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Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment

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ABSTRACT

Microalgae offer a promising technology to remove and re-use the nutrients N and P from wastewater. For effective removal of both N and P, it is important that microalgae can adjust the N and P concentration in their biomass to the N and P supply in the wastewater. The aim of this study was to evaluate to what extent microalgae can adjust the N and P concentrations in their biomass to the N and P supply in the wastewater, and to what extent supply of one nutrient influences the removal of the other nutrient. Using *Chlorella* and *Scenedesmus* as model organisms, we quantified growth and biomass composition in medium with different initial N and P concentrations in all possible combinations. Nutrient supply marginally affected biomass yield of both microalgae but had a strong influence on the composition of the biomass. The nutrient concentrations in the biomass ranged 5.0–10.1 % for N and 0.5–1.3 % for P in *Chlorella* and 2.9–8.4 % for N and 0.5–1.7 % for P in *Scenedesmus*. The concentrations of P in the biomass remained low and were relatively constant (0.6–0.8 % P) when the N concentration in the biomass was low. As a result, removal of P from the wastewater was influenced by the concentration of N in the wastewater. When the initial N concentration in the wastewater was above 40 mg L^{-1} the microalgae could remove up to 6 mg P L^{-1}, but this removal was only 2 mg P L^{-1} when the initial N concentration was below 20 mg L^{-1}. A lower N supply increased the carbohydrate concentration to about 40% and lipid concentration to about 30% for both species, compared to around 15% and 10% respectively at high N supply. Our results show that sufficiently high N concentrations are needed to ensure effective P removal from wastewater due to the positive effect of N on the accumulation of P.

1. Introduction

Although the idea to use microalgae for wastewater treatment is not new and dates back almost half a century (Oswald et al., 1957), interest in this concept has revived in recent years. Microalgae-based wastewater treatment has several advantages over conventional wastewater treatment using activated sludge. First, microalgae-based wastewater treatment has a lower energy demand because oxygen is supplied

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through photosynthesis rather than energy-intensive electromechanical blowers (Green et al., 1995). Second, microalgae not only remove nitrogen (N) and phosphorus (P) from wastewater, but also recycle these nutrients in a biomass that can be converted into energy, raw chemicals or other products (Martins et al., 2010). These advantages are very attractive in times when there is a need to increase energy efficiency and to "close the loop" by recycling elements from waste streams. In this respect, the combination of microalgae wastewater treatment with the production of microalgal biofuels has attracted much attention in recent years (Craggs et al., 2013; Pittman et al., 2011).

In conventional wastewater treatment, N and P are removed from wastewater in two separate processes. Usually, N is converted to N_2 gas through coupled nitrification-denitrification while P is precipitated with metal salts. Microalgae, on the contrary, remove N and P from wastewater in a single process. Microalgae absorb N and P from wastewater and use these nutrients to produce biomass. Because microalgae require both N and P to produce new biomass, removal of one nutrient depends on the availability of the other: microalgae cannot remove N without the presence of P in the wastewater, or vice versa, because both nutrients are essential for their growth. The concentrations of N and P in wastewater are quite variable. In domestic wastewater, N concentrations vary between 15 and 90 mg L^{-1} and P concentrations between 4 and 20 mg L^{-1} (Abdelaziz et al., 2013; Cai et al., 2013; Christenson and Sims, 2011). Because the sources of N and P in wastewater are different, N and P concentrations often vary independently from each other. Human excreta are a source of both P and N while detergents, soaps and personal care products contain P but little N (Smil, 2000; Tjandraatmadja et al., 2010). Because N and P removal are coupled in microalgae, the fact that N and P concentrations in wastewater vary independent from each other poses a challenge when engineering nutrient removal from wastewater using microalgae.

Removal of N and P by microalgae depends on the concentrations of these nutrients in the microalgal biomass. The N and P requirements of microalgae have been an active research field in microalgal ecology and physiology for over half a century. In 1958, Redfield studied nutrient concentrations in marine microalgae and found a ratio of about 106:16:1 C:N:P (molar ratio) in the biomass, a ratio that is since then known as the Redfield ratio (Redfield, 1958). It soon became apparent, however, that this ratio is not fixed and that nutrient concentrations in microalgal biomass can be quite variable, particularly in freshwater species (Rhee, 1978). Microalgae have the ability to adjust the N and P concentration of their biomass depending on the supply of nutrients in the medium, resulting in a low nutrient concentration in the biomass when the supply of a nutrient is low and a higher concentration when the supply is high (e.g. Geider and La Roche, 2002; Rhee, 1978; Sterner and Elser, 2002). The microalgal P concentration can range from 0.03% to more than 3% of dry biomass and the microalgal N concentration can range from 3% up to 12% (Reynolds, 2006). Because N and P are used by microalgae to produce various biochemical compounds, changes in the concentrations of N and P in the biomass will influence the biochemical composition of the biomass (Klausmeier et al., 2004; Loladze and Elser, 2011). Nitrogen is predominantly used for synthesis of proteins while P is mainly incorporated into ribosomal RNA. When either N or P are limiting, the protein content of the cell is reduced and cell division slows down but C acquisition through photosynthesis continues. Therefore, cells tend to accumulate C-rich metabolites such as carbohydrates or lipids when the supply of N and P is low (González-Fernández and Ballesteros, 2012; Smith et al., 2010). Induction of N or P limitation is commonly used in microalgal production for biofuels to increase the carbohydrate or lipid yield (Craggs et al., 2013).

The flexibility of the N and P concentration of microalgal biomass allows microalgae to adjust their intracellular N and P concentration to the supply of N and P in the wastewater. This flexibility is essential to ensure simultaneous removal of N and P from wastewater. Although there have been a large number of studies on N and P removal from wastewater by microalgae, relatively few studies have specifically studied the flexibility of the N and P concentration in microalgal biomass in response to the supply of N or P in the wastewater. Several studies have shown that a lower N or P supply in wastewater results in a lower N or P concentration of the microalgal biomass and this is often associated with an accumulation of carbohydrates and/or lipids (Akerström et al., 2014; Arbib et al., 2013; Aslan and Kapdan, 2006; Dickinson et al., 2013; Samorì et al., 2013; Xin et al., 2010). Other studies have investigated to what extent microalgae can accumulate excess nutrients in their biomass, known as luxury uptake, which is interesting for removal of nutrients from high-strength wastewaters. Some species are capable of accumulating P as polyphosphate granules when the P supply is high and can accumulate up to 3% P in the biomass (Eixler et al., 2006; Powell et al., 2009). Other species of microalgae, such as some diatom species, can accumulate nitrate in their central vacuole (Coppens et al., 2014). In most studies, the concentration of only one nutrient is varied while the other is maintained constant (Arbib et al., 2013; Samorì et al., 2013; Xin et al., 2010; Zhang and Hong, 2014) or the concentration of both nutrients is varied simultaneously without changing their ratio (Akerström et al., 2014; Aslan and Kapdan, 2006). No studies have investigated to what extent the supply of one nutrient influences the uptake of the other nutrient.

The aim of this study was to evaluate to what extent microalgae can adjust the concentration of N and P in their biomass to the supply of these nutrients in the wastewater. Because nutrient availability has a profound influence on the biochemical composition of microalgae, we expect that availability of one nutrient may have an influence on the uptake of the other nutrient. Therefore, we used a central composite design in which the supply of N and P to microalgae was varied independently. We tested this for the two microalgae species that are most frequently observed in microalgae-based wastewater treatment systems: *Chlorella* and *Scenedesmus* (Craggs et al., 2013; Pittman et al., 2011). We also evaluated to what extent changes in wastewater N and P supply influenced the biochemical composition of the biomass because this has important implications for the valorisation of the biomass.