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Integration of *Chlorella protothecoides* production in wastewater treatment plant: From lab measurements to process design

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ABSTRACT

The exploitation of microalgae in a wastewater treatment process is currently an open issue, and its actual applicability is still under investigation. In this work the effects of temperature, day/night irradiation and bacterial competition were studied on growth and nutrient removal of *Chlorella protothecoides* cultivated in real and unsterilized primary urban wastewater. *C. protothecoides* showed a linear dependence of growth rate on temperature under continuous irradiation with a maximum at 30 °C. Continuous flow experiment under day–night irradiation condition showed that a cyclic steady state was achieved, with significant differences in biomass concentration and nutrient removal after dark and light periods. The presence of a native microflora in wastewater did not affect the microalgal growth, both in batch and continuous flow experiments. N and P were efficiently removed in all conditions tested, while the COD was not consumed by microalgal biomass. Thus, in view of a large scale application, a two step depuration process would be required, where the photobioreactor will remove nutrients as N and P and an activated sludge reactor downstream will reduce the organic matter. The experimental data obtained were used to design a possible process of this type.

1. Introduction

It is recognized worldwide that the demand of liquid fuels is expected to grow quite fast, leading to a tremendous development of biofuel production technologies. Among these, in the last decade the biodiesel derived from the cultivation of microalgal biomass has been proposed as an effective method to produce high quality, sustainable and renewable biofuel [1]. However, industrial processes based on the use of fresh water, synthetic CO_2 and chemical fertilizers is not economically sustainable [2]. In fact, many LCA studies have shown that the whole process has a high energy demand that leads to a negative overall energy balance [3–5], and many authors have highlighted that the environmental and economic benefits of microalgae utilization are not always clear and effective when passing from laboratory to a large scale [3,6,7]. For these reasons, nowadays, there is no commercial plant producing and processing microalgae biomass into biofuels yet [2]. One of the major issues related to microalgae biomass production concerns the nutrient availability [8,9] as the cultivation of microalgae at industrial scale for biofuel production requires a large amount of nutrients, typically nitrogen and phosphorous. The idea of exploiting nutrients from wastewaters to grow microalgae goes back to the 50s, when early studies were carried out by Oswald [10], but it is only in the last decade that researches have focused on this field [11–14], due to the need to find more sustainable solutions for fuel production within a short time. In addition, coupling the microalgal production with water treatment can improve its sustainability, as the nitrification–denitrification processes usually implemented for nutrient reduction are actually high energy-intensive and require huge capital costs [15]. A carefully engineered approach could improve the overall process yield, reducing the total energy demand and, at the same time, producing a biomass feedstock with high energy content. Some key issues for industrial applicability remain still unsolved, such as environmental fluctuations of temperature, light availability and possible competition with native microflora present in wastewaters.

Along the year, wastewaters in treatment plants are characterized by significant temperature changes, depending on geographical position and climate that affect the sludge growth kinetic. Wastewater temperature is usually higher than that of the water supply thanks to the addition of solid waste and warmer water from household appliances, so that their temperatures usually range from 10 °C to 30 °C [15] at mid-latitudes.

In addition, in an actual photobioreactor operated outdoor at mid-latitudes, the light conditions are widely variable, so that a number of issues must be thoroughly addressed, i.e. reactor orientation, light variation during the day, reflection of light, light gradient in the reactor [16].

Obviously, when using wastewater as the feed for microalgae cultivation, the wastewater cannot be previously sterilized due to the enormous volumes to be processed. In these conditions, many species (including bacteria) would necessarily coexist in the culture together

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with microalgae. A classical wastewater treatment biological reactor is characterized by the presence of thousands of species belonging to almost all of biological kingdoms [15]. Due to this huge genomic and phenotypic variability, the study of this ecosystem (the so-called "activated sludge") is extremely complex [17], even if improving knowledge of the ecosystem composition could lead to a better understanding of the biochemical reactions occurring within the reactor, thus allowing the enhancement of its performance [18]. To date, some microalgal bacteria consortia have been tested by many authors with the aim of nutrient removal from water [11–14]. The feasibility of this process at industrial scale in continuous systems is not clear, in particular concerning a long term competition between bacteria and photosynthetic organisms.

The objective of this work is to assess the exploitation of wastewater as nutrient source for microalgal growth, in order to better understand this process, and to give a contribution towards its industrialization and large-scale application: the influence on growth of several parameters, as the effect of temperature and real irradiation, COD consumption and competition with native microflora were considered.

To this aim, *Chlorella protothecoides* was cultivated in real non-sterilized wastewaters, taking into account the effect on growth kinetic of actual temperature range and alternation of day–night cycle. Nutrient removal was measured: in particular we focused on nitrogen, phosphorus and chemical oxygen demand (COD) concentration, in view of developing an integrated system where bacteria and microalgae cooperate in the water treatment process. Bacteria contamination in different conditions tested was also considered as a rough measure of a possible coexistence in a continuous bioreactor. Experiments under day–night irradiation were carried out both in batch and in continuous, in order to verify the biomass productivity. Starting from experimental results, the model parameters of nutrient kinetic uptake were correlated with the measured data. Eventually, a possible integrated process was proposed and nutrient, biomass and energy balances were applied to a preliminary process design.

2. Materials and methods

2.1. Microalgae strains and growth experiments

C. protothecoides 33.80 (from SAG Goettingen, Germany), was maintained in liquid BG11 medium [19] for the pre-inoculum. Microalgae were cultured in wastewaters sampled from a treatment plant (ETRA S.p.A.) in Camposanpiero, Padova (PD), Italy, which treats domestic wastewater. The water was sampled after the primary treatment. No sterilization treatment was carried out (excluding one experiment, as reported in Section 3.3: in this case the sterilization of wastewater was performed by autoclave at 121 °C for 20 min). Water was maintained in a refrigerator before each experiment. Experiments of microalgal growth were carried out both in batch and continuous systems. Due to the nutrient content of sampled wastewater, which was not constant with time, all batch experiments were carried out with water from a single sampling from the plant, and nutrients were measured at time 0 of each growth curve, in order to verify the effective consumption by microalgae. Initial nutrient concentrations are reported in Table 1. Each batch experiment was carried out in at least two replicates, and started with an initial microalgae inoculation of $OD_{750} = 0.5$, corresponding to a cell concentration of about 20×10^6 cells mL^{-1}. As reported in Fig. 1A (data provided from the treatment plant where waters were sampled), inlet wastewaters in a treatment process are exposed to seasonal temperature fluctuations. Thus, a number of experiments were performed at 10, 15, 23 and 30 °C under continuous irradiation of 100 μmol photons m^{-2} s^{-1}. Other batch experiments were carried out to verify the effect of bubbling of CO_2 and air-only on COD consumption and bacterial competition, at 23 °C and 100 μmol photons m^{-2} s^{-1} of irradiation.

Table 1
Initial and final concentrations of nutrients in batch experiments under continuous irradiation at different temperatures.

Temperature	Total N				Total P			
	Initial		Final		Initial		Final	
(°C)	(mg L^{-1})		(mg L^{-1})		(mg L^{-1})		(mg L^{-1})	
	Mean	St.dev	Mean	St.dev	Mean	St.dev	Mean	St.dev
10	38.71	1.02	10.97	8.52	2.53	0.06	0.006	0.003
15	31.51	2.62	1.01	0.09	2.61	0.07	0.01	0.04
23	28.74	0.26	1.87	0.86	2.34	0.02	0.01	0.03
30	38.48	1.22	4.99	0.12	2.47	0.02	0.01	0.007

A continuous flow experiment was carried out in order to test *C. protothecoides* behavior in more realistic conditions at 23 °C and day–night irradiation (sunlight cycle of October in Padova, Italy). The continuous experiment was carried out with water collected during a second sampling at the water treatment plant, and the initial nutrient content was specifically measured. In order to remove the suspended solids and avoid tubes clogging in the continuous flow experiments, waters have been subjected to a first filtration treatment by paper filter of 100 μm of particle retention. This resulted in a lower COD concentration of water used in continuous experiment, with respect to those of batch experiments.

2.2. Experimental apparatus

Pre-inoculum and batch experiments were performed in glass bottles of 250 mL, continuously mixed by magnetic stirring and bubbling air enriched with 5% v/v of CO_2, which also provided a non-limiting CO_2 supply. The total gas flow rate was 1 L h^{-1} for each bottle. The temperature was controlled by an incubator (Frigomeccanica Andreaus, Padova), and artificial light (white neon lamps OSRAM) was provided continuously at an intensity of 100 μmol m^{-2} s^{-1} of PAR (photosynthetic active radiation) photons measured by a photoradiometer (Model LI-189, LI-COR, USA).

Continuous flow experiment was carried out in a flat plate vertical reactor of 250 mL of volume (see [21]), reasonably assumed as a perfectly mixed reactor (CSTR, or chemostat) as confirmed by tracer experiments [22]. Alternated day–night cycles were generated with a LED lamp (Photon System Instruments, SN-SL 3500-22). The light intensity as a function of time was simulated so as to provide the PBR with the same PAR amount of energy received under natural conditions at the selected latitude. PVGIS Solar Irradiation Data [23] is an online available database of typical day evolution of irradiation on a given surface for any earth location and time of year. This software was used as the source of irradiation data for the location of Padova, Italy (Fig. 1B). An incident angle of 35° was applied, as the default setting of the database, in order to exploit the maximum solar energy. The light intensities were measured both at the front and at the back sides of the reactor by the photoradiometer, in order to verify the actual light absorbed by the panel.

C. protothecoides was inoculated at the beginning into the reactor with non-sterilized fresh wastewater medium. To prevent the occurring of washout, the reactor operation was started in batch mode. Once it reached a significant concentration (the order of 10^8 cells mL^{-1}), the operational mode was switched from batch to continuous, feeding non-sterilized wastewater by a peristaltic pump (Sci-Q 120S, Watson Marlow, USA). Inlet wastewater storage tank was continuously mixed with a magnetic stirrer, and maintained at the same temperature of the reactor. In order to avoid any increase of contamination, the water in the inlet tank was replaced every day. The liquid level in the reactor was controlled with an overflow tube placed close to the top, and the outlet flow was collected in a bottle. So, the residence time in the reactor (τ) was directly controlled by the peristaltic pump. A flow rate of 130 mL d^{-1} was set, thus leading to a residence time (τ) of 1.9 days. Steady-state operation was reached after 5 days and maintained for

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