



## The fate of nitrogen through algal treatment of landfill leachate

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### ABSTRACT

Nitrogen was tracked through an algae-based landfill leachate remediation system. This system was designed to remove nutrients from the liquid waste via nitrogen assimilation into new algae biomass. While the nitrogen removal pathway of bio-assimilation was present, it was not the only nitrogen removal pathway in the treatment system. Weekly measurements of the dissolved inorganic nitrogen (ammonia-N, nitrate-N, and nitrite-N) and the nitrogen content of the biomass were used to track nitrogen transformation pathways during this yearlong study. During a major part of the study (83.4% of the observed weeks), all nitrogen could not be accounted for in the dissolved inorganic nitrogen or biomass portions of the system. It is hypothesized that some of the unaccounted-for nitrogen was lost due to volatilization of gaseous nitrogen species. Based upon characteristic distribution of measured dissolved inorganic nitrogen and the nitrogen content of the biomass for each week and prior metagenomic analysis of the microbial community in the treatment system, we have postulated potential scenarios of the fate of nitrogen in this algae-based wastewater treatment system. Further research is needed to identify all pathways of nitrogen conversion in algae-based wastewater remediation systems and verify our proposed scenarios.

### 1. Introduction

The use of algae in wastewater treatment has long been proposed as an efficient, sustainable, and likely cost effective alternative for removing nutrients from liquid waste streams [1–3]. Evidence suggests that algae can quickly and efficiently remove dissolved nitrogen, typically in either the form of ammonia or nitrate, from an aqueous system under a variety of conditions [4–8]. Complete nitrogen removal is often reported in algae-based wastewater treatment systems [9–12]; however nitrogen budgets of these systems are rarely able to attribute 100% of the nitrogen removed from the liquid portion of a system to biomass growth, even in axenic conditions [13–19]. Coupling algal growth with the treatment of waste streams has been studied but very few have evaluated the nitrogen budget of this system [20]. The results of the nitrogen budget by Nunez et al. showed that the majority of nitrogen starting in the aqueous phase was incorporated into biomass or volatilized as ammonia (NH<sub>3</sub>) into the gas phase [13]. Among the published literature of engineered systems using algae to treat wastewater, some studies focus on maximizing nutrient removal while others focus on accounting for the fate of nitrogen species within a system; studies with a focus on the treatment ability frequently assume that all nitrogen removed from the liquid waste stream is incorporated into new biomass, whereas studies aiming to develop a nitrogen budget for these systems do not make this assumption about the fate of nitrogen in these

systems [13].

Engineered systems using photosynthetic organisms designed to treat wastewater rely on a complex web of physio-chemical interactions and biological activity (Fig. 1). A complete nitrogen budget of these systems must include chemical and biological conversion pathways in the liquid phase, as well as mass transfer to the gaseous phase, such as off-gassing of NH<sub>3</sub> [13,14,21]. In non-axenic algal-based systems that contain other microorganisms, it is especially important to consider nitrogen transformation pathways beyond bio-assimilation, such as nitrification (the sequential oxidation of NH<sub>3</sub> to NO<sub>2</sub><sup>-</sup> and then NO<sub>3</sub><sup>-</sup>) or denitrification (the sequential reduction of NO<sub>3</sub><sup>-</sup> to N<sub>2</sub>). Inorganic nitrogen in the form of ammonia and nitrate can be used as a nutrient source for algae growth, while bacteria can often use these nitrogen sources as well as nitrite and organic nitrogen for growth. These nitrogen species are transported into the cell and then assimilated into macromolecules of organic nitrogen such as amino and nucleic acids. The dissimilatory conversion of NH<sub>3</sub> to NO<sub>2</sub><sup>-</sup> via partial nitrification is less commonly reported in bench-scale wastewater treatment publications, although it has been reported to be a common transformation in commercial treatment facilities [6,22,23]. While the production of nitrous oxide (N<sub>2</sub>O) and nitric oxide (NO) is only rarely reported, some studies have shown that this pathway of nitrogen conversion is possible in wastewater treatment [24]. Ammonia oxidizing bacteria/archaea (AOB/AOA), nitrite oxidizing bacteria (NOB), Anammox, and

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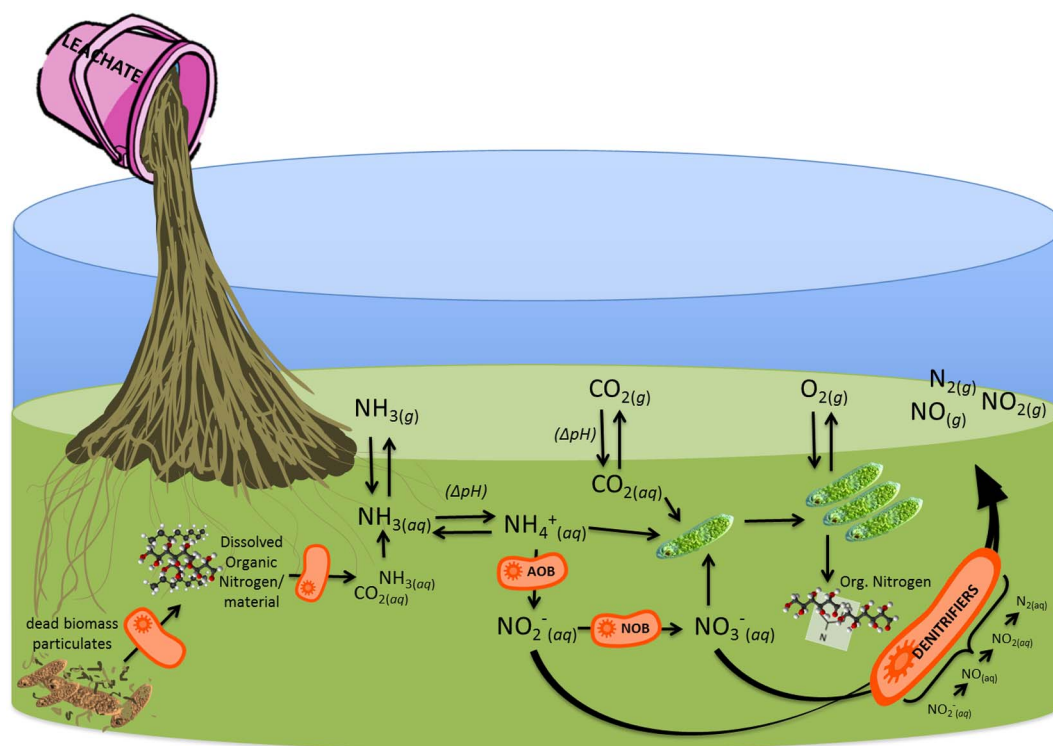


Fig. 1. Potential pathways of nitrogen transformation.

denitrifying bacteria have all been found to play an important role in nitrogen removal in wastewater treatment systems [14,16,21,25]. The sorption of ammonia on to particulates within engineered system is frequently observed in phytoremediation systems where soil or gravel is used as a medium for rooting plants but is also applicable to any system with particulate matter that could act as a sorbent [14,16,25]. The nitrogen transformation pathways introduced here are a representative but not exhaustive list of the possible nitrogen conversions that could occur in an engineered biological wastewater treatment system.

The analysis presented here attempts to track nitrogen through a landfill leachate treatment system that utilizes algae with the remediation goal of removing high concentrations of ammonia contained in the leachate influent. Most algal systems are designed on the premise that the primary mechanism for nitrogen removal is by its assimilation into growing algal biomass. Nitrogen is a critical nutrient for algal growth, required for the production of cellular proteins, chlorophyll, and structural components. This premise assumes the following null hypotheses:

**Hypothesis 1.** All nitrogen removed from the liquid phase is incorporated into new algal biomass.

**Hypothesis 2.** In the absence of biomass growth, no nitrogen is removed from the liquid phase.

This study evaluates these wastewater remediation hypotheses using data collected over the course of one year from algae-based wastewater treatment systems of different sizes, and proposes a quantitative nitrogen budget analysis to evaluate the transformation of nitrogen through a leachate remediation system.

## 2. Materials and methods

Data collected during a yearlong (February 2016 to February 2017) operation of an experimental algae-based landfill leachate treatment system designed for nitrogen removal are used for this study [4]. The treatment system used two 100 L aquarium tanks (ATs) and two 1000 L

raceway ponds (RWPs) seeded with a wild sample of a mixed algae/bacteria consortium, with untreated landfill leachate as a nutrient source. The AT and RWP vessels had working volumes of 60 L and 600 L, respectively, and were operated as semi-batch reactors on a weekly basis [26]. The untreated landfill leachate, used as the nutrient medium in this study, was collected from the Sandtown Landfill in Felton, DE. At the beginning and end of each week samples were taken from all vessels and analyzed for biomass density and dissolved inorganic nitrogen species (ammonia, nitrate, and nitrite). To prepare for the next weeks batch, one third of the liquid volume was removed and then replaced with water and leachate. Leachate additions ranged from 5 to 40 L in order to obtain observations under a variety of leachate concentrations. A far more detailed description of this study can be found in Sniffen et al. [27]. The aim of the evaluation, described herein, was to track nitrogen transformation processes through the system.

### 2.1. Liquids analysis ( $C_{N-Liquid}$ )

Dissolved ammonia-*N*, nitrate-*N*, and nitrite-*N* were measured at the beginning and end of each seven-day experiment over the course of one year. A Hach DR2400 spectrophotometer and test kits were used to measure the dissolved ammonia-*N*, nitrate-*N*, and nitrite-*N* content, using methods 10,031, 10,020, and 8153, respectively. Single samples of each test were run using sample volumes specified by each method. The  $C_{N-Liquid}$  components are included in Eq. (1), all measured in [mgN/L].

$$C_{N-Liquid} = C_{NH_3} + C_{NO_2^-} + C_{NO_3^-} \quad (1)$$

### 2.2. Solids analysis ( $C_{N-Solid}$ )

The average nitrogen content of the biomass samples was analyzed at EcoCore of the National Renewable Energy Laboratory at Colorado State University. This analysis used a LECO Tru-SPEC elemental analyzer (Leco Corp, St. Joseph, MI) to determine the nitrogen content of the solids. The solids portion of this system contained algae, bacteria, as

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