Reduction of nitrogen content in landfill leachate using microalgae

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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 BOD5 but still high contents of N-NH4+. Usually, the ammonium concentration could be reduced by nitrification processes followed by biological denitrification, but for leachate from run out landfills this step requires the addition of organic molecules (e.g. methanol or acetic acid) as carbon source. To overcome this drawback, the authors suggested the use of mixotrophic microalgae to reduce the nitrogen content from landfill leachate before and after nitrification processes. In fact, microalgae could potentially offer many advantages in leachate treatment, being able to use inorganic nutrients for their heterotrophic growth without an aerobic environment. Microalgae cultures were performed with different quantities of landfill leachate after microfiltration pretreatment in order to have different nitrogen concentrations in water. Additional runs were performed in landfill leachate after biological nitrification pretreatment. Runs were compared with those carried out in classic Bold Basal Medium taken as a control. During the growth, biomass was observed microscopically and the ammonium, nitrate, nitrite contents were determined.

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 (Renou *et al*., 2008). Landfill leachate (LL) is the result of water percolating through waste deposits that have undergone aerobic and anaerobic microbial decomposition (Chofqi *et al*., 2004). LL composition depends on the type of waste in the landfill, landfill age, climate conditions, and landfill position (Slack *et al*., 2005). 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), the control of a landfill site, and appropriate treatment of the leachate it produces, is of primary interest (Brennan *et al*., 2017). 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 (Mohammad-pajooh *et al*., 2017).

Several studies have focused their attention on microalgae cultured in wastewater, such as those from farming activities (swine, poultry and cattle), dairy, and municipality (Hoffmann, 1998). In fact, photosynthetic unicellular organisms, such as microalgae and cyanobacteria, could be a new potential treatment of landfill leachate, due to their ability to grow easily even in non-optimal conditions, like nutrient deficiency, sodium chloride excess and light intensity limitation (Casazza *et al*., 2015).

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 (Bischoff, 1963), was grown in medium enriched with different quantities of landfill leachate after microfiltration pretreatment in order to have different nitrogen concentrations in water. Additional runs were performed in landfill leachate after biological nitrification pretreatment. Runs were compared with those carried out in classic Bold Basal Medium taken as a control. During the growth, biomass was quantified spectrophotometrically and observed microscopically. The ammonium, nitrate, 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 Bold's Basal Medium (Bishoff *et al*., 1969), using carbon dioxide for the pH control.

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

	ELL	**ELL-AN**
TDS (g/L)		
pH	6.8	8.2
$N\text{-}NH_4^+$ (mg/L)	258.4	-
$N\text{-}NO_3^-$ (mg/L)	24.3	162.25
$N\text{-}NO_2^-$ (mg/L)	6.4	712.2
Cl^- (mg/L)	1241.6	1326.5
$P\text{-}PO_4^{3-}$ (mg/L)	-	-
$S\text{-}SO_4^{2-}$ (mg/L)	10.2	35.1

2.2. Culture conditions

C. vulgaris was grown under controlled temperature (24.0 ± 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 using 1.5 L of medium. 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 $\mu E/m^2$ intensity, provided by fluorescent lamps.

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 (days).

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:

$$P = \frac{Cf - Ci}{t} \quad (2)$$

The lipid content (Y) was calculated as follow (Eq. 3):

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

where M_L is the lipid content at the end of the growth and M_{DB} 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, Perkin Elmer, Milan, Italy). Biomass concentration (C) was related to OD by the following equation:

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

Biomass was observed weekly by an optical microscope DMLS equipped with a DC 200 digital camera (Leica, Wetzlar, Germany). Chlorophyll was quantified following the methodology described by Ortiz Montoya *et al*. (2014).

After growth, *C. vulgars* was collected and centrifuged at 6000 ×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 (Casazza *et al*., 2015). After the extraction process, lipids were transesterified with methanol and analysed using a gaschromatograph model Dani 1000 (Dani Instruments, Milan, Italy), equipped with a FID detector.

Ammonium was determine every two days using an appropriate kit (Nanocolor, VELP Scientifica, Usmate, MB, Italy), while nitrite, nitrate, sulfate, phosphate and chloride where determined by ionic chromatography using an IC equipped.

3. Results and Discussions

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

The main difference of ELL and ELL-AN growths respect to the Control growth was given by a lower growth rate in the first eight days of cultivation. This could be due to an initial adaptation phase of *C. vulgaris* to the new media.

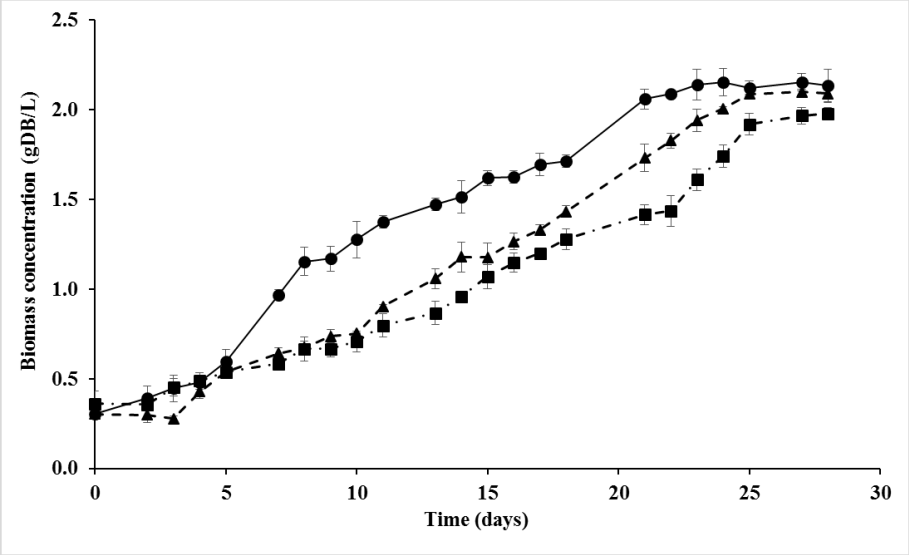

Figure 1. *C. vulgaris* growths in Bold Basal medium (●), exhausted landfill leachate (▲) and exhausted landfill leachate after nitrification pretreatment (■).

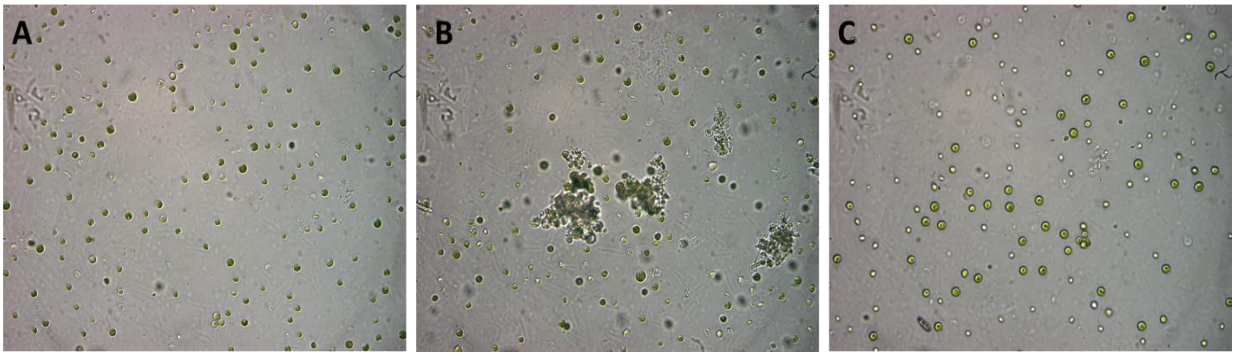

Figure 2. Light microscopy images (40×) of *C. vulgaris* after growth in BBM (Control, A), in e exhausted landfill leachate (ELL, B) and in exhausted landfill leachate after nitrification pretreatment (ELL-AN, C).

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

Run	μ (1/d)	P ($mg_{DB}/L\ d$)	C_{max} (g_{DB}/L)	Y_L ($g/100g_{DB}$)	Y_T ($g/100g_{lipids}$)	Chlorophyll ($g/100g_{DB}$)
Control	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	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	0.0610 ± 0.0064	57.8 ± 1.3	2.00 ± 0.03	18.22 ± 2.87	35.10 ± 1.51	5.84 ± 0.74

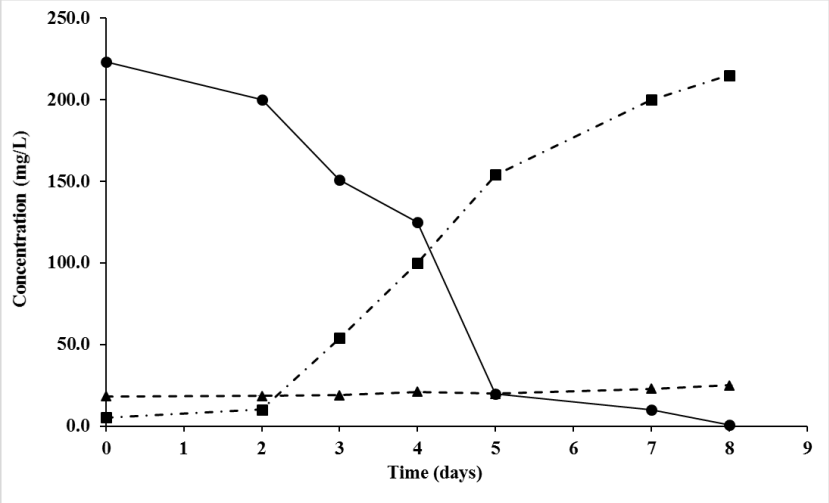

Figure 3. Concentration of $N\text{-}NH_4^+$ (●), $N\text{-}NO_2^-$ (▲) and $N\text{-}NO_3^-$ (■) in the medium during the first eight days of C. vulgaris growth in exhausted landfill leachate.

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	$NH_4^+{}_R$ (mg/L d)	$NO_2^-{}_R$ (mg/L d)	$NO_3^-{}_R$ (mg/L d)	$NO_2^-{}_P$ (mg/L d)	$NO_3^-{}_P$ (mg/L d)
Control	-	-	8.41	-	-
ELL	38.76	-	-	35.66	1.73
ELL-AN	-	1.07	5.50	-	-

The light microscopy images of *C. vulgaris* taken after 28 day of growth (Figure 2) 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.

Kinetics parameters, total chlorophyll content and lipid yields are shown in Table 2. The microalgae grown in ELL and ELL-AN have shown an higher concentration in lipids and in triglycerides 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 conduct to an increase in lipid fraction.

Moreover, lipid content was inversely proportional to the biomass specific growth rate.

Results of nitrogen removal during *C. vulgaris* growths are reported in Figure 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 % 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). While the concentration of $N\text{-}NO_3^-$ was almost the same (from 18 to 25 mg_N/L).

4. Conclusions

Chlorella vulgaris can proliferate in presence of exhausted landfill leachate and exhausted landfill leachate after nitrification pretreatment comparable to the growth in BBM. Biomass reached concentration next to 2.0 gDB/L. *C. vulgaris* presented an ammonium removal efficiency of 38.8 mg/L d. The resulting medium, rich in nitrite and nitrate could be allocate to following denitrification processes, while the produced microalgal biomass, rich in lipids, could be used for energetic purposes.

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