Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2018.22537

127 (2018) 71–74
September

Reduction of nitrogen content in landfill leachate using microalgae

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Received 8 February 2018; Accepted 22 May 2018

ABSTRACT

Landfill leachate contains large amounts of biodegradable or refractory to biodegradation organic materials, where organic and inorganic salts, ammonia-nitrogen, heavy metals, and chlorinated consist important groups. Leachate from run out municipal landfills has a lower biochemical oxygen demand after five days but still high contents of N-NH^{4+}. Usually, the ammonium concentration could be reduced by nitrification processes followed by biological denitrification, but for leachate from run out landfills, organic molecules (e.g., methanol or acetic acid) must be added as carbon source. To overcome this drawback, in this work, the use of microalgae to reduce the nitrogen content from leachate before and after nitrification processes was suggested. Microalgae cultures were performed with different quantities of leachate after a microfiltration pretreatment. Additional runs were performed in landfill leachate after biological nitrification pretreatment. Runs were compared with those carried out in classic Bold's Basal medium taken as a control. During the growth, the biomass was observed microscopically and the ammonium, nitrate, and nitrite contents were determined. Results showed that *Chlorella vulgaris* can proliferate in presence of exhausted landfill leachate and exhausted landfill leachate after nitrification pretreatment with an ammonium removal efficiency of 38.8 mg/L d. The produced microalgal biomass, rich in lipids, could be used for energetic purposes.

Keywords: Landfill leachate; Nitrification treatment; *Chlorella vulgaris*; Lipid content.

1. Introduction

The leachate production is one of the potential environmental problems caused by wastes decomposition in landfills. Leachate originates from percolated rainwater and waste decomposition [1]. Landfill leachate (LL) is the result of water percolating through waste deposits that have undergone aerobic and anaerobic microbial decomposition [2]. LL composition depends on the type of waste in the landfill, landfill age, climate conditions, and landfill position [3].

A landfill will produce leachate throughout its working life and for hundred years after its disposal. For the protection of the surrounding environment (groundwater, rivers, lakes, and soils) [4], the control of a landfill site, and appropriate treatment of the leachate it produces, is of primary interest [5]. The remaining ammonium nitrogen, contained in the leachate of exhausted landfilled, can be removed through conventional treatment processes such as nitrification/denitrification, air stripping, and struvite precipitation. Biological techniques such as nitritation/denitritation, deammonification, and anaerobic ammonium oxidation (anammox) in moving bed bioreactor configuration, or membrane-based processes such as a membrane bioreactor integrated with an anoxic tank could also been used [6].

Several studies have focused their attention on microalgae cultured in wastewater, such as those from farming activities (swine, poultry, and cattle), dairy, and municipality [7]. In fact, photosynthetic unicellular organisms, such as microalgae and cyanobacteria, could be a new potential treatment of LL, due to their ability to grow easily even in nonoptimal conditions, like nutrient deficiency, sodium chloride excess and light intensity limitation [8].

In addition to the treatment of LL, microalgal biomass produced during leachate treatment could be used for

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Presented at the 15th International Conference on Environmental Science and Technology (CEST-2017), 31 August–2 September 2017, Rhodes, Greece.

different purposes, including biogas substrate, biofuels, fertilizers, and biopolymers, which can be converted into packaging materials, and have the advantage of being renewable.

In this study, *Chlorella vulgaris*, usually cultured on the Bold's Basal medium (BBM), a nutrient solution that simulates freshwater composition, with the addition of B1, B8, and B12 vitamins [9], was grown in medium enriched with different quantities of LL after microfiltration pretreatment in order to have different nitrogen concentrations in water. Additional runs were performed in LL after biological nitrification pretreatment. Runs were compared with those carried out in classic BBM taken as a control. During the growth, biomass was quantified spectrophotometrically and observed microscopically. The ammonium, nitrate, and nitrite contents were also determined.

2. Materials and methods

2.1. Microorganisms

C. vulgaris CCAP 211 (Culture Collection of Algae and Protozoa, Argyll, UK), a eukaryotic photosynthetic microorganism, was used in this study. The microalga was maintained in BBM [9], using carbon dioxide for the pH control.

2.2. Culture conditions

C. vulgaris was grown under controlled temperature (24.0°C ± 0.5°C) employing exhausted landfill leachate (ELL) and the effluent obtained after a traditional nitrification pretreatment (ELL-AN) (Table 1). The cultures were performed in 2.0 L-vertical photobioreactor (internal diameter: mm; height: mm) using 1.5 L of medium, reaching a depth in the photobioreactor of 350 mm. ELL and ELLAN where mixed with deionized water in order to maintain the same nitrogen concentration present in the BBM (247 mg_N/L).

When the nitrogen concentration reached values next to zero, biomass was collected by centrifugation and inoculated in new medium. Air was bubbled continuously during the runs and cultures were exposed to artificial light with about 45 μE/m^2 s intensity, provided by fluorescent lamps.

Table 1
Exhausted landfill leachate (ELL) and ELL after nitrification pretreatment (ELL-AN) characterization

	ELL	ELL-AN
TDS[a] (g/L)	11.3	13.1
pH	6.8	8.2
N-NH_4^+ (mg/L)	258.4	–
N-NO_3^- (mg/L)	24.3	162.25
N-NO_2^- (mg/L)	6.4	712.2
Cl^- (mg/L)	1,241.6	1,326.5
P-PO_4^{3-} (mg/L)	–	–
S-SO_4^{2-} (mg/L)	10.2	35.1

[a]TDS: Total dissolved solids.

2.3. Kinetic parameters

The average specific growth rate (μ) was calculated by the equation:

$$\mu = \frac{1}{t} \times \ln \frac{C_f}{C_i} \quad (1)$$

where C_f and C_i are the microalgae concentrations at the end and the beginning of runs, respectively, and t is the cultivation time (d).

The average biomass productivity (P) was defined as the ratio of produced biomass per unit volume between the end and the beginning of runs to the cultivation time as follows:

$$P = \frac{C_f - C_i}{t} \quad (2)$$

The lipid content (Y) was calculated as follows:

$$Y = \frac{M_L}{M_{DB}} \quad (3)$$

where M_L is the lipid content at the end of the growth and M_{DB} is the mass of dried *C. vulgaris*.

2.4. Analytical methods

C. vulgaris concentration was determined daily by optical density (OD) at 625 nm using an UV–vis spectrophotometer (Lambda 25, PerkinElmer, Milan, Italy). Biomass concentration (C) was related to OD by the following equation:

$$Abs_{625} = 4.203\ C \quad (4)$$

Biomass at the end of the growths was observed weekly by an optical microscope DMLS equipped with a DC 200 digital camera (Leica, Wetzlar, Germany) at the end of the growths. Chlorophyll was quantified following the methodology described by Ortiz Montoya et al. [10].

After growth, *C. vulgaris* was collected and centrifuged at 6,000×*g*, using a centrifuge model PK131 (ALC, Milan, Italy). Biomass was dried at 100°C until constant weight.

Lipids were extracted using a modified version of Folch method [8]. After the extraction process, lipids were transesterified with methanol and analyzed using a gas chromatograph model Dani 1000 (Dani Instruments, Milan, Italy), equipped with a flame ionization detector.

Ammonium was determined every 2 d using an appropriate kit (Nanocolor, VELP Scientifica, Usmate, MB, Italy), while nitrite, nitrate, sulfate, phosphate, and chloride were determined by ionic chromatography.

3. Results and discussions

Results of *C. vulgaris* cultivations carried out in 2.0 L photobioreactors are reported in Fig. 1. As can be seen, the employed microalga, after 28 d of cultivation, reached a final concentration of 2.07 and 2.09 g_{DB}/L for ELL and ELL-AN growths, while the control showed a final concentration of 1.98 g_{DB}/L.

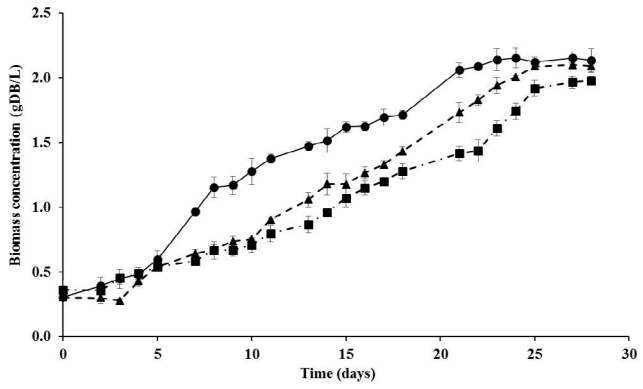

Fig. 1. *C. vulgaris* growths in Bold's Basal medium (●), exhausted landfill leachate (▲) and exhausted landfill leachate after nitrification pretreatment (■).

During the first 8 d of cultivation ELL and ELL-AN growths exhibit a lower growth rate compared with the control. This could be due to an initial period of adaptation for *C. vulgaris* into the new media.

The light microscopy images of *C. vulgaris* taken after 28 d of growth (Fig. 2) have shown the presence of single isolated cells for BBM and ELL-AN growth, while the presence of aggregates could be seen in the ELL growth, while the presence of aggregates could be seen in the ELL growth.

Kinetics parameters, total chlorophyll content, and lipid yields are shown in Table 2. The microalgae grown in ELL and ELL-AN have shown a higher concentration in lipids and in triglycerides with respect to *C. vulgaris* grown in the BBM.

Thus confirming that the growth of *C. vulgaris* in non-optimal conditions leads to a stress that lead to an increase in lipid fraction [11]. Moreover, lipid content was inversely proportional to the biomass specific growth rate.

Results about nitrogen removal during *C. vulgaris* growths are reported in Fig. 3 and Table 3. As can be seen, in the first days of *C. vulgaris* growth in presence of ELL, $N\text{-}NH_4^+$ was reduced up to 100% with respect to the initial concentration (223 mg_N/L). This reduction corresponded to an increase of $N\text{-}NO_2^-$ (from 5 to 210 mg_N/L). Instead the concentration of $N\text{-}NO_3^-$ did not noticeably vary (from 18 to 25 mg_N/L).

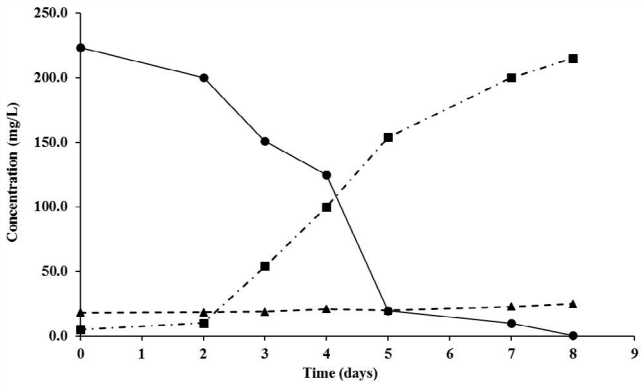

Fig. 3. Concentration of $N\text{-}NH_4^+$ (●), $N\text{-}NO_2^-$ (▲) and $N\text{-}NO_3^-$ (■) in the medium during the first 8 d of *C. vulgaris* growth in exhausted landfill leachate.

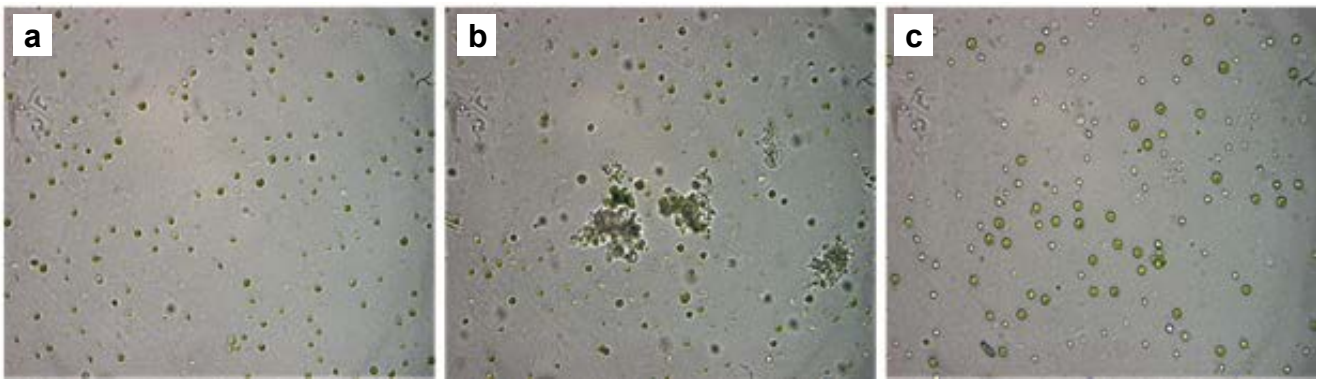

Fig. 2. Light microscopy images (40×) of *C. vulgaris* after growth (eighth day of growth) in BBM (Control, a), in an exhausted landfill leachate (ELL, b) and in exhausted landfill leachate after nitrification pretreatment (ELL-AN, c).

Table 2
pH values and kinetic parameters of *C. vulgaris* growths and total lipids, triglycerides and total chlorophyll content using Bold's Basal (control), exhausted landfill leachate (ELL) and ELL after nitrification process (ELL-AN) as growth media

Run	Initial pH (d 0)	Final pH (d 8)	μ (d^{-1})	P ($mg_{DB}/L\ d$)	C_{max} (g_{DB}/L)	Y_L (g/100 g_{DB})	Y_T (g/100 g_{lipids})	Chlorophyll (g/100 g_{DB})
Control	7.5	8.3	0.0695 ± 0.0009	65.4 ± 2.5	2.15 ± 0.08	11.02 ± 1.05	24.21 ± 1.22	6.65 ± 0.23
ELL	7.1	8.2	0.0688 ± 0.0034	63.8 ± 2.5	2.10 ± 0.02	13.25 ± 2.51	31.18 ± 0.99	7.01 ± 0.99
ELL-AN	8.3	8.7	0.0610 ± 0.0064	57.8 ± 1.3	2.00 ± 0.03	18.22 ± 2.87	35.10 ± 1.51	5.84 ± 0.74

Table 3
Ammonium removal and nitrate/nitrite production during *C. vulgaris* growth in exhausted landfill leachate (ELL) and nitrite/nitrate removal using BBM and ELL after nitrification process (ELL-AN) as growth media

Run	NH^{+}_{4R} (mg/L d)	NO^{-}_{2R} (mg/L d)	NO^{-}_{3R} (mg/L d)	NO^{-}_{2P} (mg/L d)	NO^{-}_{3P} (mg/L d)
Control	–	–	8.41	–	–
ELL	38.76	–	–	35.66	1.73
ELL-AN	–	1.07	5.50	–	–

Other authors demonstrated the capability of *Chlorella* sp. to reduce the content of $N\text{-}NH^{+}_{4}$ in LL, but they reported that leachate with 135 mg/L of $N\text{-}NH^{+}_{4}$ supported algal growth, while higher concentrations of leachate suppressed the growth of the investigated strains [12].

4. Conclusions

C. vulgaris can proliferate in presence of ELL and ELL-AN, showing a growth comparable with the one in BBM. In the former conditions, biomass was able to reach a final concentration next to 2.0 g_{DB}/L.

C. vulgaris presented an ammonium removal efficiency of 38.8 mg/L d. According to this preliminary result, it could be concluded that the resulting medium, rich in nitrite and nitrate, could be allocated to following denitrification processes, while the produced microalgal biomass, rich in lipids, could be used for energetic purposes. In fact, biomass could be treated by pyrolysis for the production of liquid and gaseous biofuels.

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