Chapter 120

Ammonianitrogen removal by *chlorella* sp. in different landfill leach dilutions

https://doi.org/10.56238/methofocusinterv1-120

Maria Celia Cavalcante de Paula e Silva
PhD in Environmental Engineering from the Graduate Program in Environmental Science and Technology of the State University of Paraíba - UEPB.
E-mail: celiadoutorado@gmail.com

Maria Virginia de Conceição Albuquerque
PhD in Environmental Engineering from the Graduate Program in Environmental Science and Technology of the State University of Paraíba - UEPB
E-mail: virginia.albuquerque@yahoo.com.br

Roberta Milena Moura Rodrigues
PhD student in Environmental Engineering at the Graduate Program in Environmental Science and Technology of the State University of Paraíba - UEPB
E-mail: robertamilena_rm@hotmail.com

Railson de Oliveira Ramos
PhD in Analytical Chemistry from the Federal University of Paraíba- UFPB
E-mail: railson_uepb@outlook.com

Evelyne Morgana Ferreira Costa
PhD student in Environmental Engineering at the Graduate Program in Environmental Science and Technology of the State University of Paraíba - UEPB
E-mail: evelyne.fcosta@gmail.com

Wilton Silva Lopes
PhD in Chemistry, Federal University of Paraíba - UFPB, Associate Professor B, Department of Sanitary and Environmental Engineering, Science and Technology Center of the State University of Paraíba - UEPB
E-mail: wiltonuepb@gmail.com

Howard William Pearson
PhD in Environmental Microbiology from the University of London, Professor of PPGEQ, Federal University of Campina Grande - UFCG
E-mail: howard_william@uol.com.br

Valderi Duarte Leite
PhD in Hydraulics and Sanitation from USP, Associate Professor C, Department of Sanitary and Environmental Engineering, Science and Technology Center, State University of Paraíba - UEPB1
E-mail: mangabeiraleite@gmail.com

ABSTRACT

Sanitary landfill leachate is a highly polluting complex matrix wastewater. It has high concentrations of ammoniacal nitrogen, phosphorus, carbonaceous matter and recalcitrant substances. The application of microalgae to remove pollutants from leachate has been investigated. The *Chlorella* sp. applied in this work, was isolated from the leachate from the sanitary landfill in João Pessoa-PB. The experimental system consisted of 3 bioreactors with a useful volume of 210 mL, being 200 mL of leachate diluted in distilled water and 10 mL of *Chlorella* sp. in stationary phase, fed in batch, photoperiod of 24 hours, temperature of 27º C, TDH of 240 hours and influent concentrations of ammoniacal-N of 46, 192 and 575 mg. L-1 and the positive control. The highest cell densities were recorded at the influential ammonia N concentrations of 46 and 192 mg. L-1, with increments greater than 250% until the 5th day of monitoring. The lowest growth was obtained at the ammoniacal nitrogen concentration of 575 mg. $^{L-1}$ with 12% increments until the 5th day. The analysis of the residual nitrogen mass calculation in the system indicated mass removal of 66, 59 and 56% for affluent inputs of 9.66; 40.32 and 120.75 mg-N for respective influent-N concentrations of 46, 192 and 575 mg. L-1. The results are indicative that *Chlorella* sp. can adapt and grow in different concentrations of ammoniacal nitrogen, having the potential to be applied efficiently in the tertiary treatment of landfill leachate.

Keywords: Nitrogen ammonia, Phytoremediation, Microalgae, Manure treatment.

1 INTRODUCTION

The generation of municipal solid waste by society represents a challenge that needs the participation of several segments to solve it. The largest fraction of these residues is arranged in controlled landfills and dumps, which, during its biodegradation, causes several problems, among these, the generation of leached, liquid residue of high contaminant power for the ecosystem in general.

Landfill leaching has high levels ofammoniacal nium (3000-5000 mg. L-1- Ammoniacal N), toxic organic compounds, dissolved xenobiotics, low ratio of demand of chemoand biochemical oxygen (BOD_5: DQO → 0.2 or less) and heavy metals (KHANZADA et al. 2018). Leachised is considered a wastewater of high ionic strength due to dissolved inorganic nutrients, which can lead to salinity with increased chloride ion levels (~ 5g Cl^-. L-1), total dissolved salts and conductivity (CHENG et al., 2011; KJELDSEN et al., 2002).

The ammoniacal nitrogen present in leachate can take ionic or free form in aqueous solution, thus depending on its pH and temperature concentration. Ammoniacal nitrogen concentration increases over time and can be one of the main long-lasting pollutants. One of the main issues related to the management of closed landfills is the elimination of leache that continues to be produced (with high concentration of NH^{4+} -N) for a long time, even after the closure of the landfill (KJELDSEN et al., 2002).

Bioremediation applyingmicroalgae, better known as phytoremediation, has been an ecological approach to comba to haveenvironmental pollution, such as wastewater treatment (MISHRA et al. , 2018). Microalgae are important in tertiary effluent treatment due to their metabolic capacity to remove nutrients, pollutants and heavy metals (PACHECO et al., 2015). Nitrogen is the main constituent of proteins, hormones, energy transfer molecules (ATP), genetic material construction, chlorophyll and enzymes involved in photosynthesis. It is responsible for 1-10% dry biomass and its availability affects microalgae photosynthesis (JIA and YUAN, 2016).

Silva et al. (2017), monitoring tubular bioreactors with a volume of 0.1L with *chlorella* sp. filling immobilized in calcium alginate spheres in final concinlet of 2, 4 and 6%, with 5-hour TDH, fed by sand filter effluent, under intermittent batch, obtained removal of 81% of total phosphorus in bioreactors with calcium alginate spheres, obtained removal of 81% of total phosphorus in bioreactors with calcium alginate spheres, obtained removal of 81% of total phosphorus in bioreactors with calcium alginate spheres, obtained removal of 81% of total phosphorus in bioreactors with calcium alginate spheres, obtained removal of 81% of total phosphorus in bioreactors with calcium alginate spheres, obtained removal of 81% of total phosphorus in bioreactors with calcium alginate spheres concentration of 2%. The removal values obtained for bioreactors with 4% and 6% alginate were significantly lower.

In a study by Silva et al. (2019), monitoring bioreactors filled with *Chlorella* sp. immobilized in a 4% calcium alginate matrix, with controlled temperature and luminosity, in the removal of Ammoniacal N, fed by substrate consisting of domestic sewage and leached from landfill with different concentrations of

ammoniacal nitrogen, 3-hour TDH and batch feeding regime, removals were recorded between 59 and 81% at the concentrations tested.

Considering the complex chemical matrix presented by leaching and the multiple impacts that this liquid residue can cause on soil and aquatic ecosystems, when released without treatment, this work aimed to study the removal of ammoniacal nitrogen *by Chlorella* sp. in different dilutions of leaching landfill to obtain an effluent with better sanitary conditions.

2 METHODOLOGY

2.1 GENERAL CONSIDERATIONS

This work was carried out in the physical facilities of the Experimental Station of Biological Treatments of Sanitary Sewage (EXTRABES), belonging to the State University of Paraíba, located in the Neighborhood of Tambor, in the city of Campina Grande - PB.

The LAS (leached landfill) was collected at the entrance of the decanting pond of the leachtreatment pond system of the landfill in the metropolitan region of the city of João Pessoa -ASMJP- PB, transported in polyethylene reservoirs of 250L to the extrabes facilities, and characterized byphys ica and chemically. Figure 1 shows an image of the aerialview of the landfill in the city of João Pessoa.

Figure 1-Aerial view of the ASMJP, with emphasis on the leach treatment ponds.

Source: Google Earth

2.2 IDENTIFICATION OF ITOPLANKTON

They were inoculated, 5 mL of leached in 10 erlenmeyer vials of 250 mL, each containing 100 mL of sterile ASM-1 medium, (GORHAM et al. 1964 and ZAGATTO and ARAGON, 1992). The samples were placed ona rotator table at Therbital Digital AM-255 with 80 rpm, temperature of 30º C and photoperiod of 24 hours. After the 7-day period, the Olympus CBA binocul ar microscopewas identified, up to 400 x increased. The classification system for classes and genera followed recommendations by Bicudo and Menezes (2006), and for the species specific identification keys of each group were used.

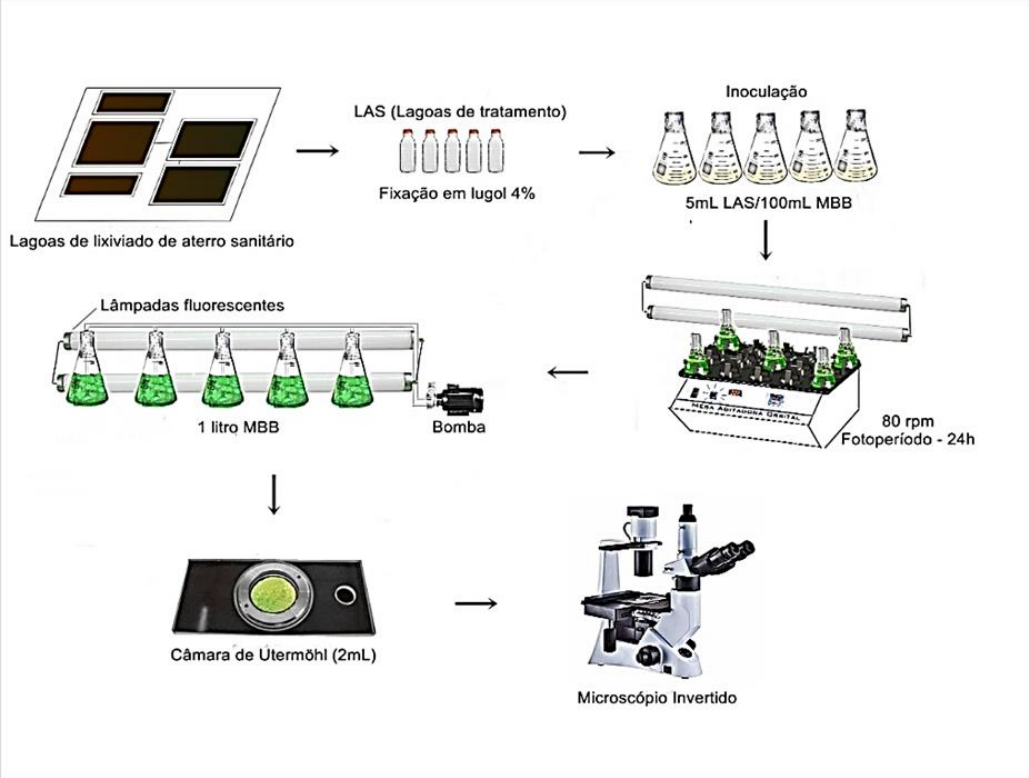
Phytoplankton counting was performed by a 2 ml Utermöhl chamber of CIENLAB, by the Utermöhl sedimentation method (1958).

2.3 *CHLORELLA* SP. ISOLATION

In view of the phytoplankton survey, *chlorella* sp. was isolated by the plate Agar method recommended by Guerrero III and Villegas (1982). The *chlorella* sp. strain was inoculated in Petri dishes, ré-sterilized containing Basal Bold Medium 's-MBB (BISCHOFF and BOLD, 1963; BOROWITZKA, 1988) with 1.5% agar. The samples were kept in chamber d andcultured with temperature of 27º C in 24-hour photoperiod, illumination of 4 fluorescent lamps, intensity of photons of 85 µE.s-1. $^{m-2}$.

After 21 days, the isolation was carried out using NETLAB glass pasteur pipette. The observation of the genus algal was carried out under an inverted microscope of the Oleman brand in a 400x objective, and this unialgal sample was chopped in erlenmeyers vials containing 50 ml of MBB. At 21 days, the microalgae were inoculated in 100 mL of MBB and placed on the rotational table with 80 rpm in erlenmeyers vials of 250 mL. After 7 days, 32 mL of culture medium was resuspended in erlenmeyer s 2L vials containing 1600mL MBB. Figure 2 shows a schematic diagram from the collection to the identification of the integral phytoplankton species.

Figure 2. Sequential steps from LAS collection to phytoplankton identification.



2.4 REAGENTS

The following chemical components were used to prepare phytoplankton culture media:

The sterile ASM-1 medium was prepared with the following reagent solutions: NaNOur (8.5 g.L-1), MgSOur ·7HurO, (2.45 g.L-1) MgClur + 6HurO (2, 05g. L-1) CaClur 2HurO (1, 45g. $^{L-1}$), KHしいPOしい, (8.7 g.L-1), Naしいhpour · 12HurO (17.8 g.L-1), HurBO (28.40 g. L-1), MnClur · 4HurO (13.9 g. L-1), FeClしい · 6HurO (10.8 g. L-1), ZnClur (3.35 g. L-1) CaClur 6HurO 0.1900, CaClur · 2HurO, 0.0140 g. L-1), EDTA titriplex (18.6 g. L-1). For each stock solution the compounds were diluted in 1,000 mL of deionized water and stored in the freezer in plastic bottles.

Bolds asal B medium (BBM) for *chlorella* sp. cultivation was composed of $NaNO_3$ (250 mg $^{L-1}$), $MgSO_4 \cdot 7H_2O$ (75 mg $^{L-1}$), NaCl (25 mg $^{L-1}$), K_2HPO_4 (75 mg $^{L-1}$), KH_2PO_4 (175 mg L $^{-1}$), $CaCl_2 \cdot 2H_2O$ (25 mg $^{L-1}$), $ZnSO_4 \cdot 7H_2O$ (8.82 mg $^{L-1}$), $MnCl_2 \cdot 4H_2O$ (1.44 mg $^{L-1}$), MoO_3 (0.71 mg $^{L-1}$), $CuSO_4 \cdot 5H_2O$ (1.57 mg $^{L-1}$), Co $(NO_3)_2 \cdot 6H_2O$ (0.49 mg $^{L-1}$), H_3BO_3 (11.42 mg $^{L-1}$), Na_2 EDTA (50 mg $^{L-1}$), KOH (31 mg $^{L-1}$), $FeSO_4 \cdot 7H_2O$ (4.98 mg $^{L-1}$).

2.5 MONITORING OF BIOREACTORS

Three bioreactors were assembled, fed under batch. These received leached from a landfill diluted in distilled water with a final volume of 200 mL, at different affluent concentrations of Ammoniacal N (C_1, C_2 and C_3) being 46, 192 and 575 mg, respectively. L-1 and um positive control containing mbb. Each bioreactor was inoculated with 10 mL of cultivation with *Chlorella* sp. in stationary phase presenting Initial Lar Celu Density(ICD) of $1.78437x10^5$ célula.mL-1. The bioreactors were kept in an environment with a photoperiod of 24 hours, controlled temperature at 27º C and TDH of 240 hours.

Three aliquots of 50mL each were collected, at time zero(0), 120 and 250 hours, to determine pH and ammoniacal nitrogen. For quantification of *Chlorella* sp. cells, a neubauer chamber count was performed in which, to determine the cell concentration, all the cells of the larger individual blocks of the Neubauer chamber applied in Equation 1 were counted, according to Tavares and Rocha (2003).

$$C\ (células/\ mL) = contagem\ total\ x\ 10^4 / n^0\ de\ blocos\ contados$$

Equation 1

The parameters of characterization of leachate and their respective analytical methods followed what is recommended in APHA (2012). For the evaluation of the ions, theshow was filtered in glass fibra membrane, gf/c-whatman grade of 0.45 and 0.22 µm for injection in ionic chromatograph Dionex ICS-1100 from marca Thermo Scientific. The operating temperature was 35º C, loop volume of 25 µL and eluent flow was 0.25 mL.min-1. The operating conditions of the columns are described in Table 1.

Table 1. Operating conditions of the ionic chromatograph.

Analysis of anions	**Parameter**	**Dionex Ion Pac AG23**
	Pre-Column Suppressive	Dionex Ion Pac AG23 ASRS 300 2 mm
	Eluent	Carbonate Solution (4.5 mM) and Baking Soda (98 mM)
	Pressure in the column and pre-column	< 1100 Psi
Cation Analysis	Chromatographic Column	Dionex Ion Pac CS 12A
	Pre-Column	CS CG 12[a]
	Suppressive	ASRS 300 2 mm
	Eluent	10 mmol solution. $^{L-1}$ of H_2SO_4.
	Pressure in the column and pre-column	< 1100 Psi

3 RESULTS AND DISCUSSION

3.1 CHARACTERIZATION OF LEACHED

The leaching applied in the research was characterized physically and chemically. Table 2 shows the characterization data of the LAS used in the cultivation of *Chlorella* sp.

Table 2: physical and chemical parameters of the LAS applied in the research.

PARÂMETRO	Magnitude
DQO total (mgO_2/L)	3647,8
DQO filtrada(mgO_2/L)	2270,6
DBO_5(mgO_2/L)	1163,2
NTK(mgN/L)	2710
N-NH_4^+(mg- NH_4^+/L)	2514
N-NO_3^- (mg N- NO_3^-/L)	7,38
N-NO_2^- (mgNO_2^-/L)	-
ST (mg/L)	16003,3
STV (mg/L)	5430
STF (mg/L)	10573,33
SST(mg/L)	210
SSV(mg/L)	193,33
SSF(mg/L)	16,67
Ortofosfato(mg Orto-P/L)	14,184
Fósforo Total(mg/L)	18,03
Cl^- (mg/L)	3619,8
Na^+ (mg/L)	2301
K^+ (mg/L)	2000
Mg^+(mg/L)	275,04
Ca^{++}(mg/L)	626,46
pH	8,0

Source: search data

In the data analysis of Table 1, a high concentration of total solids is identified, which may interfere in the passage of light to the microalgeous photosynthetic process. As for ammoniacal nitrogen, in sua greater fraction in the form of NH^{4+}, approximately 95% as a function of pH 8.0 , is the form preferably assimilated by microalgae, however, its magnitude suggests that leaching should be diluted in distilled water or sewage to reduce its toxicity potential and, favor removal. The concentration of Orthophosphate, an immediate assimilatedfraction, is in the range of 14.2 mg. L-1, which may favor the growth *of Chlorella* sp. by integrating nucleic acid molecules.

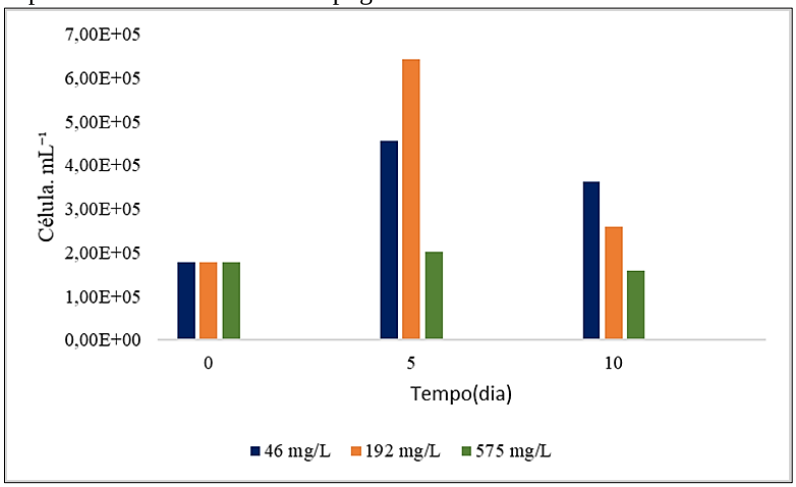
The significant availability of macronutrients such as phosphorus and nitrogen in leached may favor biomass growth, however, for the magnitude of ammoniacal nitrogen, it is recommended to dilute the residue before treatment. According to Procházková et al. (2014), for optimal growth of microalgae a number of nutrients is required, such as macronutrients such as carbon (C), nitrogen (N), oxygen (O), hydrogen (H) and phosphorus (P), as well as calcium (Ca), magnesium (Mg), sulfur (S) and potassium (K).

3.2 CELL GROWTH

Cell increment recorded ata concentration of 192 mg. L-1 of N- amoniacal affluent was 360% until the 5th day of monitoring, starting from the ICD of $1.78437x10^5$ cel. mL-1 reaching 6.43125×10^5 with removal of 30 mg of ammoniacal nitrogen.

In all concentrations studied, it was found that thecultures were in a phase of decline between the 5th and 10th day of monitoring, reductions in CD of 21, 60 and 22%, respectively forthe concentrations of affluent ammoniacal N of 46, 192 and 575 mg. L-1. In the control, an increaseof 235% was recorded until the 10th dia, reaching values of $5.97x10^5$ with complete removal of affluent ammoniacal nitrogen (2.4 mg). L-1) from day 11. Figure 3 shows the growth of *Chlorella* sp. in different concentrations of ammoniacal N in 10-day TDH.

Figure 3. Temporal behavior of *chlorella* sp. growth in different concentrations of ammoniacal N.



Source:research data

This result can be explained by a phenomenon called "hormesis" is a term used to describe a phenomenon associated with toxic compounds that, in low doses, show stimulator or beneficial effect to the exposed organism and in high doses show efeito inhibit or toxic (HASHMI et al. 2014). A similar result was obtained by El Quaer et al. (2019), when they cultivated *Chlorella* sp. in different dilutions of landfill leaching with raw leaching, presenting ammoniacal nitrogen concentrations in the range of 3595 mg. L-1. In this work, an inhibitory effect of algae growth was recorded from the leached concentration of 30%.

3.3 *PH*

In this study, progressive increments in pH were recorded in the threetreatments at a concentration of 46 mg. L-1 of affluent Ammoniacal N, the initial pH was 7.8 reaching 9.7 by the 10th day of monitoring. The less expressive elevations (0.6 unit) were observed in the concaffluent intake of 575 mg. L-1. This result suggests that even starting from a high pH, *Chlorella* sp.,through photosynthesis, assimilated CO_2 from the medium, where the bicarbonate ions present dissociated fromproducing CO_2 and OH^- raising the pH.

Andall point point to pH reduction in las phytoremediation. InJIA and YUAN work, (2016), the NH^{4+} when assimilated directly into the glutamic acid pathway, releases H+ ions, which can reduce the pH of the medium. Khanzada et al. (2018) studied *Chlorella vulgaris* and *Chlamydomonas reinhardii in* leachtreatment, and reported that the pH was constantly decreasing, reaching 5.7.

3.4 REMOVAL OF AMMONIACAL N

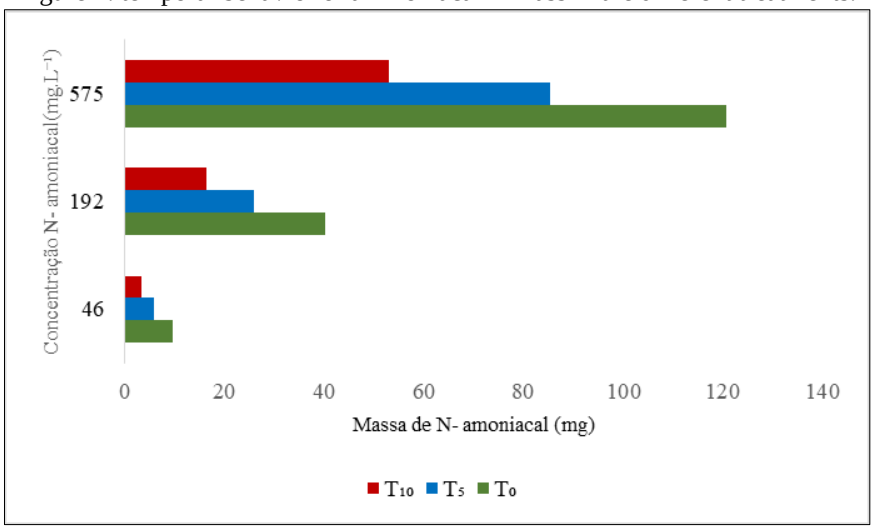
Calculating the mass of ammoniacal N in each system, approximate effluentvalues of (9.7) (40.3) and (121.0) mg-N with respective values at the end of the experiment were recorded at the end of the experiment of (3.3) (16.5) and (52.9) mg-N, representing mean removals of 66, 59 and 56% for the respective concentrations of omniacal N- am of 46, 192 and 575 mg. L-1. These data are indicative that *Chlorella* sp. is tolerant to high concentrations of Ammoniacal N, managing to incorporate nitrogen mass, to be directed mainly to the synthesis of amino acids and proteins.

Another aspect to be highlighted is related to the volume of *chlorella sp*. cell. . At the affluent concentration of 46 mg. L-1, the cells maintained their normal volumes of approximately 10µm. The reverse was observed ata concentration of 575 mg. L-1, where initial volume reduction was recorded by up to 50%. At the affluent concentration of 192 mg. L-1, the cells showed a significant increase in their volume. This result suggests that high ammoniacal nitrogen concentrations may interfere with cell division by increasing the density and size of *Chlorella* sp. These results are consistent with the registrados by Hu et al. (2012) where leaching remarkably affects the subcellular structure, reducing the volume of chloroplasts and the amount of thylaids.

Corroborating these results of interference ofammoniacal nitrogen io mass in the cell volume of *Chlorella* sp. El Quaer et al. (2019) recorded that in dilutions of leached at 10% and range of 350 mg. $^{L-1}$ of Ammoniacal N the cells appeared larger with a darker green color compared tothose observed in the control (MBB).

In the conception of Klochenko et al. (2003), the tolerance of green algae to high ammonium concentrations is due to them having greater activity of the gs/gdh enzymatic system, which therefore promotes rapid conversion of ammonium into amino acids rather than being accumulated in the cell. Corroborating this positioning, Giordano et al. (2003)states that ammonium stimulates the production of PEPCasand, an enzyme that accelerates the incorporation of ammonium in organic compounds to avoid toxicity in algae. Figure 4 shows the temporal behavior of ammoniacal N mass in the system during monitoring.

Figure 4: temporal behavior of ammoniacal N mass in the different treatments.



Source : search data

4 FINAL CONSIDERATIONS

From the analysis of these data, it can be inferred that:

•Whereas landfill leaching is a liquid residue of complex matrix, with recalcitrant substances and high turbidity, the results obtained are indicative *that Chlorella* sp. presented ammoniacal nitrogen removal efficiency in different leaching dilutions in batch-fed systems;

•Concenpulls greater than 200 mg. L-1 ammoniacal nitrogen, can produce an inhibitative effect in the cell, lacking further studies in the biological treatment of leached d and landfill, specifically in the scope of phytoremediation;

•In the current Brazilian conjuncture and in regions or cities where landfills are already installed and in operation, the choice of phytoremediation of wastewater from the degradation of solid waste from *Chlorella* sp. is a viable and efficient biotechnology in the recovery of resources. It should also be emphasized that they are systems of easy monitoring, low cost, with high sequestration power of greenhouse gas and potential production of useful biomass.

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