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Characterization of algal and microbial community growth in a wastewater treating batch photo-bioreactor inoculated with lake water

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ABSTRACT

Microalgae grown in photo-bioreactors can be a valuable source of biomass, especially when combined with wastewater treatment. While most published research has studied pure cultures, the consortia of algae and bacteria from wastewater have more complex community dynamics which affect both the biomass production and pollutant removal. In this paper we investigate the dynamics of algal and bacterial growth in wastewater treating batch photo-bioreactors. The photo-bioreactors were inoculated with water from a nearby lake. Lake water was obtained in August, November and December in order to add native algal species and study the effects of the season. The photo-bioreactors inoculated with lake water obtained in August and November produced more biomass and grew faster than those that only contained the algae from wastewater. The results indicated a rapid decline in bacterial abundance before algae began to multiply in reactors supplemented with lake water in November and December. The reactors were also successful in removing nitrogen and phosphorous from wastewater.

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1. Introduction

Recent studies have shown that cultivation of algae in wastewater may be an effective way to recover nutrients and cultivate biomass [13,18,21]. Wastewater is also readily accessible in most urban environments making its use as a growth medium possible in a wide variety of locations.

The use of wastewater as a medium introduces a whole consortium of microorganisms into the process making it more robust in terms of metabolic pathways present [10]. In addition, natural inoculants such as lake water may serve as a source for indigenous algal strains. This removes the need to find a perfect pure culture to use in the process and let the medium do the selection [14].

Compared to conventional monoculture photo-bioreactors, it is important to study the algal and microbial communities in these systems in order to understand and control the process [9,14,18]. Lakaniemi et al. [9] concluded that understanding of the interactions between microorganisms in photo-bioreactors is needed to increase the biomass production. The potential of the bacterial and algal consortium in similar biotechnological applications has also been described by Subashchandrabose et al. [19], who concluded that understanding the community relationships is crucial when algal biomass is produced in the course of different wastewater treatments and the degradation of pollutants and production of metabolites (proteins, fatty acids, steroids, carotenoids, phycocolloids, lectins, mycosporine-like amino acids, halogenated compounds, and polyketides) are highly desirable.

The aim of the present study was to investigate algal growth and nutrient removal in a photo-bioreactor using water containing indigenous algae from an inland lake in central Sweden (Lake Mälaren) and inflow wastewater to a wastewater treatment plant (WWTP) treating the water of a medium sized town in central Sweden (Västerås) as a growth medium in three different seasons. Samples were taken in August, when the algal biomass growth in the lake was high, in November, when there is no visible algal biomass growth in the lake and in December, when the lake surface had frozen. Specific objectives were to:

(1) Investigate the dynamics of the microbial and algal growth in a wastewater treating batch photo-bioreactor after the introduction of indigenous algae from a nearby inland lake taken at different seasons.

Abbreviations: DOC, dissolved organic carbon; DW, dry weight; LW, lake water; PE, purification efficiency; qPCR, quantitative polymerase chain reaction; TOC, total organic carbon; TP, total phosphorous; WW, wastewater; WWTP, wastewater treatment plant

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(2) Investigate how nutrient concentrations change in the algal cultivation process to assess the water purification efficiency in the photo-bioreactors.

2. Materials and methods

2.1. Experimental setup

2.1.1. Determining the wastewater and lake water ratio

The 70% wastewater and 30% lake water mixture was determined based on a flask experiment. Ten 250 mL flasks were set up in a climate chamber with automatic light and temperature regulation using a protocol of 12 h of light and 12 h of dark per 24 h at 23 ± 0.5 °C. Lake water and wastewater ratios of 30/70, 50/50 and 70/30 were tested. Stirring was not added; however, the flasks were manually shaken everyday. They were compared to pure lake water and wastewater. The experiment indicated that a 70/30 ratio of lake water and wastewater had the highest increase (1.6×) of optical density (at 630 nm) over 14 days of growth compared to the other samples.

2.1.2. Determining the effect of lake water addition

Three similar 16 day laboratory experiments were conducted with lake water sampled in November, December and August. Four photo-bioreactors (height: 18 cm, diam.: 10 cm) with a total volume of 1 L of water mixture were set up and the following variants were treated simultaneously in each experiment: 1) wastewater (WW), 2) 70/30 mixture of wastewater and lake water (WW + LW), and 3) 70/30 mixture of wastewater and distilled water (WW + W). A sterilized wastewater reactor was set up as a control to detect cross-contamination. For the control reactor, wastewater was sterilized by autoclaving at 121 °C for 20 min. All measurements on the sterilized wastewater were performed after the sterilization process.

The reactors were glass cylinders with stainless steel tops and bottoms. During the experiments, the reactors were closed and filter paper was used to cover openings on the top in order to allow gas exchange. The light source was above the reactors and mirrors were used to reflect light in order to increase lighting efficiency. The experimental light and temperature conditions were manipulated in order to simulate conditions that stimulate the maximum growth rate of algae during the summer. The reactors were lit by 4 fluorescent tubes (Aura Long Life 51W/830) with 16 h of light and 8 h of dark per 24 h at around 100 μmol/m^{2}s, measured from the inside wall of the reactor. The temperature in the reactors was set to 23 ± 0.5 °C [20] and the mixture was stirred with magnetic stirrer bars at around 350 rpm [20] throughout the experiment. Air was pumped into the reactors through a 0.22 μm Millipore filter (3 L/min) to optimize the gas exchange and to prevent excessive pH increases in the reactors.

100 mL water samples were taken from each reactor at 4 day intervals to determine nutrient concentrations, chlorophyll a concentration and pH. The pH of the samples was measured using a 744 pH meter (Metrohm AG, Herisau, Switzerland).

2.2. Wastewater and lake water origin and properties

Inflow wastewater obtained from the WWTP in Västerås (central Sweden) was used in this study. The plant uses a conventional treatment process, treating sewage from the equivalent of a 118 000 population, yielding 12 000 tonnes of dewatered (25% dry matter) sludge per year. In the current water treatment process, influent raw wastewater is screened, pre-precipitated with iron sulfate, and biologically treated by an activated sludge process with pre-denitrification supported with glycol as the external carbon and energy source. Wastewater for the experimental system was collected from the WWTP inflow (from the top layer of the center of the mixed basin).

Lake water for algal inoculation was taken from Lake Mälaren, which has an area of 1096 km^2, a mean depth of 12.8 m and a maximum depth of 64 m. It is the third largest lake in Sweden, with a water volume of 14 km^3 [8]. Mälaren lake water was collected from a yacht harbor next to the WWTP from the upper layer (0.5 m) of the lake.

All lake and WWTP samples were taken with sterilized equipment according to the SS/ISO 5667-3:2004 standard and were immediately transported and used in the experimental setup. For initial nutrient analysis 50 mL of the sampled waters was filtered through a Whatman GF/C filter (1.2 μm) and preserved with 0.5 mL of concentrated sulfuric acid (98%, Thermo Fisher Scientific, Waltham, MA, USA). The samples were stored at −20 °C until further analysis. Initial chemical compositions of the waters and mixtures used in the experiments are in Table 1.

2.3. Chlorophyll a and algal community analysis

Chlorophyll a concentration was measured at room temperature to assess algal biomass growth [1]. 25 mL of the water sample was filtered through a Whatman GF/C filter (1.2 μm). Chlorophyll a was extracted with acetone (99%, Thermo Fisher Scientific, Waltham, MA, USA). Absorbance was measured at 665 nm (chlorophyll a) and 750 nm (turbidity) with an Ultrospec 3000 spectrophotometer (Pharmacia Biotech, Sweden), and the chlorophyll a concentration (mg L^{-1}) was calculated using Eq. (1):

$$C = (A_{665} - A_{750}) * V/V_S * 11.3/L/1000 \quad (1)$$

where C is the chlorophyll concentration (mg L^{-1}), V is the volume of the solvent (mL), V_S is the volume of the sample (L), L is the light path (cm), A is the absorbance and 11.3 is the specific extraction coefficient for acetone.

Dry weight (DW) was assessed by filtering 25 mL of the sample through a Durapore 0.45 μm filter (Merck Millipore). The filters were dried and weighed before and after filtering and DW was calculated as the difference between the filter with and without the sample.

At each sampling time, 5 mL of the sample was taken for microscopic analysis of the algal community. 250 μL of Lugol's iodine was added to the samples which were then stored at 4 °C prior to microscopic analysis.

The algal community was studied using an Alphaphot-2 YS2 microscope (Nikon Instruments Inc., Tokyo). The samples were concentrated 5× by centrifugation. 50 μL of the sample was placed under a cover glass and studied under 60× lens. Images were taken using a Sony NEX 5N camera with an APS-C size sensor (1.5× crop factor).

2.4. Quantitative PCR and data analysis

25 mL of water was filtered through a 0.22 μm Millipore filter and stored at −20 °C for microbial community analysis.

DNA was extracted from the samples using a MoBio PowerWater DNA extraction kit (Mobio Laboratories Inc., Carlsbad, CA, USA). The extraction was performed according to the manufacturer's protocol. The quality and concentration of the extracted DNA were measured at 260 and 280 nm using an Infinite 200 PRO spectrophotometer (Tecan Group Ltd, Männedorf, Switzerland) and NanoQuant plate (Tecan Group Ltd, Männedorf, Switzerland).

The development of the bacterial community was estimated from 16S rRNA gene copy numbers. L-V6 (5′-GAACGCGARGAACCTTACC-3′) and R-V6 (5′-ACAACACGAGCTGACGAC-3′) primers were used to amplify the bacterial 16S rRNA gene 111 bp gene fragment from the V6 hypervariable region [5]. Quantitative PCR (qPCR) was performed with a Rotor-Gene Q (Qiagen, CA, USA). The qPCR program was as follows: 2 min at 95 °C, 45 cycles of 15 s at 95 °C, 30 s at 54 °C and 30 s at 72 °C. Melting curve analysis was performed at 65–90 °C. The standard curve was constructed using the standard plasmid as described by Nõlvak et al. [12]. 50 copies of the standard plasmid were diluted in 10 μL of reaction

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