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Cultivating and harvesting of marine alga *Nannochloropsis oculata* in local municipal wastewater for biodiesel

Sema Şirin *, Mika Sillanpää

Laboratory of Green Chemistry, Lappeenranta University of Technology (LUT), Sammonkatu 12, 50130 Mikkeli, Finland

HIGHLIGHTS

- The feasibility of seawater and wastewater mixture to produce algae was studied.
- High TP and TN removals were obtained with sufficient biomass concentration.
- Pre-concentration methods for the grown cultures were studied.
- Dewatering with autoflocculation is found promising.

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ABSTRACT

The feasibility of using the mixture of seawater and municipal wastewater; (1) the wastewater before activated sludge tank, just after primary settling (BAS) and (2) the wastewater after activated sludge tank, just before addition of polymer flocculants (AAS); as culture medium for the cultivation of marine microalgae *Nannochloropsis oculata* was investigated.

10% BAS, 20% BAS and 10% AAS, 20% AAS, 50% AAS, 70% AAS, 100% AAS effluent loadings were well adapted to used wastewater. Sufficient dry weights obtained (345–406 mg L^{-1}) with growth rates 0.37–0.45 for aerated cultures. High TN and TP removals (~74–90%) were achieved. Harvesting technique for grown cultures was also studied with natural sedimentation and pH induced flocculation. By alkalinity induced flocculation, at pH values of 10.50, high recovery of the cells (~80%) achieved with high sedimentation rates in 10 min. The flocculation efficiencies decreased, sedimentation rates increased with the increase of the cell concentration.

1. Introduction

With increasing concerns of global climate change and varying fossil fuel prices, alternative energy sources have attracted growing attention in industry and research. From different types of energy sources; algae biodiesel announced to be a potential source due to containing no sulfur and performing as well as petroleum diesel, while reducing emissions of particulate matter, CO, hydrocarbons, and SO_x, where emissions of NO_x may be higher in some engine type (Delucchi, 2003). It can be used in current diesel car infrastructure without major engine modifications as well (Huang et al., 2015).

Algae biodiesel is also likely to have a much lower impact on the environment and on the world's food supply than conventional biofuel-producing crops (Chisti, 2007).

For sustainable wastewater treatment systems, microalgae can offer some interesting advantages over conventional systems. Microalgae-based treatments are more cost effective to remove biochemical oxygen demand, pathogens, phosphorus and nitrogen than activated sludge process and other secondary treatment processes (Green et al., 1996). They also require low energy (Oswald, 2003) and reduce the sludge formation and GHG emission. They can also produce useful algal biomass which can be used for biofuel or biofertilizer production (Mulbry et al., 2005). One of the major requirements of wastewater treatment is the need to remove high concentrations of nutrients in particular N and P. It was observed that introducing algae pond process to conventional treatment process enhanced cost-efficiency and it had proven abilities of removing nitrogen, phosphorus, and chemical oxygen demand (Wang et al., 2010).

In this work, *Nannochloropsis oculata* was used as a microorganism in the field of marine biotechnology because of its high lipid content and it has been widely used as feedstock in aquaculture (Chiu et al., 2009).

* Corresponding author.
E-mail address: semasirin@gmail.com (S. Şirin).

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Microalgae harvesting involves the concentration of dilute microalgal culture where the key challenge especially in industrial-scale is high operational cost (at least 20–30% of the total cost) in downstream processing (Kim et al., 2005). Therefore, the harvesting strategy has to be based on a low energy method in order to overcome the problems and make algal production economically feasible. In addition, microalgae harvesting is also very important in wastewater treatment with stabilization or oxidation pond, drinking water supply and environmental management of inland water (Xu et al., 2011). Many harvesting methods have been developed for microalgae recovery, such as centrifugation, filtration, flocculation and flotation, the possibility of (auto)flocculation by pH is one of the promising biological harvesting methods.

The goal of this study is:

- To determine the effects of different ratios of municipal wastewater collected from two different points on growth of *N. oculata*. Biomass concentrations, F_v/F_m ratios, dry weight and ash free dry weight, TN and TP removal of the cultures were studied. Zeta potentials of growth microalgae were also examined.
- To determine a simple, rapid and an efficient pre-concentration method – preferentially cost effective – through testing sedimentation by gravity and sedimentation by induced pH (simulation of autoflocculation). Sedimentation rates were also obtained to assist the designs to scale-up processes.

2. Methods

2.1. Microalgal strain and culture media

The marine algae *N. oculata* SCCAP K-1281was purchased from The Scandinavian Culture Collection of Algae and Protozoa at the University of Copenhagen. Artificial seawater (SW) was prepared by aquarium salt (Red Sea's Coral Pro Salt). In order to reach required salinity, the amount of aquarium salt dissolved in a certain volume of MilliQ water. Salinity was checked with Atago Master-S/Mill M series refractometer. L1 medium was used in test tubes with 20 mL and in conical flasks with 200 mL (in 500 mL flasks) medium cultures to cultivate the alga specie. General experimental conditions for 200 mL cultures, unless specified otherwise, included incubation in 25 ppt sterilized SW at 25 ± 1 °C under continuous illumination of 50 μmol photons $m^{-2}s^{-1}$. All cultures were stationary without aeration. To prevent sedimentation, flasks were shaken by hand 2–3 times daily. Other kinds of growth medium, Walne's medium and enriched SW, were also tested for cultivation of *N. oculata* species for 200 mL cultures. The enriched seawater was artificial seawater supplemented with additional basic elements containing 300 mg L^{-1} urea, 45 mg L^{-1} $NaH_2PO_4{\cdot}2H_2O$, 190 mg L^{-1} $NaSiO_3{\cdot}5H_2O$, 70 mg L^{-1} Na_2EDTA and 6 mg L^{-1} $FeCl_3$.

Table 1
Characteristics of municipal waste water samples.

Parameters	Waste water before activated sludge tank (BAS)	Waste water after activated sludge tank (AAS)*
pH	7.4–7.6	6.8–7.1
Turbidity (NTU)	140–160	3–5
TOC (mg L^{-1})	201–311	8–26
COD	190–310	24–99
Total suspended solids (mg L^{-1})	95.8 ± 4.9	–
TP (mg L^{-1} P–PO_4^{-3})	4.4 ± 3.4	2.3 ± 1.35
TN (mg L^{-1} TN_b)	57.9 ± 7.6	12.1 ± 2.9
Ammonium (mg L^{-1} NH_4–N)	45.6 ± 7.9	10 ± 3.8

* Sedimented and filtered.

2.2. Municipal wastewater (MW) samples

Wastewater samples were collected from two different points of municipal wastewater plant located in Mikkeli, Finland. The two wastewater samples were; (1) the wastewater before activated sludge tank, just after primary settling: (BAS); (2) the wastewater after activated sludge tank, just before addition of polymer flocculants: (AAS). For algal cultivation experiments, the samples were filtered using glass microfiber to remove large particles and indigenous bacteria. Characteristics of BAS and AAS samples are shown in Table 1.

2.3. Cultivation of N. oculata in MW

Growth of *N. oculata* (in 500 mL flasks with 200 mL medium) was tested in the mixture of MW and SW with the ratios of 10%, 20%, 50%, 70% and 100% for both BAS and AAS. The salinity of the media was adjusted with aquarium salt. Sodium silicate were added as the same ratio of Walne's medium due to use of aquarium salts for salinity. All the media were autoclaved prior to experiments. The culture grown with Walne's medium was served as control.

At the end of adaptation experiments, depending on the growth performance in the media, larger volume cultures were grown in 2 L volumetric flasks. Continuous illumination with daylight fluorescents TDL24W-840 with a 70–100 μmol photons $m^{-2}s^{-1}$ was used. A stream of air was injected with flow rate of 9 L min^{-1} to provide CO_2 and culture agitation.

2.4. Analytical methods

2.4.1. Growth and biomass analyses

To see the growth performance and/or the adaptation of algae to different media, pH, change in cell density (cells L^{-1}) and the maximum yield of photosystem II (F_v/F_m) were measured daily. pH of the cultures were measured daily during the experiments with WTW pH-730 pHmeter.

DELTA OHM – HD 2302.0 – Photo-Radiometer was used to check the light transmission during the growth of algal cultures.

Cell density: Cell growth was monitored by means of daily cell counts with a flow cytometer (BD Accuri™ C6). Daily samples were also monitored with a UV–visible spectrophotometer (Perkin Elmer LAMBDA 45 UV/Vis) using optical density (OD) measurements at 750 nm. The relationship between cell density and optical density was calculated by means of least square regression fitting by inter-plotting absorbance levels at 750 nm obtained by correlating cell counts taken with flow cytometer. The correlation of the measurements is described by the equation $y = 58.318 * 10^7x + 235286$ ($R^2 = 0.9986$) for the wavelength of 750, where y represents the cell density (cells mL^{-1}) and x is the absorbance value at the required wavelength.

Ash-free dry weight (AFDW): Aliquots of algal suspension were filtered through preweighed, precombusted (400 °C, 2 h) glass fiber filters (Whatman GF/F Glass Microfiber filter 4.7 cm, nominal pore size 0.7 μm) to obtain dry weight and ash free dry weight. Details of filtration and dry weight calculations can be find in elsewhere (Sirin et al., 2012).

Efficiency of photosystem II: The maximum photochemical quantum yield of PSII (F_v/F_m) was measured using the Pulse Amplitude Modulated (PAM) (Walz GmbH-PAM control WATER-ED) with 30 min dark adapted samples.

The effect of harvesting treatments on the photosynthetic activity of the phytoplankton was also measured by the chlorophyll fluorescence produced by light pulses generated from light-emitting diodes, LEDs. Details of the procedure can be found in elsewhere (Sirin et al., 2015).