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Microalgal bioremediation of nitrogenous compounds in landfill leachate – The importance of micronutrient balance in the treatment of leachates of variable composition

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ABSTRACT

Landfill leachate is a type of wastewater which is challenging to treat. Phycoremediation has been proposed as an alternative biological treatment for removal of ammonia nitrogen. Several studies have shown microalgae based bioremediation to be possible with ammonia tolerant microalgal species, provided that an optimal dilution is used and the initial molecular N:P ratio is adjusted.

The composition of landfill leachate varies between sites and throughout the year. The performance of selected microalgal strains and their susceptibility to variation in landfill leachate composition is poorly understood. This study compares the growth of *Chlamydomonas* sp. strain SW15aRL in a variety of leachate samples. The leachate samples are from different sites including leachate sampled on different occasions from the same site. These substrates were diluted to obtain ammonia nitrogen concentration within the range of 30 to 220 $mg \cdot l^{-1}$.

Results showed that strain SW15aRL was capable of growth in a variety of leachates but was dependent on the overall composition profile of the landfill leachate rather than just its dilution. Growth was negatively affected in two of the leachates tested, due to metal toxicity and mineral bioavailability or deficiency. Phosphate addition was essential for growth in the landfill leachates even though precipitation occurred in some instances. Ammonia nitrogen decrease varied between 70% and 100% in the substrates where microalgae could successfully grow.

This study indicates that due to their overall mineral profile some landfill leachates are more suited for microalgae based remediation than others. Furthermore, this study indicates that a better understanding of other physicochemical processes that take place concurrently during the growth of microalgae in landfill leachate and which contribute to overall nutrient reduction is required.

1. Introduction

Phycoremediation generally refers to a type of biological treatment of wastes in which algae remove inorganic and simple organic compounds for their growth while some more complex substances can undergo a certain degree of biotransformation. The studies having assessed the viability of such technology have mostly been conducted in countries with plentiful supply of light and in warm climates. These latter conditions are typically reflected in the laboratory conditions used in previous experimental work. There is therefore limited data on growth rates, nutrient removal and uptake, and energy consumption for such applications for countries with temperate climates where attaining high light and temperature conditions would come at substantial extra cost.

The possibility of landfill leachate phycoremediation has previously been shown with various chlorophytes [1–6]. However, there are few follow up studies comparing the performances of the microalgae strains used with a variety of leachate samples [5,7]. Landfill leachate is known for its variable composition depending on the landfill site, age of landfill and weather conditions, while certain trends in physicochemical parameters still apply [8,9]. Chemical loading within this type of wastewater is usually high and requires dilution to make the growth of microalgae possible. Nutrient proportions in landfill leachate can also be seen as disadvantageous. The molecular N:P ratio tends to be high

Abbreviations: TAN, total ammonia nitrogen; TON, total oxidised nitrogen; APHA, American Public Health Association; TSS, total suspended solids

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with phosphorus being a limiting growth nutrient [10]. While small scale, short term studies have shown that landfill leachate constitutes a potentially rich source of nutrients for microalgal growth, little is known of the effects that variable leachate compositions can have over time on the sustainable growth of microalgae. Taking into account microalgal nutrient requirements which can be related to composition of this type of wastewater, mineral imbalances have been implied based on calculations rather than experimental observations [11]. Minerals such as Fe, Co, Mg, Mo, Mn, Zn, Cu and Ni fulfil important physiological functions and their amounts as well as their chemical speciation in solution do matter for the successful growth of microalgae and thus the overall remediation effectiveness [12,13]. Wastewaters are complex in nature and the proportions of free metal ions rather than their absolute concentrations are important in terms of bioavailability and also toxicity [12]. Complexation with organic matter or formation of insoluble hydroxides reduces free metal ions in solutions, which can cause mineral deficiencies in plants as well as in microalgae [14,15].

The present study involved conducting experiments in batch cultures to verify the capacity of *Chlamydomonas* sp. strain SW15aRL to survive, grow and bioremediate a range of landfill leachate samples collected from different sites or on varying occasions. In addition, the effects of mineral nutrient modulation on the growth and remediation potential of the strain were evaluated.

2. Materials and methods

2.1. Microalgae strain

Strain *Chlamydomonas* sp. SW15aRL was isolated from a sample of raw leachate (landfill site in Northern Ireland) in 2014. The ability of this strain to deplete nutrients from landfill leachate was previously studied [10]. The strain stocks were maintained in landfill leachates prior to the nutrient depletion experiments.

2.2. Landfill leachate

Landfill leachate was collected at four different sites during 2015 (Tables S1 and S2) either directly from the leachate collection system or from holding tanks. The leachate was collected into plastic bottles and stored at < 5 °C until used.

2.3. Physicochemical analysis

Physicochemical properties were determined according to published methods [16]. Nutrient profiles (PO_4^{3-}-P, TON, TAN, Cl^-, SO_4^{2-}) were determined spectrophotometrically with an Aquakem 250 autoanalyser on samples filtered through 0.45 μm filter (VWR, Cat. No. 28145-503) prior to analysis. Conductivity and pH were measured electrochemically (HACH conductivity meter sensION5 and 713 pH Meter Metrohm). Colour was estimated by spectrophotometric (VARIAN Cary 50 UV–Visible Spectrophotometer) measurement at λ = 455 nm after filtration through 0.45 μm filter. Alkalinity was determined titrimetrically with 0.1 N HCl to pH 4.5 (using Metrohm 713 pH Meter). The metal profiles were determined on raw leachates and also leachates filtered through 0.7 μm glass filter as all the leachate samples were filtered prior to microalgae remediation experiments in this manner. Leachate samples were digested using a microwave digestion system (Milestone Ethos Plus) with HNO_3 (ROMIL-UpA™) according to Method 3015A [17] prior to trace element analysis. Several trace elements (i.e. Fe, Mn, Zn, Co, Cu, Mo, Al, Cr, Ni, Cd, Pb) were determined by ICP-MS (Varian 820MS). Ca, Na, K were measured by flame photometry (Sherwood 360) and Mg was determined by flame AAS (Agilent 200 AA). Suspended solids were quantified gravimetrically by filtering a known volume of sample through 0.7 μm glass filter (VWR, Cat. No. 516-0345) and drying at 105 °C until constant weight. Chemical Oxygen Demand (COD) was determined spectrophotometrically after sample digestion using HACH Lange Ltd test kits.

2.4. Growth of microalgae in six different leachate samples

Experiments were conducted with raw or diluted leachate using autoclaved deionised water as diluent. The dilution factor depended on nutrient loading with the aim of having a final nitrogen concentration under or near 250 $mg \cdot l^{-1}$. Leachate samples were filtered through a glass fibre filter (VWR 1.6 μm pore size followed by 0.7 μm).

Tests were conducted in 250 ml Erlenmeyer flasks with 150 ml volume of leachate-microalgae mixture in stationary flasks. Flasks were inoculated at approximately the same initial biovolume of 0.15 $mm^3 \cdot ml^{-1}$ (~100,000 $cells \cdot ml^{-1}$). All experiments were conducted in triplicate. Controls with no microalgae were set up to monitor losses of nutrients due to reasons other than microalgal assimilation. All solutions were phosphorus supplemented (1000 $mg \cdot l^{-1}$ PO_4^{3-}-P prepared from K_2HPO_4) to achieve a molecular ratio 16:1 N:P in the final volume. Aliquots of 2 ml were sampled at intervals and analysed for cell number, TAN, TON and orthophosphate. The incubation conditions were as follows: a temperature of 15 °C, a light cycle of 14:10 h (light:dark) and a photosynthetic photon flux density (PPFD) of 22 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

The growth progress through the experiments was monitored by cell counts using a haemocytometer. Nutrient concentration changes (PO_4^{3-}-P, TON, TAN) were determined spectrophotometrically with Aquakem 250 autoanalyser on samples filtered through 0.45 μm filter. Variation in pH during the experiments was estimated using pH indicator strips (Merck MColorpHast™ pH 5.0–10, pH 7.5–14, Δ0.5 pH, Dosatest® pH 7.0–10.0, Δ0.3 pH) with small aliquots of culture removed from the flasks.

2.5. Growth of microalgae in leachate S1 with three different starting cell concentrations

This was carried out to verify if nutrient removal could improve with increasing starting microalgae concentration. The experiment was set up as per Section 2.4 with leachate S1 (100%) except that phosphate was adjusted to a molecular ratio ~ 32:1 N:P in the final volume due to the previous observation of extensive precipitation. Three starting cell concentrations were used: 100,000, 200,000 and 500,000 $cells \cdot ml^{-1}$.

2.6. Growth of microalgae in three leachate samples supplemented with minerals

This experiment was set up to examine if the addition of specific micronutrients could influence microalgal growth and macronutrient content removal in leachates during the remediation. Three samples were chosen: S3 (10%) where microalgae initially grew but started dying off, S2 (20%) where microalgae growth was slow and S6 (30%) where microalgae grew well. The experiment set up was similar to that in Section 2.4 with the addition of extra sets of microalgae treated samples and controls supplemented with iron ($FeCl_3 \cdot 6H_2O$) and magnesium ($MgSO_4 \cdot 7H_2O$), which were monitored alongside the mineral non-supplemented flasks. While growth only at one concentration was monitored in S2 (20%) and S3 (10%), several concentrations were monitored in leachate S6 (30%) (Table 1). The choice of these two minerals was made based on the leachate chemical profile as discussed in Results and discussion section.

2.7. Biomass and precipitate dry weight determination

Microalgal dry weight and precipitates in the controls were determined by gravimetric quantification by filtering a known volume of sample through 0.7 μm glass filter (VWR, Cat. No. 516-0345) and drying at 105 °C until it attained constant weight. As attempts to wash