Phosphorus Recovery from Microbial Biofuel Residual Using Microwave Peroxide Digestion and Anion Exchange

by

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ABSTRACT

Biofuel from microbial biomass is a viable alternative to current energy production practices that could mitigate greenhouse gas levels and reduce dependency on fossil fuels. Sustainable production of microbial biomass requires efficient utilization of nutrients like phosphorus (P). P is a limited resource which is vital for global food security. This paper seeks to understand the fate of P through biofuel production and proposes a proof-of-concept process to recover P from microbial biomass. The photosynthetic cyanobacterium *Synechocystis* sp. PCC 6803 is found to contain 1.4% P by dry weight. After the crude lipids are extracted for biofuel processing, 92% of the intercellular P is found within the residual biomass. Most intercellular P is associated with nucleic acids which remain within the cell after lipids are extracted. Phospholipids comprise a small percentage of cellular P. A wet chemical advanced oxidation process of adding 30% hydrogen peroxide followed by 10 min of microwave heating converts 92% of the total cellular P from organic-P and polyphosphate into orthophosphate. P was then isolated and concentrated from the complex digested matrix by use of resins. An anion exchange resin impregnated with iron nanoparticles demonstrates high affinity for P by sorbing 98% of the influent P through 20 bed volumes, but only was able to release 23% of it when regenerated. A strong base anion exchange resin sorbed 87% of the influent P through 20 bed volumes then released 50% of it upon regeneration. The overall P recovery process was able to recover 48% of the starting intercellular P into a pure and concentrated nutrient solution available for reuse. Further optimization of elution could improve P

recovery, but this provides a proof-of-concept for converting residual biomass after lipid extraction to a beneficial P source.

DEDICATION

This thesis is the third greatest thing I've helped create over the last two years. I dedicate it to the two greatest things; my children Blaine and Christine.

ACKNOWLEDGMENTS

Thank you to my advisor Dr. Paul Westerhoff for the consistent direction and feedback. Thank you to my committee members Dr. Bruce Rittmann and Dr. Ravi Vanella for guidance in the world of biotechnology. Thank you to Jie Sheng who provided training on lipid extraction and biofuel processing. Funding for this study was provided by Dean's Fellowship from the Ira A. Fulton Schools of Engineering at Arizona State University.

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Chapter 1

INTRODUCTION

Our future energy cannot be derived from fossil fuels, which release known and suspected human carcinogens (USEPA 1994) and create greenhouse gases (IPCC 2007). Biofuel provides renewable energy with net greenhouse gas emissions significantly lower than fossil fuel (Batan et al. 2010). Microbial biofuel may be preferred over terrestrial plants in the future because it does not compete with food crops, does not require arable land, and may be continuously harvested (Rittmann 2008).

Microbial biofuel production requires inputs and generates residuals. Microbes require water, sunlight, carbon dioxide, and nutrients, particularly nitrogen (N) and phosphorus (P). When microbial biomass is processed for extraction of lipids, most of the N and P incorporated into the cellular structures reside in biomass residue. New nutrients for subsequent biomass growth create a huge nutrient demand. Capturing and recycling nutrients represents a significant opportunity for improving the overall sustainability of large-scale cultivation of photosynthetic biomass (Clarens et al. 2010).

Unlike N, which can be fixed from the atmosphere through the Haber-Bosch method (Huo et al. 2012), P is mined from ore that has finite stocks. World reserves of accessible P are estimated as 65,000 million metric tons (USGS 2011). Some have speculated that economically viable P would be exhausted within the next 50 to 100 years (Smil 2000; Steen and Agro 1998), and others project that global production capacity will peak within 25 years and subsequently decline

(Cordell et al. 2009). Depletion of readily available P would bring about an international crisis due to the essential role of P fertilizer for global food production (Soil Association 2010). The stability of socio-economic systems depends on secure access to nutritious food (Roy et al. 2006), and farmers in developing countries would be disproportionately harmed by a shortage of P fertilizer (Childers et al. 2011). In order to be truly sustainable from a global perspective, biofuel production must not compete with food production for new P resources. Sustainable microbial biofuel production demands efficient nutrient recycling to prevent biofuel from becoming an enormous P demand.

This research develops a P recovery process from microbial biomass for biofuel at the proof-of-concept level. The fate of P through biofuel production is tracked to focus recovery efforts. The research objective is to recover P from residual biomass in a reusable form by using advanced oxidation to release the P for efficient ion exchange capture. This recovery of P occurs in a four step process shown in Figure 1. Microbial biomass is first grown and lipids extracted and refined for biofuel production. Second, the residual biomass is digested to oxidize organically held P to more bioavailable inorganic forms. Third, the P is then isolated from the resulting complex solution into a pure and concentrated form. Last, this solution of bioavailable P can then be used for subsequent biomass growth and lipid extraction.

The microorganism selected for this work is the cyanobacterium *Synechocystis* sp. PCC 6803. *Synechocystis* is a photosynthetic autotroph, Gram negative, prokaryotic, able to withstand a wide range of environmental conditions,

and has an extensive network of thylakoid membranes within which oxygenic photosynthesis and respiration are performed (Sheng et al. 2011a; van de Meene et al. 2006; Vermaas 2001). Lipids in the thylakoid membranes are diacylglycerols. Compared to triacylglycerols in algae, diacylglycerols are more polar and inherently more incorporated into cell structure, interfering with lipid extraction (Sheng et al. 2011b). The entire genome, comprising over 3.5 million base pairs, has been sequenced (Kaneko 1996), allowing for genetic manipulation of specific traits favorable for biofuel production and is a likely candidate future utilization in large scale biomass cultivation.

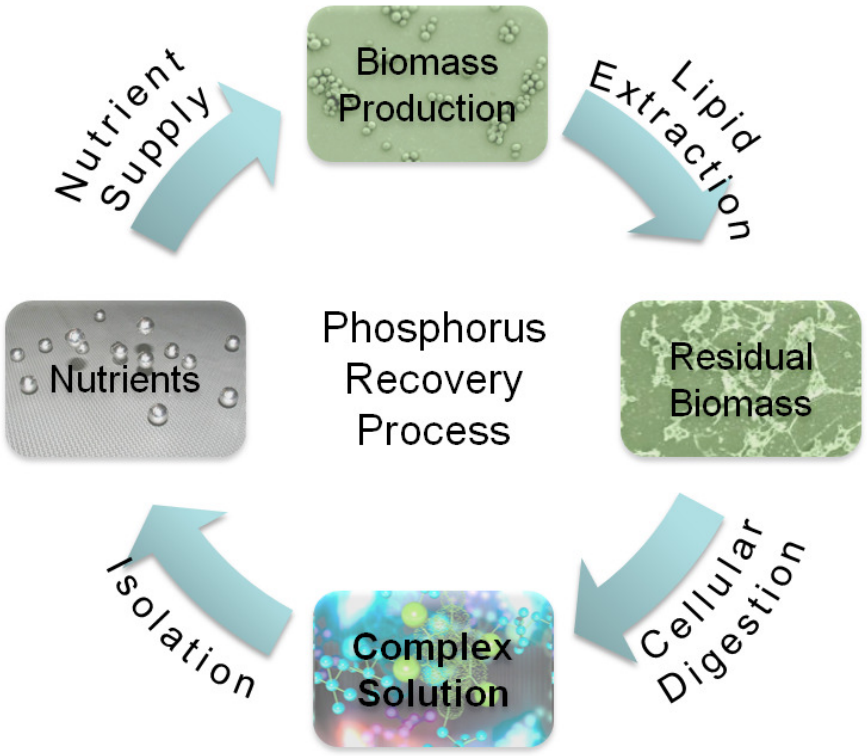
Numerous processes can be used to convert organic P (cellular material) to inorganic P (Rittmann et al. 2011). Here we selected a chemical based method because of its standard use in laboratories. Release of P from primary residual was achieved by an advanced oxidation process using a microwave and hydrogen peroxide. This creates hydroxyl free radicals that are highly effective for the destruction of organic matter, which should also release orthophosphate (ortho-PO_4^{-3}) from organic-P and polyphosphate (Liao et al. 2005). This transformation may include both oxidation and hydrolysis.

Two anion exchange resins were tested to separate ortho-PO_4^{-3} from the digested sample. A hybrid anion exchange resin (HAX) impregnated with iron (hydr)oxide nanoparticles (Layne RT, Layne Christensen) was tested because it has a high sorption capacity and selectivity for ortho-PO_4^{-3} (Sengupta and Pandit 2011) and the ability to release a high concentration ortho-PO_4^{-3} solution upon regeneration (Midorikawa et al. 2008). A strong base anion exchange resin

(SBAX) with quaternary amine exchange functional group in a chloride ion form also was tested (21K-XLT, Dowex). It has an anion exchange capacity of 1.4 equivalents/L and has been effectively used for uranium (Stucker et al. 2011) and chromium (Rees-Nowak et al. 2005) control but has yet to be tested for phosphate recovery.

Figure 1

Conceptualization of Phosphorus Recovery Process



Phosphorus in microbial biofuel production is found primarily in the cellular byproducts which if wasted can contribute to mineral depletion and water body eutrophication. Phosphorus can be beneficially recovered by oxidizing cellular material and isolating with anion exchange resin.

Chapter 2

MATERIALS AND METHODS

Strain, Growth Conditions, and Biomass Production

Synechocystis sp. PCC 6803 was grown in BG-11 growth media (Rippka et al. 1979) modified to have five times the normal amount of phosphate (added as K_2HPO_4) (Kim et al. 2010) in a bench top photobioreactor in semi-continuous growth mode. To separated biomass from the growth medium 50 mL aliquots were centrifuged at 1,500 g for 20 min. The cell pellet was resuspended in 1 mM sodium bicarbonate (Sigma-Aldrich) to rinse away residual medium. The centrifuging and rinsing was repeated two times, and then the final pellet was freeze dried (Labconco Freezone 6) for 2 days at 0.013 mbar and -50ºC. Enough biomass was collected to perform all lipid extraction and P recovery experiments at least in duplicate.

Lipid Extraction and Transesterification

Lipid extraction was accomplished using the Folch Method (Folch et al. 1957) using a 2:1 (V:V) mixture of chloroform (Mallinckrodt) and methanol (Fisher Scientific), since it has a high extraction efficiency for *Synechocystis* lipids (Sheng et al. 2011b). A 300 mg (all weights given as dry weight) sample was ground with agate mortar and pestle, suspended in 60 mL of Folch solvent, and placed on a shaker table at 175 rpm for 2 days. The suspension was then filtered through a glass fiber filter (Whatman GF/B) and then a 0.2 µm polytetrafluoroethylene filter (Whatman). The biomass retained on both filters

was the primary residual, and the filtrate contained the extracted crude lipid. For samples undergoing transesterification, the solvent was evaporated from the crude lipid under N gas to avoid oxidation of lipids. For samples where no further lipid processing was carried out, solvent was evaporated by heating on hotplate.

Transesterification of the crude lipid (Sheng et al. 2011b) was performed by adding 1 mL of methanolic hydrochloric acid (Supelco) and heating the mixture in an 85ºC water bath for 2 h. After cooling to room temperature, 0.5 mL of deionized (DI) water and 1 mL of hexane were added, the mixture was shaken by hand for 30 s, and the phases were allowed to separate. The hexane layer containing partitioned fatty acids was collected. The addition of hexane, mixing, separation, and extraction was repeated two additional times then all the hexane was pooled. The extracted hexane contained the fatty acid methyl esters (FAME) and the remaining water contained the secondary residual.

For fate tracking experiments, two samples of biomass were analyzed for total starting P, two samples were lipid extracted to find total P in crude lipid and primary residual, and two samples were extracted then transesterified to find total P in FAME and secondary residual. For recovery experiments, the primary residual was oxidized and isolated as described in the following paragraphs and analyzed for total P and ortho-PO_4^{-3}. Since little P was found within the crude lipid, transesterification and recovery from secondary residual was not performed.

P Release

Primary residual was scraped from the dried filters and added to 60 mL (3.6gVSS/L) of 30% ultrapure H_2O_2 solution (JT Baker Ultrex II) before 1 h of pre-digestion at room temperature. Samples were then digested in a microwave (CEM MARS XPress) at 400 W by ramping the temperature up to 160ºC for 10 min then to 170ºC for 10 min per method SW846-3015 (USEPA 2008). High pressure microwave vessels were utilized due to tendency to release high gas volume.

Phosphate Separation

Preliminary screening of the P separation capacity of the anion exchange resins was completed by placing 3.5 g of fresh resin in a 1.5 cm inner diameter glass column giving a bed depth of 3.0 cm. The resin was supported by glass beads to ensure even flow distribution. Before the ortho-PO_4^{-3} exchange experiment, 100 mL of DI water was flushed through the column, and air bubbles were allowed to escape. A solution of monobasic sodium phosphate (Mallinkrodt ACS) in DI water (concentration 80 mgP/L) was pumped through the column at 3.2 mL/min to give an empty bed contact time of approximately 2 min (loading rate of 4.4 mgP/s/g resin). Effluent samples were taken periodically for P analysis, and the experiment was continued until the effluent P concentration stabilized near the influent P concentration. The P was then desorbed from the resin using a strong regeneration solution described below at a pump rate of 0.5 mL/min (empty bed contact time of approximately 10 min) until the effluent P

concentration stabilized at nearly zero. The strong regeneration solution used for the HAX resin was 0.1 N potassium hydroxide (EMD), and for the SBAX resin it was 0.1 N sodium chloride (Sigma Aldrich).

Each resin was then tested with oxidized biomass by using 2.0 g of fresh resin for a bed depth of 1.7 cm. 60 mL of digested sample (20 bed volumes) was pumped through the column at 1.4 mL/min to give an empty bed contact time of approximately 2 min. The effluent was collected and pumped through the column two more times to ensure complete capture of phosphate onto the resin. Retained ortho-PO_4^{-3} was then recovered by removing the resin from the column and placing it in 33 mL (11 bed volumes) of strong regeneration solution, which was heated on a 95ºC hotplate, shaken for 24 h, and then decanted. The elution and decanting was repeated two times and elution solutions pooled so that the serial batch elution mimicked a continually stirred tank mixer (CSTM) in series (n=3). The total volume of 100 mL (33 bed volumes) was analyzed for pH, electroconductivity, total P, and ortho-PO_4^{-3}.

Sample Analysis

Total P was assayed after persulfate digestion (Standard Method 4500-P.B.5) (Miner 2006) followed by ICP-OES. Samples were suspended in 50 mL DI water plus 1 mL of concentrated sulfuric acid (JT Baker ultrapure). Then 0.4 mg of ammonium persulfate (Malinckrodt) was added to each sample. The sample was autoclaved for 30 min at a pressure of 1.05 kg/cm^2 and a temperature of

122ºC. Analysis for total P was conducted by inductively coupled plasma optical emission spectroscopy (Thermo iCAP6300) read at a wavelength of 213.6 nm.

Ortho-PO_4^{-3} was determined by spectrophotometer (HACH DR5000) using PhosVer 3 Method that is equivalent to Standard Method 4500-P.E (Miner 2006). Reagent powder was added to 5 mL of sample and given 2 min of reaction time with results measured at 880 nm.

Chapter 3

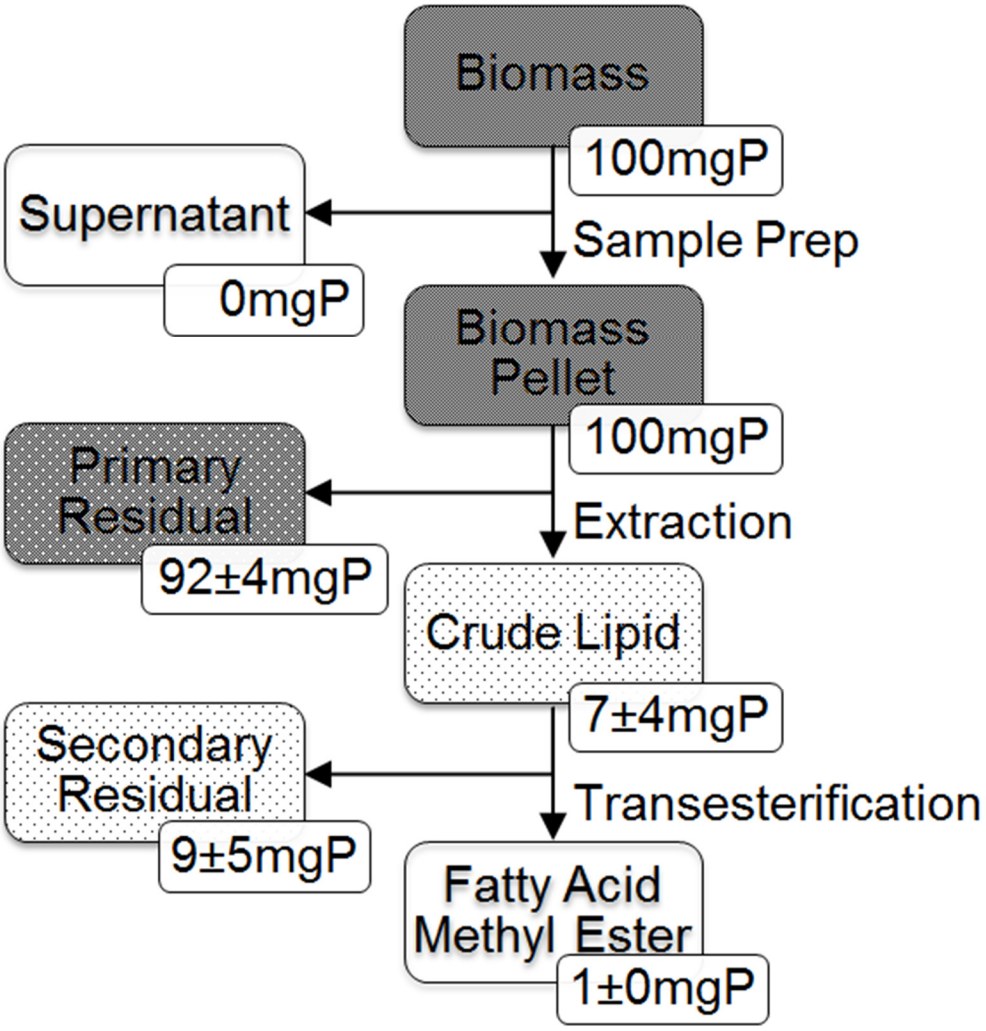
RESULTS

Fate of P through Lipid Extraction

Freeze dried *Synechocystis* sp. PCC 6803 biomass contained 1.39%±0.28% total P by mass (all weights given by dry weight), which is consistent with previous findings of 1.5% (Kim et al. 2010). In lipid extracted biomass samples, primary residual contained 1.50%±0.36% total P. Figure 2 summarizes the fate of P through lipid extraction normalized to 100 mg of total P in the starting biomass. The primary residual contained 92±4.3 mg total P. Crude lipid contained 7.3±4.2 mg total P. For transesterified samples, total P in the FAME was 0.5±0.1 mg total P. Total P in the secondary residual was found to be 9.5±5.3 mg. Thus, nearly all of the starting organic P was found in the primary residual after lipid extraction. Of the small amount left in the crude lipids, nearly all of it was found in the secondary residual. Essentially no P (<1% of the starting P) was found in the transesterified FAME.

Figure 2

Fate of Phosphorus through Lipid Extraction



The fate of P through the lipid extraction process normalized to 100 mg of total starting P. A majority of the P remains with the biomass in the primary residual, but some associated with lipid goes to the secondary residual. Essentially no P is found in the FAME.

Oxidation of Organic P to Ortho-PO_4^{-3}

Since only small amounts of the starting P were found in the crude lipid and subsequent lipid processing, the primary residual became the focus of the recovery effort. Primary residual is dry, green cellular material retained on filter. Prior to treatment with H_2O_2 and microwave heating, this primary residual contained 82±1 mg total P with 0.2 mg of it as ortho-PO_4^{-3}. After treatment, samples contained 90±12 mg total P including 75±6 mg as ortho-PO_4^{-3}. Therefore this process recovered 106±17% of the total P and converted a vast majority of it to ortho-PO_4^{-3}.

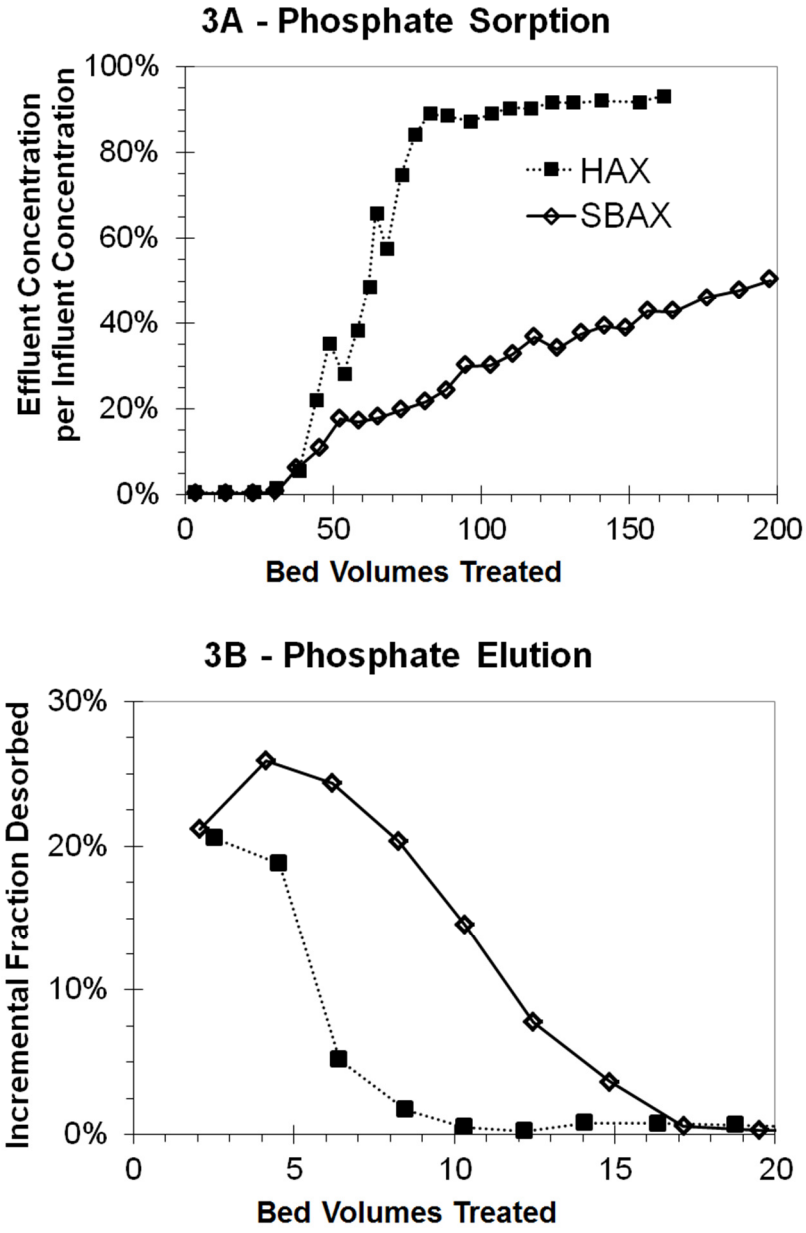
Recovery of Ortho-PO_4^{-3} by Resins from DI Water

Screening the ability of the two resins to absorb P in DI water is shown in Figure 3A. Both resins could capture all of the P up to 30 bed volumes. The HAX resin then began a sharp breakthrough and reached complete saturation near 80 bed volumes. The SBAX resin began a gradual breakthrough, reaching 50% saturation around 200 bed volumes and 80% saturation around 500 bed volumes. The total mass of P sorbed to each resin was found by summing the difference between the influent concentration and the effluent concentration for each sample multiplied by the volume treated in the time period (area above the curve times flow rate). The HAX resin sorbed a total mass of 38 mg of P, giving a sorption capacity of 33.4 mgP/g resin. The SBAX resin sorbed a total mass of 140 mg of P, giving a sorption capacity of 122.3 mgP/g resin.

All of the P that could be released from the resin was eluted from both of the resins within the first 20 bed volumes of regeneration with no additional P eluted when eluted out to 30 bed volumes (Figure 3B). The fastest rate of P elution was observed for the SBAX resin around 5 bed volumes and for the HAX resin around 8 bed volumes. A total of 19 mg of P was eluted from the HAX resin, meaning 51% of the total sorbed was recovered. A total of 167 mg of P was eluted from the SBAX resin, meaning 119% of the total sorbed was recovered.

Figure 3

Phosphate Sorption and Elution



Performance of an iron hydr(oxide) impregnated anion exchange (HAX) resin (squares) and a strong base anion exchange (SBAX) resin (diamonds) for recovering phosphate from DI water. 3a) Uptake of phosphate by fresh resin in column test. 3b) Desorption of phosphate from resin by 0.1 normal hydroxide for HAX or 0.1 normal chloride for SBAX.

Recovery of Ortho-PO_4^{-3} by Resins from Oxidized Biomass

Oxidized primary residual was pumped through the columns with enough resin to give about 20 bed volumes and ensure complete capture of the P. The HAX column effluent contained 1.7±0.3 mg of P, indicating a vast majority of the P sorbed to the resin. After elution, 16.7±0.0 mg P was found in the 100 mL elute. Of this, 14.9±0.1 mg was ortho-PO_4^{-3}. The pH of this solution was 12.4±0.5. This process with this resin recovered 23%±0.2% of the influent P.

The SBAX column effluent contained 20.9±7.6 mg of P indicating a majority of the P sorbed to the resin. After elution, 54.4±8.9 mg of P was found in the 100 mL elute. Of this, 53.0±8.2 mg was ortho-PO_4^{-3}. The pH of this solution was 6.6±0.1, and the electroconductivity was 9.7±0.2 mS/cm. This process with this resin recovered 50%±5% of the influent P.

The summary results of each process step in the overall recovery process are in Table 1 and shown graphically in Figure 4. Since the lipid extraction, cellular oxidation, and nutrient isolation processes were respectively able to recover 92%, 106%, and 50% of the starting P, the overall process can recover 50% of the starting P.

Table 1

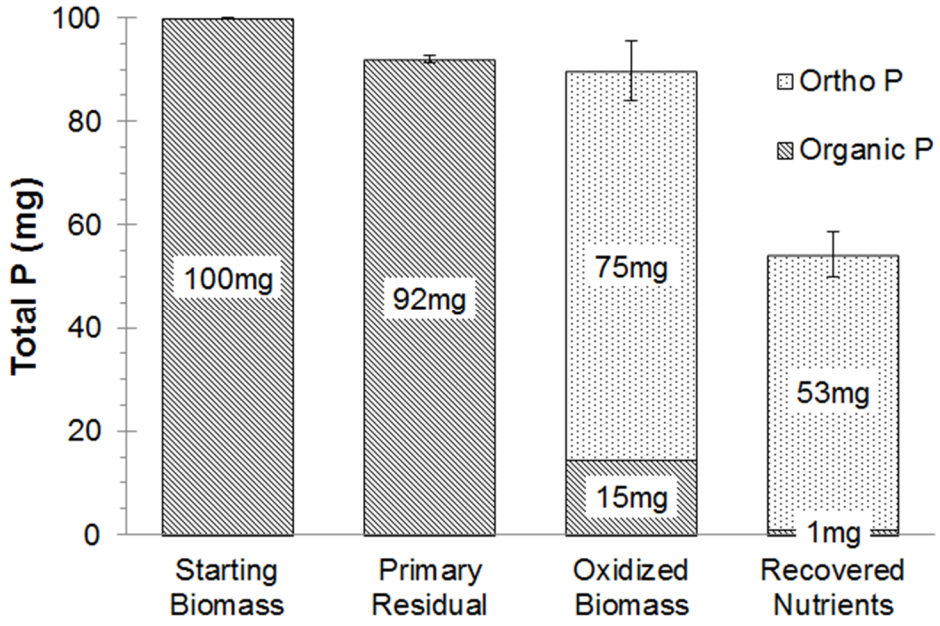
Phosphorus Recovery Yields

Step	Sample Description	Sample 1	Sample 2	Sample 3	Sample 4	Average	
Starting Biomass	Dry Organics	100	100	100	100	100	
Extracted Lipids	Solvent Suspension	1.5	2.2	4.2	3.7	7.3±4.2	
Primary Residual	Dry Organics	93.7	95.1	89.5	-	93±1.5	
Before Oxidation	Complex Solution	80.9 (0.2)	83.0	82.2	-	82±0.5 (0.2)	
After Oxidation	Complex Solution	72.8 (67.3)	70.2 (61.9)	118.9 (83.9)	97.6 (87.1)	90±12 (75±6.2)	
Column Influent	Complex Solution	HAX 72.8	HAX 70.2	SBAX 118.9	SBAX 97.6	HAX 72±0.9	SBAX 108±7.5
Column Effluent	Treated Solution	1.3	2.2	10.1	31.6	1.7±0.3	21±7.6
Eluted from Resin	Eluted Solution	16.8 (15.0)	16.7 (14.8)	66.9 (64.6)	41.8 (41.4)	17±0.0 (15±0.1)	54±8.9 (53±8.2)

Recovery of P through the cycling process using microwave peroxide oxidation and resins. Results normalized to 100 mg of starting P and given as total P and (ortho-PO_4^{-3}). Samples 1 and 2 use HAX resin and Samples 3 and 4 use SBAX resin.

Figure 4

Phosphorus Recovery Process Yields



Summary of the P yields through the P recovery process normalized to 100 mg of starting P. The portion of the original starting P available after each of the process steps and its partitioning to ortho-PO_4^{-3} or organic P is shown.

Chapter 4

DISCUSSION

P Fate through Lipid Extraction

Evaluating why a majority of the P was found in the primary residual requires understanding the biological composition of *Synechocystis*. P within a microbial cell may be located within many biological structures, including nucleic acid, lipids, and adenosine triphosphate (ATP). While ATP contains over 18% P by weight (CAS Registry Number 56-65-5), it is consumed quickly after production and is assumed not to be a major contribution of the cellular storage of P. The major contributors of P within a microbe are therefore DNA, RNA, and lipids.

The P content associated with DNA and RNA can be estimated by comparing its biological composition with its elemental composition. *Synechocystis* sp. PCC 6803 is approximately 3% DNA and 17% RNA by weight (Shastri and Morgan 2005). It is reported that DNA is 10% P by weight (Dell' Anno and Danovaro 2005) and that P contributes 9% of the total mass of either nucleic acid (Sterner and Elser 2002). It is therefore calculated that P associated with DNA comprises 0.3% of the total cell weight, and that P associated with RNA is 1.7% of the total cell weight. As a comparison, studies done on diverse biota, including lake bacteria, suggest that P associated with RNA comprises 49% ± 1.9% of the total cell P (Elser et al. 2003). It is further noted that allocation to RNA is the primary determinant of variation in biomass P content (Elser et al. 2003).

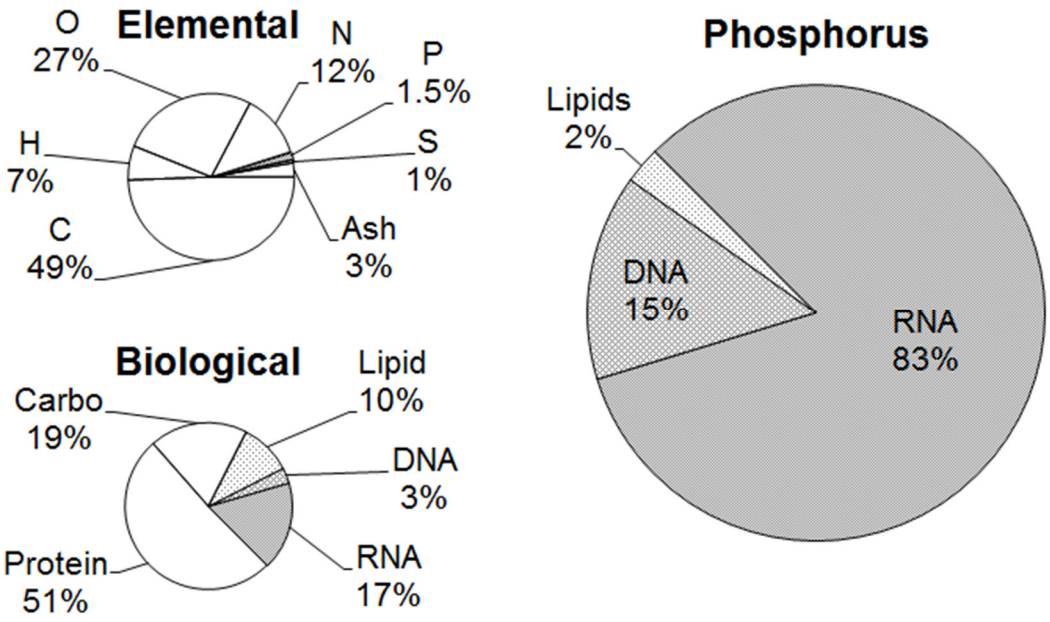
The P content associated with lipid is a function of phospholipid fraction of all lipid and P within phospholipid. The predominant phospholipid head within cyanobacteria is phosphatidylglycerol (PG) and is the only phospholipid associated with thykaloid membranes in *Synechocystis* sp. PCC 6803 (Hajime and Murata 2007). It has an elemental composition of $C_8H_{12}O_{10}P$. The most prevalent fatty acid chain in *Synechocystis* is C16:0, or palmitic acid (Sheng et al. 2011b), which has an elemental composition of $C_{16}H_{32}O_2$. Assuming that all phospholipids within *Synechocystis* are the diacylglycerol PG with two palmitic acid molecules, the overall elemental formula for phospholipid can therefore be approximated as $C_{40}H_{76}O_{14}P$. This means that phospholipid is estimated to be 3.8% P by weight. It is reported that PG based lipids comprise approximately 14% of all lipids in *Synechocystis* (Sakurai et al. 2006). Lipids represent approximately 10% of the biological makeup of the overall cell (Shastri and Morgan 2005). Combining these estimates gives the theoretical amount of P associated with lipid in *Synechocystis* sp. PCC 6803 as 0.053% of the total cell weight. A genetically altered high lipid strain containing 50% crude lipids could then have as high as 0.27% of the total cell weight be P associated with lipid.

Figure 4 summarizes the computed amount of P in a *Synechocystis* cell. The total cell P is found to be dominated by RNA with only small amounts stored in phospholipids. This explains the large fraction of P in the primary residual, since P associated with phospholipid would be expected to partition to the crude lipid during extraction, while P associated with nucleic acid would remain in the primary residual. The increase in P content from dry cells (1.39±0.28%) to

primary residual (1.50±0.36%) further demonstrates the disproportional storage of P in non-lipid structures. The 92±4% of P found in the residual correlates with the estimated 98% P associated with nucleic acid. The small amount of P found in the fatty acids is only due to impurities from incomplete partitioning or analytical margin of error.

Figure 5

Phosphorus Speciation in *Synechocystis* PCC6803



Summary of the composition of *Synechocystis* sp. PCC 6803 from an elemental analysis (top left, Kim et al. 2010) and a biological analysis (bottom left, Shastri and Morgan 2005). The location of the P within the cell is then estimated (right). A majority of cellular P is found within RNA and only small amounts are in phospholipids. This explains why a majority of P was located within the primary residual after lipid extraction and not the crude lipid. All numbers given are percent by weight of the total biomass or total P in the biomass.

Resin Comparison

Comparison of the two resins tested indicates that the HAX resin had higher affinity for P demonstrated by the lower amount of P located in the column effluent, the sharp breakthrough curve showing a short saturation zone, and higher sorption capacity. However it demonstrated lower recoverability since a smaller fraction of P was eluted. A previous study had shown that 80% of the P could be released in the first bed volume of elution (Martin et al. 2009), but only 51% could be recovered in this study. Here the HAX resin sorbs P more strongly, but doesn't fully release it once sorbed. This might indicate that at least part of the sorbed P was irreversibly adsorbed by the impregnated iron (hydr)oxide nanoparticles instead of sorbed entirely by anion exchange. So while the SBAX resin had a lower capacity to sorb P, it may be more suitable for recovery uses since the P can be eluted and captured.

However the high pH of the HAX column eluent indicates the P was likely in the form of HPO_4^{-2}, which is the same as the original BG-11 nutrient media. Additionally it was eluted with K^+ which is also the same as BG-11. The lower pH of the SBAX elute indicates the P was in form of $H_2PO_4^{-1}$, which is different from BG-11 and may not be as bioavailable. Additionally a high saline concentration due to elution with Na^+ in the case of the SBAX is undesirable for microbial growth reuse.

Both resins were only able to desorb about half of the P loaded from oxidized biomass as opposed to loaded from DI water (HAX went from 51% to 23%, SBAX went from 119% to 50%). It is possible that the complex solution

from the oxidized biomass contained other constituents, such as sulfate or nitrate, that were sorbed and eluted by the resins too. The remaining P not found in the effluent nor the elute is assumed to still be sorbed to the resin and unable to be recovered.

Comparison to Alternate Recovery Methods

Although P was effectively recycled in this experiment, the proposed process should be viewed as a proof-of-concept only. Future work will be to reduce the energy requirement for the organicP to ortho-PO_4^{-3} conversion, to improve the resin selection for a high capacity to reversibly sorb P, and to raise the recovered P concentration. The current process has limited ability to recover concentrated P since both peroxide oxidation and resin elution require volumes of liquid proportional to the mass being treated. Possible alternate methods for future exploration are discussed here.

A wide range of P recovery methods have been proposed. More complete discussions of P recovery techniques may be reviewed in the literature (de-Bashan and Bashan 2004; Morse et al. 1998; Rittmann et al. 2011). A majority of these studies have been performed on wastewater biosolids or animal solid waste as opposed to biofuel residual. While these two biomass sources are similar in that they are each composed of microbial cells and complex organic matrices, they differ in that biofuel residual will not have contamination from pathogens, heavy metals, and persistent organic pollutants. Additionally the wastewater biosolids

will contain a fraction of active cells whereas biofuel residual will be completely inactivated and devoid of lipid content.

An alternate method for conversion of organic P to ortho-PO_4^{-3} is anaerobic digestion. This is a series of microbe induced reactions that decompose organic matter to simple mineral forms and generally produces net energy (Rittmann and McCarty 2001). While a good deal of energy is available from digestion of primary residual, it may be lower than expected due to lack of high energy lipid structures. Low energy yields have been observed from anaerobic digestion of cyanobacteria attributed to polysaccharides and poor carbon to N ratio (Quintana et al. 2011). This might be overcome by blending with additional waste feeds like waste activated sludge (Parameswaran and Rittmann 2012). Additionally some energy must be reinvested to maintain the high temperature required for anaerobic digestion, pumping and mixing. While this method of P transformation is certainly preferable from an energy expenditure standpoint, it may pose challenges for P cycling in biomass production since a significant fraction of P is still in a complex organic matrix with organically bound P that is lost when solids are removed. It would be difficult to prevent the anaerobic digestion microbes from contaminating the lipid production microbes when reused as feedstock. Additionally the digester influent would be diluted to a high degree, making difficult to recover a concentrated solution of P. Similar challenges exist for removal by enhanced biological P removal techniques. Some of these challenges may be mitigated by use of a microbial fuel cell (Rittmann 2008).

An alternate P isolation technique is precipitation. Precipitation with ammonium and magnesium yields the mineral struvite ($MgNH_4PO_4 \cdot 6H_2O$) and with calcium yields apatite ($Ca_x(PO_4)_y(OH)_z$), both considered to be good slow release fertilizers (de-Bashan and Bashan 2004). Struvite may occur when equimolar quantities of magnesium and ammonia and phosphate are available at a slightly basic pH (Zhang et al. 2010). Digested primary residual would likely contain stoichiomentric excess of N over P and only trace magnesium, and struvite precipitation would likely be spurred by addition of magnesium. Apatite precipitation occurs with calcium and magnesium present at basic pH and increased temperature (Maurer and Boller 1999). Since only trace amounts of calcium would be available in the digested primary residual, its formation may be instigated by calcium addition. While the insoluble products of precipitation are still considered bioavailable (Johnston and Richards 2003), they may not be suitable for use as nutrient supply for aqueous microbes that undergo exponential growth. Since the solubility of struvite and apatite minerals increases at low pH (Maurer and Boller 1999), this challenge might be addressed by re-dissolving in acidic conditions via carbon dioxide diffusion.

A second common P isolation technique is adsorption onto iron or aluminum precipitates, which have been widely used in municipal wastewater treatment due to P selectivity (Safferman et al. 2004). Magnetite, steel slag, and titanium dioxide are also being explored (Karapinar 2004, Bowden 2009, Nagamine 2003). However the complexation with metal oxides renders the P in a

form that is extremely difficult to recycle (de-Bashan and Bashan 2004) and not bioavailable for subsequent microbial growth.

Broader Impacts

The proposed P recovery process allows for residual biomass to be viewed as a resource instead of a waste byproduct. While the focus of this study has been residual cyanobacteria in biofuel production, it might have application for wastewater biosolids or even solid animal waste handling. Oxidation and hydrolysis of organic material allows for multiple nutrients such as P, N, potassium and possibly even trace metals like titanium to be accessible and in a form that may be useful for subsequent reuse. Though the current process may not yet be cost effective or energy saving, the alternative is to continue current industry practice of landfilling or agricultural land application. Reuse of the biomass through resource recovery prevents over fertilization and eutrophication often associated with land application, as well as possible future social recoil to application of genetically modified organisms into the natural environment.

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APPENDIX A

DETAILED LARGE SCALE PHOSPHORUS RECOVERY EXPERIMENTAL

Biomass Harvesting

- BG-11
- Aeration
- Inoculate
- Monitor OD730
- Alternately
    - Obtain 50L of culture at OD730 of 1 abs

Biofuel Processing

- Centrifuge
    - Centrifuge 1L aliquots at 1,500g for 20 minutes
        - A large volume centrifuge is located in Dr. Vanella's lab
    - Decant supernatant
    - Prepare 1mM HCO3 rinse by adding 84mg NaHCO3 to 1L of nanopure
    - Resuspend in 1mM HCO3 and mix well
    - Centrifuge, decant, and resuspend a second time
    - Centrifuge and decant a third time. Use only enough rinse to move the cell pellet into a freeze dry flask
- Freeze Dry
    - Fit as many cell pellets in freeze dry flasks as possible. Each flask can hold half its volume (ie. A 600mL flask can hold up to 300mL of cell pellets). Use as many flasks as needed to hold it all.
    - Put rubber lids on flasks and parafilm the opening to prevent contamination. Place each flask in a freezer overnight. Lay them on an angle as to maximize the liquid/air interface surface.
    - Activate freeze dryer. Allow temperature to reach below -50°C and pressure less than 0.1mbar. Remove first sample from freezer, remove parafilm and attach to freeze dryer. Open sample port slowly as to avoid sudden expansion which will break the beaker and lose the sample. Allow freeze dryer to return to -50°C and 0.1mbar. Repeat for all samples, one at a time.
    - Allow to operate until dry weight stabilizes, typically two to three days. All solid chunks will be gone. Cells will appear as dark green flakes, but should not be sticky.
    - Close sample valves slowly one at a time then shut off freeze dryer.
    - Grind cell powder on an agate mortar and pestle. (The white porcelain ones will cause high sample loss.) Cells will be dry, dark green, fine powder.
    - Combine all cell powder. Take a 50mg sample for starting P (Sample 1). Suspend in 50mL nanopure and store in fridge to prevent mold growth. Measure 10g (100~150mgP) of starting mass for cycling experiment. Store remainder in a sealed container in a freezer.

- Lipid Extraction
    - Prepare Folch solvent by combining 1.33L chloroform with 0.67L methanol. Caution! These are toxic chemicals and should be treated with great care and kept in the hood. Only use glassware or PTFE plastic.
    - Suspend the dry cells in the solvent. Place on a shake table at a sufficient speed to maintain suspension. Ensure secondary containment in case of spill. Let extract for two days.
    - Filter the solvent to separate the residual from the lipids in solvent. Use a large glass filter like a GF/B in series with a 0.45um PTFE filter. Only do small aliquots at a time and replace the filters.
    - Keep all of the residual on the filters and let dry in a hood overnight. Scrape off a small amount and measure mass (Sample 2). Suspend sample in 50 mL nanopure water and store in fridge.
    - Take a 50mL sample of the filtered solvent (Sample 3) and record the total volume filtered. Evaporate off the solvent from the sample by heating on a hotplate under a hood. Do not heat for so long that the lipids bake onto the glass. Resuspend in 50mL nanopure.
    - Discard remaining crude lipid by use of a hotplate in a hood for solvent evaporation. Do not heat for so long that the lipids bake onto the glass.

Cellular Oxidation

- Suspend the dry residual from the filters in 2L of 30% hydrogen peroxide (3.6gVSS/L). Take a 5mL sample (Sample 6) and dilute to 50mL. Immediately test ortho P and COD.
- Allow to predigest in a hood overnight.
- Mix well and extract 38 aliquots of 20 mL each into high pressure microwave digestion vessels.
- Prepare a blank by putting 20 mL of 30% hydrogen peroxide in a digestion vessel.
- Prepare a control by adding 20uL of 1000ppmP glycerol diphosphate to 20 mL of hydrogen peroxide and placing in a digestion vessel.
- Microwave 40 vessels using method EPASW846-3015, which ramps to 160°C in 10 minutes then to 170°C in the next ten minutes.
- Allow to cool to room temperature. Open vessels under hood with cap pointed away from self. Combine 38 samples. Preserve the blank (Sample 7) and the control (Sample 8).
- Repeat for next 38 vessels with additional blank and control. Microwave, cool, and combine with previous oxidized residual (preserve Samples 9 and 10). Repeat for remaining vessels with additional blank and control. Microwave, cool, and combine with previous oxidized residual (preserve Samples 11 and 12). Take 5mL sample of combined oxidized residual (Sample 13) and dilute to 50 mL.

Nutrient Isolation

- Prepare an ion exchange column with a 2.54 cm inner diameter. Place glass beads, then glass fiber, then resin, then glass fiber, then glass beads to fill the column completely. Use 75mL resin to maintain treated volume below 30 bedvolumes, which is about 50 g of resin. Use either Layne RT or Dowex 21K-XLT resin. Pump nanopure water in up flow mode through the column before sealing the top to allow air bubbles to escape.
- Treat Sample
    - Pump sample at a rate of 40mL/min to give at least 2 minutes of EBCT.
    - Collect the first 30mL (~1 column volume) of effluent. Set aside. This is the rinse.
    - Collect the next 2L of column effluent. Should take ~50 minutes. Periodically verify flow rate. This is the treated sample. When the influent becomes depleted, place the intake tube into the rinse, taking care to not introduce air bubbles to the intake tube. The rinse should become depleted at the same time the effluent collection hits 2L.
    - Repeat treatment two times.
    - Collect a 5mL sample of column effluent, dilute to 50 mL. (Sample 14)
- Elute
    - Prepare 1.5L (20BV) of strong elute solution. For Layne RT use 0.1N KOH. For Dowex 21K-XLT use 0.1N KCl.
    - Warm regenerant solution on a hot plate to 90°C. Pump regenerant solution through column at a rate of 8mL/min for 10min EBCT.
    - Collect and set aside the first 30mL of effluent. Collect the next 2L as the recovered P solution. When the influent becomes depleted use the rinse to complete the 2L of collected effluent.
    - Collect a 5mL sample of regenerant solution, dilute to 50 mL (Sample 15).
    - Assuming a 50% process efficiency, the column elute should contain ~75mgP, or ~50mgP/L

Nutrient Reuse – outsource this step

- Prepare BG-11 no P
- Prepare normal BG-11
- Prepare BG-11 with recovered P

Sample Analysis

- Analyze all samples for:
    - Ortho P
        - IC (also observe NO_3^{1-}, SO_4^{2-})

    - Total P
        - Persulfate digestion
        - ICP-OES
    - COD
    - pH
    - Conductivity
- General Notes
    - During data processing, remember to adjust results for mass lost to sampling, and to adjust total P results for dilution due to acid addition.
    - Take duplicates of a few samples if warranted.
    - Store samples in ≥100mL glass containers with air tight lids

Table 2

Summary of Samples

	Description	Form
Sample 1	Freeze dried cells	50 mg in 50mL water
Sample 2	Lipid extraction residual	20 mg in 50mL water
Sample 3	Crude lipids	Trace in 50mL water
Sample 4	Residual pre-oxidation	50 mL
Sample 5	Digestion 1 blank	20 mL
Sample 6	Digestion 1 control	20 mL
Sample 7	Digestion 2 blank	20 mL
Sample 8	Digestion 2 control	20 mL
Sample 9	Digestion 3 blank	20 mL
Sample 10	Digestion 3 control	20 mL
Sample 11	Residual post-oxidation	50 mL
Sample 12	Column Effluent	50 mL
Sample 13	Column Regenerant	50 mL

Summary of samples taken in large scale phosphorus recovery process.

BIOGRAPHICAL SKETCH

Mr. Gifford is a native Arizonan dedicated to a career in water resource engineering. He graduated magna cum laude in Civil Engineering from the University of Arizona in 2006. He worked for four years in consulting engineering designing hydrologic and hydraulic infrastructure. He then became a doctoral student in Civil and Environmental Engineering at Arizona State University focusing in water quality and treatment processes as well as sustainable technology and management. His research focuses on resource recovery technologies as well as developing small drinking water treatment systems with novel sorbents and monitoring technologies. He is involved in the Boy Scouts of America.