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Development of an Integrated Process for Bio-Oil Production from Microalgae

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In this paper an integrated process for bio-oil, fine chemicals and energy production from microalgae in photobioreactors is proposed. The proposed process is aimed to: fix carbon dioxide coming from thermovalorization plants; use the industrial effluents containing organic carbon and nitrogen for the heterotrophic growth of microalgae; obtain bio-oil as raw material for biodiesel production; produce high value-added fine chemicals; increase the thermal value of the solid residue for energy production.
The selected strains (*Chlorella vulgaris* and *Nannochloropsis oculata*) were used in lab-scale reactors in order to optimise the operating conditions for maximising oil production.

1. Introduction

The development of new technologies producing alternative and renewable energy is a priority target of institutional governments and private enterprises due to the depletion of fossil oil reservoirs and the dramatic increase of energy requirements from emerging countries. The Mediterranean Basin is characterized by wide regions with significant solar irradiation which could be exploited for the production of renewable combustibles and, among then, oil for biodiesel and hydrogen (Scott et al., 2010).
Biodiesel can be produced by alkali treatment with methanol of different kinds of oils as raw material. Oils are mainly obtained by agro-industrial productions (first generation biocombustibles). Actually in Europe, and in particular in Italy, biodiesel is produced using palm oil imported from Asia making rise the global impact associated to the production of this biocombustible due to energy consumption for the transport. The low yield of oil from agricultural cultures along with the growing need of fields to be used for food production, make microalgae a good alternative candidate for oil production. Some microalgae can give up to 70% of oil as dry weight; they grow easily and rapidly if exposed to the proper solar irradiation, and oil extraction can be simply obtained by pressing them (Wijffels and Barbosa, 2010).
The availability in the Mediterranean Basin of oil as raw material for biodiesel production will contribute to the diffusion of this biocombustible by diminishing its price (today up to 90% of production costs for biodiesel can be due to raw material). In addition building such full-plants in geographical regions that are without alternative use, will be a way to valorize them promoting the technological, economical and social development of the area.

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In this context, this work is aimed to develop an integrated process for bio-oil, fine chemicals and energy production from microalgae in photobioreactors. To this purpose, an economic funding from the Italian Ministry for the Environment and Territory was obtained.
The proposed process is aimed to: fix carbon dioxide coming from thermovalorization plants (about 2 Kg/m2 of CO2 per day); use the industrial effluents containing organic carbon and nitrogen for the heterotrophic growth of microalgae; obtain bio-oil as raw material for biodiesel production; produce high value-added fine chemicals (i.e. sterols, carotenoids, proteins, polyphenols); increase the thermal value of the solid residue (about 0.6 Kg/m2 of dry biomass per day) for energy production.
The lab-scale optimisation of the operating conditions was carried out. In particular the selected strains (*Chlorella vulgaris* and *Nannochloropsis oculata*) were used in lab-scale reactors in order to optimize the operating conditions for maximizing biomass and oil production. Specifically the effect of strain type and feed composition (concentration of nitrate and addition of organic substrates) were addressed.

2. Materials and Methods

2.1 Microalgae cultivation

Two microalgal species were used in this study, specifically *N. oculata* and *C. vulgaris* (from SAG Culture Collection, University of Göttingen, Germany). Both microalgae are eukaryotic photosynthetic microorganisms that grow rapidly due to their simple structure (Converti et al., 2009). *C. vulgaris* was grown in the Bold's Basal Medium and *N. oculata* on the Guillard f2 using carbon dioxide (supplied with a CO_2 cylinder, 98%) and $NaNO_3$ as the sole sources of carbon and nitrogen, respectively.

2.2 Culture system

Growth experiments were performed at different concentrations of nitrogen and glucose in 0.5 L-Erlenmeyer flasks. These tests were performed using both strains (*C. vulgaris* and *N. oculata*). The medium and flasks were sterilized in an autoclave for 30min at 120 ◦C in order to prevent any contamination during the early stages of growth.
The growth was done using a thermostated incubator equipped with artificial lighting; the carbon dioxide was supplied with a cylinder for 4 hours a days (CO_2 at 98%). For the remaining time growth was done using the CO_2 contained in air (about 300 ppm), continuously injected through pumps. Each batch cultivation was carried out in duplicate for about 30 days.
Nitrogen and glucose concentrations in the medium were selected on the basis of literature references (Converti et al., 2009; Xiaoling and Qingyu, 2006).
Because the literature suggested that nitrogen limitation could exalt the lipid content and the growth of many microalgae, the central concentrations of nitrate in medium (0.15 g L^{-1} for both *N. oculata* and *C. vulgaris*) were selected according to Bold's Basal Medium and Guillard f2 Medium, and additional cultivations were run at 0.1 and 0.75 g L^{-1}.
Heterotrophic growth for both species was stimulated by adding glucose (1% w/v). For comparison purpose control tests were performed using standard growth conditions.

2.3 Determination of microalgae concentration

The microalgae concentration was determined daily by optical density measurements at 690 nm by a UV–vis spectrophotometer, model Cary 50 (VARIAN, INC). All measures were carried out in triplicate, and biomass concentration (cell*10^6 mL^{-1}) was related to optical density by the equation $y = 1.188 \cdot x$ (R^2 = 0.968) for *C. vulgaris* and $y = 1.101 \cdot x$ (R^2 = 0.951) for *N. oculata*, respectively. These relations were determined by a direct microscopic count with a LEITS Laborlux 12 POL microscope.

3. Results and Discussion

The effects of nitrate concentration and glucose presence on batch growth of the two selected microalgae were studied.

3.1 Effect of nitrate concentration on microalgae growth

Firstly, an investigation was carried out on the effect resulting from a reduction of the nitrogen concentration in the medium. Nitrogen limiting conditions were in fact reported to significantly increase the biomass production and the lipid fraction of many microalgae (Converti et al., 2009). For this purpose, the concentration of nitrate in both media for *N. oculata* and *C. vulgaris* batch growth was reduced to half and third of the standard media described in Section 2 while the light intensity and the CO_2/air flux were kept the same trough the experiments.

The effect of a reduction of $NaNO_3$ concentration on *C. vulgaris* growth is reported in Figure 1, showing that biomass increases as nitrate decreases. The same result was obtained for *N. oculata* (Figure 2). Literature reports that nitrogen limitations also cause an increase in the lipids production. Tests for the selection of the best lipid extraction methodology are in progress.

Generally, it can be observed that biomass production is higher for *N.oculata* than for *C.vulgaris.*

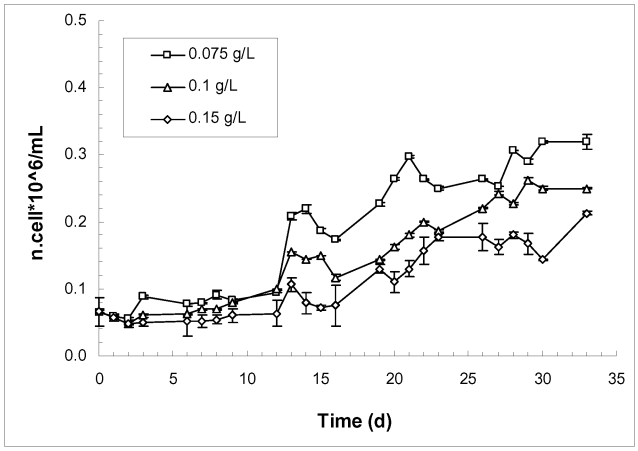

Figure 1: Growth of C. vulgaris at different $NaNO_3$ concentration in the medium.

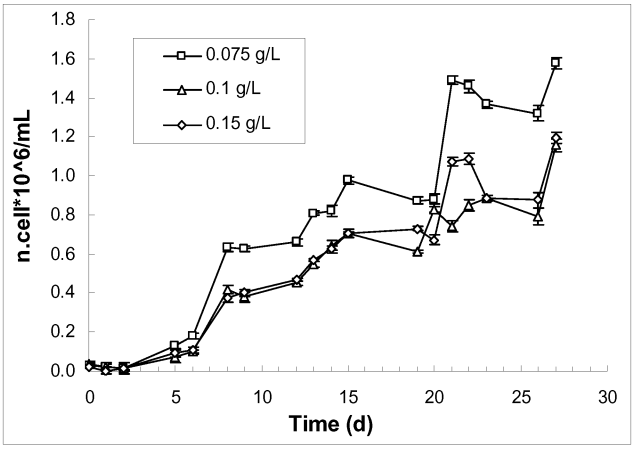

Figure 2: Growth of N. oculata at different $NaNO_3$ concentration in the medium.

3.2 Effect of glucose on microalgae growth

Heterotrophic growth of some microalgae has been used for efficient production of biomass and some metabolites such as lipid which can reduce the cost of microalgal biomass production and microalgal oil production (Xiaoling and Qingyu, 2006).
Heterotrophic growth for both species was evaluated by adding glucose (1% w/v). For comparison purpose control tests were performed using standard growth conditions. The light intensity and the CO_2/air flux were kept the same trough the experiments.
The effect of glucose addition on *C. vulgaris* growth is reported in Figure 3, showing that biomass production increases in a significant way adding the organic substrate. The same result was obtained for *N. oculata* (Figure 4).
Generally, it can be observed that biomass production is higher for *N.oculata* than for *C.vulgaris.*

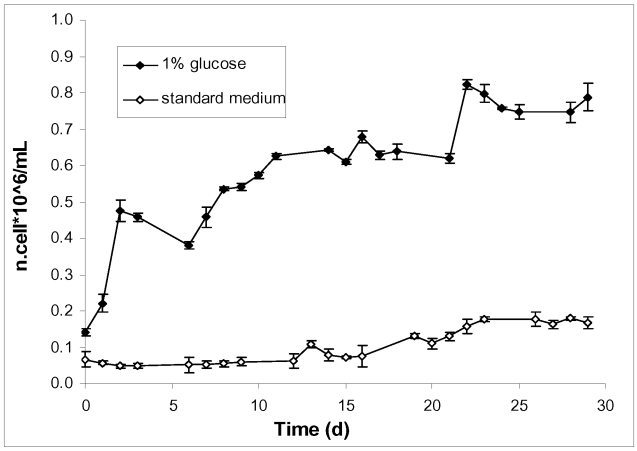

Figure 3: Growth of C. vulgaris in presence and in absence of glucose in the medium.

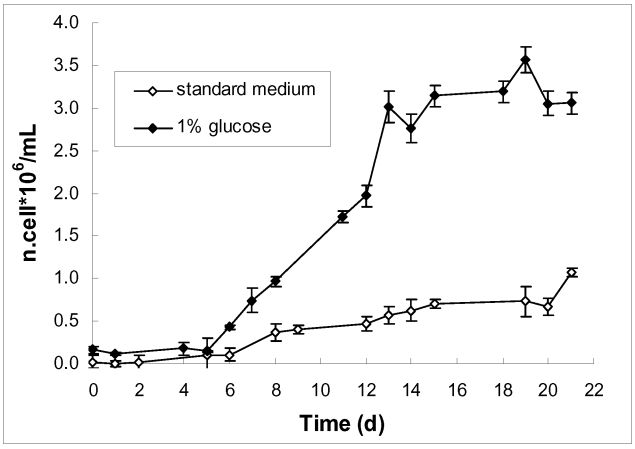

Figure 4: Growth of N.oculata in presence and in absence of glucose in the medium.

The next step will be the use of industrial effluents containing organic carbon and nitrogen, instead of glucose, for the heterotrophic growth of microalgae.
Subsequently, we are going to verify if these optimized conditions are also able to lead to an higher lipid production.

4. Conclusion

In this paper an integrated process for bio-oil, fine chemicals and energy production from microalgae in photobioreactors is proposed. Two microalgal species were used in this study, specifically *N. oculata* and *C. vulgaris.* The selected strains were used in lab-scale reactors in order to optimize the operating conditions for maximizing biomass and oil production. Specifically the effect of nitrate concentration and addition of organic substrates were addressed. The variation of parameters tested strongly influenced the microalgae production. In particular nitrogen limiting conditions and organic substrates stimulate biomass growth.

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