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The distribution of phosphorus and its transformations during batch growth of *Synechocystis*

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ABSTRACT

Phosphorus (P) is an essential nutrient that affects the growth and metabolism of microalgal biomass. Despite the obvious importance of P, the dynamics of how it is taken up and distributed in microalgae are largely undefined. In this study, we tracked the fate of P during batch growth of the cyanobacterium *Synechocystis* sp. PCC 6803. We determined the distribution of P in intracellular polymeric substances (IPS), extracellular polymeric substances (EPS), and soluble microbial products (SMP) for three initial ortho-phosphate concentrations. Results show that the initial P concentration had no impact on the production of biomass, SMP, and EPS. While the initial P concentration affected the rate and the timing of how P was transformed among internal and external forms of inorganic P (IP) and organic P (OP), the trends were the same no matter the starting P concentration. Initially, IP in the bulk solution was rapidly and simultaneously adsorbed by EPS (IP_{EPS}) and taken up as internal IP (IP_{int}). As the bulk-solution's IP was depleted, desorption of IP_{EPS} became the predominant source for IP that was taken up by the growing cells and converted into OP_{int}. At the end of the 9-d batch experiments, almost all P was OP, and most of the OP was intracellular. Based on all of the results, we propose a set of transformation pathways for P during the growth of *Synechocystis*. Key is that EPS and intracellular P pool play important and distinct roles in the uptake and storage of P.

1. Introduction

Fossil fuels provide at least 80% of energy demand worldwide (Goldemberg et al., 2004), but the combustion of fossil fuels is increasing the concentration of atmospheric CO_2, resulting in global warming and climate change (Rittmann, 2008). Photosynthesis captures photons from sunlight, takes up CO_2, and generates plant, algae, and cyanobacterial biomass (Kim et al., 2011b; Rittmann, 2008; Rittmann et al., 2006). Since its lipid portion can be converted to biodiesel and the non-lipid proportions can be used to produce methane, hydrogen and electricity (Chisti, 2007; Kim et al., 2011a; Rittmann, 2008), cyanobacterial has been studied extensively in recent decades.

Among the factors that affect the growth of cyanobacterial biomass, phosphorus (P) is an important nutrient that regulates growth and metabolism (Borovec et al., 2010; Theodorou et al., 1991; Zevin et al., 2015). At the metabolic level, many studies have focused on the effect of P on the activity of extracellular phosphatases, the P-uptake rate, and the role of P in controlling the rate of photosynthesis (Fredeen et al., 1990; Goldstein et al., 1989; Huang et al., 2015; Lefebvre et al., 1990; Rivkin and Swift, 1985; Yao et al., 2011). For example, low-P may diminish ribulose-1,5-bis-phosphate (RuBP) regeneration and, hence, photosynthetic CO_2-fixation by reducing Calvin-cycle enzyme activity (Fredeen

Abbreviations: P, Phosphorus; IP, inorganic phosphorus; OP, organic phosphorus; TP, total phosphorus; BS, bulk solution; EPS, extracellular polymeric substances; IPS, intracellular polymeric substances; SMP, soluble microbial products; IP_{EPS}, inorganic P in EPS; IP_{BS}, inorganic P in the bulk solution; IP_{int}, intracellular inorganic P; OP_{SMP}, OP in SMP; OP_{EPS}, OP in EPS; OP_{int}, intracellular OP; PG, phosphatidylgcerol; ADP, adenosinediphosphate; ATP, adenosinetriphosphate; OD, optical density; DW, dry weight; PCOD, particulate chemical oxygen demand; SCOD, soluble chemical oxygen demand; BSA, bovine serum albumin.

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et al., 1990). Understanding the effect of P distribution on the growth rate of microalgae will be of value for stimulating microalgae growth in cultures used for biofuel production and other valuable products and for minimizing their undesired growth in water bodies.

Total P in aquatic systems consists of inorganic phosphate (IP) and P-containing organic compounds (OP) (Francko and Heath, 1979), both of which can be further partitioned into an extracellular P pool (P not inside microorganisms) and an intracellular P pool (P inside microorganisms) (Cembella et al., 1984; Yao et al., 2011). The uptake of inorganic P (IP) by biomass also involves its adsorption by extracellular polymeric substances (EPS), a process that is distinct from and independent of its uptake as intracellular P (Yao et al., 2011; Zhang et al., 2013). EPS are microbe-produced solids that are located outside the cell and are comprised of protein, carbohydrate, and other organic components (Adav and Lee, 2008). A common functional group in protein, quaternary ammonium ($-NH_3^+$), can complex with negatively charged phosphate (Wingender et al., 1999; Zhou et al., 2017). For example, when the pH was greater than 8, the key surface ligand for the EPS of *Shewanella alga* strain BrY was $-NH_3^+$ (Deo et al., 2010). EPS-adsorbed P was 60–90% of total cellular P in different algal species (Sañudo-Wilhelmy et al., 2004).

Within the cells, IP from the bulk solution (IP_{BS}) can be transported across the cellular membrane and become part of intracellular IP (IP_{int}) (Cembella et al., 1984). Part of IP_{int} is IP that is adsorbed by the $-NH_3^+$ groups of organic components in intracellular polymeric substances (IPS) (Deo et al., 2010). Another part of IP_{int} is non-adsorbed IP that participates in conversion of adenosinediphosphate (ADP) to adenosinetriphosphate (ATP) (Novikoff et al., 1952). IP_{int} can be transformed into intracellular OP (OP_{int}) during biomass synthesis (Kim et al., 2011b).

Fig. 1 illustrates and defines the locations in which IP and OP exist in cyanobacterial biomass or the liquid medium. The biomass contains EPS and IPS, which are located outside and inside the cell membrane, respectively (Adav and Lee, 2008). SMP are soluble cellular components that are released from the biomass (Wingender et al., 1999). IP can be free or adsorbed. Free IP exists dissolved in the bulk solution (IP_{BS}) and inside the cell (IP_{int}), such as for use to convert ADP to ATP (Novikoff et al., 1952). Adsorbed IP is present in SMP, EPS, and IPS due to it complexation with $-NH_3^+$ functional groups (Deo et al., 2010). P in nucleic acids and lipids is part of OP, and it can be found in soluble microbial product (SMP), EPS and intracellular P pool (Youngburg and Youngburg, 1930).

Despite the obvious importance of P partitioning and transformation, little is known about these factors during the growth of microalgae. In this study, we carried out batch growth experiments with *Synechocystis* sp. PCC 6803, a well characterized cyanobacterium that has been widely used as a model organism in a variety of molecular and engineering studies (Kim et al., 2011b; Zevin et al., 2015). Establishing a complete P mass balance, we systematically investigated how added inorganic phosphate was transformed and distributed among the IP and OP components as *Synechocystis* grew in batch culture. No matter the staring P concentration, sorption of IP was the dominant mechanism at the beginning of batch-growth studies, but most P was transformed to OP by the end of the batch growth. We translate the results into a model for the dynamics of P uptake and distribution.

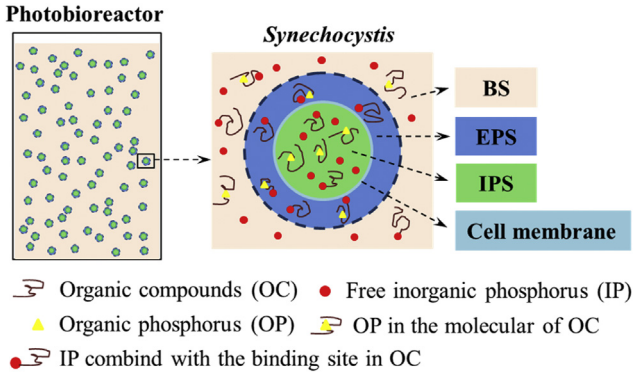

Fig. 1. Definitions of the various forms of inorganic and organic P among the components of *Synechocystis*.

2. Materials and methods

2.1. Synechocystis sp. PCC 6803 cultures and growth experiments

Stock cultures of wild-type *Synechocystis* sp. PCC 6803, provided by the laboratory of Dr. Willem F. J. Vermaas (School of Life Sciences, Arizona State University), were maintained in 500-mL (working volume) Erlenmeyer flasks containing standard BG-11 medium (Rippka et al., 1979) (composition in Table S1) and bubbled with air filtered through a 1.0-μm air filter (Pall, Port Washington, NY, U.S.). An aliquot from a flask culture was diluted to an optical density (OD) of ~0.6 to initiate a batch growth experiments. Table 1 summarizes characteristics of the *Synechocystis* inoculate at the start of batch experiments.

Erlenmeyer flasks with a volume of 1000 mL were used for batch growth experiments. A constant temperature of 30 °C was maintained by 3 × 12-W automated-air fans (Minebea-Matsushita Motor Corp., Japan) (Nguyen and Rittmann, 2016a), and pure CO_2 was supplied by sparging with humidified air filtered through the 1.0-μm air filter (Pall, Port Washington, NY, USA). The incident light intensity was 276 μE/m^2/s, measured using a PAR sensor (Agilent, U1252A, USA), and it was provided from T5 fluorescent plant-grow lamps (Envirogro Hydrofarm, USA). The pH value of the culture was maintained at 8.5 using a pH-Stat that automatically sparged CO_2 when the pH rose above 8.51 (Nguyen and Rittmann, 2015). Fig. S1 is a schematic diagram of the set up for the growth experiments.

Previous study (Kim et al., 2011b) measured the elemental composition of *Synechocystis* sp. PCC 6803 and found it to be

Table 1
Characteristics of the *Synechocystis* inoculum used to initiate growth experiments.

Parameter	Value
OD_{730}[a]	0.59 ± 0.04
PCOD[b] (mg COD/L)	250 ± 11
SMP[c] (mg COD/L)	6.3 ± 0.45
EPS[d] (mg COD/L)	14.8 ± 0.38
Protein in SMP (mg COD/L)	1.68 ± 0.21
Carbohydrate in SMP (mg COD/L)	3.68 ± 0.49
Protein in EPS (mg COD/L)	8.47 ± 0.39
Carbohydrate in EPS (mg COD/L)	5.32 ± 0.42
IP[e] in SMP (mg P/L)	0.078 ± 0.011
OP[f] in SMP (mg P/L)	0.032 ± 0.007
IP in EPS (mg P/L)	0.141 ± 0.028
OP in EPS (mg P/L)	0.093 ± 0.006
IP in IPS (mg P/L)	0.062 ± 0.010
OP in IPS (mg P/L)	0.335 ± 0.031
TP[g] of *Synechocystis* (mg P/L)	0.752 ± 0.079

[a] OD_{730} is the optical density at 730 nm.
[b] PCOD is particulate chemical oxygen demand.
[c] SMP is soluble microbial products.
[d] EPS is extracellular polymeric substances.
[e] IP is inorganic phosphorus.
[f] OP is organic phosphorus.
[g] TP is total phosphorus.