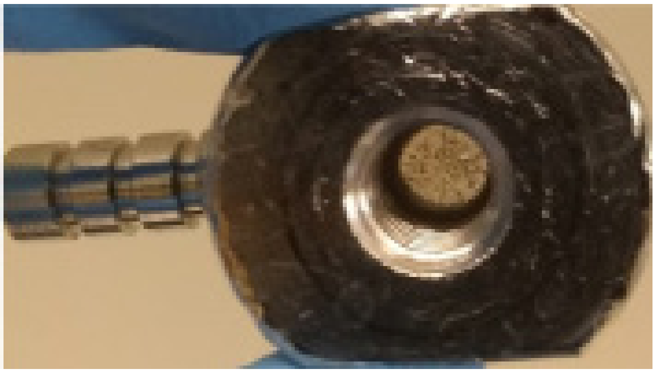

Figure 2. KBr pellet press used in the third method of our experiment. The translucent window was made by compressing the powder mixture of the biosolid and KBr using two bolts and wrenches.

RESULTS

In the experiment, the independent variable is each of seven biosolids resulting from unique processing at their respective wastewater treatment plant (**Table 1**); the dependent variable is phosphorus forms indicated by peaks in each FTIR spectrum. We compared three different FTIR techniques: the attenuated total reflectance (ATR) method, the NaCl salt plates with mineral oil method, and the pressed KBr pellet method. Since the KBr FTIR method displayed the most amount of information without extraneous noise in the spectra data as shown in **Figure 3**, we chose the KBr FTIR method as the final method to analyze organic and inorganic pure phosphorus compounds (see **Figure 4** for example of organic and inorganic phosphorus compound structures), which were used as controls to determine where we expected to see organic and inorganic P species in the FTIR spectra, and seven Wisconsin biosolids.

The FTIR spectra are shown for two of the organic P standards (phytic acid and RNA) and two of the inorganic P standards (K_2HPO_4 and Na_2HPO_4) in **Figure 5**, where the blue arrows indicate possible inorganic and the green arrows indicate possible organic phosphorus components. In an FTIR spectrometer, a chemical bond would vibrate if it absorbs an infrared photon with a frequency (proportional to a wavenumber) that is equal to its natural vibrational frequency. This would result a spectral plot with wavenumber on the axis and transmittance on the y-axis. Since the spectral plots all involved transmittance, the peaks pointed downward, indicating the energy of the photons absorbed by the vibrations of the chemical bonds at specific wavenumbers. The two

Biosolid Characteristic	Madison Metrogro	Madison Cake	Madison Compost	Delafield	East Troy	Fort Atkinson	Mukwo-nago
Dry Matter (%)	7.7	24.5	47.7	75.7	19.2	14.8	26.5
Total Nitrogen (% of DM)	5.4	4.4	6.5	2.8	5.4	7.7	3.6
Phosphorus (% of DM)	2.5	2.9	2.0	3.9	3.1	1.27	1.18
Calcium (% of DM)	3.1	4.8	3.9	5.40	2.24	2.01	3.22
Magnesium (% of DM)	0.82	0.69	0.98	0.75	0.46	0.83	0.95
Iron (mg/kg DM)	15947	41854	23994	9027	4648	6952	31705
pH				6.1	6.8	6.3	6.6
Type	liquid	solid	solid	solid	solid	solid	solid
Digestion	anaerobic	anaerobic	anaerobic	anaerobic	aerobic	aerobic	anaerobic
P Removal Treatment	biological	biological	biological	alum	alum	biological	ferrous chloride

Table 1. Selected biosolid characteristics of the Metrogro (liquid), cake, and compost samples taken from the Madison Metropolitan Sewerage District (MMSD) in January 2018 and from four Wisconsin wastewater treatment plants in April 2014. “DM” in the table stands for dry matter. “P” stands for phosphorus. “Biological” refers to P removal done by microorganisms. “Alum” is an aluminum sulfate compound. “Ferrous chloride” is an iron chloride compound.

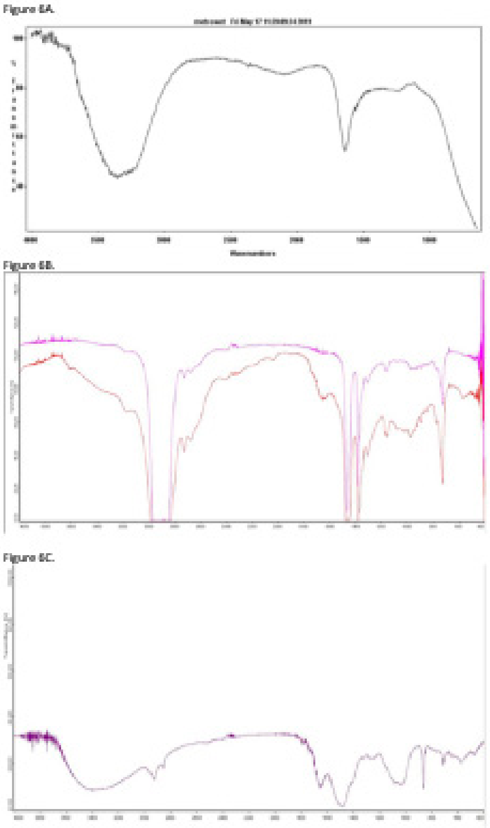

Figure 3. Three spectral data of Madison Metrogro biosolid collected by different FTIR techniques: Attenuated Total Reflectance FTIR (A), NaCl salt plate FTIR (Metrogro biosolid (red), mineral oil blank (pink)) (B), and KBr pellet FTIR (C).

major peaks that appeared in all spectra of pure phosphorus compounds (**Figure 5**) were O-P-O deformational bending vibrations (typically found between 500 cm^{-1} and 670 cm^{-1}) and the P-O asymmetric stretching vibrations (typically found between 1010 cm^{-1} and 1080 cm^{-1}) (11-13). Comparing the pure organic phosphorus compound spectra to the inorganic spectra, we found different locations of the peaks indicating the P-O asymmetric stretching vibrations. For the organic compound spectra, we observed a slight shift in the peak to the right which is closer to 1000 cm^{-1} wavenumber (**Figure 5C** and **3D**). For the inorganic compound spectra, we found a slight shift in the peak to the left, closer to 1100 cm^{-1}. (**Figure 5A** and **3B**).

We analyzed seven samples of biosolids collected from five different Wisconsin Wastewater Treatment Plants (**Figure 6**), including three samples collected from different stages of the wastewater treatment process in the Madison Metropolitan Sewerage District (MMSD) (**Figure 6A**, **5B**, and **5C**), along with ones collected from Ft. Atkinson, East Troy, Mukwonago, and Delafield (**Figure 6D**, **5E**, **5F**, and **5G**, respectively). Among these seven types of biosolids, ones from Madison Metrogro, Cake, Compost, and Fort Atkinson are biologically treated, whereas those from Delafield, East Troy, and Mukwonago are treated with metals, as shown in **Table 1** (14).

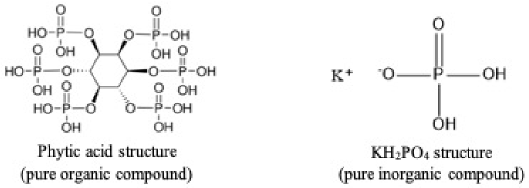

Figure 4. Example of organic phosphorus compound (phytic acid) and inorganic phosphorus compound (potassium phosphate) structures. Typically, an organic phosphorus compound is larger than an inorganic phosphorus compound.

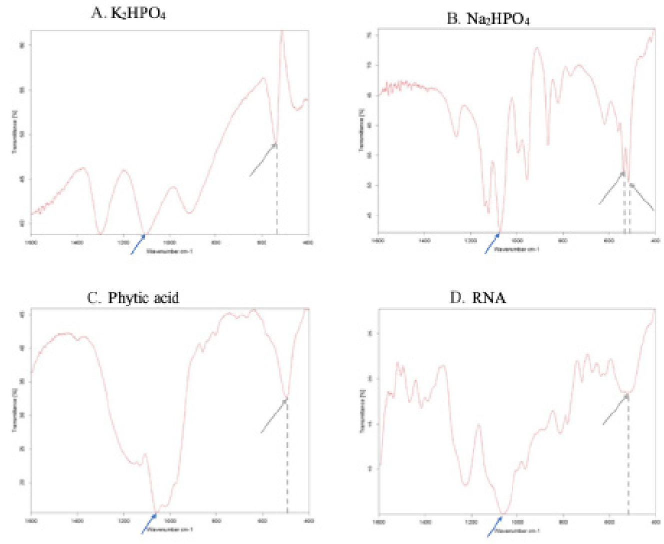

Figure 5. Comparison of Pure Organic and Inorganic Phosphorus Compounds: A) K_2HPO_4, B) Na_2PO_4, C) phytic acid, D) RNA. K_2HPO_4 (A) and Na_2PO_4 (B) are inorganic phosphorus compounds; phytic acid (C) and RNA (D) are organic phosphorus compounds. All spectra were collected by FTIR using pressed KBr pellet method. The blue arrows point towards P-O asymmetric stretching vibrations (typically found between 1010 cm^{-1} and 1080 cm^{-1}); the green arrows point towards O-P-O deformational bending vibration (typically found between 500 cm^{-1} and 670 cm^{-1}).

We reported characteristics of each type of biosolid, including percentages of dry matter (7.7-75.7%), amount of nitrogen (2.8-7.7% of dry matter), phosphorus (1.2-3.9% of dry matter), calcium (2.0-5.4% of dry matter), magnesium (0.46-0.98% of dry matter), and iron (4648-41854 mg/kg dry matter) (**Table 1**). The pH of most of the biosolids was between 6 and 7. Biosolids from Madison Metrogro, Cake, Compost, Delafield, and Mukwonago undergo anaerobic digestion, which means that the treatment process takes place in the absence of oxygen; those from East troy and Fort Atkinson undergo aerobic digestion, which means the treatment process takes place in the presence of oxygen.

The two major peaks identified in the pure compounds we also observed in the biosolids' spectra, indicating O-P-O deformational bending vibrations (typically found between 500 cm^{-1} and 670 cm^{-1}) and the P-O asymmetric stretching vibrations (typically found between 1010 cm^{-1} and 1080 cm^{-1}). The main response of the dependent variable to the different biosolid processing methods seems to be in the P-O asymmetric stretching. There are two distinct peaks in both Madison cake (**Figure 6A**) and compost (**Figure 6B**), one at about 1000 cm^{-1} and the other one at about 1100 cm^{-1} wavenumber. For Madison Metrogro (**Figure 6C**) and Ft. Atkinson (**Figure 6D**), the peak is closer to 1000 cm^{-1}. For the biosolids collected from East Troy, Mukwonago, and Delafield (**Figure 6E**, **5F**, and **5G**, respectively), the peaks are closer to 1100 cm^{-1} wavenumber.

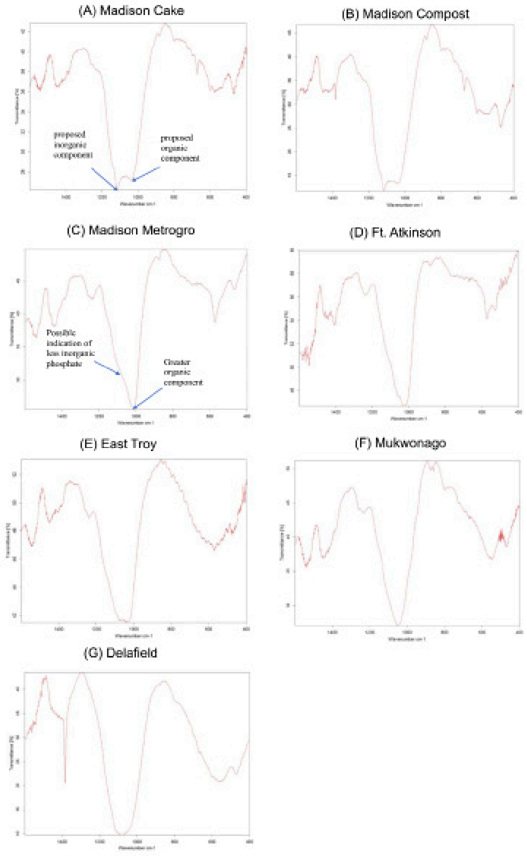

Figure 6. FTIR spectra of seven biosolids from different Wisconsin wastewater treatment plants: (A) Madison Cake, (B) Madison compost, (C) Madison Metrogro, (D) Ft. Atkinson, (E) East Troy, (F) Mukwonago, (G) Delafield. Spectra were collected by FTIR using pressed KBr pellet method. The arrows in (A) indicate that we proposed there are approximately equal amounts of inorganic and organic phosphorus compounds found in the biosolid because of the two distinct peaks. The arrows in (C) shows that there is a greater component of organic phosphorus than inorganic phosphorus because the peak is closer to 1000 cm^{-1}.

DISCUSSION

The results show that there appeared to be a slight shift to the left for phosphorus vibrational peaks with a wavenumber closer to 1000 cm^{-1} in the spectra of inorganic compounds; whereas in those of organic compounds, there appeared to be a slight shift to the right with a wavenumber closer to 1100 cm^{-1}. One possible explanation is that the overall weight and size of inorganic and organic compounds differ; out-of-phase P-O-C stretches from 920-1088 cm^{-1} are observed near the P-O asymmetric vibrations in some organic samples (11). For example, phytic acid, an organic phosphate compound, is substantially larger than potassium dihydrogen phosphate (KH_2PO_4), an inorganic phosphate compound (see **Figure 4** for example P structures). Thus, the larger and heavier the phosphate compound is, the slower the P-O asymmetric stretching vibration is, indicated by a lower wavenumber on the spectral plot. Based on the results, our hypothesis that shifts in the peaks on the spectra can distinguish between the organic and inorganic species of phosphorus was supported by our data (**Figure 6**). One possible explanation for the finding that there are two distinct peaks in Madison cake and compost, is that there are approximately equal amounts of inorganic and organic phosphorus compounds found in the biosolid. For Madison Metrogro (**Figure 6C**) and Ft. Atkinson (**Figure 6D**), since the peak is closer to 1000 cm^{-1}, it suggests that there is a greater organic component in the biosolid than the inorganic component. This finding matches with the information about the treatment of the biosolids from Madison and Ft. Atkinson are mainly treated biologically (**Table 1**). For the biosolids collected from East Troy, Mukwonago, and Delafield (**Figure 6E**, **5F**, and **5G**, respectively), the peaks are closer to 1100 cm^{-1} wavenumber. indicating a greater percentage of inorganic components. The results are consistent with the fact that these biosolids are produced using inorganic compounds to remove P out of the wastewater: alum (a compound containing aluminum) or ferrous chloride (a compound containing iron) as shown in **Table 1** (14). There appeared to be a shift to the left in the peaks on spectra of biosolids treated with metal, and there appeared to be a shift to the right in the peaks on spectra of the biologically-treated biosolids. However, further research with more variation of phosphorus species needs to be done to give more confidence on the statement that these shifts and their relative ratio of abundance on the spectra can indicate phosphorus availability in the biosolids. Based on the difference between inorganic and organic peaks, it may be possible to develop a fast, effective IR spectroscopy method scanning for phosphorus species in biosolids. Such information can be used to inform land application rates to minimize the environmental risk of phosphorus runoff and maximize agricultural outputs. Based on previous biosolid and soil incubations, biosolids treated only with biological P removal (resulting in more organic compounds in the biosolids) may have a higher risk for runoff than those treated with iron and aluminum chemicals (resulting in more inorganic compounds in the biosolids) (unpublished data from Angela Ebeling's research lab). However, over time the microbes decompose the organically bound phosphorus into inorganic phosphate which is plant available. Thus, organic phosphorus could provide available phosphorus to the crop over the course of the growing season. After conducting a soil test and knowing available phosphorus levels of the soil, we can apply spectra results to guide the appropriate applications of biosolids to land fields by modifying the Wisconsin Phosphorus Index (7) to account for the inorganic and organic composition of individual biosolids. For example, if the soil has a low level of bio-available phosphorus, biosolids that contain a greater composition of organic phosphorus compounds can be applied because the phosphorus would become plant-available.

One limitation of the FTIR technique is that it does not provide quantitative measurement of any particular phosphorus species. It can only provide comparisons of relative peak intensity. Also, FTIR spectra can show general trends of peaks that indicate organic and inorganic phosphorus species, but it is hard to assign peaks to specific chemical species because even a slight change in the surrounding chemical species would have impact on the wavenumber of peaks. In the future, comparing the FTIR, ^{31}P NMR results, and chemical extractions will be necessary in order to provide both qualitative and quantitative information that helps describe the inorganic and organic phosphorus forms and species in the biosolids that have gone through different treatments or production processes. This can provide farmers with more accurate information for the proper application of biosolids as fertilizers in order to maximize crop yields while simultaneously protecting water quality.

MATERIALS AND METHODS

Three different FTIR methods were used for the analysis of biosolids: attenuated total reflectance (ATR), NaCl salt plates with mineral oil, and KBr pellets. The cake, Metrogro, and compost biosolids from Madison, WI, along with inorganic spikes of KH_2PO_4 and 50% phytic acid were utilized in all three methods. Since the KBr method was chosen for further experimentation, it also involved biosolids taken from Ft. Atkinson, Delafield, East Troy, and Mukwonago. Delafield, East Troy, Fort Atkinson, and Mukwonago were collected from the respective cities' wastewater treatment plants on April 8th, 2014. The Madison cake, compost, and Metrogro biosolids were collected from the Madison Metropolitan Sewerage District on January 8th, 2018. Biosolids were placed in 5-gallon buckets for transporting, and then were separated into smaller homogenized samples in which particles were mixed well and distributed uniformly. The samples were then frozen (approximately $-18^{o}C$) until FTIR analysis. Subsamples of each biosolid were sent to the University of Wisconsin Soil and Forage Analysis Lab to be characterized (**Table 1**). Both inorganic and organic pure phosphorus compounds were also analyzed by FTIR for comparison. The inorganic compounds included potassium dihydrogen phosphate (KH_2PO_4), sodium hydrogen phosphate (Na_2HPO_4), calcium phosphate ($CaHPO_4$), and ammonium dihydrogen phosphate ($NH_4H_2PO_4$). The organic compounds included 50% phytic acid ($C6H_{18}O_{24}P_6$), ribonucleic acid (RNA), D-glucose 6-phosphate ($C_6H_{13}O_9P$), 2-aminoethyl phosphonic acid ($C_2H_8NO_3P$), and adenosine 5'-triphosphate ($C_{10}H_{16}N_5O_{13}P_2$).

Attenuated Total Reflectance (ATR)

We used a Mattson (4020 Galaxy FTIR) to conduct the ATR analysis. The biosolids were placed on top of the ATR crystal and pressed down using a small metal cylinder. This method does not require much sample preparation. However, as shown in **Figure 3A**, it did not show much useful information because it does not provide the spectral details that were seen in the other FTIR techniques we used.

NaCl Salt plates with mineral oil

The second method of FTIR involving NaCl salt plates required a greater amount of sample preparation. Each biosolid sample was placed between two NaCl salt plates with a thin layer of Nujol mineral oil (2-3 drops). The sample with salt plates was then placed into the Bruker Tensor 27 to run the FTIR as shown in **Figure 1**. The extraneous noise in this spectra data which were produced by bond vibrations in mineral oil, as shown in **Figure 3B**, overpowered the sample peaks, making spectra difficult to interpret. Even though signal processing may be able to remove the mineral oil footprint, the practice will be hard because it would accidentally create spurious peaks or miss small features.

Pressed KBr pellet

The third method used IR-grade (>99%) potassium bromide (KBr), a transparent chemical in the IR spectrum, and a pellet press to produce a "window." Each biosolid sample or pure phosphorus compound and KBr were mixed at an approximately 1:50 ratio by mass. The sample and KBr were ground with a mortar and pestle. The powder was transferred to a metal press and compressed into a translucent pellet (**Figure 2**) by using two bolts and wrenches and tightening the bolt tips together. When the bolts were removed, the press was placed in the Bruker FTIR (**Figure 1**). Sixteen scans were performed and averaged for each sample. This method was the best method among three alternatives because it provided the greatest number of clear peaks (**Figure 3C**).

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