Bioresource Technology 202 (2016) 238–243

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Inhibition of nitrification in municipal wastewater-treating photobioreactors: Effect on algal growth and nutrient uptake

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HIGHLIGHTS

- Microalgae were able to grow faster when nitrification was inhibited.
- The inhibited reactors had *Cryptomonas* and *Chlorella* as the dominant genera.
- Inhibition of nitrification did not affect the uptake rate for N and P.
- Inhibition of nitrification affected N speciation in the reactors.

ARTICLE INFO

Article history:
Received 22 September 2015
Received in revised form 30 November 2015
Accepted 8 December 2015
Available online 17 December 2015

Keywords:
Photobioreactors
Microalgae
Wastewater treatment
Nitrification
Algal–bacterial interactions

ABSTRACT

The effect of inhibiting nitrification on algal growth and nutrient uptake was studied in photobioreactors treating municipal wastewater. As previous studies have indicated that algae prefer certain nitrogen species to others, and because nitrifying bacteria are inhibited by microalgae, it is important to shed more light on these interactions. In this study allylthiourea (ATU) was used to inhibit nitrification in wastewater-treating photobioreactors. The nitrification-inhibited reactors were compared to control reactors with no ATU added.

Microalgae had higher growth in the inhibited reactors, resulting in a higher chlorophyll a concentration. The species mix also differed, with *Chlorella* and *Scenedesmus* being the dominant genera in the control reactors and *Cryptomonas* and *Chlorella* dominating in the inhibited reactors. The nitrogen speciation in the reactors after 8 days incubation was also different in the two setups, with N existing mostly as NH_4-N in the inhibited reactors and as NO_3-N in the control reactors.

1. Introduction

Wastewater treatment has been a subject of interest in microalgae research for more than half a century (Beuckels et al., 2015). However the complexity and potential for real-world use of such systems has increased over the past two decades (Muñoz and Guieysse, 2006). While the initial focus was on tertiary treatment and the use of high rate algal ponds, currently photobioreactors are implemented as secondary treatment of municipal wastewater (Ruiz-Martinez et al., 2012; Su et al., 2011). In this application, the uptake of nutrients such as nitrogen and phosphorous has become an important factor in photobioreactor research.

Nitrogen is taken up from the wastewater by algae and cyanobacteria in various forms, with ammonium being preferred to nitrate (Dortch, 1990). As municipal wastewaters contain high concentrations of ammonium and low concentrations of nitrate, they are ideal for algae. Most types of wastewater contain a large variety of different microorganisms (Saunders et al., 2015), so every microalgae system treating unsterilized wastewater will inevitably contain a mixed consortium of algae, bacteria and other microorganisms. This means that up to 81–85% of the ammonium in a wastewater-treating photobioreactor is nitrified by the bacteria present in the wastewater, rather than being taken up by algae (Karya et al., 2013). In addition, the availability of nitrogen in the reactor influences the uptake of phosphorous (Beuckels et al., 2015). The dynamics of these nutrients are therefore strongly

Abbreviations: ATU, allylthiourea; DO, dissolved oxygen; DOC, Dissolved Organic Carbon; LW, lake water; NH_4-N, ammonium nitrogen; NO_2-N, nitrite nitrogen; NO_3-N, nitrate nitrogen; TOC, Total Organic Carbon; TP, Total Phosphorous; WW, wastewater; WWTP, wastewater treatment plant.

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http://dx.doi.org/10.1016/j.biortech.2015.12.020
0960-8524/

coupled to each other, and to the metabolic processes present in the photobioreactors.

The understanding of the interactions between bacteria and microalgae is still very limited (Unnithan et al., 2014), but recent research has provided a glimpse into the many possible processes taking place. While some interactions can be relatively straight forward such as the production of different metabolites such as vitamin B12 (Krohn-Molt et al., 2013), others can be more complex. For example, Choi et al. (2010) demonstrated in a bioreactor that algae and cyanobacteria can inhibit nitrifying bacteria growth by a factor of 4. Similar results have also been reported by Huang et al. (2015) who showed that the growth and activity of nitrifying bacteria was inhibited when grown together with algae. This, in turn can have an effect on the algal growth and community composition as it changes the speciation of nitrogen in the system and it is known that algae prefer some forms of nitrogen to others (Dortch, 1990). There is currently little information available on how the inhibition of nitrification affects the algal growth and nutrient reduction in wastewater-treating photobioreactors.

The present study aims to determine whether nitrification inhibition has an effect on the growth and community composition of microalgae and the uptake of nutrients in a lab-scale municipal wastewater-treating photobioreactor. Reactors were set up with a mixed algae community from an inland lake in Sweden (Lake Mälaren) and municipal wastewater, and compared to reactors where nitrification was inhibited with allylthiourea (ATU). The focus was on algal growth, differences in algae communities and the removal of nitrogen and phosphorous. Similar setups have been successful in producing a complex community of bacteria and algae in previous studies (Krustok et al., 2015a,b).

2. Methods

2.1. Origin and properties of wastewater and lake water

The wastewater used in the experiments was obtained from the wastewater treatment plant (WWTP) in Västerås, Sweden. The plant treats sewage from a local population of 118,000. The treatment process consists of screening, pre-precipitation with iron sulphate and biological treatment of the inflowing raw municipal wastewater. Wastewater used in the experiment was collected from the top layer of the mixing basin at the inflow of the WWTP after iron sulphate was added.

To introduce more algae to the reactors, lake water was collected from the upper layer (0.5 m) of a yacht harbor on Lake Mälaren in March 2015. The lake has seasonal algae blooms in spring and late summer involving a variety of diatoms, green algae and cyanobacteria. Previous studies in which wastewater-treating photobioreactors have been inoculated with water from Lake Mälaren have produced a community containing mostly *Scenedesmus*, *Desmodesmus* and *Chlorella* species, with *Scenedesmus obliquus* being dominant (Krustok et al., 2015b).

All lake and wastewater samples were collected in sterile vessels, stored at 4 °C and used on the same day. The nutrient content of the wastewater and lake water used in the experiments was analyzed and is shown in Table 1.

2.2. Experimental setup

Four photobioreactors with a height of 18 cm, a diameter of 10 cm and a working volume of 1 L were used in the experiments. The reactors were composed of glass cylinders with steel tops and bottoms. During the experiment the reactors were closed with only a small opening at the top to allow for sampling and gas exchange.

Table 1
Chemical parameters of the wastewater used as a growth substrate (WW) and lake water used to introduce algae (LW). Average values and standard deviations of the parameters in the three different experiments are shown. Abbreviations: TOC – Total Organic Carbon, DOC – Dissolved Organic Carbon, TP – Total Phosphorous.

Parameter	WW	LW
pH	7.5 ± 0.3	7.3 ± 0.4
Chl a (mg L^{-1})	0.02 ± 0.01	0.07
TOC (mg L^{-1})	224 ± 68	16
DOC (mg L^{-1})	25 ± 1	12
NH_4-N (mg L^{-1})	40.9 ± 1.5	0.7
NO_3-N + NO_2-N (mg L^{-1})	0.5 ± 0.1	5.4
TP (mg L^{-1})	3.2 ± 3.2	0.05
Dissolved P (mg L^{-1})	0.5 ± 0.2	<0.05

The water temperature in the reactors was maintained at 23 °C and the contents were stirred throughout at around 350 rpm. While this speed could potentially disrupt algae cells it was chosen in order to keep the culture homogenous. At lower speeds the stirring mechanism was more prone to clogging and stopping.

The reactors were lit from above by 4 fluorescent tubes (Aura Long Life 51W/830) in a 16 h on/8 h off cycle. Mirrors were placed at a 45° angle next to the reactors to direct light into the glass cylinders. This was done because the reactors had metal tops that reduced the amount of light available to the algae. The light intensity in the reactor was measured at around 100 μmol $m^{-2}s^{-1}$.

Pure food grade CO_2 (AGA AQVIA) was pumped into the reactors by a control valve that opened when the pH exceeded 8. pH was measured using metric FermProbe pH sensors (Broadley-James). The control valve was closed via relays when the pH meter detected a level below 8 and the inflowing gas reduced the pH to around 7 with each time the valve opened. This was done to prevent high pH from inhibiting both the algal growth and nitrification.

The reactors were set up with a mixture of 70% wastewater (WW) and 30% lake water (LW) by volume. This mixture was previously shown to produce a complex community with a variety of bacteria and microalgae present (Krustok et al., 2015b). Two of the reactors were set up as controls with only WW and LW for normal operation while two had 0.05 g of ATU added to inhibit nitrification. ATU inhibits ammonia oxidation most likely by chelating the copper of the ammonia monoxygenase active site (Ginestet et al., 1998).

Reactors were sampled daily to determine NH_4-N and NO_3-N + NO_2-N concentrations. 10 ml was sampled, filtered through a 1.2 μm filter (Whatman GF/C) and stored at −20 °C until measurement. Every other day 200 ml was sampled for chlorophyll a, ammonium nitrogen (NH_4-N), sum nitrate and nitrite nitrogen (NO_3-N + NO_2-N), dissolved phosphorous (DP) concentrations and potential nitrification measurements. Dissolved Organic Carbon (DOC), Total Organic Carbon (TOC) and Total Phosphorous (TP) concentrations were measured in the beginning and the end of the experiment. The water collected from the reactors was replaced with fresh wastewater to achieve a total volume of 1L again. In the inhibited reactors an additional 0.05 g of ATU was added when wastewater was added.

2.2. Chemical analysis

TOC and DOC were measured from the initial waters using HACH LANGE cuvette tests LCK380 and LCK381. Dissolved oxygen (DO) was measured every other day using a HACH LANGE LD101 DO probe.

For NH_4-N and NO_3-N + NO_2-N, 25 mL of each water sample was filtered through a 1.2 μm filter (Whatman GF/C). The filtered water samples were stored at −20 °C until analysis was performed