

# A Combined Evaporation and Microalgal Cultivation Approach for Efficient Treatment of Landfill Leachate: Batch and Continuous Experiments and Process Simulation

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Leachate from landfill is one of the most relevant unsolved environmental issues, in particular related to the high ammonia content. In this work, the possibility of treating this waste stream with a combined approach that includes both physical and biological treatment was assessed. The process is composed by a first evaporation/condensation step in an innovative industrial technology (WastWa) developed by Solwa SrL, that allows separating an aqueous stream in which about half of the water volume and most of the ammonia are recovered, and a concentrated leachate stream. The ammonia-rich water stream can be then sent to a microalgal cultivation step for N removal, while the concentrated waste can either be partially used to provide micronutrients for microalgal growth or delivered to phytodepuration. The feasibility of the process was tested at lab scale by carrying out both batch and continuous experiments with *Acutodesmus obliquus*, a microalgal species isolated from a pond containing pretreated leachate from an urban landfill located in Lazio (Italy). This strain was cultivated both in untreated leachate and in the aqueous outlet stream of the WastWa unit. A simulation model of the microalgal growth process was implemented in Aspen Plus, keeping into account the chemical equilibrium and ionic speciation of the nutrients in solution as well as the pH. The model was able to reproduce well the experimental data, and can therefore be a useful tool to evaluate the feasibility of the proposed process at large-scale.

## 1. Introduction

The liquid phase generated from landfill, known as leachate and defined as “a liquid that has passed through or emerged from solid waste and contains soluble, suspended or miscible materials derived from such waste” (US Code of Federal Regulations, 1980), is one of the most challenging and complex wastes to treat. It is estimated that roughly 50-250 Mton year<sup>-1</sup> of leachate are produced in Europe (Brennan et al., 2016). The main concern over this waste is related to the extremely high concentration of ammonia nitrogen (ranging from 30-5000 mg L<sup>-1</sup>) (Paskuliakova et al., 2016) and the deleterious effects it can cause to the environment. A number of biological and physico-chemical methods for leachate treatment already exist, but they are generally quite costly. For this reason, alternative processes are continuously investigated.

In the recently emerging context that aims at integrating waste management with the production of valuable resources, the use of microalgae for N removal from landfill leachate appears particularly fit, by producing biomass that can be destined to various applications. These photosynthetic microorganisms are already widely employed in the treatment of urban, industrial and agricultural wastewaters, thanks to their high efficiency in removing nutrients (N and P), as well as other contaminants and heavy metals from such streams (Abdel-Raouf et al., 2012). Some studies have tested the capability of different microalgal strains to grow in raw leachate. However, due to the nature of this substrate, characterized by dark colour and high concentration of toxic compounds, dilutions of at least 1:10 are generally required to obtain satisfactory performances (Sforza et al., 2015; Lin et al. 2007). A possible way to avoid or reduce the issues related to direct cultivation in raw leachate could be the integration of microalgae with an innovative, low-energy

technology developed by Solwa Srl for leachate treatment, i.e. the WastWa process. In this process, the collected leachate flows through a solar greenhouse, which allows evaporating roughly 50% of the water and 98% of the nitrogen (NH<sub>3</sub>), subsequently condensed into an ammonia-rich, clear aqueous stream. Such a system exploits, and is completely sustained by, solar radiation as energy source. Currently, the ammonia-rich aqueous stream is sent to a scrubber, where ammonium salts are precipitated through acidification of the solution, while the concentrated leachate, with an adequate C/N ratio, is delivered to a phytodepuration unit. In this work, the possibility of integrating microalgae cultivation in the WastWa process for N removal was assessed by performing batch and continuous growth experiments in different combinations of the liquid streams involved, and evaluating the performances in terms of biomass production and N uptake. In addition, a simple process simulation model was implemented, to be used as a tool for large-scale material balances evaluation.

## 2. Materials and methods

### 2.1 Algae strain and culture media

The microalga used in this study is *Acutodesmus obliquus* RL01, a species that was isolated from a pond containing pre-treated leachate from a landfill located in Lazio, Italy (Ferrigo et al. 2015). This species was chosen because of its high tolerance to high ammonia concentrations, and for its high growth rate and lipid content even in this type of wastewater (Sforza et al. 2015).

The culture was maintained in BG11 medium with 1.5 g L<sup>-1</sup> NaNO<sub>3</sub> and 30.5 mg L<sup>-1</sup> K<sub>2</sub>HPO<sub>4</sub>, used also for control experiments. Leachate was sampled from an Italian landfill located in Lovadina (TV), Italy, managed by Contarina SpA, and was characterized by concentrations of 832 mg L<sup>-1</sup> NH<sub>3</sub>-N and 6.25 mg L<sup>-1</sup> PO<sub>4</sub>-P, and pH = 7.8. The NH<sub>3</sub>-rich water, obtained from a different leachate sample, was provided by Solwa Srl, and had a concentration of 510 mg L<sup>-1</sup> NH<sub>3</sub>-N and pH = 9.34. Both liquids were stored in a refrigerator (-20°C) before being used for each experiment. No sterilization was carried out on the media prior to algal inoculation.

### 2.2 Experimental set-up and analytical measurements

Microalgal growth experiments were carried out both in batch and continuous mode. In both cases the temperature was kept constant at 28 ± 1°C in a refrigerated incubator. The culture pH was adjusted manually by addition of HCl or NaOH in order to keep its value between 7.5 and 8. A constant light intensity of 100 μmol m<sup>-2</sup> s<sup>-1</sup> of PAR (Photosynthetically Active Radiation, 400-700 nm) was provided by fluorescent lamps and measured with a photoradiometer (Delta OHM HD 2102.1). Air enriched with 5% v/v CO<sub>2</sub> was continuously supplied to the cultures at a total gas flow-rate of 1 L h<sup>-1</sup>.

Batch experiments were carried out, at least in duplicate, in Drechsel bottles of 250 mL, mixed by a magnetic stirrer to avoid sedimentation, and inoculated with an initial microalgae concentration of OD<sub>750</sub> = 0.5, corresponding to 2·10<sup>6</sup> cells mL<sup>-1</sup>. Continuous experiments were conducted in a vertical thin polycarbonate flat-panel photobioreactor (PBR), having a volume of 290 mL and 1.4 cm thickness. The culture was perfectly mixed by air-CO<sub>2</sub> bubbling aided by a magnetic stirrer, so that the reactor can be considered as a CSTR. The inlet medium was continuously supplied at a constant flow-rate by means of a peristaltic pump (Watson Marlow Sci-Q 400), and the culture volume was kept constant by an over-flow tube. The residence time of the culture inside the reactor was then given by:

$$\tau = V_R / Q \quad (1)$$

where  $V_R$  is the reactor volume (mL) and  $Q$  the volumetric flow-rate (mL d<sup>-1</sup>). After an initial transient period observed when changing experimental conditions, the biomass concentration and the other biochemical characteristics reached a stable steady-state.

The microalgal concentration was monitored daily by measuring the optical density at 750 nm (OD<sub>750</sub>) with a spectrophotometer (UV-Visible UV 500 by Spectronic Unicam, UK) and the cell concentration using a Bürker Counting Chamber (HBG, Germany). The specific growth rate ( $\mu$ , d<sup>-1</sup>) in batch experiments was calculated as the slope of the straight line interpolating 3 to 5 points of the logarithmic phase of growth. The biomass concentration was also measured in terms of g L<sup>-1</sup> as dry weight (DW) at the end of each batch curve, and daily in continuous experiments. DW was determined gravimetrically by filtering 5 mL of sample with previously weighted 0.2 μm filters, then dried in a laboratory oven at 80°C for 4 h. The nutrients analysed in the inlet and outlet medium were NH<sub>3</sub>-N and PO<sub>4</sub>-P. The former was measured using standard test kits (HYDROCHECK SPECTRATEST, Reasol®), by reaction with Nessler reagent in alkaline conditions, while orthophosphates were detected by a modified ascorbic acid method, described in APHA-AWWA-WEF, 1992.

## 2.3 Simulation model

A simple model to simulate the growth of *A. obliquus* in continuous systems was developed using Aspen Plus software® (v. 8.2). In order to keep into account the chemical equilibrium and ionic distribution of nutrients in aqueous solution as well as pH, *ElecNRTL* (Electrolyte Non-Random Two-Liquid) was selected as thermodynamic model, considering the equilibria of ammonium/ammonia, carbonates and orthophosphates. Prior to the PBR, a FLASH unit was set, to reproduce the equilibrium between the NH<sub>3</sub>-rich aqueous stream (pH = 9.34), K<sub>2</sub>HPO<sub>4</sub> added as P source, and a 5% v/v CO<sub>2</sub>-air gaseous stream, which serves as C source for microalgae as well as for regulating the pH to a value of around 8. The liquid stream exiting the FLASH represents the inlet medium for the PBR.

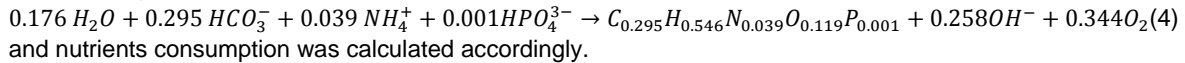
The PBR was modelled as 4 CSTR reactors in series that approximate the behaviour of a PFR, more feasible at large-scale than a single CSTR. Each reactor was represented by the following material balance equation (at steady-state):

$$0 = c_{x,e} - c_x + r_x \tau \quad (2)$$

where  $c_x$  and  $r_x$  are the biomass concentration (g L<sup>-1</sup>) and growth rate (g L<sup>-1</sup> d<sup>-1</sup>), and the subscript "e" refers to inlet conditions. Assuming a first order kinetics with respect to the biomass concentration ( $r_x = kc_x$ ), eq. 2 can be re-written as:

$$c_x = c_{x,e} / (1 - k\tau) \quad (3)$$

A value of  $k = 0.506$  d<sup>-1</sup> was taken for this species, based on experimental results. A partial recycle of the outlet biomass from the last CSTR unit was considered in order to have an inlet concentration  $c_{x,e} = 0.25$  g L<sup>-1</sup>, to avoid wash-out of the culture. Finally, based on the elemental composition of the microalga, the stoichiometry of biomass production was written as:



## 3. Results and discussion

### 3.1 Batch experiments

A first set of experiments was carried out cultivating *A. obliquus* in raw leachate, according to the conceptual design shown in Figure 1A, whose aim is to reduce the concentration of N, P and other micro-pollutants prior to the evaporation and phytodepuration steps. In the preliminary experiments, three different dilutions were applied to leachate, namely 1:1 (raw), 1:2 and 1:3. As reported in Figure 1B, even though a slight microalgal growth was measured with increasing dilution, it was still much lower compared to that achieved in BG11. Consequently, nitrogen uptake was also relatively little (Table 1). The reason of such reduced growth is likely due to the dark colour of the medium, which strongly limited light penetration, or to the presence of possible toxic compounds at high concentrations. This confirms that stronger dilutions would be required to achieve acceptable performances, which is not preferable from the industrial and environmental standpoints in terms of water consumption, so that alternative configurations should be employed for an efficient treatment.

In this regard, it appears preferable to place the microalgal cultivation system after the WastWa evaporation unit, according to Figure 2A, so that the PBR is fed with a clear medium, still containing most of the N. A first experiment was hence carried out cultivating *A. obliquus* in the NH<sub>3</sub>-rich water, diluted 2-fold with tap water to avoid possible toxicity effects related to high concentrations of free-ammonia (Azov and Goldman, 1982).

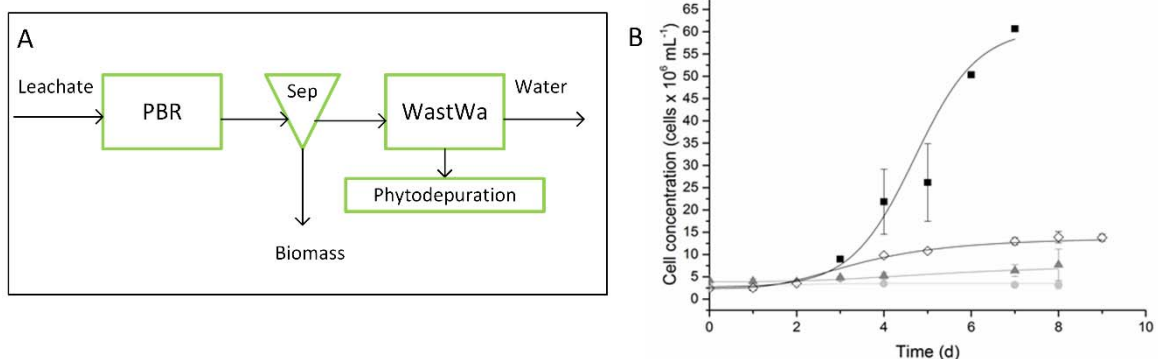


Figure 1: Growth of *A. obliquus* in raw leachate. Conceptual design (A) and growth curves (B). In Fig. 1B, light grey circles refer to 1:1 dilution, grey triangles 1:2, open diamonds 1:3, while black squares are control BG11.

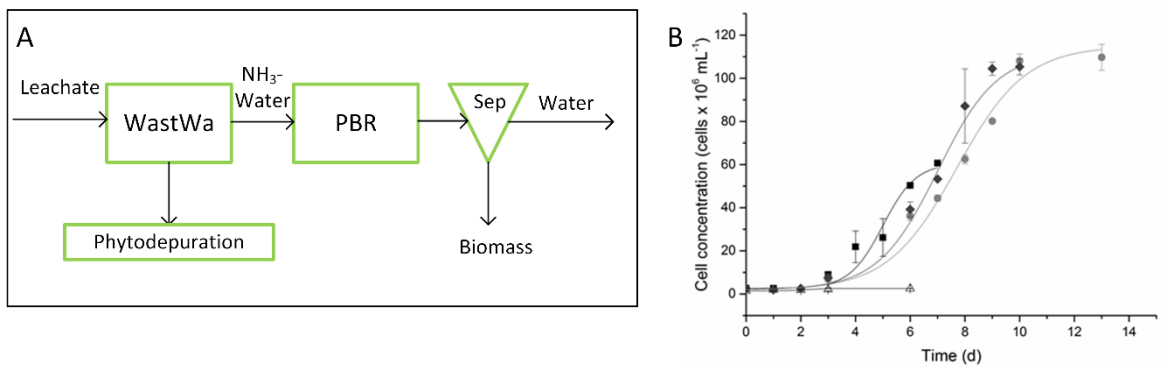


Figure 2: Growth of *A. obliquus* in  $\text{NH}_3$ -rich water. Conceptual design (A) and growth curves (B). In Fig. 2B, open triangles refer to 1:2  $\text{NH}_3$ -rich water diluted with tap water, light grey circles to 1:2  $\text{NH}_3$ -rich water diluted with BG11<sub>0</sub>, grey diamonds to undiluted  $\text{NH}_3$ -rich water with BG11 nutrients, while black squares are control BG11.

Reasonably, no growth could be obtained in these conditions as, apart from N, no other nutrient is dissolved in this aqueous medium (Figure 2B). On the other hand, by using BG11<sub>0</sub> (i.e. BG11 without  $\text{NaNO}_3$ ) to dilute the  $\text{NH}_3$ -water to the same extent, the growth performances were greatly improved compared to direct cultivation in leachate, achieving a specific growth rate similar to that of the control ( $0.59 \text{ d}^{-1}$ ), and 73.5% N consumption from the medium (Table 1). In addition, considering that *A. obliquus*, being isolated from a leachate-containing pond, should have a higher tolerance to elevated  $\text{NH}_3$  concentrations, a third experiment was carried out still supplying other nutrients to match BG11 concentrations, but without diluting the  $\text{NH}_3$ -water medium. Under these conditions, growth performances equal to the previous case (Figure 2A) were obtained, confirming that *A. obliquus* can indeed tolerate higher ammonia concentrations, and no dilution was required. Moreover, even though the percent nitrogen consumption is reduced (47%), the net consumption is higher ( $226 \pm 21 \text{ mg L}^{-1}$ ). Therefore, even though the main goal of integrating microalgae cultivation in the WastWa process is that of removing  $\text{NH}_3$ -N, while the other pollutants are dealt with by phytodepuration, nutrients are required in the medium for microalgae to grow and up-take the nitrogen. By considering that the main issue of direct cultivation in the leachate was related to the need of stronger dilutions, but that overall it contained nutrients concentrations sufficiently high to sustain growth, the two process schemes investigated could be integrated, as shown in Figure 3A. Here, a small percent of influent leachate by-passes the WastWa evaporation unit and is mixed with the  $\text{NH}_3$ -water stream to supply the required nutrients, which is preferable than providing them by external supplies.

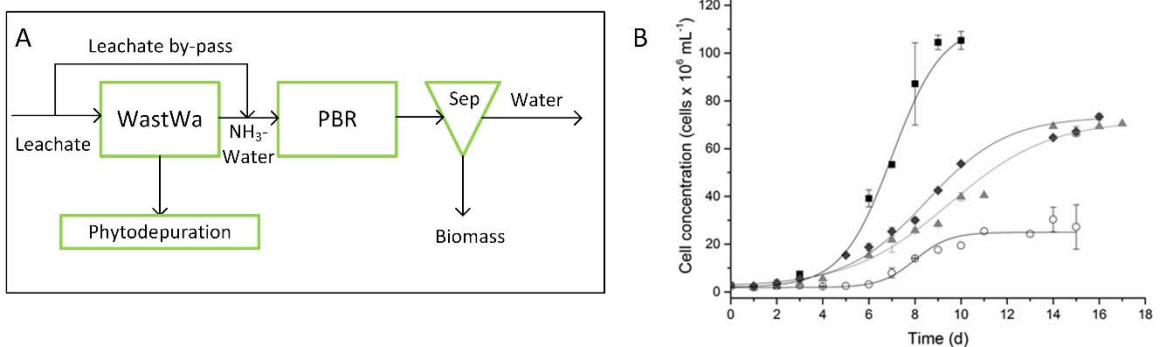


Figure 3: Growth of *A. obliquus* in  $\text{NH}_3$ -rich water + leachate. Conceptual design (A) and growth curves (B). In Fig. 3B, open circles refer to 10% leachate, light grey triangles to 5% leachate, grey diamonds to 3% leachate, while black squares are growth in undiluted  $\text{NH}_3$ -water supplied with BG11 nutrients, as a reference.

The maximum amount of leachate that can be by-passed according to the proposed scheme is set by the discharge limit for chloride ions: these in fact are not up-taken by microalgae, and since no further treatment is considered after the PBR, their concentration prior to the cultivation system needs to be lower than such limit. Hence, for the specific leachate used in this study, a maximum of 10% could be deviated. Accordingly, by-pass percentages of 10%, 5% and 3% v/v were used to assess microalgal growth performances and N uptake. In each case, an appropriate amount of P ( $\text{K}_2\text{HPO}_4$ ) was added to keep a non-limiting N/P ratio, equal

to that of BG11. This macronutrient is in fact generally limiting in leachate (Paskuliakova et al., 2016). The results are shown in Figure 3B. Table 1 summarizes all the growth experimental runs performed. As it can be seen, when 10% of the leachate was mixed with the NH<sub>3</sub>-water, a long lag-phase was observed, and a lower final cell concentration was reached compared with the other two cases. However, the specific growth rate and the final biomass dry weight were comparable, and so was the N uptake, which gets around 50% (220-250 mg L<sup>-1</sup>) for all the conditions investigated. In fact, under 10% leachate by-pass, microalgal cells were noticed to be larger, which might indicate a stress condition. Overall, even if the specific growth rate is lower compared to that of external micronutrients supply, the results obtained are promising, showing satisfactory performances while minimizing water and external nutrients supply.

Table 1: Summary of batch experiments (\*NG = No Growth)

| Medium                                       | $\mu$ (d <sup>-1</sup> ) | DW (g L <sup>-1</sup> ) | N <sub>in</sub> (mg L <sup>-1</sup> ) | N <sub>fin</sub> (mg L <sup>-1</sup> ) |
|--|--------------------------|-------------------------|---------------------------------------|--|
| BG11   | 0.58                     | 2.98 ± 0.38             | -                                     | -                                      |
| Leachate 1:1                                 | *NG                      | *NG                     | 832 ± 16                              | *NG                                    |
| Leachate 1:2                                 | 0.1                      | 0.61 ± 0.13             | 388 ± 23                              | 319 ± 31                               |
| Leachate 1:3                                 | 0.3                      | 1.13 ± 0.04             | 263 ± 7                               | 141 ± 0.34                             |
| NH <sub>3</sub> -water 1:2                   | *NG                      | *NG                     | 255 ± 3                               | *NG                                    |
| NH <sub>3</sub> -water/BG11 <sub>0</sub> 1:2 | 0.59                     | 1.84 ± 0.08             | 227 ± 15                              | 60 ± 6.5                               |
| NH <sub>3</sub> -water+nutrients             | 0.59                     | 2.4 ± 0.05              | 484 ± 12                              | 258 ± 9                                |
| NH <sub>3</sub> -water+10% leachate          | 0.38                     | 2.2 ± 0.09              | 500 ± 7                               | 247.5 ± 0.6                            |
| NH <sub>3</sub> -water+5% leachate           | 0.4                      | 2.85 ± 0.11             | 478 ± 15                              | 241 ± 2                                |
| NH <sub>3</sub> -water+3% leachate           | 0.42                     | 2.69 ± 0.04             | 424 ± 5                               | 207 ± 2.9                              |

### 3.2 Continuous experiments

The batch experiments showed promising results and allowed identifying a possible process scheme to integrate microalgal cultivation in leachate treatment. However, from a practical perspective it is more interesting to operate in a continuous system. Continuous experiments were hence carried out in different media (NH<sub>3</sub>-water with 5% leachate and NH<sub>3</sub>-water with external nutrients supply), at different values of residence time. The results obtained are summarized in Table 2. It can be noticed that, when feeding the NH<sub>3</sub>-water solution mixed with 5% leachate to the PBR, a residence time of 1.8 d was not sufficient to sustain growth, and wash-out of the culture occurred. It is then necessary to operate the PBR at higher values of  $\tau$ . Using a residence time of 2.3 d, on the other hand, allowed obtaining a biomass concentration of 0.78 g L<sup>-1</sup>, and a corresponding productivity  $P_x = DW/\tau$  of 0.339 g L<sup>-1</sup> d<sup>-1</sup>, which were even slightly higher when using a  $\tau$  of 3.3 d. To verify whether some micronutrients present in the leachate in insufficient amounts limited biomass productivity and N consumption, an experiment was carried out supplying all the other nutrients of BG11 to the NH<sub>3</sub>-water, at  $\tau = 2.3$  d. Indeed, the biomass productivity and N consumption resulted almost 50% higher than the previous case, highlighting that micronutrients composition is a relevant factor also in continuous cultivation. Unfortunately, the outlet N concentration under the conditions tested resulted higher than the discharge limit of 11.67 mg L<sup>-1</sup> NH<sub>3</sub>-N. However, it must be considered that most likely the light intensity used (100  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) was limiting, and that higher values should be used to improve the performances of the system. Overall, this work shows that *A. obliquus* is able to grow and uptake nutrients from leachate also in continuous systems, reaching stable steady-state. These results are promising and establish the potential for further investigation and application of the proposed process at large scale.

Table 2: Results of continuous experiments (\*WO = Wash-out)

| Medium                             | $\tau$ (d) | DW (g L <sup>-1</sup> ) | $P_x$ (g L <sup>-1</sup> d <sup>-1</sup> ) | N <sub>in</sub> (mg L <sup>-1</sup> ) | N <sub>out</sub> (mg L <sup>-1</sup> ) |
|------------------------------------|------------|-------------------------|--|---------------------------------------|--|
| NH <sub>3</sub> -water+5% leachate | 1.8        | *WO                     | *WO  | *WO                                   | *WO                                    |
|                                    | 2.3        | 0.78 ± 0.1              | 0.339 ± 0.043                              | 455 ± 21                              | 366 ± 7                                |
|                                    | 3.3        | 1.2 ± 0.02              | 0.364 ± 0.006                              | 447 ± 30                              | 297 ± 12                               |
| NH <sub>3</sub> -water+nutrients   | 2.3        | 1.12 ± 0.07             | 0.487 ± 0.03                               | 388 ± 18                              | 255 ± 3                                |

### 3.3 Simulation results

Given the high variability in leachate composition, which depends on many environmental factors, a tool predict the performances of the system and evaluate proper operating conditions prior to carrying out actual experiments is indeed very useful. Accordingly, the main goal of the simulations was to verify that the model

proposed in Section 2.3 could reproduce well the experimental data in terms of biomass growth and nutrients consumption in the continuous PBR. Major focus was paid to the chemical equilibrium of macronutrients (C, N, P) in the PBR inlet stream, calculated by means of the FLASH unit. It was verified that, for an inlet flow-rate of 100 L h<sup>-1</sup> of NH<sub>3</sub>-water (510 mg L<sup>-1</sup> of N and 11.1 mg L<sup>-1</sup> of P) at pH = 9.34, between 30 and 50 L h<sup>-1</sup> of air enriched with CO<sub>2</sub> 5% v/v are required to lower the pH to a value between 7.8 and 8, suitable for algal growth. In these conditions, NH<sub>4</sub><sup>+</sup> and HCO<sub>3</sub><sup>-</sup> are the predominant ionic species for N and C, while H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and HPO<sub>4</sub><sup>2-</sup> are present in almost equal proportion. The overall residence time was set to a value of  $\tau = 2.3$  d. Results are displayed in Table 3. It can be seen that the model outputs reproduce quite well the experimental data, and could therefore be used as a preliminary tool for large-scale material balances evaluation. On the other hand, the model could be quite easily improved by considering the effect of light on the growth rate constant  $k$ , which is worth further investigation.

Table 3: Results of simulation and comparison with experimental data

|   | Simulation | Experimental |
|---|------------|--------------|
| Outlet biomass concentration (g L <sup>-1</sup> )         | 1.08       | 1.12 ± 0.07  |
| Biomass productivity (g L <sup>-1</sup> d <sup>-1</sup> ) | 0.451      | 0.487 ± 0.03 |
| N consumption (mg L <sup>-1</sup> )                       | 101        | 133 ± 21     |

#### 4. Conclusions

The results obtained in this work outlines a promising process flowsheet for leachate treatment that integrates microalgal cultivation for NH<sub>3</sub>-N removal and biomass production with an innovative, low-energy process (i.e. WastWa). *Acutodesmus obliquus*, a microalgal species isolated from landfill, showed good growth rates (0.4-0.6 d<sup>-1</sup>) in batch experiments, as well as stable steady-state production in continuous systems, even under high ammonia concentrations. Considering the experimental results obtained, the most effective process scheme comprises microalgal cultivation following the NH<sub>3</sub>-water evaporation step in a solar greenhouse, exploiting a small fraction of the inlet leachate to provide other nutrients to the medium that are required for microalgal growth. However, P and possibly some trace elements will be needed to make-up suitable concentrations. Unfortunately, under the conditions investigated the N consumption was not sufficient to guarantee outlet concentrations that meet the law discharge limits. It is however worth highlighting that higher light intensities could improve the performances, possibly leading to positive results. The simulation model implemented reproduced quite well the experimental results obtained in the continuous system. Therefore, it would be of much interest to study the effect of light from both the experimental and simulation point of view, before scaling the process up to industrial applications.

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