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Phosphorus recovery from microbial biofuel residual using microwave peroxide digestion and anion exchange

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ABSTRACT

Sustainable production of microalgae for biofuel requires efficient phosphorus (P) utilization, which is a limited resource and vital for global food security. This research tracks the fate of P through biofuel production and investigates P recovery from the biomass using the cyanobacterium *Synechocystis* sp. PCC 6803. Our results show that *Synechocystis* contained 1.4% P dry weight. After crude lipids were extracted (e.g., for biofuel processing), 92% of the intracellular P remained in the residual biomass, indicating phospholipids comprised only a small percentage of cellular P. We estimate a majority of the P is primarily associated with nucleic acids. Advanced oxidation using hydrogen peroxide and microwave heating released 92% of the cellular P into orthophosphate. We then recovered the orthophosphate from the digestion matrix using two different types of anion exchange resins. One resin impregnated with iron nanoparticles adsorbed 98% of the influent P through 20 bed volumes, but only released 23% during regeneration. A strong-base anion exchange resin adsorbed 87% of the influent P through 20 bed volumes and released 50% of it upon regeneration. This recovered P subsequently supported growth of *Synechocystis*. This proof-of-concept recovery process reduced P demand of biofuel microalgae by 54%.

1. Introduction

There is an urgent need to find energy replacements for fossil fuels, whose combustion releases known and suspected human carcinogens and greenhouse gases into the atmosphere. One promising alternative is biofuel, which provides renewable energy with net greenhouse gas emissions significantly lower than fossil fuel (Batan et al., 2010). Biofuel derived

Abbreviations: ATP, adenosine triphosphate; DI, deionized water; EBCT, empty bed contact time; FAME, fatty acid methyl esters; HAX, hybrid anion exchange; ortho-PO_4^{3-}, orthophosphate; P, phosphorus; PG, phosphatidylglycerol; SBAX, strong base anion exchange.

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from microalgae offers several advantages over biofuel from terrestrial plants: it does not compete with food crops for arable land, it can be continuously harvested, and it provides a much higher areal yield (Rittmann, 2008; Schenk et al., 2008).

Microalgae biofuel production requires several inputs, including water, sunlight, carbon dioxide, and nutrients – particularly nitrogen (N) and phosphorus (P). During lipid extraction from microalgae biomass for liquid fuels, most of the N and P are discarded, requiring new nutrients for subsequent growth. Should microalgae become a significant replacement for fossil fuel in the future, the requirements for biomass growth would create a huge nutrient demand, rivaling that of agriculture (Erisman et al., 2010). Thus, capturing and recycling nutrients represents a significant opportunity for making large-scale cultivation of microalgae more sustainable (Clarens et al., 2010).

Nutrient recycling is particularly essential for P. 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), and these are non-renewable and not substitutable. Depletion of economically affordable P may bring about international crises due to the essential role of P fertilizer for global food production (Cordell et al., 2009). Farmers in developing countries could be disproportionately harmed (Childers et al., 2011). Sustainable microbial biofuel production demands efficient nutrient recycling to prevent biofuel from becoming an enormous P demand competing with food production.

This research develops a proof-of-concept process for P-recovery from microalgae after extraction of lipids. The research objective is to track P through biofuel production and then recover P from residual biomass in a reusable form by using advanced oxidation to release the P for efficient ion exchange capture. The reusable form provides bioavailable P that supports microalgae growth.

We selected cyanobacteria for this work because it is an excellent candidate for future utilization in large-scale biomass cultivation, particularly when energy efficiency in biosynthesis of fatty acids is crucial (Wijffels et al., 2013). Specifically we use *Synechocystis* sp. PCC 6803, which is a prokaryotic autotroph, Gram negative and able to withstand a wide range of environmental conditions. Lipids in the form of diacylglycerols are available in an extensive network of thylakoid membranes (van de Meene et al., 2006; Vermaas, 2001). It may be genetically manipulated for specific traits favorable for biofuel production such as high lipid content (Vermaas, 1996) because the entire genome has been sequenced (Kaneko et al., 1996).

1.1. P recovery

To recover P from microbial biomass we first release organic-bound P as inorganic orthophosphate (ortho-PO_4^{3-}). This is necessary to improve the efficiency of the subsequent capture since ortho-PO_4^{3-} is more reactive. It also mitigates heterotrophic contamination of the biomass culture, which can occur after long run periods or with accumulation of inactive cells (Mata et al., 2010). Subsequently, we selectively capture the ortho-PO_4^{3-} from the liquid in a usable form. This is necessary to isolate and purify the ortho-PO_4^{3-}, allowing accurate and controlled dosing into the aqueous growth media during reuse. It also concentrates the ortho-PO_4^{3-} solution to minimize handling or hauling. This subsection gives the impetus for the technologies we selected to accomplish those goals.

Many P-recovery methods are available (de-Bashan and Bashan, 2004; Morse et al., 1998; Rittmann et al., 2011). We selected an advanced oxidation process using hydrogen peroxide and microwave heating to release organic P from the residual biomass. Advanced oxidation creates hydroxyl free radicals that are highly effective for attacking organic matter to release ortho-PO_4^{3-} (Liao et al., 2005). This transformation may involve oxidation and hydrolysis reactions. While it may be possible to find technologies that are less energy-intensive, such as enzymatic hydrolysis or microbial fuel cells (Rittmann et al., 2011), or that do not dilute the biomass with additional liquid such as supercritical carbon dioxide (Blocher et al., 2012; Soh and Zimmerman, 2011), advanced oxidation demonstrates the principle for releasing PO_4^{3-}.

We capture ortho-PO_4^{3-} using ion exchange since it recovers a liquid concentrate that is preferable for nutrient reuse during aquatic microalgae production. Other common recovery techniques such as aluminum adsorption or struvite precipitation (de-Bashan and Bashan, 2004) produce complex or low solubility solids which may be better suited for agricultural application. We evaluated two anion-exchange resins having distinctly different properties. The first was a hybrid anion exchange resin (HAX) impregnated with iron (hydr) oxide nanoparticles (Layne RT, Layne Christensen). It is reported to have a high sorption capacity and selectivity for ortho-PO_4^{3-} (Sengupta, 2013) and the ability to release a high concentration ortho-PO_4^{3-} solution upon regeneration (Blaney et al., 2007; Midorikawa et al., 2008). The second was a type-1 strong-base anion exchange resin (SBAX) with quaternary amine functional groups in chloride ion form (21K-XLT, Dowex). It has a general anion-exchange capacity of 1.4 equivalents/L. It has previously been used for uranium (Stucker et al., 2011) and chromium (Rees-Nowak et al., 2005) removal, but has yet to be tested for phosphate recovery.

While the individual P recovery technologies employed in this study are not novel by themselves, their usage together such that the P completes an entire use and reuse cycle is. It is also the first study we know of to apply these technologies in the context of microbial biofuel production. Thus this study serves as a proof-of-concept that proposes an approach and can inform future optimization.

1.2. Microbial P

To focus the recovery efforts properly, this subsection estimates where P in *Synechocystis* is located based on literature review. Others have done this for several marine microalgae (Geider and La Roche, 2002; Sterner and Elser, 2002) but not specifically for *Synechocystis*. Biochemical fractions in cells can vary based on growth conditions (Sheng et al., 2011a) but this provides clues for understanding the fate of P after lipid processing. Fig. 1 summarizes the expected location of P in a *Synechocystis* cell. P may be located within adenosine