CHEMICAL ENGINEERING TRANSACTIONS
VOL. 43, 2015
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Chief Editors: Sauro Pierucci, Jiří J. Klemeš

ISBN 978-88-95608-34-1; **ISSN** 2283-9216
DOI: 10.3303/CET1543063

Exploitation of Urban Landfill Leachate as Nutrient Source for Microalgal Biomass Production

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Increasing concern over climate change and the impact of greenhouse gas emissions as well as diminishing global oil reserves has pushed research into alternative energy sources. Among these options, reducing the cost of microalgae cultivation, a promising source for renewable energy, is a key step in commercializing biodiesel production. Microalgae also have a two-fold advantage thanks to the capability to treat waste streams by reducing contaminants via bioremediation.
In this work, the possibility of exploiting nutrients from landfill leachate was tested. Some microalgal species are able to grow in the leachate as a substrate, but the chemical composition of this waste stream often leads to growth inhibition. Thus, using strain directly isolated from the target environment could avoid problems of inhibition, in order to obtain higher biomass productivity and nutrient, i.e. pollutant, removal.
Acutodesmus obliquus is a microalgal species isolated from a pond containing pretreated leachate from an urban landfill located in Lazio (Italy). This strain was cultivated in leachate from a landfill located in Istrana (TV), Italy. The leachate was diluted with distilled water and the growth of the species was measured. Several dilution ratios were tested and the maximum growth rate was obtained with 10 % of leachate in water, even though an acceptable growth was also observed with 1:2 dilution. Lipid content of about 38 – 48 % dry weight was measured. Nutrients uptake in the form of ammonia from the various leachate dilutions ranged from 30 - 97 % and, generally, it was >90 % in the case of phosphorus, which was assessed as the limiting nutrient for growth.
The outcome of this work has established the real potential of utilizing ammonia-N enriched leachate as the nutrient source for yielding high lipid content algal biomass, by using native strains. Based on experimental results, preliminary calculations on photobioreactor design to treat leachate from urban landfill were carried out.

1. Introduction

Microalgal biodiesel is becoming widely accepted as a viable competitor to traditional crude oil derived diesel. Advantages of non-arable land cultivation, short reproduction cycle and carbon neutrality has increased its international image as a leading alternative energy source (Chisti, 2013). However, for commercial viable biofuel production to be achieved, cost effective upstream and downstream avenues must be explored, exploited and established (Chisti, 2013). More specifically, nutrients availability is one of many aspects currently being investigated, with particular focus on wastewater-based cultivation (Komolafe et al., 2014). In particular, published works in this area have focused on the success of algal cultivation in municipal, industrial (Mata et al., 2014) and agricultural wastewater, all of which have high concentration of the two fundamental elements required by algae, nitrogen and phosphorous (Sforza et al., 2014). An alternative source of nutrients with similar economical and environmental advantages is landfill leachate, which is defined as “a liquid that has passed through or emerged from solid waste and contains soluble, suspended or miscible materials removed from such waste” (US Code of Federal Regulations, 1980). Leachate is typically composed by organic matter, decomposition products, organic chemicals and dissolved metals: leachate has an extremely

Please cite this article as: Sforza E., Khairallah Al Emara M.H., Sharif A., Bertucco A., 2015, Exploitation of urban landfill leachate as nutrient source for microalgal biomass production, Chemical Engineering Transactions, 43, 373-378 DOI: 10.3303/CET1543063

high concentration of nitrogen, ranging from 1,000 to 5,000 mg L^{-1} as well as phosphorus and heavy metals amongst others (Zhao et al., 2014). Thus, there is a common concern over the deleterious effect of this waste stream on the environment, and leachate streams must always be contained and treated. In this context, the use of a microalgae-based bioremediation appears a viable method for treating leachate and simultaneously produce valuable biomass (Richards and Mullins, 2013). On the other hand, the high concentration of free ammonia in leachate might be an inhibiting factor for microalgal growth, as reported by Cheung et al. (1993) for *Chlorella* sp., *Dunaliella* sp and *Scenedesmus,* Lin et al. (2007) for *Chlorella pyrenoidosa* and Zhao et al. (2014) for a microalgae-bacteria *consortium*. This problem is usually solved by diluting the leachate in order to reduce the ammonia toxicity. Alternatively, a promising strategy is to exploit ammonia-tolerant microalgae for cultivation, usually directly isolated from landfill ponds (Cheung et al., 1993).
In this work, an endogenous species isolated from landfill (Ferrigo et al., 2014) was cultivated in leachate, under different dilutions, the growth rate and the nutrient uptake were measured to assess the biomass production and bioremediation potentials. In addition, the viability of leachate based cultivation was assessed by applying mass and energy balances on a hypothetical integrated biomass production and leachate treatment plant.

2. Materials ad Methods

2.1 Experimental procedures and equipment

Acutodesmus obliquus RL01 was isolated from a pond containing pretreated leachate from an urban landfill located in Lazio, Italy (Ferrigo et al., 2014) and was maintained in liquid BG11 medium for the pre-inoculum. Microalgae were cultured in leachate sampled from an Italian landfill in Istrana, Treviso (TV), Italy. No sterilization treatment was carried out. Water was maintained in refrigerator (-20 °C) before each experiment. Experiments of microalgal growth were carried out in batch systems. Due to the nutrient content of sampled wastewater, which was not constant with time, all batch experiments were carried out with water from a single sampling from the landfill, and nutrients were measured at time 0 of each growth curve, in order to verify the actual consumption by microalgae. Initial nutrient concentrations are reported in Table 1. Each batch experiment was carried out in at least two or three replicates, and started with an initial microalgae inoculation of OD_{750} = 0.5, corresponding to a cell concentration of about 3 x 10^6 cells mL^{-1} (about 0.15 g L^{-1}).
Pre-inoculum and batch experiments were performed in glass bottles of 250 mL, continuously mixed by magnetic stirring and bubbling air enriched with 5% v/v of CO_2, which also provided a non-limiting CO_2 supply (total gas flow rate of 1 L h^{-1}). The temperature was controlled by an incubator (Frigomeccanica Andreaus, Padova), and artificial light (white neon lamps OSRAM) was provided continuously at constant intensity of 100 µmol m^{-2} s^{-1} of PAR (Photosynthetic Active Radiation) photons, measured by a photoradiometer (Model LI-189, LI-COR, USA).
The biomass concentration was monitored daily by spectrophotometric analysis of the optical density (OD_{750}) (UV-Visible UV 500 from Spectronic Unicam, UK) and correlated to cell concentration, measured with a Bürker Counting Chamber (HBG, Germany). Specific growth rates were determined by linear regression of three to to six experimental points of logarithmic phase of growth. The concentration of biomass was also gravimetrically measured as dry weight (DW) in terms of g L^{-1}, in cells previously harvested with a 0.22 µm filter, and then dried for 4 h at 80 °C in a laboratory oven. The nutrients analysed were ammonium ($N\text{-}NH_4$) and phosphate ($P\text{-}PO_4$), measured using standard methods in water and wastewater (Ramos Tercero et al., 2013), as free dissolved nutrients in samples of 0.2 µm filtered culture. Ammonium was measured by test kits provided by St. Carlo Erba Reagenti (Italy) (code 0800.05405), while orthophosphates were detected by a modified ascorbic acid method described in APHA-AWWA-WEF, 1992.
Total lipids were extracted from dried cells of A. obliquus using ethanol-hexane (2.5:1 vol/vol) in a Soxhlet apparatus for 10 h, following a homogenization step in a mortar. The total lipid mass was measured gravimetrically after removal of the solvent by a rotary evaporator.

2.2 Mass and energy balance

Microalgal photobioreactor (PBR) design was based on biomass mass balance, and experimental phosphorous and nitrogen yields. In particular the reactor was assumed as a perfectly mixed reactor (CSTR), working at steady state. Thus, by considering an accumulation term and an inlet biomass concentration equal to zero, the biomass balance can be expressed as:

$$0 = -C_{xo} + \mu * \tau \quad [1]$$

where τ is the residence time and μ the specific growth rate. The subscript "*o*" refers to outlet conditions. The residence time is calculated as:

$$\tau = V_R/Q \quad [2]$$

where V_R is the reactor volume and Q the inlet volumetric flow rate. In the case of biological reactor, the biomass growth rate corresponds to the dilution rate, which is the inverse of the residence time:

$$\mu = D = 1/\tau \quad [3]$$

According to Eq(3), a minimum residence time was assumed in our calculations, based on the experimental maximum specific growth rate.

The biomass production can be linked to the nutrient specific production rate through the Biomass/Nutrient yield ($Y_{x/_i}$):

$$-\frac{dC_x}{dC_i} = Y_{x/_i} \quad [4]$$

The N and P yields were calculated as the averaged ratio of biomass concentration on actual nutrient consumption of experiments carried out with leachate, in the case of non-inhibited growth. Accordingly, on the base of the volumetric flow rate of leachate stream and its nutrient content, the biomass concentration C_{xo} - from Eq(4) and the biomass productivity P_X (t y^{-1}) can be calculated, according to:

$$P_x = C_{xo} \cdot Q \quad [5]$$

Concerning the energy balance, by considering that biomass productivity is directly linked to the specific energy irradiation available, the biomass production depends on the incident solar energy: 22 MJ of energy from solar irradiation are needed to produce 1kg of biomass, according to the lower heat value (LHV) of microalgae (Gons and Mur, 1980). Data of solar irradiation (E_{PD}) were obtained from PVGIS (http://re.jrc.ec.europa.eu/pvgis/) for the location of Padova, Italy, and were used to calculate the area A (m^2) required to obtain the target biomass productivity needed to consume all the nutrient contained in the leachate:

$$A = \frac{P_x \cdot LHV}{E_{PD} \cdot \eta} \quad [6]$$

A photosynthetic efficiency (η) of 7 % was considered (Chisti, 2013).

Finally, on the base of the volumetric flow rate of wastewater in real landfills (Q), the height of the flat plate PBR results:

$$H = \frac{\mathrm{Q} \cdot \tau}{A} \quad [7]$$

3. Results and discussion

3.1 Growth of *A. obliquus* in different leachate dilutions

Two control batches were initially carried out to provide baseline data for theoretically ideal and most hostile medium base, to establish the high and low limits for the batches to be undertaken at different leachate dilutions. The earlier one comprised of cultivation in sterile BG11 medium and the latter one in pure leachate, which was referred to as 100 % leachate run. The experimental set up was chosen to investigate cultivation potential at four different ratios of pure unfiltered and unsterilized leachate in deionized water to give dilutions of 1:2, 1:3, 1:4 and 1:10, equating to 50 %, 34 %, 25 % and 10 % fractions of leachate in water. Growth rates were calculated after the beginning of exponential phase of growth. Samples were taken daily to monitor the growth and calculate specific growth rates of cell duplication ($\mu_{duplication}$) and biomass ($\mu_{biomass}$). Results of specific growth rate and final biomass concentration are reported in Figure 1, where zero concentration refers to the control curve carried out in BG11 medium. The general trend suggests an increase in average specific growth rate at higher dilution for both number of cells and dry weight. In particular, the specific growth rate under 10% of dilution (1:10) resulted very close to the BG11 control, even if the final biomass concentration (Fig 1B) is lower, probably due to lower content of phosphorus after dilution. The trends of $\mu_{duplication}$ and $\mu_{biomass}$ were found quite similar, with a $\mu_{biomass}$ generally lower than the duplication. Under 10% of dilution, a larger difference between $\mu_{biomass}$ and $\mu_{duplication}$ was observed, probably due to the presence of cells generally smaller than under the BG11 control.

A review of limited literature involving microalgal cultivation in leachate showed similarities to data attained in this study, with overall better performances recorded in our experiments, especially at lower dilutions (25 % and 34 % leachate in water). More specifically, Zhao et al. (2014) conducted investigation into growth potential of *Chlorella pyrenoidosa* in leachate concentrations of 5 %, 10 %, 15 % and 20 % in municipal wastewater, resulting in growth rates of 0.268, 0.280, 0.251, 0.244 d^{-1}. Similarly, Lin et al. (2007) also reported no significant growth for leachate isolated strains *Chlorella pyrenoidosa* and *Chlorella snowiae* at dilutions containing more than 10 % of leachate. The drop in biomass productivity reported at higher leachate concentration (Lin et al., 2007) are likely caused by an algal growth inhibition under high levels of ammonium (Przytocka-Jusiak et al., 1984). Other studies in the past also reported such excess ammonia associated inhibitory effects on algal growth (Svenson, 2003, Källqvist and Svenson, 2003; Wong et al., 1984).

The lipid content was found quite similar for each dilution, ranging from 38 to 48 % of dry weight (DW). Such a scarce influence of cultivating conditions on the biochemical composition of *A. obliquus* is promising in the perspective of a large-scale application. In fact, while other species show higher lipid contents, these are normally achieved by applying nutritional stress to the culture (in particular N-deprivation), which often also affects overall biomass productivity. A good stable lipid accumulation under a variety of conditions is a valuable property because it may result in a steady production of biofuels.

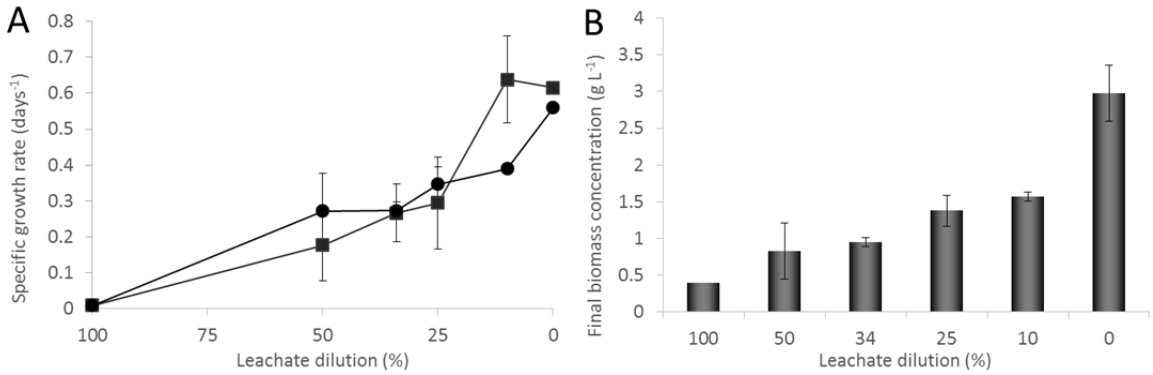

Fig 1: A - Growth rate of A. obliquus calculated as number of cell (square) and dry weight (circles), under different dilution of leachate in distilled water. "0" dilution indicate the control curve carried out in BG11 medium. B - The corresponding final biomass concentration

3.2 Nitrogen and phosphorus consumption by microalgae

The N and P consumption was measured in all batch experiments. The initial concentration of nutrients in the raw leachate were 2,168 mg L^{-1} and 3.56 mg L^{-1}. Table 1 summarizes in detail the results for pure and diluted leachate runs. A no-growth (*NG) was recorded for the pure batch thus no samples were taken forward for nutrient analysis. The algal uptake from various leachate dilutions ranged from between 30-97% for nitrogen, and it was always >90 % for phosphorus. In particular, the highest ammonia uptake was recorded at 216 mg L^{-1} (97 %) for 10 % leachate dilution, while the phosphorus consumption was almost total in each case. This suggests that the main limiting nutrient for algal growth, in this leachate, is phosphorus. Other studies indicated similar results at 10 % leachate dilution, with up to 99 % total nitrogen uptake from a starting concentration of 183.2 mg L^{-1} (Zhao et al., 2014), but with a higher initial P concentration than in our study.

*Table 1: Breakdown of initial and final concentrations of ammonia and phosphorous with consumption percentage at different dilutions of leachate to water *NG = No Growth*

Dilution	Leachate %	Ammonia			Phosphorus		
		Initial (mg L^{-1})	Final (mg L^{-1})	Consumed (%)	Initial (mg L^{-1})	Final (mg L^{-1})	Consumed (%)
Pure	100 %	2,168.10	*NG	*NG	3.56	*NG	*NG
1:2	50 %	1,084.05	763.16	30	1.78	0.12	93
1:3	34 %	780.89	-	-	1.21	0.04	97
1:4	25 %	542.03	191.96	65	0.89	0.03	97
1:10	10 %	216.81	5.60	97	0.36	0.01	97

Based on experimental data of nutrients consumption (Table 1) and final biomass obtained (Figure 1B), the N and P yields were calculated, by considering only the cases on non-inhibited growth. These number resulted 0.19 mg_N mg_X^{-1} for nitrogen and 0.0021 mg_P mg_X^{-1} for phosphorus, which are in agreement with the current literature available (Sforza et al., 2014).

3.3 Design of an integrated leachate treatment and algal biomass production plant

Based on experimental measurements reported in previous sections, a combined process with microalgae production and leachate treatment is proposed: *A. obliquus* was found able to efficiently remove N and P from

leachate, achieving a good biomass production, but only when a proper dilution was set, in order to avoid inhibitory effects of high concentration of free ammonia.
The microalgal reactor design was based on nutrient yields expressed by Eq(4). A CSTR working at steady-state conditions was assumed. Microalgal reactor design can be based on phosphorus or nitrogen balances, depending on whichever of these is more precautionary: in this work, the reactor design was firstly based on P balance, which resulted to be the limiting nutrient in the wastewater considered. Nutrients and flow rates vary significantly depending on the landfill considered. Accordingly, a first base case was assumed for calculations, corresponding to the data of landfill where the leachate used in this study was sampled. The values of parameter used as the basis for all calculation are reported in Table 2. In particular, a residence time of 2.56 days was set, corresponding to the maximum specific growth rate obtainable, according to Eq(3). The mass balance is based on DW, thus the maximum specific growth rate chosen was the $\mu_{biomass}$ measured under 10 % of leachate dilution (section 3.1).

Table 2: Values of parameters used for calculations

$Y_{x/_N}$ ($mg_N\ mg_X^{-1}$)	$Y_{x/_P}$ ($mg_P\ mg_X^{-1}$)	LHV ($MJ\ kg^{-1}$)	E_{PD} ($MJ\ m^{-2}\ y^{-1}$)	η (-)	$\mu_{biomass}$ (d^{-1})	τ (d)
0.19	0.0021	22	4500	0.07	0.39	2.56

By applying the P balance from Eq. (4) on the yield values reported in Table 2 and N and P concentration and flow rate of Table 3, the biomass concentration C_{Xo} can be calculated as 170 mg L^{-1}. A dilution of 10 was chosen, basing on the best results obtained in experimental runs reported in section 3.1. The energy balance was also considered, by assuming an annual solar irradiation at middle latitudes of about 4,500 MJ $m^{-2}\ y^{-1}$, and a photosynthetic efficiency of 0.07: accordingly, the area required to ensure the biomass productivity resulted 864 m^2, obtained by applying Eq. 6. Volumetric biomass productivity, associated to the values of residence time set, resulted about 0.07 g $L^{-1}\ d^{-1}$, which is quite low, due to the low content of P in the water solution. In fact, the corresponding nitrogen outlet concentration from the microalgal reactor, calculated by Eq. (4) on N basis, is very high, corresponding to 63% of the initial concentration, suggesting that without any addition of P to leachate it is not possible to efficiently remove the N content of the waste stream.

Table 3: Starting data and results of calculation for the base case, basing on the P yield

C_{Pi} (mg L^{-1})	C_{Ni} (mg L^{-1})	Q ($m^3\ d^{-1}$)	Dilution factor	C_{Xo} (g L^{-1})	P_X (t y^{-1})	Area (m^2)	H (cm)	V_{PBR} (m^3)
3.56	2168	20	10	0.170	12.4	864	59.3	512

To overcome this problem, the addition of an external P source is required or, possibly, the treatment of the leachate before algal cultivation, in order to make available the organic P contained in the raw material, which is generally higher than inorganic ortophosphate content. Accordingly, other calculations were carried out based on the N balance, and by considering a non-limiting P concentration (due to the external addition required). In Table 4, results of calculation are reported, for the base case and for other four cases at two selected N concentration and flow rates, in the range of real data reported for landfills. A 95 % reduction of total initial nitrogen was assumed for all cases.

Table 4: Starting data and results of calculation for the base case and other assumed, basing on the P yield

CASE	C_{Ni} (mg L^{-1})	Q ($m^3\ d^{-1}$)	Dilution factor	C_{Xo} (g L^{-1})	P_X (t y^{-1})	Area (ha)	H (cm)	V_{PBR} (m^3)
base	2,168	20	10	1.0	75	0.524	9.8	512
I	100	10	-	0.5	1.8	0.013	20.1	25.6
II	100	60	-	0.5	11	0.075	20.1	153.8
III	3,000	10	10	1.5	54.8	0.38	6.7	236.4
IV	3,000	60	10	1.5	323.5	2.29	6.7	1536

In the base case, a higher productivity was achieved, corresponding to 0.4 g $L^{-1}\ d^{-1}$, with respect to the calculation carried out without P addition. Looking at the results obtained for the other cases assumed, a larger area is generally required at high N content, which also allows to obtain a high biomass concentration, while the flow rate of leachate affects the height of the reactor, because a constant value of residence time was assumed.

4. Conclusions

The outcome of this work has established two-fold capability for the real potential of utilizing ammonia-N enriched leachate as nutrient source in order to yield high lipid content algal biomass and consequently to reduce the selected leachate waste streams of nitrogen and phosphorus contents. *A. obliquus*, a microalgal species, isolated from landfill, showed a remarkable growth rate in the presence of leachate. Even though the higher growth rate was observed at 10 % of dilution, significant growth was also identified at higher leachate concentrations then previously reported, suggesting that the exploitation of strains isolated from the target environment could be a viable strategy to avoid inhibiting effect. The N consumption by microalgae was high at all dilutions tested, but it was found dependent on the P concentration, which was the limiting nutrient in this waste stream. The lipid content of algal biomass was found stable at all dilution tested, and remarkably high (about 45 % DW). According to experimental results, some preliminary calculation of an integrated process for microalgal biomass production and leachate treatment were carried out. In this work, we demonstrated that a sufficient N abatement from leachate is not achievable without any addition of P to the waste. If P is provided in sufficient concentration, by applying mass and energy balances, the operating variables and the area required for the PBR were calculated, depending on the N load and the flow rate of leachate stream.

Acknowledgments

Authors would thank the Istrana landfill (TV) for providing samples of leachate exploited in this work, and the Solwa SrL staff for the valuable support.

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