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Nutrient uptake and lipid yield in diverse microalgal communities grown in wastewater

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ABSTRACT

Microalgae show great potential as feedstock for biofuel production. Some of the advantages they offer over terrestrial energy crops are a high productivity and the possibility to use waste streams (e.g., wastewater for inorganic nutrients and flue gas for CO_2 input). Most of the published literature on microalgal cultivation has focused on monocultures of a single strain. An alternative approach would be to cultivate multispecies communities, which can increase production if the different species in the culture have different requirements and complement each other. To study the effect of multispecies communities, we cultivated 12 different species in monocultures and randomly selected communities of three, five and seven species. This was performed using municipal wastewater, with no further nutrient addition. Growth rate, biomass production, nutrient depletion and lipid production were measured. In general, it was observed that the mean growth and nutrient depletion increased with increasing number of species in the community. The variability (e.g., nutrient removal, resource use efficiency) between communities decreased with an increasing number of species. Biomass production in communities with the strongest growth was similar to, but not higher than the production in monocultures with the strongest growth (i.e., non-transgressive overyielding). However, the highest lipid content was obtained from some of the multispecies communities, which indicates transgressive overyielding in terms of lipid production. Combining communities randomly is probably not the best approach to increase algal biomass production, carefully selecting species, based on prior knowledge of physiology and ecology, might be a more effective approach to increase productivity.

1. Introduction

Microalgae have received much attention as a potential raw material for biofuels in the last decade [1–3]. One of the benefits of using algae is that they can be grown using wastewater. Wastewater provides high amounts of inorganic nutrients necessary for algal growth, and provides a cultivation system that does not compete directly for freshwater and nutrient resources important for traditional agriculture [4].

Using algae for the bioremediation of wastewater revives an old idea [5] and most of the published literature describes studies that test algal growth in different wastewater streams ranging from municipal wastewater [6] to the treatment of animal manure [7]. Generally, algae can remove a high percentage of available nutrients [8] and environmentally hazardous components, such as heavy metals [9,10].

Recent development has focused on high rate algal ponds (HARPs), which have achieved high recovery rates of nutrients, in particular when coupled with additional CO_2 input [11]. Municipal and agricultural wastewater streams especially are rich in macronutrients (N and P) and other essential nutrients for algal growth (e.g., Fe, Cu, Mn, Zn) [12], and the nutrients are suspended in water, which is ideal for growing algae. However, algal cultivation depends a lot on the specific wastewater source, as e.g., rainfall can considerably dilute nutrients and industrial wastewater would contain compounds very different from natural sources. However, the most promising factor in this case is that nutrients in wastewater can be obtained at very low or no cost; they are in most cases not desired in water, because of problems with eutrophication and the pollution of aquatic ecosystems (if wastewaters are discharged without treatment). For these reasons, wastewater streams provide a cost-efficient way to cultivate and grow algae for bioenergy and/or biofuels purposes, without the need to purchase fertilizers.

Algae have been shown to take up nutrients rapidly and efficiently (ca. 90% of NH_4-N and PO_4-P) [13] and to accumulate high amounts of biomass with a high nutrient content, and simultaneously provide an environmental service by removing nutrients from wastewater. Diversity in algal species (species richness) is positively correlated with a higher uptake rate of certain nutrients [14,15]. Recent studies have demonstrated the potential for increasing production by including a community of species in the cultivation [16,17], while monocultures of selected microalgal strains might not be superior diverse microalgal communities in terms of lipid production. In controlled growth

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experiments, diverse microalgal communities showed a higher lipid-/biomass production and biomass-specific lipid content than corresponding monocultures [18,19]. Introducing multispecies cultivation offers the potential to increase microalgal production. However, a more mechanistic insight into the connections between biodiversity and lipid productivity is needed, to incorporate these findings into any larger scale production system.

In addition to biomass productivity, another critical requirement in the mass cultivation of microalgae is stability [20,21]. Studies in terrestrial primary producer communities have shown that communities with higher diversity provided a more stable biomass even after distinct disturbances [22]. A higher diversity in primary producer communities provides a greater variety in traits [23], which reduces variability on a temporal scale [14,21,24].

In a recent study, Corcoran and Boeing [25] showed that diversity is very important for stability and consistent biomass yields. Changing environmental conditions, for example light and temperature, throughout the year can be tolerated more easily by a diverse community of algae that contains different species and a wide range of traits.

We have previously suggested that microalgal diversity is positively linked to microalgal biomass and lipid production [18,19]. Wastewater streams are heterogeneous, e.g., they contain a complex composition of various nutrients compared with the traditional cultivation media used in algal growth experiments. To test the effect of algal diversity on biomass growth and nutrient removal from wastewater, we set up an experiment with 12 monocultures and randomly selected three, five and seven species to generate communities in municipal wastewater. Our initial hypotheses were: (1) nutrient uptake and growth are more rapid in more diverse communities; (2) an increase in microalgal diversity leads to higher lipid production; (3) variability in productivity and/or nutrient removal is lower in more diverse communities.

2. Methods

2.1. Experimental design

We performed experiments using laboratory microalgal communities that contained different levels of species richness (representing the major taxonomic groups of microalgae: Chlorophyta, Bacillariophyta, Cyanophyta and Chrysophyta). We used a set of 12 microalgal strains (SAG Culture Collection of Algae, Göttingen; UTCC, Toronto; Max Planck Institute (MPI) for Limnology, Plön) (Table S1). Each microalgal strain was pre-cultured as a monoculture in modified Woods Hole growth medium (WC-medium) [26] for several months before initiating the experiment. A diversity gradient of all monocultures and polycultures of three, five and seven different species was established. Each diversity level was replicated four times, with different randomly selected species compositions, which resulted in 24 experimental microalgal communities (Table S2).

The experiment was conducted at Suomenoja municipal wastewater treatment plant, in the city of Espoo, Finland. The experiment was performed using wastewater from an incoming line used for research purposes, originating from approximately 40 households, with no industrial wastewater included. Particulates were removed by filtration (Whatman GF/F, pore size 0.7 μm) before the cultivation of algae. During the pre-cultivation, we observed that not all the algal species could grow in undiluted wastewater, probably due to a high ammonium concentration. Therefore, we diluted the wastewater in the experiments with deionized water to a final concentration of 10% wastewater.

The initial, total microalgal biovolume was adjusted to be identical (2.4×10^6 fL mL^{-1}) in all treatments. The experiment was arranged in a batch-mode of cultivation in 650 mL cell culture flasks (CELLSTAR, Greiner bio-one) using 520 mL working volume. Cultures were maintained at ambient room temperature directly at the wastewater facility for 19 days. Constant light exposure (90 μmol photons m^{-2} s^{-1}, PAR) in 12:12 h light:dark cycle was maintained throughout the experiment. Each bottle received a constant, 0.2 μm filtered air supply (without any additional CO_2) with an air:culture volume ratio of 8 per minute (min; VVM).

2.2. Measurements

At the beginning of the experiment, the total microalgal biovolume and cell densities were estimated using a cell counter (CASY®-Cell-Counter, Schärfe-System, Germany). During the experiment, biomass was estimated fluorimetrically using an AquaPen-C AP-P 100 (Photosystem Instruments—PSI, Czech Republic). The fluorescence minimum (F_0) was used as a proxy for biomass. Biomass was estimated daily during the first week, and thereafter on average every three days. Inorganic nutrients were determined on a weekly basis according to standard colorimetric methods [27]

On the final day of the experiment, the dry weight (DW) was determined by filtration onto pre-weighted GF/F filters (Whatman, Buckinghamshire, UK) that were subsequently dried overnight at 60 °C. The filters were reweighed after drying and the DW was calculated from the difference between the filter weight with and without algal biomass.

Te microalgal lipid content was estimated by staining neutral lipids with Nile Red. To stain the algae, 1 mg fine-grained Nile Red (9-diethylamino-5 H-benzo[α]phenoxazine-5-one; HPLC grade, Sigma Aldrich, Germany) was dissolved in 4 mL acetone (HPLC grade) [28]. Subsequently, 12 μL Nile Red solution was added to 3 mL microalgal solution (pre-frozen at −20 °C), and incubated for 30 min in a darkened container. Fluorescence was measured with a spectrofluorometer (Cary Varian Eclipse, Agilent Technologies, Inc., Colorado, USA) at an excitation peak wavelength of 530 nm and an emission peak wavelength of 580 nm [29]. Fluorescence readings were corrected for the background fluorescence of Nile Red in the culture medium and for microalgal fluorescence.

At the end of the experiment, we fixed algal mixtures with Lugol's iodine to estimate the species-specific composition using the standard Utermöhl technique [30]. The cell biovolume was determined by measuring live pictures using analySIS software (Pro 2.11.006, soft-Imaging software GmbH, Germany) and calculating the biovolume according to geometric shapes [31].

Microalgal resource use efficiency (RUE) was calculated as the biomass dry weight (mg) (at the end of the experiment) as a proportion of the initially available nutrients (as a sum of the initially available orthophosphate (μmol) and inorganic nitrogen (μmol)) according to Ptacnik et al. [15].

Statistical analyses were performed using the values of each monoculture, and polycultures for each diversity level [32]. Statistical analyses were performed using linear regression and hyperbolic function methods from the values of each diversity level. Differences between groups in depleting inorganic nitrogen and orthophosphate conditions on day 10 were described using One-way ANOVA with the Holm–Sidak Post hoc test and ANOVA on Ranks with Dunn's pair wise comparison.

3. Results

3.1. Growth, nutrient uptake and quantum yield

Mixed communities removed inorganic nutrients more rapidly than the mean of single-species, and increasing species richness led to a more rapid nutrient depletion for both orthophosphate (Fig. 1A) and inorganic nitrogen (Fig. 1B). This was reflected in the growth, where increasing species richness increased the growth rate (Fig. 2). The mean growth rate, based on the F_o measurements during exponential growth was 0.23 ± 0.17 (SD), 0.31 ± 0.06 (SD), 0.40 ± 0.08 (SD) and 0.43 ± 0.04 (SD) d^{-1} for the monocultures and mixed communities of three, five and seven species, respectively. The largest variability in the growth