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Microalgae cultivation in urban wastewater: Nutrient removal and biomass production for biodiesel and methane

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ABSTRACT

The freshwater microalgae species *Chlorella kessleri* and *Chlorella vulgaris*, and the marine microalgae species *Nannochloropsis oculata* were cultivated in urban wastewater. The freshwater species demonstrated the possibility of growing in urban wastewater reaching high biomass production and nutrient removal when cultured in batch mode using a flat-panel airlift photobioreactor. Both microalgae species reached high biomass dry weights, 2.70 ± 0.08 g/L and 2.91 ± 0.02 g/L respectively, accompanied by nitrogen concentration reduction around 96% and 95%, and a phosphorous concentration reduction around 99% and 98% respectively. *N. oculata* was able to uptake nutrients from wastewater to grow but with less efficiency, indicating the need of microalgae acclimation or process optimisation to achieve high nutrient removals. During *C. kessleri* and *C. vulgaris* cultivation, the nitrogen consumption led to a progressive N-starvation process which increased the microalgae potential for biofuels production; both species produced 346 ± 3 mL_{CH_4}/g_{VS} and 415 ± 2 mL_{CH_4}/g_{VS} during anaerobic digestion, and 7.4 ± 0.2 $g_{Biodiesel}/100\ g_{VS}$ and 11.3 ± 0.1 $g_{Biodiesel}/100\ g_{VS}$ respectively.

1. Introduction

Microalgae are able to convert solar energy and carbon dioxide into energy as a result of their photosynthetic activity; when converted into biodiesel or methane, this energy could meet the population energy needs [1]. The microalgae cultivation with energetic purposes started years ago when microalgae, able to accumulate a high amount of lipids, appeared as promising crop substitute in biodiesel production [2]. Currently, crops are the common feedstocks in the biodiesel production industry but also in the food industry, thus the crop-based biofuels production has created increased food prices [3]. A wide variety of microalgae species reach higher lipid productivities than crops [2], becoming potential substrates to alleviate the referred to "food-versus-fuel competition" [3]. Despite the efforts made, the industrial biodiesel production from microalgae is not economically viable nowadays due especially to the high costs for drying and lipid extraction among other costs [4]. On the contrary, the anaerobic digestion process converts wet biomass into methane [5], and energy is recovered not only from the lipid fraction.

The anaerobic digestion process is a widely known technology, currently used in the wastewater treatment plants (WWTPs). The sewage sludge, a waste produced during the wastewater treatment, generates high operating costs in its final disposal [5]; the anaerobic digestion process converts the sludge into a stable product with simultaneous generation of a valuable by-product such us methane. Methane utilisation contributes favourably to reduce the high operating costs generated by the final disposal of sewage sludge [5].

The nutrient level in wastewaters might be another problem in WWTPs. The water discharge with high nutrient levels cause eutrophication problems, thus the Directive 98/15/EEC establishes nutrient levels or minimum percentage of reduction before water discharge [6]. Although the nutrient concentration varies from a WWTP to another, and between the wastewater streams in the process, the stream generated during the digested sludge dewatering process always has higher nitrogen and phosphorus concentrations than in any other streams [7]. This stream, named centrate, is usually recycled for further treatment to avoid environmental problems, but it increases the WWTP costs [7]. Microalgae cultivation offers a solution to reduce the high nutrient content in centrate since microalgae consume nutrients when growing [8]. Moreover, in a waste-to-value approach, the produced biomass constitutes a by-product which could be used for various purposes, including biofuels.

Some freshwater microalgae species have already been cultured in different wastewater streams, with the purpose of reducing the nutrient concentration in wastewater or producing lipids for biofuels [7,9–13]. Similarly, seawater microalgae species have also been cultured using wastewaters despite the salinity requirements [14,15]. However, the centrate utilisation for microalgae cultivation is scarce; most of the studies were done using wastewater with a low nutrient content compared to the centrate. The use of centrate as nutrient medium allows coupling the wastewater treatment and the microalgae cultivation process with a

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minimum modification in the WWTP facilities. Wastewater treatment by microalgae cultivation is still limited, and the waste activated sludge (WAS) process is the conventional treatment in WWTP [8]. The substitution of the WAS process implies a full modification of the WWTP scheme, whereas the centrate utilisation in microalgae cultivation only needs a cultivation unit coupling.

This study analyses the possibility of coupling microalgae cultivation in WWTPs, removing nutrients from the centrate while producing biomass with energy recovery purposes. Due to the high nutrient level in the centrate which can inhibit the cell growth, the first studies aimed to determine the most suitable media for *C. kessleri*, *C. vulgaris* and *Nannochloropsis oculata* cultivation. The microalgae species were cultivated in different dilutions of the centrate; the dilutions were carried out with wastewater before the primary settling tank for the cultivation of freshwater microalgae species, or with natural seawater for the marine microalgae species. After the culture medium screening, the microalgae species were cultured in the most suitable medium, where the nutrient removal and the biomass production were evaluated. Finally, the harvested biomass was used for methane and biodiesel production.

2. Materials and methods

2.1. Pre-treatments and characteristics of the wastewaters

Wastewater samples from the WWTP of Saint Nazaire (ACCUEIL CARENE, Saint-Nazaire, France) were used as cultured medium for microalgae cultivation; they consisted of the centrate and the wastewater from a line before the primary settling tank. For marine microalgae cultivation, natural seawater was collected from the coastal area of Saint-Nazaire in France.

Large solid particles were first removed by centrifugation and then, the samples were filtered through a 0.45 μm pore size filter to remove undesirable small particles. The total nitrogen (TN), ammonia nitrogen (NH_3-N), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), phosphate phosphorus (PO_4-P), and chemical oxygen demand (COD) were determined following the Hach DR 2800 Spectrophotometer Manual using the HACH LANGE cuvette tests and following the procedure specified for each test (Hach, 2008). The characteristics of the wastewaters and the natural seawater can be observed in Table 1.

As can be observed, the composition of the wastewater sampled before the primary settling tank composition is given as a range rather than the average value with the standard deviation; two different samples collected in different opportunities were used in the experiments. These differences in the nutrient content were taken into account for the cultured medium preparation.

Table 1
Characteristics of the wastewater and the natural seawater used for microalgae cultivation.

Parameter	Water for microalgae cultivation		
	Before the primary settling tank [a]	Centrate	Natural seawater
pH	7.42–7.79	8.2 ± 0.1	7.7 ± 0.1
COD (mg/L)	95–169	706 ± 9	–
TN (mg N/L)	39–65	1233 ± 78	n.d.
NH_4^+ (mg NH_4-N/L)	36–62	1198 ± 81	n.d.
NH_4-N/TN (%)	95 ± 2	98 ± 1	–
NO_3^- (mg NO_3-N/L)	0.1–0.9	2.34 ± 0.11	6.2 ± 0.1
NO_2^- (mg NO_2-N/L)	0.1–0.4	0.14 ± 0.02	n.d
P (mg PO_4-P/L)	3.1–5.4	11.90 ± 0.10	n.d.
Salinity (‰)	–	–	28 ± 0.5

n.d.: not detected.
[a] Parameter ranges from two different samples collected in two different opportunities.

2.2. Microalgae cultivation

2.2.1. Shake flasks

Three different microalgae species were cultured. The freshwater microalgae species *Chlorella kessleri* (strain UTEX2229) and *Chlorella vulgaris* (strain CCAP211/19) were obtained from the collection of algae at the University of Nantes; and the marine microalgae species *N. oculata*, from the Alphabiotech collection (Asserac, France).

The microalgae were first inoculated in shake flasks. The freshwater microalgae species in 250 mL Erlenmeyer flask containing a modified Bold Basal Medium (BBM) and the marine microalgae species, in filtered and sterilised seawater with salinity adjusted at 25‰ and enriched with Conway medium (3 mL/L of seawater). The detailed composition of the modified BBM and Conway medium is given in Pruvost et al. [16,17].

2.2.2. Culture medium screening in Efficient Overproducing Screening System-Photobioreactors (EOSS-PBR)

The EOSS-PBR was especially developed for the fast screening of culture media and microalgae species in conditions representative of PBR cultivation. It consisted of six small-scale photobioreactors (bubble columns) run in parallel, each tube having a volume $V_r = 3 \cdot 10^{-5}$ m^3, an illuminated area of $S_L = 0.008$ m^2 and a specific illuminated area of $a_{light} = S/V_r$ of 266.7 1/m. A full description of the EOSS-PBR is done in Taleb et al. [18].

The culture medium was prepared by diluting the centrate to reduce the high TN concentration, either with wastewater or with natural seawater (Table 1). Before inoculation, the culture medium was filtered through a 0.45 μm pore size filter to remove undesirable small particles. The TN concentrations in the culture medium for the freshwater microalgae species were 30 mg N/L (0.002 mol/L), 140 mg N/L (0.010 mol/L), 260 mg N/L (0.019 mol/L), 490 mg N/L (0.035 mol/L), 700 mg N/L (0.050 mol/L) and 1200 mg N/L (0.086 mol/L). For the marine microalgae species cultivation, the TN concentrations were 6 mg N/L (<0.001 mol/L), 71 mg N/L (0.005 mol/L), 135 N mg N/L (0.010 mol/L), 265 mg N/L (0.019 mol/L), 524 mg N/L (0.037 mol/L) and 782 mg N/L (0.056 mol/L).

The pH in the culture medium was around 7.5 for the freshwater microalgae species and 8 for the marine microalgae species, according to Pruvost et al. and Taleb et al. [17,18]. The culture agitation was provided by continuous injection of air with 2 vol.% CO_2 at a flow rate of 3 mL/min; the incident photon flux density (PFD), by a set of 6 fluorescent white tubes was ~150 μmol/m^2·s. The temperature was regulated at 25 °C by ambient air flow. The reactor was operated in batch mode.

The microalgae growth was evaluated following the evolution of the number of cells as a function of time (t). Cell concentration N expressed as number of cells per millilitre of culture was determined under an optical microscope (Axiostar-Plus, Carl Zeiss, Germany) using Malassez counting cell. The chlorophyll fluorescence in the microalgae was observed in the microscope, using the green filter set 530–585 (BP 530–585 as exciter filter, FT 600 as chromatic beam splitter and LP 615 as barrier filter; Zeiss, Oberkochen, Germany). The algal dry weight concentration (Cx) was determined at the end of the experiment, by filtration through a pre-dried and pre-weighed glass-fibre filter (Whatman GF/F). The filters were dried for 24 h at 105 °C, cooled down in a desiccator and then weighed again. The total pigment content was determined using a spectrophotometric method, following the procedure described in [17].

2.2.3. Microalgae cultivation in flat-panel airlift photobioreactor (PBR)

Once the culture medium was defined, microalgae were cultured in batch mode using a flat-panel airlift PBR with $V_r = 10^{-3}$ m^3, and depth of culture of $L_z = 3 \cdot 10^{-2}$ m, a $S_L = 8 \cdot 10^{-3}$ m^2 and a $a_{light} = 33.3$ 1/m. Before inoculation, the cells were centrifuged for 3 min at 6000 ×g at room temperature, and the cell pellets were washed with the decided culture medium twice before finally suspending cells.