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Mixed microalgae culture for ammonium removal in the absence of phosphorus: Effect of phosphorus supplementation and process modeling

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ABSTRACT

Microalgal growth and ammonium removal in a P-free medium have been studied in two batch photobioreactors seeded with a mixed microalgal culture and operated for 46 days. A significant amount of ammonium (106 mg NH_4-Nl^{-1}) was removed in a P-free medium, showing that microalgal growth and phosphorus uptake are independent processes. The ammonium removal rate decreased during the experiment, partly due to a decrease in the cellular phosphorus content. After a single phosphate addition in the medium of one of the reactors, intracellular phosphorus content of the corresponding microalgal culture rapidly increased, and so did the ammonium removal rate. These results show how the amount of phosphorus internally stored affects the ammonium removal rate. A mathematical model was proposed to reproduce these observations. The kinetic expression for microalgae growth includes a Monod term and a Hill's function to represent the effect of ammonium and stored polyphosphate concentrations, respectively. The proposed model accurately reproduced the experimental data ($r = 0.952$, P-value <0.01).

1. Introduction

Interest on microalgae has increased during the last decades as they constitute a promising alternative for obtaining value-added products and biofuels such as biodiesel, biohydrogen biogas or biocrude. Moreover, microalgal systems for wastewater treatment have long been proposed and studied [1]. These systems range from open-pond cultures to closed photobioreactors [2] and focus primarily on the removal of inorganic nutrients such as ammonium, nitrate and phosphate.

Several studies have proved the suitability of microalgal cultures for nutrient removal in diverse wastewaters. These studies, which showed different degrees of nutrient removal efficiencies, generally agree that the most important advantages of microalgae utilization for this purpose are CO_2 abatement and the possibility of reusing biomass as fertilizer or as renewable source of energy [3–5]. On the other hand, the process spares the otherwise necessary cost of nutrients for algae cultivation. Currently, a rather extended opinion in the scientific community is that the production of algae-based biofuels, at least in the short-term, is neither economically nor energetically feasible without simultaneous wastewater treatment [6].

Phosphorus is an essential component of microalgae. According to the Redfield ratio [7], it constitutes 0.87% of its dry weight. Phosphorus is present in basic cell constituents such as phospholipids, nucleic acids or nucleotides. It can also be accumulated to higher levels inside the microalgal cells, where inorganic polyphosphate serves as reservoir. As reviewed by Powell et al. [8], there are two mechanisms involved in this accumulation: over-compensation, which occurs after re-exposure to phosphorus following a starvation phase, and luxury uptake, where microalgae accumulate much more phosphorus than it is needed for their survival without previous exposure to P-poor medium.

Different studies, which aimed at defining the polyphosphate accumulation and phosphate uptake dynamics, have shown a relationship between phosphorus stress in the medium and low polyphosphate content in the cells, together with recovery of polyphosphate levels after addition of phosphorus [9,10]. It is also known that starvation enhances the phosphate uptake rate. The effect of P-starvation on ammonium uptake rate is, however,

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less known. Previous studies did not focus on the influence that polyphosphate content exerts on the nitrogen uptake velocity, as these studies were not undertaken with a wastewater treatment approach.

In the wastewater treatment field mathematical models are useful tools for process design, WWTP scale-up or upgrade, or water quality prediction. Up to now, microalgal growth modeling has been tackled with a diversity of approaches. There are various examples of different complexity-level models which determine phytoplankton evolution in the ecosystems [11–13], content and evolution of intracellular components of interest such as lipids or sugars [14], specific metabolism of single species [15], microalgal production inside photobioreactors [16] or others.

The present work was designed to study the ammonium removal process in a phosphate-free medium and the relationship between the microalgal intracellular phosphorus content and the ammonium removal rate, with a view to designing suitable strategies for wastewater treatment. Therefore it is also the aim of this work to define a kinetic expression for microalgae growth considering the effect of ammonium concentration in the medium and the amount of internally stored polyphosphate on the rate of this process. To this aim, a mathematical model considering microalgae growth and death was proposed and model parameters were obtained by minimizing differences between experimental data and model predictions. This model should be useful for prediction of ammonium removal rates in wastewater treatment systems.

A microalgal culture was fed only with ammonium in a lab-scale photobioreactor (PBR) and afterwards separated into two identical PBRs. Phosphate was supplied only to one of them. Nutrient uptake kinetics of the two PBRs were studied, as well as biomass composition (%N and %P). Microalgae production – in terms of chemical oxygen demand and suspended solids – was assessed. The experimental data obtained was successfully reproduced by the proposed model. This model can be useful for designing strategies and predicting the behavior of wastewater treatment systems where nutrient removal is achieved by microalgal growth.

2. Materials and methods

2.1. Experimental setup

Three identical PBRs were used in this study (*initial reactor*, *Nitrogen only reactor* and *Nitrogen and phosphorus reactor*, as it will be explained in Section 2.2). Each PBR consisted of a cylindrical, transparent methacrylate tank (20 cm internal diameter) with a total volume of 10 l (see Fig. 1a). The PBRs were closed and the algae culture was mixed by recycling the headspace gas through four fine bubble diffusers mounted at the bottom. Both PBRs were equipped with electronic sensors in order to obtain online measurements of conductivity, oxidation reduction potential, temperature, pH and dissolved oxygen. The probes were connected to a multiparametric analyzer (CONSORT C832, Belgium) and an oximeter (Oxi 320, SET WTW, Germany), respectively. These devices were in turn connected to a PC for data monitoring and storage. Data sampling was conducted every 60 s.

pH in the PBRs was maintained around 7.5 to avoid undesirable processes such as phosphate precipitation and free ammonia stripping. Pure CO_2 (99.9%) from a pressurized cylinder was injected into the gas flow whenever pH exceeded the setpoint of 7.5. Recycling gas from the headspace contributes to minimize the CO_2 requirements for pH control. Since the reactors were closed CO_2 stripping was also minimized but since they were not hermetically sealed extreme overpressure and overaccumulation of oxygen were avoided. Four arrays of 3 vertical fluorescent lamps (Sylvania Grolux, 18 W) 10 cm apart from each other continuously illuminated each PBR from a minimum distance of 10 cm. Photosynthetically active radiation (PAR) of $153 \pm 16\ \mu E\ m^{-2}\ s^{-1}$ was measured at the surface of the reactors as the arrow in Fig. 1b) indicates. The PBRs were placed inside a climatic chamber with air temperature control set to 20 °C. Due to the constant illumination the temperature in the culture resulted in 25.5 °C.

A phosphate-free medium, adapted from [17] was used in this study, 1 l of which was composed of 115 g $(NH_4)_2SO_4$, 150 mg $CaCO_3$, 400 mg $CaCl_2{\cdot}H_2O$, 400 mg $Na_2SeO_3{\cdot}5H_2O$, 350 mg $MgSO_4{\cdot}7H_2O$, 54 mg $(NH_4)_6Mo_7O_2{\cdot}4H_2O$, 30 mg $ZnCl_2$, 30 mg HBO_3, 30 mg $NiCl_2{\cdot}6H_2O$, 18 mg $CuCl_2{\cdot}2H_2O$, 12 mg K_2SO_4, 1.2 mg $FeCl_3{\cdot}4H_2O$, 1.2 mg $CoCl_2{\cdot}6H2O$, 0.6 mg EDTA, 0.3 mg $MnCl_2{\cdot}4H_2O$.

2.2. Operation

7 l of a microalgal culture was maintained for 19 days in ammonium-rich and phosphate-free medium in a lab-scale PBR as described in Section 2.1, called *initial reactor*. Ammonium in the form of $(NH_4)_2SO_4$ was manually added at the beginning of the experiment and when its concentration dropped below 4 mg NH_4-N l^{-1} (day 7). On day 19, when ammonium concentration had reached again 4 mg NH_4-N l^{-1}, the 7 l culture was split into two PBRs, with a working volume of 3.5 l each. These two PBRs will henceforth be called *NOP* (*Nitrogen only reactor*) and *N&PR (Nitrogen and phosphorus reactor*) and were not carried out in duplicate. Immediately after the splitting, ammonium in the form of $(NH_4)_2SO_4$ was added into *NOR*, reaching a concentration of 28 mg NH_4-N l^{-1}, and phosphate in the form of KH_2PO_4 was added into *N&PR*, reaching a concentration of 12 mg PO_4-P l^{-1}. From then on, both reactors were operated for 27 days. Ammonium was added again in both reactors when its concentration dropped below 4 mg NH_4-N l^{-1} (day 29 in *NOR* and days 20, 22 and 29 in *N&PR*).

2.3. Microorganisms

The *initial reactor* was seeded with microalgae isolated from the walls of the secondary clarifier in the Carraixet WWTP (Valencia, Spain) and maintained in the laboratory under semi-continuous feeding conditions with a cellular retention time of 4 days and continuous illumination varying between 114 and 198 $\mu E\ m^{-2}\ s^{-1}$. The effluent of a submerged anaerobic membrane bioreactor (SAnMBR, described in [18]) was used as growth medium. This effluent displays a variable N/P ratio and has been proved to sustain algal growth [5]. Microalgae from the *Chlorococcum* genus together with cyanobacteria (*Spirulina* sp. and *Pseudoanabaena* sp.) were identified as the main groups present.

2.4. Analytical methods

Nutrient removal was evaluated by regular measurements of inorganic nitrogen and phosphorus levels in the samples taken from the PBRs. Ammonium (NH_4-N), nitrite (NO_2-N), nitrate (NO_3-N) and phosphate (PO_4-P) were determined according to Standard Methods [19] (4500-NH3-G, 4500-NO2-B, 4500-NO3-H and 4500-P-F, respectively) in a Smartchem 200 automatic analyzer (Westco Scientific Instruments, Westco). Total nitrogen in the algae culture was measured using standard kits (Merck, Darmstadt, Germany, 100613). The acid peroxodisulphate digestion method [19] was used for total phosphorus (TP) measurements. The nitrogen content of the algae biomass was calculated as the difference between total nitrogen and soluble nitrogen. Likewise, the phosphorus content of the algae biomass (total suspended phosphorus, TSP) was calculated as the difference between total phosphorus and orthophosphate concentration. Total and volatile suspended solids (TSS and VSS), as well as chemical oxygen demand (COD) were determined according to Standard Methods [19].

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