Ecotoxicology and Environmental Safety 147 (2018) 622–630

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Phycoremediation of landfill leachate with the chlorophyte *Chlamydomonas* sp. SW15aRL and evaluation of toxicity pre and post treatment

Andrea Paskuliakova[a,*], Ted McGowan[b], Steve Tonry[a], Nicolas Touzet[a]

[a] Centre for Environmental Research Innovation and Sustainability (CERIS), Institute of Technology Sligo, Ash Lane, Sligo, Ireland
[b] School of Science, Department of Life Science, Institute of Technology Sligo, Ash Lane, Sligo, Ireland

ARTICLE INFO

Keywords:
Microalgae
Landfill leachate
Phycoremediation
Toxicity testing
Biomass generation

ABSTRACT

Landfill leachate treatment is an ongoing challenge in the wastewater management of existing sanitary landfill sites due to the complex nature of leachates and their heavy pollutant load. There is a continuous interest in treatment biotechnologies with expected added benefits for resource recovery; microalgal bioremediation is seen as promising in this regard.

Toxicity reduction of landfill leachate subsequent to phycoremediation was investigated in this study. The treatment eventuated from the growth of the ammonia tolerant microalgal strain *Chlamydomonas* sp. SW15aRL using a N:P ratio adjustment in diluted leachate for facilitating the process. Toxicity tests ranging over a number of trophic levels were applied, including bacterial-yeast (MARA), protistean (microalgae growth inhibition test), crustacean (daphnia, rotifer) and higher plant (monocot, dicot) assays.

Ammonia nitrogen in the diluted landfill leachate containing up to 158 mg l^{-1} NH_4^+-N (60% dilution of the original) was reduced by 83% during the microalgal treatment. Testing prior to remediation indicated the highest toxicity in the crustacean assays *Daphnia magna* and *Brachionus calyciflorus* with EC50s at 24 h of ~ 35% and 40% leachate dilution, respectively. A major reduction in toxicity was achieved with both bioassays post microalgal treatment with effects well below the EC20s. The microalgae inhibition test on the other hand indicated increased stimulation of growth after treatment as a result of toxicity reduction but also the presence of residual nutrients. Several concurrent processes of both biotic and abiotic natures contributed to pollutant reduction during the treatment. Modifying phosphate dosage especially seems to require further attention. As a by-product of the remediation process, up to 1.2 g l^{-1} of microalgal biomass was obtained with ~ 18% DW lipid content.

1. Introduction

Landfill leachate is high strength wastewater saturated with various compounds leaching out of decomposing municipal waste. Its composition is very complex because of the wide range of toxicants it contains (Cecilia and Junestedt, 2008). Ammonia nitrogen is considered one of the main toxicants therein and can be present at very high concentration, as can many other inorganic compounds. Other organic and metal-organic compounds are present at very low concentrations and are often difficult to detect by standard analytical procedures; with the possibility that many have not yet even been identified (Cecilia and Junestedt, 2008). Many of the 'priority' or 'priority hazardous' substances listed in Directive 2008/105/EC (Daughter Directive to the Water Framework Directive) typically do not exceed the limit values within landfill leachates, which can still exhibit high toxicity as shown by ecotoxicological testing. It is thought that the combined toxic effects of many compounds at sub-detection levels are the causes (Brito-Pelegrini et al., 2007; Płaza et al., 2011).

Biological testing has been used as an indicatory means of evaluating the ecotoxicological impact of complex wastewater matrices such as landfill leachate. Multispecies assays that cover a number of trophic levels are usually recommended. In this way, different groups of pollutants can be detected by species sensitive to them. Several standardised and/or commercially available bioassays currently exist (Brito-Pelegrini et al., 2007; Bernard et al., 1996; Persoone and Gillett, 1990). According to Bernard et al. (1996) the toxicity of the majority of leachate samples can be assessed with a battery of tests including bacterial assays, protozoan assays and microalgae assays jointly with higher

Abbreviations: MARA, microbial assay for risk assessment; TSS, total suspended solids; OECD, organisation for economic co-operation and development; COD, chemical oxygen demand; TAN, total ammonia nitrogen; DW, dry weight; EC20, 20% maximal effective concentration; EC50, half maximal effective concentration

* Corresponding author.
E-mail address: andrea.pask@ymail.com (A. Paskuliakova).

http://dx.doi.org/10.1016/j.ecoenv.2017.09.010
Received 28 March 2017; Received in revised form 3 September 2017; Accepted 5 September 2017
Available online 10 October 2017
0147-6513/

plants, rotifers or crustaceans. The methods for these tests are well established. The OECD publishes procedures (i.e. Guidelines for the Testing of Chemicals) that are generally accepted internationally as standard methods for assessing the potential risk of chemicals on the environment and these procedures can also be used for the testing of multi-constituent matrices such as landfill leachate. Toxicological tests have also been carried out using aquatic vertebrate models such as the zebrafish or the frog embryo (FETAX) assay. Fish cell line tests exist as alternative to animal testing (Burýšková et al., 2006; Bols et al., 2005; Hollert et al., 2003). Genotoxicity assays can be also applied to ecotoxicity testing and have been employed to ascertain the risk associated with wastewater treatment (Kumari et al., 2016; Tice et al., 2000).

Biological treatments are extensively used in wastewater management. Some are well established, such as bacterial activated sludge processes. Others, such as wet lands employing planted vegetation for biofiltration of pollutants, fungi or microalge which can be immobilised or grown in suspended cultures similarly to bacterial, are becoming increasingly researched. These treatments employ organisms from distinct phylogenetic groups with specific metabolisms, which can be more effective for the degradation or removal of certain pollutants than others depending on their required nutrient source. Microalgae have been explored for wastewater treatment purposes for over two decades and are actively considered for biofuel production. The most successful species usually come from the chlorophytes group, including *Scenedesmus* sp., *Chlorella* sp. or *Chlamydomonas* sp., but cyanobacteria or other phylogenetic groups appear occasionally within the literature (Choudhary et al., 2016; Kothari et al., 2013; Mandal and Mallick, 2011; Zhou et al., 2011). Microalgae can be used to effectively remove ammonia nitrogen and other inorganic constituents to build their biomass (Sutherland et al., 2014; Sforza et al., 2015; Prajapati et al., 2014; Zhao et al., 2014; Mandal and Mallick, 2011; Lin et al., 2007), ammonia being one of the main pollutants in landfill leachate. Some studies have reported that certain microalgal species are capable of removing, biodegrading or biotransforming organic compounds (Li et al., 2009; Lima et al., 2004; Yan and Pan, 2004; Hirooka et al., 2003; Pinto et al., 2003) and also extracting metals from solutions (Li et al., 2015; Thongpinyochai and Ritchie, 2014). However, it has also been shown that remediation of landfill leachate with microalgae can require a certain process control such as pH adjustment (Edmundson and Wilkie, 2013) or the need for nutrient compensations (Pereira et al., 2016; Paskuliakova et al., 2016a) to facilitate growth of the microalgal cells. It is therefore sometimes a requirement to add certain chemicals to overcome these limitations. Whilst this can initially increase the pollution load, it also aids the overall remediation process by overcoming the limitations identified. Toxicological assays can be subsequently employed to demonstrate that the treatment process not only removes specific pollutants of interest but also reduces the overall ecotoxicity of treated wastewaters. This has previously been demonstrated for landfill leachate in small scale microalgae-based remediation experiments (Kumari et al., 2016; Lin et al., 2007).

Associating microalgal remediation to biomass valorisation for biofuel production has been suggested to increase the economic viability of 3rd generation biofuels (Pittman et al., 2011). Several options for energy generation from microalgal biomass have been described depending on its composition (Juneja et al., 2013; Brennan and Owende, 2010). The use of microalgal biomass grown on possibly toxic wastewaters such as landfill leachate for other than bioenergy purposes is however limited due to the possible accumulation of toxicants within the biomass.

Phosphate supplementation appears to be essential for the successful use of microalgae in the treatment of landfill leachate (Paskuliakova et al., 2016a). The present study compared phosphoric acid to dipotassium hydrogen phosphate for their suitability as a phosphorus source for microalgal growth in landfill leachate. In addition to nutrient reduction, the remediation capability of *Chlamydomonas* sp. strain SW15aRL was evaluated by assessing the toxicity of the leachate. The toxicity was determined both pre- and post-treatment using assays spanning several trophic levels. The lipid content achieved in the microalgal biomass was also determined post treatment in order to verify its potential for possible conversion into bioenergy commodities.

2. Materials and methods

2.1. Landfill leachate

The landfill leachate samples S7 and S8 were collected in October 2015 and January 2016, respectively, from a site in the Republic of Ireland and stored at < 5 °C until use.

2.2. Physicochemical analyses

Physicochemical properties were determined according to published methods (APHA American Public Health Association, 2005). Nutrient profiles (PO_4^{3-}-P, TON, TAN, Cl^-, SO_4^{2-}) were determined spectrophotometrically with the Aquakem 250 autoanalyser on samples passed through 0.45 µm filters (VWR, Cat. No. 28145-503) prior to analysis. Conductivity (using HACH conductivity meter sensION5) and pH (using Metrohm 713 pH Meter) were measured electrochemically. Lovibond® test discs were used to categorise the colour of the samples after filtration through 0.45 µm filters. Alkalinity was determined titrimetrically with 0.1 N HCl to pH 4.5 (using Metrohm 713 pH Meter). The metal profiles were determined on both, the raw leachates and leachates filtered through 0.7 µm (VWR, Cat. No. 516-0345) glass filters, given that all the leachate samples were filtered through 0.7 µm glass filters prior to microalgae remediation experiments. Leachate samples were processed by microwave digestion (Milestone Ethos Plus) with HNO_3 (ROMIL-UpA™) according to Method 3015A (US EPA, 2007) prior to trace element analysis. Several trace elements (i.e. Fe, Mn, Zn, Co, Cu, Mo, Al, Cr, Ni, Cd, Pb) were determined by inductively coupled plasma mass spectrometry (ICP-MS, Varian 820). Major elements (i.e. Ca, Na and K) were determined by flame photometry (Sherwood 360) while Mg was determined by flame Atomic Absorption Spectroscopy (Agilent 200 AA). Suspended solids were quantified gravimetrically by filtering a known volume of sample through a 0.7 µm glass filter and drying it at 105 °C until constant weight. Chemical Oxygen Demand (COD) was determined spectrophotometrically after sample digestion with HACH Lange Ltd test kits.

The variation in pH during the experiments was estimated with small aliquots of culture 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).

2.3. Microalgal strain

The *Chlamydomonas* sp. strain SW15aRL (previously isolated from a sample of raw leachate in 2014 from a landfill site in Northern Ireland) was maintained in raw leachate or diluted raw leachate samples with a phosphate concentration adjusted to a molar N:P ratio ~ 16:1 prior to the experiments.

2.4. Growth in leachate S7 with two different phosphate sources

Leachate S7 was filtered through a 1.2 µm glass filter (VWR, Cat. No. 516–0869) followed by filtration through a 0.7 µm glass filter (VWR, Cat. No. 516-0345). Dilution to 30% with autoclaved deionised water was carried out to decrease the inhibitory effect of TAN on microalgal growth. The experiment was set up in triplicate in 250 ml conical flasks stoppered with cotton plugs and covered by tin foil. The total volume of leachate-microalgal mixture was set to 150 ml and the flasks were incubated stationary, homogenised only for sampling at intervals to monitor nutrient depletion and microalgae growth. The