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Microalgae growth and phosphorus uptake in wastewater under simulated cold region conditions

Jordan J. Schmidt, Graham A. Gagnon, Rob C. Jamieson *

Centre for Water Resources Studies, Dalhousie University, 1360 Barrington Street, Halifax, NS, Canada

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ABSTRACT

Facultative waste stabilization ponds (WSP) are a common form of wastewater treatment in cold regions. However, cold region WSPs have been found to have highly variable and inconsistent microalgae growth and phosphorus removal. This study investigated whether facultative WSPs can be used to provide biological phosphorus removal in cold regions by evaluating maximum specific growth rates and phosphorus removal pathways under simulated cold region summer (ice-free) conditions. A factorial experiment was conducted in order to determine the main effects and interactions of temperature (10, 15 °C), photosynthetically active radiation (PAR) (100, 150 μmol/m^2/s) and initial phosphorus concentration (7.5, 15 mg P/L) on microalgae growth and phosphorus uptake. Maximum specific growth rates varied from 0.029 to 0.058/h. PAR and temperature had a statistically significant negative and positive effect, respectively, on growth rates. Initial phosphorus concentration had no statistical effect on growth rates under the studied ranges. Growth rates were similar to those observed at temperate climates. Luxury uptake was a significant phosphorus removal mechanism as it accounted for 53 ± 8% (g P/g P) of biomass phosphorus. Biomass phosphorus concentrations were positively affected by PAR and initial phosphorus concentration while temperature had no effect. A crossover interaction between temperature and initial phosphorus concentration was found to have a negative effect on biomass phosphorus concentration. Under cold region conditions biomass phosphorus concentrations were 45% greater than under warm climate conditions. Ultimately, it is expected that climate should not hinder microalgae production in cold region WSPs during the summer months when temperatures exceed 10 °C and the surface is ice-free. Cold region conditions appear to aid in phosphorus removal by increasing biomass phosphorus concentrations.

1. Introduction

Facultative waste stabilization ponds (WSPs) are a common wastewater treatment strategy for communities in cold regions. This is due to their low operating cost, minimal required technical proficiency, and low energy and chemical demand. WSPs can also be designed for long retention periods (~6–12 months) with intermittent discharge, which are necessary in communities that cannot have continuous discharge due to extended ice cover during the winter (Heaven et al., 2003). Facultative WSPs rely on microalgae to provide aeration. Microalgae are directly or indirectly related to the removal of a number of parameters including organics, nitrogen and phosphorus.

Recent research conducted in the Canadian territory of Nunavut showed that there was significant variance in the design, operation and treatment performance of four WSPs (Ragush et al., 2015). WSPs were effective at removing suspended solids but were not capable of achieving secondary wastewater treatment objectives for biochemical oxygen demand. Three of the four WSPs studied were operating anaerobically, with little microalgae production. The lack of microalgae production was found to be associated with design (depth), operational parameters (organic loading rate) and climate. Ultimately, Ragush et al. (2015) concluded that it is possible to operate a WSP facultatively in cold regions, if different design guidelines were implemented.

The removal of phosphorus is of particular interest due to its role in receiving water eutrophication. Localized nutrient enrichment has been documented in cold regions where WSP effluent enters marine receiving water environments (Krumhansl et al., 2014). Limited research has been conducted on phosphorus concentrations and removal rates in cold region treatment systems. Total phosphorus concentrations in WSPs in cold regions have been shown to exceed 7 mg P/L, while lower effluent concentrations (3.5 mg P/L) have been observed during a microalgae bloom

* Corresponding author.
E-mail address: jamiesrc@dal.ca (R.C. Jamieson).

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(Schmidt et al., 2016). In Scandinavian countries, use of WSPs has been limited since the 1960s. Precipitation ponds, or fellingsdams, are a common treatment option for small, remote communities. However, due to economic and environmental disadvantages associated with chemical precipitants there has been a renewed interest in biological treatment during ice-free periods (Ødegaard et al., 1987; Hanaeus et al., 2010). Pilot scale high rate algal ponds evaluated in a sub-arctic climate in Sweden achieved phosphorus removal of approximately 20% while daily mean temperatures were below 10 °C (Grönlund et al., 2010).

Phosphorus uptake by microalgae occurs via two mechanisms. In the first mechanism, phosphorus is assimilated into the microalgae biomass through the construction of organic cellular components such as phospholipids. The second microalgae mechanism is referred to as luxury uptake. Luxury uptake occurs when microalgae uptake and store excess phosphorus as inorganic polyphosphate granules. Polyphosphate granules can be acid soluble or insoluble. Acid soluble polyphosphate is associated with metabolism while acid insoluble polyphosphate is considered to be a storage product for when external phosphorus is limiting (Miyachi et al., 1964). Limited research has been conducted on luxury uptake in wastewater systems, however its potential has been documented. For example, Powell et al. (2008) found that by manipulating temperature (15–25 °C) and light intensity (60–150 μmol/m^2/s) biomass phosphorus percentage can be increased from 0.4 to 3.2%. Previous studies have shown that the critical growth level for microalgae is 1% phosphorus (Borchardt and Azad, 1968). The difference from this critical value represents the potential increase in removal performance due to luxury uptake. The biomass phosphorus concentrations shown by Powell et al. (2008) below the critical level may be related to the composition of the microalgae community studied. The microalgae community studied by Powell et al. (2008) was dominated by *Scenedesmus* spp., a genus of microalgae previously found to have a relatively low minimum phosphorus quota (Gotham and Rhee, 1981).

Light, temperature and external phosphorus concentration have been shown to affect biomass phosphorus concentrations (Brown and Shilton, 2014). Light has been shown to have negative (Hessen et al., 2002; Powell et al., 2008; Sterner et al., 1997) or no (Frost and Elser, 2002) effect on biomass phosphorus concentrations. Based on previous studies, light effects depend on other interactions such as external phosphorus concentration and stage of growth. For instance, under low external phosphorus concentrations, as light increases, phosphorus becomes limiting and cells become carbon rich (Sterner et al., 1997). While it is not expected that this will occur in wastewater systems, as nutrient availability is usually high, previous research has suggested a negative effect may still occur (Powell et al., 2008). It has also been hypothesized that higher light intensities lead to higher growth rates and quicker consumption of stored polyphosphates leading to lower biomass phosphorus concentrations (Powell et al., 2009). Temperature has been shown to have a positive effect on biomass phosphorus concentrations, however previous studies were conducted outside the range experienced in cold regions (Powell et al., 2008). External phosphorus concentrations have been shown to have a positive (Frost and Elser, 2002; Hessen et al., 2002) or no (Powell et al., 2008) effect on biomass phosphorus concentrations. While current research in this area demonstrated the potential for biological phosphorus removal in WSPs, the ranges of tested conditions for temperature and external phosphorus concentration were outside the typical ranges experienced by communities in cold regions (Ragush et al., 2015; Schmidt et al., 2016). Therefore, exact effects and interactions need to be experimentally determined in order to effectively create predictive tools to determine system reliability.

Table 1
Simulated wastewater recipe for microalgae cultivation and growth experiments.

Compound	Concentration (mg/L)
K_2HPO_4	84[a], 42[b]
$MgCl_2$	45
NH_4Cl	350
$CaCl_2{\cdot}2H_20$	38
$NaHCO_3$	47
$C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$(Na-EDTA)	280
Trace Metals Solution	1 mL/L
Trace Metals Solution (×1000 Stock)	
$MnCl_2{\cdot}4H_2O$	300
$AlCl_3{\cdot}6H_2O$	1700
$ZnSO_4$	200
$Na_2MoO_4{\cdot}2H_2O$	24
$CoCl_2{\cdot}6H_2O$	12
$CuSO_4$	20
$FeSO_4{\cdot}7H_20$	3000
$C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$(Na-EDTA)	5000

[a] Concentration for cultivation and high phosphorus condition.
[b] Low phosphorus condition.

Table 2
The tested levels/concentrations for each experimental factor.

Factor	High	Low
Temperature	15 °C	10 °C
Initial Phosphorus Concentration	15 mg P/L	7.5 mg P/L
Photosynthetically Active Radiation	150 μmol/m^2/s	100 μmol/m^2/s

Current studies have shown that microalgae growth and phosphorus removal in cold region wastewater systems are inconsistent and not well understood (Ragush et al., 2015; Schmidt et al., 2016). Therefore, the objective of this study is to determine microalgae growth rates and phosphorus uptake under simulated cold regions conditions. A lab scale factorial experiment was used to evaluate multiple conditions and their associated interactions. The conditions studied were temperature, photosynthetically active radiation (PAR) and phosphorus concentration. Luxury uptake was quantified in order to determine predominate removal mechanisms. *Chlorella vulgaris* and *Chlamydomonas reinhardtii* were used, as they were previously identified as two prominent microalgae species in facultative WSPs operated in cold climates.

2. Materials and methods

2.1. Algae cultivation

Chlorella vulgaris and *Chlamydomonas reinhardtii* strains were obtained from the National Research Council of Canada. Strains were cultivated in 250 mL erlenmeyer flasks under constant illumination using a modified Bold 3 N medium developed by UTEX. These cultures were used to seed a 10 L chemostat receiving simulated raw wastewater as growth medium. A chemostat was used in order to maintain a consistent inoculant for experiments. The simulated wastewater recipe is shown in Table 1. No organic carbon was added in order to minimize bacterial growth. Sodium EDTA was added as a chelating agent to prevent metal precipitation. The chemostat was under constant illumination using fluorescent lights resulting in a PAR of approximately 150 μmol/m^2/s on the immediate surface.

2.2. Experimental approach

Temperature, initial phosphorus concentration and PAR were tested to determine their influence on microalgae growth rates and phosphorus uptake. A summary of the levels used for each factor is shown in Table 2. PAR and temperature levels were representa-