Prospects of integrating algae technologies into landfill leachate treatment

Review Article

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Abstract

Landfilling of municipal waste, an environmental challenge worldwide, results in the continuous formation of significant amounts of leachate, which poses a severe contamination threat to ground and surface water resources. Landfill leachate (LL) is generated by rainwater percolating through disposed waste materials and must be treated effectively before safe discharge into the environment. LL contains numerous pollutants and toxic substances, such as dissolved organic matter, inorganic chemicals, heavy metals, and anthropogenic organic compounds. Currently, LL treatment is carried out by a combination of physical, chemical, and microbial technologies. Microalgae are now viewed as a promising sustainable addition to the repertoire of technologies for treating LL. Photosynthetic algae have been shown to grow in LL under laboratory conditions, while some species have also been employed in larger-scale LL treatments. Treating leachate with algae can contribute to sustainable waste management at existing landfills by remediating low-quality water for recycling and reuse and generating large amounts of algal biomass for cost-effective manufacturing of biofuels and bioproducts. In this review, we will examine LL composition, traditional leachate treatment technologies, LL toxicity to algae, and the potential of employing algae at LL treatment facilities. Emphasis is placed on how algae can be integrated with existing technologies for biological treatment of LL, turning leachate from an environmental liability to an asset that can produce value-added biofuels and bioproducts for the bioeconomy.

Keywords

Landfill leachate; algae; wastewater treatment; remediation

Introduction

As human population continues to grow, so do human activities, generating increasing amounts of municipal and industrial waste. In the United States alone municipal solid waste (MSW) annual generation increased from 88.1 to 267.8 million tons between 1960 and 2017 (EPA, 2019). To date, landfilling remains the most common municipal waste disposal practice worldwide. In the US 139 million tons, comprising 52.1% of MSW generated in 2017, ended up in landfills (EPA, 2019). This is due to landfilling being relatively inexpensive and technically simple compared to other disposal options, such as recycling, combustion for energy production, and composting. However, the formation of landfill leachate (LL) is an inevitable consequence of landfilling raising serious environmental concerns. For example, in the State of Florida it is estimated that 7,000 gallons of LL are generated per day per acre of landfill, equivalent to about 24 million liters per hectare per year (Meeroff et al. 2016). LL contains hazardous components that can contaminate drinking water and are toxic to microbial flora and fauna (Cheung et al. 1993; Ernst et al. 1994; Ferrari et al. 1999; Lin et al. 2007; Plotkin and Ram 1984; Ward et al. 2002). As a result, contamination of ground and surface waters by LL has become a major environmental concern for governments and communities around the world. In an effort to address this issue, modern landfills are engineered with liners to protect ground water from infiltration by landfill effluents. Additionally, LL must be treated prior to its discharge to reduce the concentration of numerous pollutants. The discharge limits mandated by government agencies, such as the Environmental Protection Agency (EPA) in the US, are becoming increasingly more stringent. Consequently, there is strong interest in novel treatment methods of high efficiency, easy applicability, and long-term sustainability, like algae technologies.

In this review, we will examine the nature of LL, provide an overview of current leachate treatment technologies, analyze algal physiology in the context of its cultivation on leachate and related wastewaters, and outline the development of outdoor algal cultivation systems that can be applied to landfills to synergistically assist with leachate treatment, while generating value-added algal products for the economy. Emphasis is placed on the potential of integrating algae with existing technologies for remediation of LL, thus turning landfill leachate from an environmental liability to a low-cost bioresource for sustainable production of value-added algal bioproducts.

Landfill Leachate generation and composition

Landfills are sites where municipal waste is buried and mixed with soil for disposal and decomposition purposes. Landfilling is an old process that remains the most common method of waste disposal in many countries worldwide because it is logistically and technically simple and relatively inexpensive compared to other treatment options. However, landfills are plagued by environmental issues, including the formation of leachate, which is a dark liquid formed when rainwater, inherent moisture, and water generated from waste decomposition reactions percolate through the waste and contaminate ground and surface water bodies with pollutants and toxins. The composition of LL depends on many factors including the nature of waste present at a given landfill, weather, moisture, practices applied at the landfill, and the age of leachate (Kjeldsen et al. 2002).

The lifecycle of such landfills and the composition of leachate have been reviewed extensively elsewhere (Christensen et al. 2001; Kjeldsen et al. 2002). Through the lifetime of a landfill, various decomposition reactions, chemical and biological, take place in sequential phases until the waste is fully decomposed (stabilized), resulting in a wide variability in LL composition over time at different locations even within the same landfill. Waste decomposition starts with an initial aerobic phase in which refuse is aerobically oxidized

releasing carbon dioxide and heat. This phase is very short and lasts for a few days only until oxygen is depleted in the buried refuse, at which point decomposition becomes anaerobic. Under anaerobic conditions, an acidic phase takes place in which fermentation of organic compounds prevails, resulting in the formation of organic acids (such as acetic acid) and a significant drop in pH. Generation of acids raises the ratio of biological oxygen demand (BOD) to chemical oxygen demand (COD) and increases the solubility of numerous pollutants in water, raising their concentrations in LL. Consequently, leachate becomes highly toxic and chemically more reactive in this phase. A methanogenic phase follows in which other bacteria of the natural consortium start converting the accumulated organic acids into methane and carbon dioxide, which are collectively termed biogas. Consumption of acids raises the pH, changing the conditions favorably for methanogenic bacteria to produce more methane. Meanwhile, the BOD/COD ratio decreases as acids are being consumed. At the end of methanogenesis the remaining refuse is dominated by recalcitrant matters, such as fulvic and humic acids, with the BOD/COD ratio typically dropping below 0.1. Additional phases beyond the stable methanogenic phase have been speculated (Bozkurt et al. 2000; Christensen and Kjeldsen 1995). Undoubtedly, full decomposition of the biodegradable part of MSW requires a consortium of microorganisms.

The constituents of LL can be classified into four main groups: dissolved organic matter, dissolved inorganic matter, heavy metals, and xenobiotics (Christensen et al. 2001; Kjeldsen et al. 2002). The composition and concentration of organic matter depend on the decomposition stage of the landfill. Common organic compounds found in LL range from carbohydrates, proteins, and fatty acids to the long-chain recalcitrant fulvic and humic acids (Chian and DeWalle 1977). Similarly, the composition of inorganic matter depends on the decomposition stage of the waste. Inorganic compounds include various anions (bicarbonate, chloride, phosphorus, sulfate) and cations (ammonium-N, calcium, iron, magnesium, potassium, sodium). Heavy metals, such as cadmium, chromium, copper, lead, nickel, and zinc are also present, but usually at lower concentrations, as metals get immobilized through sorption to colloids and precipitation, but they can leach through formation of complexes with dissolved organic matter (Baun and Christensen 2004). Xenobiotics, such as monoaromatic and halogenated hydrocarbons (Baun et al. 2004), are usually found at very low concentrations due to natural sorption, precipitation, and volatilization effects at the landfill, but they still pose a significant contamination risk for water resources despite efforts to limit the permissible disposal of xenobiotics in MSW landfills.

In general, the concentration of pollutants in LL decreases over time. On the contrary, ammonia released from the decomposition of organic waste remains at high levels, because there is no mechanism for degrading ammonia during the methanogenic stage (Kulikowska and Klimiuk 2008). It should be noted that ammonia toxicity is acute in its unionized form (NH_3), which happens to be the prevalent one in leachate under the increasing pH conditions of the stable methanogenic phase (Clément and Merlin 1995; Ernst et al. 1994; Rutherford et al. 2000). As a result, ammonia is considered the most dangerous leachate component posing long-term environmental concerns to water resources.

Leachate treatment technologies

Leachate must meet certain standards before discharge into surface water, underground injection into the water table or transfer to remote treatment facilities is allowed. Transferring LL to off-site locations is both risky and adds to the treatment cost. Therefore, when applicable, on-site LL treatment is favored. A big challenge to LL treatment is the wide variation of LL composition from site to site and over time within each site. Variability of composition and the increasingly stringent discharge regulations have sparked a search for effective LL treatment methods.

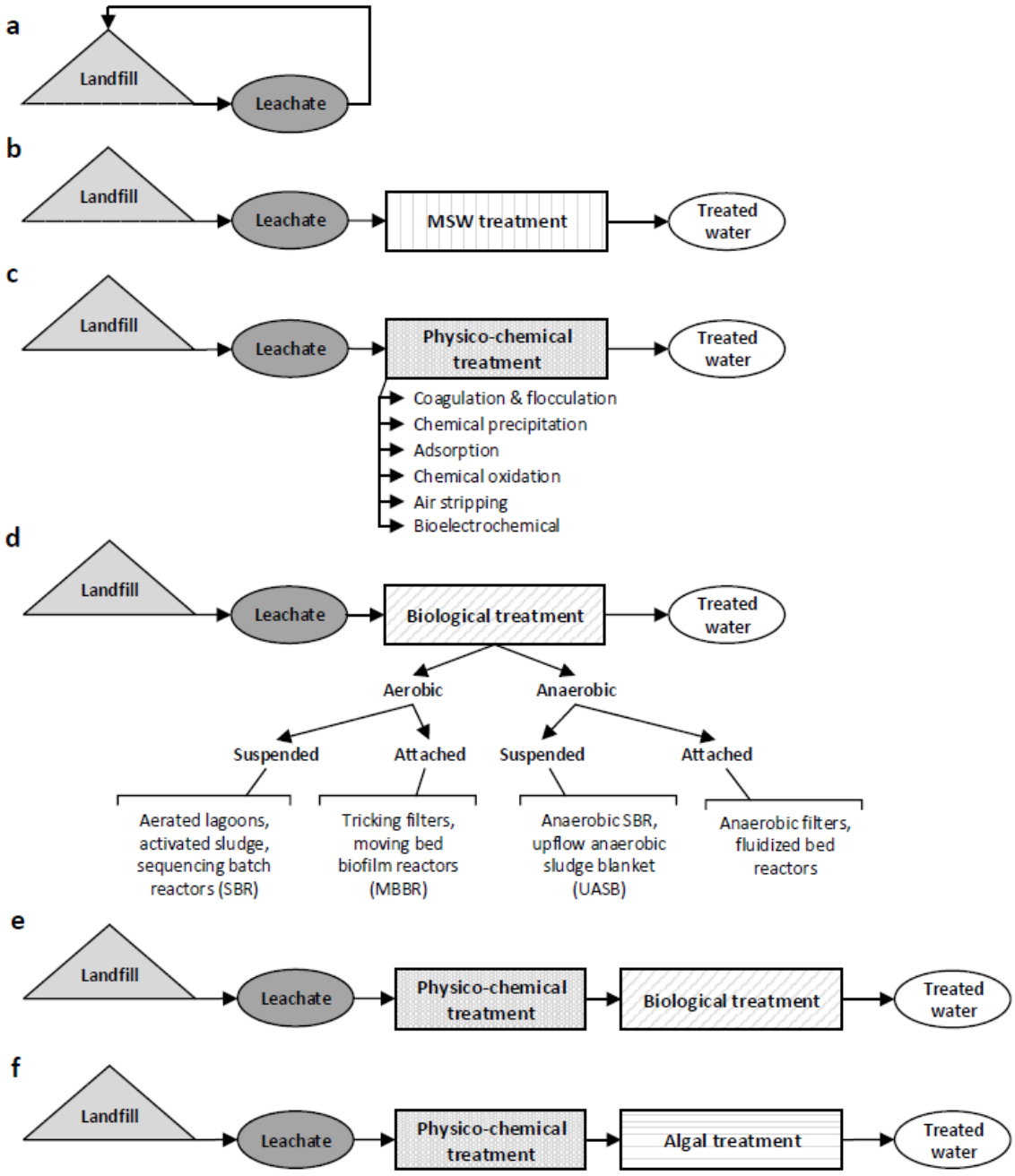

Fig 1. Summary of main landfill leachate treatment methods: a) recirculation; b) mixing with municipal solid waste (MSW); c) physico-chemical treatments; d) biological treatments; e) combination of physico-chemical and biological treatment; f) combination of physico-chemical and algal treatment.

The technologies employed for treatment of LL can be divided into two main categories: biological and physico-chemical. Biological methods are more effective in the presence of large loads of biodegradable matter, as in young landfills. On the other side, physico-chemical methods are employed for the removal of refractory matter that is otherwise not biodegradable in older landfills. Biological methods include aerobic

and anaerobic systems operating in either suspended or attached growth modes. Physico-chemical methods include coagulation and flocculation, chemical precipitation, adsorption, chemical oxidation, air stripping, and bioelectrochemical means. Oftentimes, no single method is effective on its own, so a combination of biological and physico-chemical methods is applied. LL treatment has been extensively reviewed elsewhere (Bove et al. 2015; Gao et al. 2015; Lippi et al. 2018; Peng 2017; Renou et al. 2008; Wiszniowski et al. 2006). The main LL treatment methods are summarized in Fig. 1 and a brief overview of the most practiced methods is presented next.

Transfer for co-treatment with sewage

If economically feasible, LL can be transferred to nearby sewage treatment facilities for co-treatment (Fig. 1b). An advantage of this approach is that LL and sewage contain mostly nitrogen and phosphate, respectively, complementing each other during biological treatment. A major disadvantage is inhibition of natural decomposing microorganisms by refractory compounds and heavy metals in LL. Therefore, the ratio of LL to sewage must be optimized, which will depend largely on LL composition. A study reported that leachate should not exceed 20% of the mixture (Çeçen and Çakıroğlu 2001), whereas another study recommended 10% unless powdered activated carbon was also used to help limit the toxicity of LL (Çeçen and Aktaş 2004).

Recirculation

Leachate recirculation through the waste of a landfill (Fig. 1a) is an inexpensive option that reportedly raises the moisture level within the landfill refuse and provides additional nutrients to degrading microorganisms, thus accelerating waste decomposition and leachate stabilization (Reinhart and Basel Al-Yousfi 1996). As in co-treatment with sewage, leachate toxicity to natural microorganisms is a major concern. Therefore, recirculation rate and frequency must be carefully controlled to avoid accumulation of microbial inhibitors in the refuse (Šan and Onay 2001; Sponza and Ağdağ 2004).

Biological treatment

Biological treatment is considered highly efficient and inexpensive compared to other treatment technologies. However, treatment efficiency is hampered by the presence of toxic compounds or high concentrations of refractory matter, which inhibit biological activity. Hence, this technology is well suited for leachate with high BOD content corresponding to BOD/COD ratios higher than 0.5, which is typical of young or immature leachates. As mentioned earlier, biodegradation of organic compounds in MSW is brought about by microorganisms first aerobically to carbon dioxide and then anaerobically to biogas. Microorganisms for LL treatment are grown either in suspension mode or in attached mode to a matrix under aerobic or anaerobic conditions (Fig. 1d). Attached growth systems are generally advantageous in terms of retaining microbial cell mass and shielding microorganisms to some extent from the detrimental effects of inhibitors.

Aerobic suspended growth systems include aerated lagoons (Maehlum 1995; Maynard et al. 1999; Mehmood et al. 2009; Robinson and Grantham 1988) and activated sludges (Wang et al. 2018), which are becoming less popular, and sequencing batch reactors (SBRs) (Lim et al. 2016; Sivic et al. 2017), which are more widely applied. SBRs allow aerobic oxidation of organics to carbon dioxide in addition to ammonia nitrogen removal through a microbial nitrification-denitrification process. Aerobic attached growth systems include trickling filters (Hongjiang et al. 2009; Langwaldt and Puhakka 2000) and moving bed biofilm reactors (MBBR) (Hajipour et al. 2011; Xiong et al. 2018) that utilize suspended porous materials, such as granular activated carbon, on which microorganisms form biofilms. Anaerobic suspended growth systems include anaerobic SBRs (Timur and Özturk 1999; Timur et al. 2000) and up-flow anaerobic sludge blanket (UASB) reactors (Berrueta and Castrillón 1992; Fonseka et al. 2016; Kettunen and Rintala 1998; Timur et al. 2000;

Wei et al. 2017). UASB is a relatively newer system and reportedly offers superior decomposition performance to SBR, but it remains sensitive to inhibitors. Anaerobic attached growth systems include anaerobic filters (Henry et al. 1987; Inanc et al. 2000), hybrid bed filters that are a combination of anaerobic sludge blanket with anaerobic filter (Bello-Mendoza and Castillo-Rivera 1998; Fernández et al. 1995; Timur et al. 2000), and fluidized bed reactors (Nelson et al. 2017). The latter allows adsorption as well as biodegradation of organic matter, which makes it more effective than the former two in treating old leachates.

Physico-chemical treatment

A wide array of physical and chemical processes exists in this category (Fig. 1c), with a main goal to reduce the non-biodegradable content of leachate (Kurniawan et al. 2006). Hence, they are more suited for old leachates with high loads of refractory matter, but low loads of organic matter. Nonetheless, they can also be applied as pre-treatment or final polishing steps before LL discharge to the environment.

Coagulation-flocculation is common in LL treatment. Commonly used coagulating agents include aluminum sulfate, ferrous sulfate, ferric chloride, and ferric chlorosulfate. Iron salts are reported as more efficient than aluminum ones, but major drawbacks include sludge formation and increased iron and aluminum concentrations in leachate (Amokrane et al. 1997; Aziz et al. 2007; Ghafari et al. 2009; Tatsi et al. 2003).

Chemical precipitation is used as a pretreatment step, when leachate contains high ammonia nitrogen. It is possible to precipitate ammonia in the form of magnesium ammonium phosphate (MAP) using $MgCl_2 \cdot 6H_2O$ and $Na_2HPO_4 \cdot 12H_2O$ at Mg^{2+}:NH_4^+:PO_4^{3-} molar ratio of 1:1:1 at pH 8.5-9 (Li et al. 1999). A more recent investigation recommended 1.15:1:1 molar ratio and pH of 9.5 (Zhang et al. 2009).

Adsorption can be utilized to reduce COD content, whether biodegradable or non-biodegradable, and to reduce color (Foo and Hameed 2009). Activated carbon is considered one of the best adsorbents for LL treatment. However, the need for frequent regeneration of columns and the consumption of large quantities of activated carbon are considered major process drawbacks. Typically, adsorption and biological treatments are combined. It is believed that activated carbon improves treatment by providing an attachment surface for biodegrading microorganisms and by helping cells floc (Çeçen et al. 2003; Foo and Hameed 2009).

Chemical oxidation is performed to oxidize recalcitrant matter or to enhance biodegradability prior to biological treatment. Recently, the focus has been on advanced oxidation processes (AOP) in which free radicals, mainly $OH^{\cdot}$, are generated using synergistic combinations of strong oxidizing agents, such as ozone, UV, H_2O_2, Fenton's reagent, ultrasonication, electron beam, and photocatalysts (Deng and Zhao 2015; Wang et al. 2003). In general, large doses of oxidizing agents are needed to fully oxidize (mineralize) recalcitrant matter, which means high energy consumption and treatment cost.

Air stripping has proven effective in eliminating high ammonia concentrations (Campos et al. 2013; Ferraz et al. 2013) and almost 100% removal has been reported (Silva et al. 2004). High pH is used to release ammonia gas, which is subsequently neutralized with acids to form ammonium salts. The main drawback of air stripping is the risk of ammonia gas release into the atmosphere, if not fully contained by acid treatment.

Microbial electrochemical systems (MESs) have emerged as a promising platform technology. MESs use microbes capable of converting the chemical energy stored in organic and inorganic substrates into electrical energy through microbial anaerobic respiration. In general, a MES system consists of two main compartments, anodic and cathodic. A shared common principle in almost all MESs is that biodegradable substrates (electron donors) are oxidized at the anodic end by microorganisms releasing electrons. On the

other hand, a variety of reduction-based reactions can take place at the cathodic end using various electron acceptors resulting in a range of applications, such as microbial fuel cells (MFCs) for electricity generation, microbial electrolysis cells (MECs) for production of value-added chemicals, microbial desalination cells (MDCs) for water desalination, and microbial remediation cells (MRCs) for remediation of contaminants in wastewaters. To date MESs have been successfully applied in the bioelectrochemical remediation of wastewaters, including removal and/or recovery of nitrogen, phosphorus, and perchlorate among other contaminants (Kelly and He 2014; Sevda et al. 2018) at both laboratory and pilot scale.

Finally, membrane technologies, such as microfiltration, ultrafiltration, nanofiltration and reverse osmosis are widely applied in LL treatment (Peng 2017; Renou et al. 2008; Wiszniowski et al. 2006). Microfiltration is used to remove suspended and colloidal matter. Ultrafiltration additionally allows fractionation of suspended matter based on molecular size. Nanofiltration has the advantage of removing both suspended and colloidal matter and microorganisms. These filtration techniques are usually employed as pretreatment before reverse osmosis or other treatment methods. Reverse osmosis is considered the most promising of all membrane technologies with near 100% COD removal efficiencies reported (Chianese et al. 1999; Linde et al. 1995; Ushikoshi et al. 2002). However, a major drawback of reverse osmosis and other membrane technologies is membrane fouling that necessitates downtime for cleanup or replacement. In addition, retained liquids become highly concentrated and may need further treatment before eventual disposal. All of these drawbacks raise the cost of treatment.

Technology combinations

As outlined earlier, LL is complex in nature. As a result, no sole treatment method is effective enough in stabilizing LL to meet discharge limits. Combinations of biological and physico-chemical methods in integrated multi-stage processes can provide a solution (Fig. 1e). Examples reported include: physico-chemical and biological combinations (Chemlal et al. 2014; Colombo et al. 2019; Del Moro et al. 2013; Huang et al. 2017; Klauson et al. 2015; Oller et al. 2011; Smaoui et al. 2019), AOP combinations (Goi et al. 2009; Silveira et al. 2018; Soubh and Mokhtarani 2016), physico-chemical combinations (Amor et al. 2015; Joshi and Gogate 2019; Kılıç et al. 2007; Li et al. 2010; Ntampou et al. 2006; Pi et al. 2009; Trebouet et al. 2001), and multi-process combinations (Cammarota et al. 2009; Guo et al. 2010; Hasar et al. 2009).

Several factors are taken into account before determining which LL treatment method should be applied at a specific landfill. A key factor is the treatment cost which varies among methods (biological, physico-chemical or combinations) and even within each method depending on the composition of LL and local environmental regulations. In general, physico-chemical methods tend to be costlier than biological methods because of the higher capital cost and the maintenance cost of such facilities (Kurniawan et al. 2006). Other important factors include land cost and operating cost (Daskalopoulos et al. 1997; Heyer et al. 2001; Tsagarakis et al. 2003). Typically, larger scale operations and integration with energy recovery or co-product generation lower the total cost thanks to economies of scale and co-product revenue.

Notably, because discharge limits enforced by regulatory entities are becoming more stringent over time, more competent novel treatments are always in demand. Exploiting the potential of algae in LL treatment is one such novel approach currently gaining interest. At the same time, algae are a promising renewable resource (feedstock) for producing biofuels and bioproducts. One of the biggest challenges to commercial production of algal bioproducts is the high water and energy demand. As stated earlier, LL is rich in various organic and inorganic components, which can be utilized by algae, with or without prior treatment. Hence, LL can serve as a sustainable source of water and nutrients in the manufacture of algal biofuels and bioproducts. In fact, using LL to grow algae fulfills two goals simultaneously, namely bioremediation of

leachate and algal biomass and bioproduct synthesis (Dogaris et al. 2019; Matsakas et al. 2017; Rawat et al. 2011). From a sustainability standpoint, use of algae in LL treatment offers a number of advantages: valorizing low-value waste matter and wastewater, reducing potable water consumption, and co-locating bioremediation and biomass production processes, which minimizes land use and land use change from a sustainability perspective. For these reasons, growing algae on LL has recently received increased attention from researchers (Edmundson and Wilkie 2013; Kumari et al. 2016; Mustafa et al. 2012; Zhao et al. 2014), including our own work (Dogaris et al. 2019), as summarized next.

Use of algae in landfill leachate treatment

Microalgae have been previously reported to grow in LL, generally after some LL pretreatment or dilution, but mostly in small-scale experiments, while a few algal species have been explored for biological treatment of LL effluents at larger-scale outdoor ponds and raceways. Cultivating algae in landfill wastewater can accomplish a dual goal of producing algal biomass for energy and bioproducts and at the same time remediating low-quality wastewater (LL) for recycling and reuse. Next, we provide an overview of reports on the toxicity of LL to algae, the use of LL as algal nutrient source, common algal cultivation technologies that can be integrated into LL treatment facilities, and LL treatment findings using algae and algae-bacteria consortia.

Toxicity of LL to algae

Algae are organisms that grow rapidly in inexpensive media and play important ecological roles thanks to photosynthesis, but are sensitive to environmental factors. As a result, use of algae is common in environmental studies as a means of testing water toxicity and has been applied in screening wastewaters, including LL (Hassan et al. 2016). The ISO (International Association for Standardization) test 8692 was developed to assess water quality using a freshwater algal growth inhibition test with unicellular green algae (ISO 1989). Other toxicity assessments using microalgae include esterase inhibition, ATP energy loss, motility inhibition, and chlorophyll fluorescence (Hassan et al. 2016). All these tests are typically carried out in small volumes, such as shake flasks, microplate wells or glass vials. The microalgal species *Chlamydomonas variabilis*, *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *C. pyrenoidosa*, *C. kessleri*, *Monoraphidium pusillum*, *Scenedesmus quadricauda*, *S. subspicatus*, and *S. capricornutum* have been extensively used in algal toxicity assays (Hassan et al. 2016).

In general, LL toxicity to algae is a potential limitation that needs to be considered in advance of integrating algae cultivation with LL treatment. Plotkin and Ram (1984) tested the toxicity of LL on *S. capricornutum* and found that algal growth was inhibited above 10% LL load. Cheung et al. (1993) assessed the toxicity of leachates from two landfills with four different green algal species using the ISO 8692 method and found an inhibitory effect on growth at 50% LL, while reporting that susceptibility to LL increased among algae species in the order *Chlorella pyrenoidosa*, *Scenedesmus* sp., *C. vulgaris*, and *Dunaliella tertioleeta* (Cheung et al. 1993). Baun et al. (2000) tested 27 samples of leachate from a municipal landfill using *S. capricornutum* and concluded that LL groundwater collected close to the landfill was toxic, but the toxicity decreased with distance away from the landfill. Marttinen et al. (2002) screened various physical-chemical treatments (nanofiltration, ammonia stripping, ozonation) to remove pollutants and then assessed the effluent toxicity on *Raphidocelis subcapitata* growth, which showed that none of the tested treatments was effective in eliminating LL toxicity to algae. Waara et al. (2003) observed acute toxicity of untreated as well as biologically treated LL to *Pseudokirchneriella subcapitata*. The use of *P. subcapitata* (also known as *S. capricornutum* or *R. subcapitata*) has become a very popular tool for monitoring the toxicity of landfill effluents (Ghosh et al. 2017). One bioassay kit based on this alga is commercially available under the name

Algaltoxkit F and has been used in many LL assessment studies (Ghosh et al. 2017; Kokkali and van Delft 2014; Thomas et al. 2009).

Jemec et al. (2012) reported the use of the alga *Desmodesmus subspicatus* CCAP 276/22 as biotoxicity marker in biologically treated LL. Although an increase in algal growth at low LL concentrations was observed, *D. subspicatus* cells were inhibited at more concentrated LL. Jurkoniene et al. (2004) developed a bioassay based on enzymatic activity (ATPase) in a preserved cell fraction of the freshwater alga *Nitellopsis obtuse* to produce an "on demand" toxicity test. This algal fraction toxicity test showed that LL toxicity was decreasing with an increasing dilution with fresh water, and the results were comparable with other toxicity standard tests.

LL as algal nutrient and carbon source

The ability of algae to grow in wastewater sources depends on pH, temperature, availability of light, O_2, and CO_2, presence of inhibitors, and importantly the concentration of essential nutrients, such as nitrogen and phosphorus (Pittman et al. 2011). Reviews by Cai et al. (2013) and Gonçalves et al. (2017) describe in detail the N, P, and carbon assimilation paths in algal cells. Briefly, autotrophic algae fix CO_2 from the atmosphere via photosynthesis and take up soluble carbonates from the solution to convert them into CO_2. Then, CO_2 enters cellular metabolism and is converted into organic compounds by the enzyme rubisco (ribulose biphosphate carboxylase oxygenase). Other algae are heterotrophic, instead of autotrophic, hence being able to use organic carbon (such as sugars) or mixotrophic, using carbon from both CO_2 and organic compounds. In the case of nitrogen, only the prokaryotic algae (cyanobacteria) can fix molecular nitrogen (N_2-N) from the atmosphere and assimilate it into ammonia to meet their metabolic needs, such as protein synthesis. The eukaryotic algae, on the other hand, have the capacity to take up only fixed forms of nitrogen in the form of ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-). Nitrate and nitrite first need to be reduced by enzymes (nitrate reductase and nitrite reductase, respectively) to ammonium, which then enters the metabolism. Phosphorus is also important in algal metabolism for energy synthesis (ATP) and nucleic acid synthesis. It enters algal cells in the form of phosphate (PO_4^{3-}) by active transport and is incorporated directly into various metabolic substrates, such as ATP. The removal of PO_4-P from wastewater by algae is affected by pH and O_2, with precipitation occurring at higher pH and oxygen levels.

LL in general is reported to contain significant amounts of carbon, nitrogen, and phosphorus. Table 1 presents the compositions of reported effluents from numerous landfills in terms of organic carbon, nitrogen, and phosphorus, demonstrating that LL composition varies significantly among landfill sites and treatment technologies. Carbon content fluctuates considerably ranging from 32 to 21,475 mg L^{-1} COD (average 3206 mg L^{-1}). NH_4^+ seems to be the most prevalent form of nitrogen, especially in raw LL, at an average concentration of 626 mg L^{-1} and reaching up to 2,589 mg L^{-1}, followed by NO_3^-, at an average 327 mg L^{-1} (up to 1,471 mg L^{-1}), and NO_2^- at an average 229 mg L^{-1} (up to 712 mg L^{-1}). Phosphorus levels in LL are usually lower than nitrogen at 19 mg L^{-1} on average and reaching up to 270 mg L^{-1}.

[Table 1 position]

Overview of algal cultivation systems

Integration of algae into current LL treatment processes will require the use of cultivation systems. Microalgae are cultivated in open systems, such as ponds and raceways, or in closed photobioreactors (PBRs) (Fig. 2). In PBRs the use of high-cost materials of construction and peripheral equipment for providing gases (CO_2, air) and chemicals increases the capital and operating costs compared to open systems. On the other hand, the enclosed design of PBRs protects the culture from the environment (contaminants) and enables

better control of growth conditions leading to higher productivity. PBRs can be constructed from rigid glass or plastic or from flexible and transparent plastic films. Many PBR designs have been reported (Fig. 2a-d), including flat panel, tubular, horizontal, inclined or vertical, spiral, manifold or serpentine, floating, membrane, and hybrid systems (Zittelli et al. 2013). Some are still in research phase, while a few have been used at commercial scale.

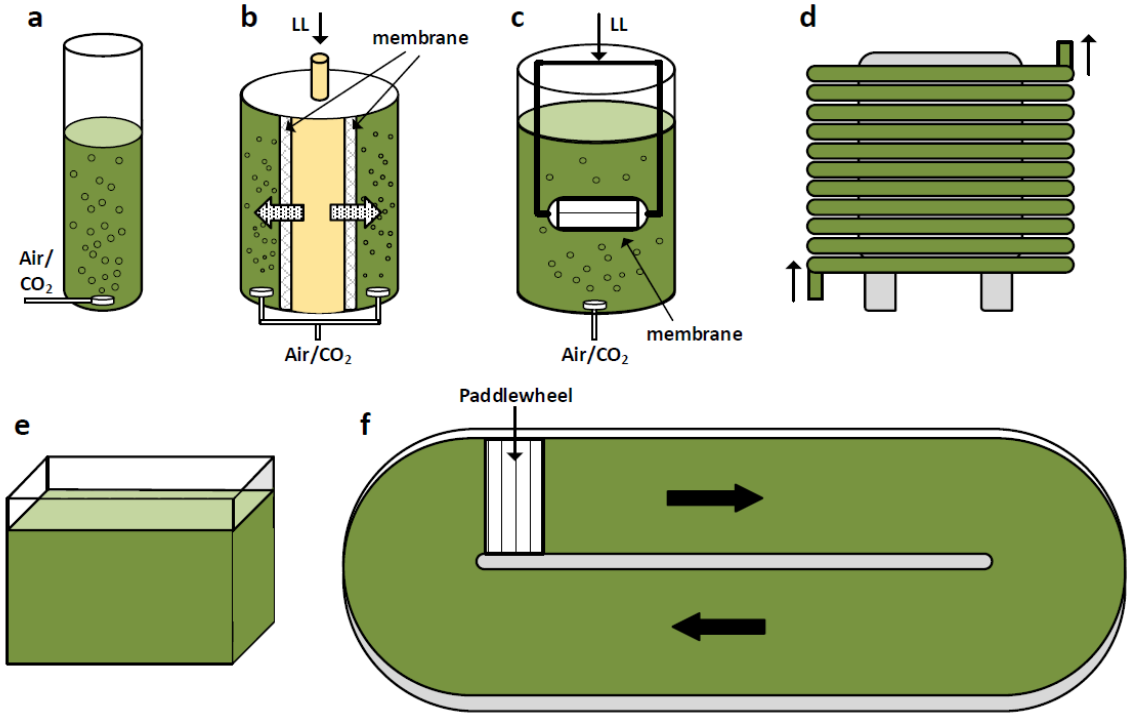

Fig 2. Simplified schematics of algal cultivation systems reported in landfill leachate (LL) treatment trials: a) tubular PBR; b) membrane PBR; c) oscillating-membrane PBR; d) 'bio-coil' tubular PBR; e) open tank; f) open raceway algal pond.

For economic reasons most large-scale cultivations of microalgae are in open systems (Borowitzka and Moheimani 2013; Wijffels and Barbosa 2010). A review by Borowitzka and Moheimani (2013) provides examples of large-scale open systems for algal cultivation, important operational characteristics, culture management practices, and ways to improve productivity. Open pond culture systems include simple ponds, lagoons or tanks, inclined (cascade) systems, circular (central-pivoting) ponds, and raceway ponds (Fig. 2e-f). Adequate mixing in the pond is essential to ensure that the algae do not settle (thus becoming unproductive), nutrients, pH, and O_2 are evenly distributed, and all cells get enough light exposure. However, contamination problems are often reported in open systems, which could be reduced with the use of rapidly growing algae species that outcompete contaminants and adequate mixing (Borowitzka and Moheimani 2013). Algal open cultivation systems have been employed in municipal, industrial, and agricultural wastewater treatment facilities, mainly in the form of stabilization ponds/lagoons and raceway ponds known as high rate algal ponds (HRAP). The use of HRAP in wastewater treatment enables the synergistic action of algae and bacteria for breaking down pollutants, effectively combining secondary and partial tertiary wastewater treatment (Sutherland et al. 2015). In such symbiosis, oxygen generated by algae through photosynthesis enables aerobic bacteria to degrade organic compounds in wastewater to CO_2 that is in turn used as carbon source by the algae. Hence, photosynthesis plays a key role in algal wastewater treatment, but

is subject to limitations by light availability, temperature, nutrient and carbon load, and pH (Sutherland et al. 2015).

Finally, integration of algae with microbial electrochemical systems (MESs) has also been reported (Sevda et al. 2019). One of the main advantages of bioelectrochemical remediation systems is that they combine contaminant removal and/or recovery with the production of energy and/or value-added chemicals (Wang and Ren 2013).

Therefore, the technologies utilized in large-scale cultivations of algae (e.g. open raceways) and in algal wastewater treatment (e.g. HRAP) are very similar, and the main requirements for algal growth (i.e. light, nitrogen, and phosphorus) apply to both cases. However, significant pre-treatment of the wastewater (e.g. filtering, air stripping or dilution with clean water) seems to be necessary to prevent extensive equipment fouling, minimize algal growth inhibition, and avoid culture crashes, as will be discussed in the following section. Another difference is the assortment of organisms present in each process: a plethora of organism types, including bacteria, protists, and fungi, may co-exist with algae in wastewater treatment operations, while single-species cultures are preferred in dedicated algal cultivations. Furthermore, it is expected that the quality of water (e.g. presence of toxic compounds, heavy metals, high solid content) will limit the end-uses of the generated algal biomass at landfills. Microalgae are a source of high-value natural compounds, such as carotenoids, antioxidants, ω-3 fatty acids, proteins, polysaccharides, which are used commercially in human food, animal feed, nutraceuticals, and cosmetics (Borowitzka 2013; Draaisma et al. 2013; Yen et al. 2013; Koller et al. 2014). These applications require 'food-grade' biomass, which necessitates a clean enough water source to avoid causing health issues to humans and animals. On the other hand, 'lower-grade' algal biomass, such as the one originating from wastewater treatment operations, can be processed to biofuels and other bio-based chemicals for industrial uses (Wijffels and Barbosa 2010; Koller et al. 2014), which can add value and generate additional income for water treatment facilities.

Algae for biological treatment of LL

Several small-scale studies have reported on the successful use of the green alga *Chlorella vulgaris* (Chlorophyta) to treat LL to effectively reduce N and P levels. Casazza and Rovatti (2018) studied the reduction of ammonia, nitrate, and nitrite levels of landfill leachate during biological denitrification treatment of LL. The growth experiments were conducted in a vertical 1.5 L PBR with air bubbling and artificial light by fluorescent lamps (Fig. 2a). High biomass concentration was attained (2 g L^{-1}) after 28 days of cultivation with almost 100% reduction of ammonia and nitrate, but partial removal of nitrite. Chang et al. (2018) adopted a membrane PBR (m-PBR) to alleviate some of the inhibitory effects of LL on algal cells (Fig. 2b). The membrane permitted only inorganic ions (such as ammonium and phosphate) to diffuse from the LL chamber to the algae chamber, hence blocking any suspended solids from passing through or the accumulation of high ammonia levels, which can be inhibitory to the algae. They also compared the nutrient removal, biomass production, and lipid profile of *C. vulgaris* FACHB-31 grown in the m-PBR versus the traditional tubular PBR (t-PBR) using biological effluent of LL as growth medium. The m-PBR performed better than the t-PBR in terms of higher N and P removal efficiency (close to 100%), higher biomass yield (reaching close to 1 g L^{-1}), and a lipid profile with better biodiesel quality potential.

In another study by Chang et al. (2019), *C. vulgaris* FACHB-31 was cultivated in untreated LL using a t-PBR and a scalable m-PBR (Fig. 2b). The untreated LL was found to be inhibitory to algal growth with the culture dying within just two days, which was attributed to the high nitrogen concentration (particularly ammonia) and the high color value of the LL. The m-PBR seemed to alleviate these inhibitory effects, resulting in more than 2 g L^{-1} of biomass and 57.5% removal of nitrate, 83.0% of ammonia, and 100% of

phosphate. Pereira et al. (2016) assessed the removal of nitrogen and phosphorus from three different batches of pretreated LL using *C. vulgaris* CCAP 211/11B grown in flasks for 10 days. The leachates had very low P content, so some tests included external addition of K_2PO_4 to support algal growth. Adding phosphorus to LL seemed to promote the growth and nutrient removal performance of *C. vulgaris*, which reached 1.7 g L^{-1} and removed up to 100% of ammonia, 27% of nitrate, and 100% of phosphate. Thongpinyochai and Ritchie (2014) tested *C. vulgaris* in 150-mL flasks for treating leachate collected from high-pollution (garbage pit) and low-pollution (landfill base) locations in the same landfill. The LL from the garbage pit was too toxic to support algal growth above a 30% load, while the performance of *C. vulgaris* in the less polluted LL was better at all loads tested. Nutrient removal after 9 days of treatment reached 65% of ammonia-nitrogen, 40% of nitrate-nitrogen, and 65% of phosphorus.

Desai (2016) investigated the use of *Chlorella* sp. in biological treatment of raw and pretreated LL at different dilutions in 1-L flasks. Nitrogenous compounds were effectively removed from both LL types, while COD remained above safe discharge levels, suggesting a multi-species treatment could be more effective. The growth of *Chlorella* sp. isolated from a clean lagoon was unhindered at 10% of untreated LL load, while culture adaptation was necessary above 30% LL load for the algae to be able to remove all the $NH4^+$-N in flask culture experiments (El Ouaer et al. 2016; El Ouaer et al. 2019). In another study by the same group using the same isolated strain and LL source, the *Chlorella* sp. population declined when raw LL was used, while slow growth was observed along with the removal of more than 60% of $NH4^+$-N (El Ouaer et al. 2017). A marine *Chlorella* sp. was cultivated in a 'Biocoil' tubular PBR (Fig. 2d), using diluted LL to assess the feasibility of reducing organic and metal loads (Reis et al. 2016). The Biocoil system comprised a 20-L tank that was connected to a 24-L tubular PBR, which was artificially illuminated. No N and P removal information was reported, but *Chlorella* cells were able to remove 60% of total organic carbon (TOC), 68% of COD, and almost 100% of boron and iron.

Chlorella pyrenoidosa has also been utilized in treatment of LL. Fan et al. (2018) reported on the treatment of a saline LL using the salt-tolerant *C. pyrenoidosa* FACHB-28 in a 30-L oscillating membrane PBR designed to reduce the biofouling of the membrane (Fig. 2c). The LL was first diluted (to about 25% load) to match the maximum salinity tolerance of the alga. *C. pyrenoidosa* grew well in diluted LL reaching 0.63 g L^{-1} and removed more than 90% of $NH4^+$-N. Lin et al. (2007) explored the feasibility of *C. pyrenoidosa* and *Chlamydomonas snowiae*, isolated from a high-ammonia leachate pond, to grow in LL and remove N and P. Flask experiments at various LL dilutions showed that algal growth was suppressed in LL loads above 10%, but removal of $NH4^+$-N and ortho-P continued to occur even at higher LL loads without significant differences between the strains. Nair et al. (2019) applied *C. pyrenoidosa* NCIM 2738 cultivation as tertiary treatment for pretreated leachate (after coagulation and air-stripping) in a 3-L tubular PBR (Fig. 2a). High algal growth was achieved (up to 2.9 g L^{-1}) and up to 86% and 96% removal of total N and phosphates, respectively, was reported.

Besides *Chlorella* species, algae belonging to the genera *Chlamydomonas, Scenedesmus,* and *Oscillatoria* have been isolated from landfill leachates and could be potential candidates for LL biological treatment. Cheng and Tian (2013) studied the use of *Scenedesmus* sp. CHX1 for biological treatment of LL. Leachate was filtered, sterilized, and fed to the algae in small flasks at different dilutions from 2% to 20% LL load. They reported better biomass yield (up to 0.75 g L^{-1}) and nutrient removal efficiencies (up to 95%) at lower LL loads, while 20% LL inhibited algal growth and resulted in only 16% nutrient removal. Chu et al. (1996) investigated the efficiency of two algal species, *Scenedesmus sp.* isolated from a local arable land drain and *C. pyrenoidosa* isolated from leachate runoff, for removing nutrients from leachates at two landfill sites. Flasks containing 400 mL of untreated (raw) LL were inoculated with algae and incubated for 12 days. No

growth was observed in untreated LL compared to pretreated LL in which both species grew and removed up to 30% of ammonia-nitrogen and up to 94% of phosphorus. Nordin et al. (2017) examined the biomass production and nitrate removal of three algae, *Chlorella* sp., *Scenedesmus* sp. and *Oscillatoria* sp., isolated from local landfill sites. The LL used was first nitrified (in a 2-L bioreactor using activated sludge) and then fed at 10-30% load to the algae cultivated in 350-mL flasks for 14 days. Biomass production ranged from 0.09-0.81 g L^{-1}, while nitrate removal ranged from 13-84%, with the highest overall performance observed with *Oscillatoria* sp.

Edmundson and Wilkie (2013) reported the growth of *C. ellipsoidea* and *S. rubescens*, isolated from a landfill site in Florida, in raw but pH-adjusted LL. Significant biomass yield and productivity was achieved, up to 1.3 g L^{-1} and 550 mg L^{-1} d^{-1}, respectively, by *S. rubescens*, which was 4-5 times higher than *C. ellipsoidea* under the same conditions. Paskuliakova et al. (2016) screened algal populations from 34 isolates from different environments in Ireland, including landfill sites, for their ability to grow in LL. Cultures of *Chlamydomonas* sp. SW15aRL, which was isolated from untreated LL, achieved the highest removal of ammonia-nitrogen (up to 91% when supplemented with phosphate) and generated the most biomass. In follow-up studies using that strain, the researchers compared the effect of different types of phosphorus supplementation and LL biotoxicity reduction (Paskuliakova et al. 2018b) and evaluated the treatment of a range of LL samples collected from four different sites (Paskuliakova et al. 2018a). There was no significant difference in nutrient removal (reaching up to 83% $NH4^{+}$-N) or biomass yield (up to 1.2 g L^{-1}) when the *Chlamydomonas* sp. flask cultures with diluted LL (60% load) were supplemented with exogenous P (Paskuliakova et al. 2018b). However, the composition of the LL (nitrate, ammonia, phosphate, and toxic metals) seemed to greatly affect growth and nutrient removal by *Chlamydomonas* sp. SW15aRL.

Richards and Mullins (2013) investigated the use of mixed cultures of marine algae in two cylindrical PBRs (2.5 and 12.5 L, Fig. 2a) for removing toxic metals from hyper-saline leachates and developed models for metal uptake kinetics. The marine species *Nannochloropsis gaditana*, *Pavlova lutheri*, *Tetraselmis chuii*, and *Chaetoceros muelleri* were exposed to filtered LL for 10 days in PBRs and the mixed culture was able to remove over 95% of metals, but no information on N and P was reported. By the end of the cultivation, *N. gaditana* and *C. muelleri* dominated the algal population and had the highest lipid content for biofuel production.

Studies on algae treatment of LL have been conducted either in flasks or in small-scale bioreactors, which are far from commercial implementation. However, there are a few reports on larger-scale outdoor cultivations ranging from 40 to 2,000 L, which can serve as basis for pilot testing before commercial deployment. The authors demonstrated growth of the marine strain *Picochlorum oculatum* in pretreated LL (Dogaris et al, 2019), using a novel horizontal bioreactor (HBR), which is a modular low-cost cultivation system that resembles an enclosed raceway pond (Fig. 2f). High biomass productivity (ranging from 37 up to 256 mg L^{-1} d^{-1}) and yield (up to 1.9 g L^{-1}) was achieved in 150-L and 2,000-L HBRs operating outdoors over prolonged periods of time (19-73 days) using pretreated LL as a non-potable and low-cost water source. The HBR is currently being scaled-up for future commercial deployment at a projected capital cost of $25,000 per hectare (Dogaris et al, 2015). Khanzada and Övez (2018) investigated the use of mixed cultures of *C. vulgaris* and *Chlamydomonas reinhardii* to treat ultra-filtered LL in 200-L open raceway ponds (Fig. 2f) and observed growth of both strains (up to 1.0 g L^{-1}) in LL, but no nitrate-nitrogen or ammonia-nitrogen was removed. Mustafa et al. (2012) used a mixture of five microalgae, *C. vulgaris*, *Scenedesmus quadricauda*, *Euglena gracilis*, *Ankistrodesmus convolutes*, and *Chlorococcum oviforme*, as well as lake water with natural populations of algae, to remove nutrients from treated LL in a 40-L open raceway pond (Fig. 2f) by progressively increasing the LL load (1-4% of medium replaced by LL daily). The five-species consortium

achieved higher biomass production (up to 5 g L^{-1}) than the natural population, but the nutrient removal rates were similar in the two ponds, almost 100% of $NH4^{+}$-N and 86% of ortho-P. These pilot studies using open raceway ponds indicate that algae cultivation systems can be suitable and cost-effective for integration with conventional LL treatment processes. Open ponds (also known as HRAP) are easy to construct and to scale up and have been employed commercially in treatment of other types of wastewater, such as municipal, agricultural, and industrial wastewaters to some extent (Sutherland et al. 2015).

Table 2 summarizes the growth and pollutant removal efficiencies of algal species in LL reported in the literature. In general the studies support the prospect of using algae in LL treatment, although universally dilution and/or pretreatment (through filtration or other physico-chemical process) of the raw leachate are typically necessary for successful LL treatment. The exact performance of algae during LL and other wastewater remediation seems to depend on the species used and cultivation conditions (Cheah et al. 2016).

[Table 2 position]

Algae-bacteria consortia for synergistic treatment of LL

In addition to algae strains, algae-bacteria consortia have also been studied for application to LL treatment. A consortium of synergistic microbial communities typically has the ability to regulate the processes occurring during biological treatment of wastewaters. As a result, algae-bacteria consortia can synergistically remove pollutants from wastewater by recycling the major nutrients N, P, and carbon. During such symbiotic action, phototrophic algae grow using sunlight and generate oxygen, which, in turn, is used by heterotrophic bacteria to oxidize organic matter, as mentioned earlier, thus achieving breakdown of the complex organic compounds found in LL.

Fernandes et al. (2013) studied a series of experimental stabilization ponds 5-8 m^3 (Fig. 2e), filled with LL and recharged daily, in order to characterize the microbial population dynamics. Through various molecular techniques and optical microscopy the researchers identified a small number of algae species, mainly belonging to the genera *Chlamydomonas* and *Cryptomonas*, while the identified bacteria species belonged to *Planctomycetales*, *Verrucomicrobiales*, *Desulfovibionaceae* (sulfate reducing bacteria), and *Pseudomonas*. Although a satisfactory removal efficiency was reported by this system (82% of ammonium and 56% of TOC), additional conventional treatment was deemed necessary to meet local limits before discharging treated LL to surface waters. In follow-up studies by the same group, where this stabilization pond system was modified to include aeration, recirculation, and filtering, higher removal rates were achieved, up to 82% of TOC and 99% of ammonia, thus meeting discharge limits (Costa et al. 2014; Martins et al. 2013). The main route of N removal was by the settling of dead or inert algae (64-79%), followed by volatilization of ammonia (12-27%), while a small part (1-6%) was attributed to assimilation by algae (Martins et al. 2013).

Kumari et al. (2016) investigated the synergistic effect of algae and bacteria on removing toxic organic compounds and heavy metals present in LL. The bacterial strain *Paenibacillus* sp. ISTP10, previously reported to degrade the banned insecticide endosulfan, was inoculated in 20% LL (v/v) or combined with the alga *Scenedesmus* sp. ISTGA1. The shake flask experiments showed that the algae-bacteria co-culture was more efficient in removing toxic organic compounds and heavy metals. Zhao et al. (2014) cultivated *C. pyrenoidosa* FACHB-9 in 500-mL flasks with a mixture of municipal wastewater and LL (0-20% load) to assess nutrient removal and algal lipid production. The algae-bacteria consortium was able to remove up to 90% of total nitrogen and 95% of phosphorus and accumulated up to 20.8% total lipids. Nair and Nagendra (2018) also combined LL with municipal sewage to effectively increase the bacterial load in the treatment by *C. pyrenoidosa* and observed removal of up to 70% of nitrogen and 89% of phosphate, while generating

2.8 g L^{-1} of total microbial biomass in a 3-L tubular PBR (Fig. 2a). Tighiri and Erkurt (2019) collected algal and bacterial populations from a wastewater treatment plant and mixed them in a 3-to-1 mass ratio to treat 10% (v/v) LL. The main microalgae identified in the consortium were the cyanobacteria *Microcystis* sp. and *Oscillatoria* sp. and the algae *Chlorella* sp., *Scenesdesmus* sp., and *Stigeoclonium* sp. In the two batches of LL treatment in a 10-L PBR (Fig. 2a), 100% removal of ammonia and above 90% reduction in nitrate, COD, and phenol were reported. Sardi Saavedra et al. (2016) studied the diversity of the algal communities that exist in a 300-L HRAP system (Fig. 2f) used for treating LL and identified 28 species of algae with *Chilomonas insignis* and *Euglena* sp. being the dominant ones. In their 2018 study, the researchers were able to predict the algal species composition in response to environmental changes.

Sniffen et al. (2015) explored the use of an algae-bacteria consortium collected from a local pond for the removal of nitrogen from raw LL in 114-L open tanks (Fig. 2e). The authors reported N removal rates up to 9.2 mg L^{-1} d^{-1} and biomass concentration up to 0.48 g L^{-1}. However, the nitrogen removal was inhibited at initial NH_4^+-N concentrations above 80 mg L^{-1}. They also studied nitrogen removal by algae-bacteria consortia at larger scale 1,000-L raceway pond (Fig. 2f) operating in a 7-day semi-batch mode in a greenhouse and compared the results with controlled flask experiments (Sniffen et al. 2017). Biomass concentration reached up to 2.1 g L^{-1} in the raceway pond, while N removal rate was generally lower in the larger scale system (1.6 mg L^{-1} d^{-1}) than in the flasks (3.2 mg L^{-1} d^{-1}). The comparison of N removal and growth rate showed a significant difference between small and larger scale, indicating the need for large-scale data to conduct techno-economic analysis of LL treatment by consortia before commercial development.

As with other industries, there is unfortunately a scarcity of published information on the performance of commercial landfills. As a result, the data compiled in Table 2 come from research studies rather than existing large-scale operations. Nevertheless, in our private conversations with landfill managers we detected an interest in considering the incorporation of algae technologies into traditional LL treatment processes. The main perceived advantage is the ability of algae to practically eliminate nitrogen and phosphorus from LL at low concentrations, hence complementing conventional physico-chemical processes that operate well at high concentrations, but become uneconomical at low concentrations. Another advantage is the ability of algae to sequester carbon dioxide released from landfill gas-powered electricity generators, hence reducing the carbon footprint (both methane and carbon dioxide) of landfills. The main drawback is the apprehension of landfill managers about the performance of algae at large scale, as the algae industry itself is still at its commercial infancy.

Conclusion

Algae have been shown to remove nitrogen, phosphorus, and other nutrients from wastewaters and thus act as a natural filtration mechanism for cleaning up polluted sources of water. In the last two decades a significant number of mostly small-scale studies has reported the ability of algae to grow in landfill leachate with simultaneous significant removal of pollutants. Almost universally, full-strength LL was inhibitory to algal growth, but when LL was diluted or pretreated or the algae were first subjected to LL adaptation, a wide range of algae species were successfully cultivated in LL and were able to remove pollutants to a significant or full extent. Hence, by scaling up and integrating algae technologies with current treatment methods it is possible to significantly improve the effectiveness of LL treatment. However, the use of algae for treating LL has not been practiced commercially yet at landfills because it is deemed uneconomical on its own, when compared to established treatment technologies. Recent advances in the algae sector indicate that these photosynthetic organisms can serve as a promising resource for production of sustainable biofuels, such as biodiesel, aviation fuel, and biogas, and high-value bioproducts, such as nutraceuticals, cosmetics, fertilizers,

animal feed, and fishmeal. As a result, if commercial LL remediation processes are integrated with large-scale outdoor algae cultivation (raceways) for biofuel/bioproduct manufacture, then potentially cost-effective and sustainable processes could be engineered and deployed at landfills around the world to combine environmental and societal benefits in the form of wastewater (LL) cleanup and CO_2 sequestration with the economic benefits derived from the production of biofuels and bioproducts with the use of LL.

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Author contributions
George Philippidis conceived the idea for the review article, contributed, and edited it. Ehab Ammar performed the literature search for sections 1-3 and drafted the respective sections. Ioannis Dogaris performed the literature search and drafted the text for section 4, and prepared all figures and tables. All authors contributed to the revision of the manuscript and read and approved the final manuscript.

Conflict of Interest
The authors declare that they have no conflict of interest.

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Table 1. Compositional profile of landfill leachate effluent after conventional treatments that may serve as input to algae cultivation.

Landfill location	LL treatment	Carbon (mg L^{-1})		Nitrogen (mg L^{-1})			Phosphorus (mg L^{-1})	Ref.
		TOC	COD	NH_4^+-N	NO_3^--N	NO_2^--N	P-PO_4^{3-}	
n.a.	Microfiltration	n.a.	n.a.	258.4	24.3	6.4	n.a.	(Casazza and Rovatti 2018)
n.a.	Microfiltration and biological nitrification	n.a.	n.a.	0	162.3	712.2	n.a.	(Casazza and Rovatti 2018)
Chongqing, China	Biological effluent	550.4	341.5	105.7 [a]	142.8 [a]	n.a.	5.0 [a]	(Chang et al. 2018)
Chongqing, China	Untreated	1630.4	1446.5	197.2 [a]	475.7 [a]	n.a.	6.1 [a]	(Chang et al. 2019)
Hangzhou, China	Filtration and autoclaving	n.a.	14427	842.4	948 [b]	n.a.	5.67 [c]	(Cheng and Tian 2013)
Junk Bay, China	Untreated	n.a.	595	724	752 [b]	n.a.	2.87	(Cheung et al. 1993)
Gin Drinkers' Bay, China	Untreated	n.a.	140	147	152 [b]	n.a.	0.34	(Cheung et al. 1993)
Junk Bay, China	Untreated	n.a.	462	680	0.87 [d]	n.a.	4.58	(Chu et al. 1996)
	Free stripped	n.a.	317	217	1.13 [d]	n.a.	1.27	
	Air stripped	n.a.	339	41	1.27 [d]	n.a.	1.39	
Gin Drinkers' Bay, China	Untreated	n.a.	455	561	57 [d]	n.a.	3.73	
	Free stripped	n.a.	257	146	61 [d]	n.a.	1.27	
	Air stripped	n.a.	241	39	68 [d]	n.a.	1.04	
Biguaçú, Santa Catarina, Brazil	Untreated	n.a.	1789	1108	1471 [b]	n.a.	n.a.	(Costa et al. 2014)
Punta Gorda, Florida, USA	Treated effluent from confined deep well	1.0	32	<0.1	<0.1	<0.1	<0.1	(Dogaris et al. 2019)
Archer, Florida, USA	Untreated	n.a.	2109.3	980	n.a.	n.a.	13.2	(Edmundson and Wilkie 2013)
Tunis, Tunisia	Untreated	10920	21475	2570	320 [d]	n.a.	n.a.	(El Ouaer et al. 2016; El Ouaer et al. 2019)
Tunis, Tunisia	Untreated	10920	23926	2589	415 [d]	n.a.	n.a.	(El Ouaer et al. 2017)
Taizhou, China	n.a.	n.a.	304	230	n.a.	n.a.	n.a.	(Fan et al. 2018)
Slovenia	Untreated	n.a.	3766	909	633	64	53	(Jemec et al. 2012)

Odayeri, Turkey	Ultrafiltration	90 [e]	386	760	800 [b]	n.a.	5.42	(Khanzada and Övez 2018)
Guangzhou, China	Untreated	n.a.	1280	1046	68.4	n.a.	5.16	(Lin et al. 2007)
Selangor, Malaysia	Aerobic treatment	n.a.	4293	152	n.a.	n.a.	8.2	(Mustafa et al. 2012)
Chennai, India	Pre-treated (coagulation and air-stripping)	586	1700	n.a.	228 [b]	n.a.	10.3	(Nair and Nagendra 2018)
Chennai, India	Coagulation and air-stripping	697	1800	182	228 [b]	n.a.	7.0	(Nair et al. 2019)
Selangor, Malaysia	Nitrification	n.a.	1624	n.d.	549	n.d.	18.3	(Nordin et al. 2017)
Northern Portugal	Aeration, biological oxidation, coagulation-flocculation, and photo-oxidation; batch 1	n.a.	n.a.	15	144	n.a.	<0.1	(Pereira et al. 2016)
Northern Portugal	Aeration, biological oxidation, coagulation-flocculation, and photo-oxidation; batch 2	n.a.	n.a.	67	136	n.a.	1	(Pereira et al. 2016)
Northern Portugal	Aeration, biological oxidation, coagulation-flocculation, and photo-oxidation; batch 3	n.a.	n.a.	75	153	n.a.	1	(Pereira et al. 2016)
São Paulo, Brazil	Diluted 10 times	95.7	251.8	n.a.	n.a.	n.a.	n.a.	(Reis et al. 2016)
Northern Ireland	Untreated	n.a.	n.a.	88	0.1 [d]	n.a.	1	(Paskuliakova et al. 2016)
Northern Ireland	Biological pretreatment and filtration	n.a.	n.a.	<0.05	85 [d]	n.a.	0.5	(Paskuliakova et al. 2016)
Northern Ireland	Untreated	n.a.	97-5030	98-2510	0.1-1280	n.a.	0.05-16.5	(Paskuliakova et al. 2018a)
Northern Ireland	Untreated	n.a.	290-469	261-367	0.1-89 [d]	n.a.	0.9-1.2	(Paskuliakova et al. 2018b)
Phuket, Thailand (from garbage pit)	Untreated	n.a.	3000-9000	593-1590	14-260	n.a.	60-270	(Thongpinyochai and Ritchie 2014)
Phuket, Thailand (from base of landfill)	Untreated	n.a.	32-160	132-199	14-49	n.a.	5.6-36.6	(Thongpinyochai and Ritchie 2014)

West Australia	Filtered	668	1008	n.a.	n.a.	n.a.	n.a.	(Richards and Mullins 2013)
Cyprus	Filtered	n.a.	9361	2568	875	362	97.5	(Tighiri and Erkurt 2019)
Shanghai, China	Filtered	294	n.a.	1381	1786 [b]	n.a.	3.2	(Zhao et al. 2014)

n.a. not available (not reported).

n.d. not detected (below detection limit).

[a] in mg of NH_4^+, NO_3^-, and PO_4^{3-}, respectively.

[b] total nitrogen or Kjeldahl nitrogen.

[c] total phosphorus.

[d] oxidized nitrogen ($N\text{-}NO_3^-$ and $N\text{-}NO_2^-$).

[e] approximately (as derived by the authors from graphs included in the noted literature).

Table 2. Key characteristics and performance data from studies of algae cultivation for treatment of LL.

Microorganism	Cultivation system and operation mode	LL type	Cultivation time (d)	Nitrogen removal			P removal	Algal growth, in g L^{-1} (dry basis) or cells mL^{-1}	Biomass productivity (mg L^{-1} d^{-1})	Reference
				NH_4^+-N	NO_3^--N	NO_2^--N				
Chlorella vulgaris CCAP 211	2 L-vertical PBR; batch	After microfiltration; supplemented with BBM	28	223 mg L^{-1} or 38.8 mg L^{-1} d^{-1}	(+)[a] 7 mg L^{-1} or 1.7 mg L^{-1} d^{-1}	(+)[a] 205 mg L^{-1} or 35.7 mg L^{-1} d^{-1}	n.a.	2.10 g L^{-1}	63.8	(Casazza and Rovatti 2018)
Chlorella vulgaris CCAP 211	2 L-vertical PBR; batch	After microfiltration and biological nitrification; supplemented with BBM	28	n.a.	5.5 mg L^{-1} d^{-1}	1.1 mg L^{-1} d^{-1}	n.a.	2.00 g L^{-1}	57.8	(Casazza and Rovatti 2018)
Chlorella vulgaris FACHB-31	Membrane PBR with two 2-L chambers; batch	Biological effluent of LL	8	135.9 mg L^{-1}	622.1 mg L^{-1}	n.a.	15.3 mg L^{-1}	0.95 g L^{-1}	240	(Chang et al. 2018)
Chlorella vulgaris FACHB-31	2-L tubular PBR; batch	Biological effluent of LL	8	135.9 mg L^{-1}	385.2 mg L^{-1}	n.a.	15.3 mg L^{-1}	0.66 g L^{-1}	150	(Chang et al. 2018)
Chlorella vulgaris FACHB-31	3 sets of membrane PBRs in series (in total 1.5-L for LL and 3-L for algal growth); batch	Untreated LL	12	210.5 mg L^{-1} (83.0%)	1211.2 mg L^{-1} (57.5%)	n.a.	18.6 mg L^{-1} (100%)	2.13 g L^{-1}	n.a.	(Chang et al. 2019)
Chlorella vulgaris FACHB-31	3-L tubular PBR; batch	Untreated LL	2	85.2 mg L^{-1} (33.6%)	522.4 mg L^{-1} (24.8%)	n.a.	7.4 mg L^{-1} (40.6%)	No growth	No growth	(Chang et al. 2019)
Scenedesmus sp. *CHX1*	250-mL flasks; batch	After filtration; autoclaving, and dilution (2% loading)	20	16.8 mg L^{-1} (84%) [b]	17.7 mg L^{-1} (75%) [b]	n.a.	0.22 mg L^{-1} (80.5%) [c]	0.75 g L^{-1}	37.5	(Cheng and Tian 2013)
Scenedesmus sp. (isolated from local arable land drain)	500-mL flasks; batch	Untreated; Gin Drinker's Bay Landfill	12	8.8%	7.3% [d]	n.a.	18%	No growth	No growth	(Chu et al. 1996)
		Untreated; Junk Bay Landfill	12	5.2%	6.4% [d]	n.a.	29%	No growth	No growth	
		After free	12	15%	7.8% [d]	n.a.	43%	10^6 cells mL^{-1} [e]	n.a.	

		stripping; Gin Drinker's Bay Landfill								
		After free stripping; Junk Bay Landfill	12	12%	8.0% [d]	n.a.	73%	10^6 cells mL^{-1} [e]	n.a.	
		After air stripping; Gin Drinker's Bay Landfill	12	29%	+2.3% [a d]	n.a.	86%	$1.2 \cdot 10^6$ cells mL^{-1} [e]	n.a.	
		After air stripping; Junk Bay Landfill	12	31%	9.0% [d]	n.a.	86%	$1.2 \cdot 10^6$ cells mL^{-1} [e]	n.a.	
Chlorella pyrenoidosa (isolated from LL runoff)	500-mL flasks; batch	Untreated; Gin Drinker's Bay Landfill	12	6.4%	5.3% [d]	n.a.	18%	No growth	No growth	
		Untreated; Junk Bay Landfill	12	6.3%	+10% [a d]	n.a.	26%	No growth	No growth	
		After free stripping; Gin Drinker's Bay Landfill	12	15%	5.0% [d]	n.a.	47%	$0.8 \cdot 10^7$ cells mL^{-1} [e]	n.a.	
		After free stripping; Junk Bay Landfill	12	12%	12% [d]	n.a.	71%	10^7 cells mL^{-1} [e]	n.a.	
		After air stripping; Gin Drinker's Bay Landfill	12	32%	+6.9% [a d]	n.a.	94%	$0.8 \cdot 10^7$ cells mL^{-1} [e]	n.a.	
		After air stripping; Junk Bay Landfill	12	26%	+6.3% [a d]	n.a.	81%	$0.8 \cdot 10^7$ cells mL^{-1} [e]	n.a.	
Chlorella sp.	1-L flasks	Untreated; 85% load	20	1175 mg L^{-1} (100%)	n.a.	n.a.	n.a.	n.a.	n.a.	(Desai 2016)
Chlorella sp.	1-L flasks	Pretreated; 85% load	10	n.a.	250 mg L^{-1} [e] (99%)	n.a.	n.a.	n.a.	n.a.	(Desai 2016)
Picochlorum oculatum UTEX LB 1998	150-L horizontal bioreactor; fed-batch; 3 cycles	Treated effluent from confined deep well	18-37	n.a.	n.a.	n.a.	n.a.	1.5-1.9 g L^{-1} (1.2-$1.7 \cdot 10^9$ cells mL^{-1})	37-55	(Dogaris et al. 2019)
P. oculatum UTEX LB 1998	2000-L floating or ground-based horizontal bioreactor; batch	Treated effluent from confined deep well	19-23	n.a.	n.a.	n.a.	n.a.	1.9 g L^{-1} (0.73-$0.95 \cdot 10^9$ cells mL^{-1})	75-80	(Dogaris et al. 2019)
C. cf. *ellipsoidea*	125-mL flasks; batch	Untreated; pH-adjusted	3	n.a.	n.a.	n.a.	n.a.	0.29 g L^{-1}	100	(Edmundson and Wilkie 2013)

S. cf. *rubescens*	125-mL flasks; batch	Untreated; pH-adjusted	4	n.a.	n.a.	n.a.	n.a.	1.3 g L^{-1}	550	(Edmundson and Wilkie 2013)
Chlorella sp. (isolated from a clean lagoon)	500-mL flasks; batch	Sterilized; 10-100% load	36	up to 100%	n.a.	n.a.	n.a.	up to 4.2 $\cdot 10^7$ cells mL^{-1} (for 10% load)	n.a.	(El Ouaer et al. 2016)
Chlorella sp. (isolated from a clean lagoon)	500-mL flasks; batch	Sterilized; 10-100% load	24	up to 90%	n.a.	n.a.	n.a.	up to 2.9 $\cdot 10^7$ cells mL^{-1} (for 10% load)	n.a.	(El Ouaer et al. 2017)
Chlorella sp. (isolated from a clean lagoon)	500-mL flasks; batch	Untreated; 10% load	28	100%	n.a.	n.a.	n.a.	3 $\cdot 10^7$ cells mL^{-1} [e]	n.a.	(El Ouaer et al. 2019)
C. pyrenoidosa FACHB-28	30-L membrane PBR; batch	About 25% load	30	>90%	n.a.	n.a.	n.a.	0.41-0.63 g L^{-1}	60[e]-80	(Fan et al. 2018)
C. vulgaris and *Chlamydomonas reinhardii*	500-mL bottles; batch	Ultrafiltration; 10-100% load	28-60	<70%	No removal	n.a.	n.a.	0.46-1.5 g L^{-1}	n.a.	(Khanzada and Övez 2018)
C. vulgaris and *Chlamydomonas reinhardii*	200-L open raceway pond; 5 runs; batch	Ultrafiltration	32-54	No removal	No removal	n.a.	n.a.	0.68-1.03 g L^{-1}	160-440 (23-64 mg m^{-2} d^{-1})	(Khanzada and Övez 2018)
C. pyrenoidosa (isolated from leachate)	300-mL flasks; batch	Filtered; 10-100% load	12	5-80% [e]	n.a.	n.a.	5-80% [e]	up to 4.1 $\cdot 10^6$ cells mL^{-1} (for 10% load)	n.a.	(Lin et al. 2007)
C. pyrenoidosa (isolated from clean river)	300-mL flasks; batch	Filtered; 10-100% load	12	5-80% [e]	n.a.	n.a.	5-70% [e]	up to 5.3 $\cdot 10^6$ cells mL^{-1} (for 10% load)	n.a.	(Lin et al. 2007)
Chlamydonomas snowiae (isolated from leachate)	300-mL flasks; batch	Filtered; 10-100% load	12	5-80% [e]	n.a.	n.a.	5-60% [e]	up to 1.8 $\cdot 10^5$ cells mL^{-1} (for 10% load)	n.a.	(Lin et al. 2007)
Scenedesmus quadricauda UMACC 039, *Euglena gracilis* UMACC 058,	40-L open raceway pond; semi-continuous	Pre-treated (mechanical aeration); 1-4% medium replaced with LL daily.	351	99.9%	n.a.	n.a.	86%	up to 5.5 g L^{-1}	n.a.	(Mustafa et al. 2012)

C. vulgaris UMACC 001, *Ankistrodesmus convolutus* UMACC 101, *Chlorococcum oviforme* UMACC 110										
C. pyrenoidosa NCIM 2738	3-L tubular PBR; batch	Pre-treated (coagulation and air-stripping)	18	90%	86% [b]	n.a.	96%	2.9 g L^{-1}	260	(Nair et al. 2019)
Oscillatoria sp. (isolated from leachate)	350-mL flasks; batch	Pre-treated (nitrification); 10-30% load	14	n.a.	27.8-86.5 mg L^{-1} or 2.0-6.2 mg L^{-1} d^{-1} (43-84%)	n.a.	n.a.	0.43-0.81 g L^{-1}	44-107	(Nordin et al. 2017)
Chlorella sp. (isolated from leachate)	350-mL flasks; batch	Pre-treated (nitrification); 10-30% load	14	n.a.	21.0-83.8 mg L^{-1} or 1.5-6.0 mg L^{-1} d^{-1} (13-77%)	n.a.	n.a.	0.09-0.43 g L^{-1}	18-66	
Scenedesmus sp. (isolated from leachate)	350-mL flasks; batch	Pre-treated (nitrification); 10-30% load	14	n.a.	18.3-86.9 mg L^{-1} or 1.3-6.2 mg L^{-1} d^{-1} (35-66%)	n.a.	n.a.	0.16-0.24 g L^{-1}	37-46	
C. vulgaris CCAP 211/11B	1-L flasks; batch	Pre-treated; 3 LL batches; Supplemented with KH_2PO_4	10	22-100%	0-27%	n.a.	38-100%	0.81-1.71 g L^{-1}	20-110	(Pereira et al. 2016)
Chlorella sp. (marine)	24-L tubular PBR; batch	Diluted 10 times and autoclaved	3	n.a.	n.a.	n.a.	n.a.	2.2-2.6·10^6 cells mL^{-1} [e]	n.a.	(Reis et al. 2016)
Chlamydomonas sp. SW13aLS	250-mL flask; batch	Untreated; 10% load	30	0-11.4%	n.a.	n.a.	100%	no growth	no growth	(Paskuliakova et al. 2016)
	250-mL flask; batch	Permeate (biological pretreatment and filtration); 10% load	30	n.a.	41.4% [d]	n.a.	100%	0.6 mm^3 ml^{-1} [e]	n.a.	
Scenedesmus sp. OT08aTL	250-mL flask; batch	Untreated; 10% load	30	40-55% [e]	n.a.	n.a.	75%	no growth	no growth	
	250-mL flask; batch	Permeate (biological pretreatment and filtration); 10% load	30	n.a.	20% [d e]	n.a.	100%	0.3 mm^3 ml^{-1} [e]	n.a.	

Scenedesmus sp. OT11aTL	250-mL flask; batch	Untreated; 10% load	30	40-50% [e]	n.a.	n.a.	87%	no growth	no growth	
	250-mL flask; batch	Permeate (biological pretreatment and filtration); 10% load	30	n.a.	25-38% [d e]	n.a.	100%	0.4 mm^3 ml^{-1} [e]	n.a.	
Chlamydomonas sp. SW15aRL	250-mL flask; batch	Untreated; 10% load	30	50-92% [e]	n.a.	n.a.	100%	1.6 mm^3 ml^{-1} [e]	n.a.	
	250-mL flask; batch	Permeate (biological pretreatment and filtration); 10% load	30	n.a.	5-10% [d e]	n.a.	100%	no growth	no growth	
Chlamydomonas sp. SW15aRL	150-mL flask; batch	Filtered and diluted (0-100% load)	40	16.8-133.2 mg l^{-1} (15-100%)	up to 21 mg l^{-1} (8%) [d]	n.a.	8-18 mg l^{-1} (25-90%) [e]	up to $1.8 \cdot 10^6$ cells mL^{-1} [e] (no growth in 100% load)	n.a.	(Paskuliakova et al. 2018a)
Chlamydomonas sp. SW15aRL	150-mL flask; batch	Filtered and diluted (30-60% load)	35	131 mg l^{-1} (83%)	0% [d e]	n.a.	95% [e]	1.21 g L^{-1}	n.a.	(Paskuliakova et al. 2018b)
C. vulgaris	150-mL flask; batch	Untreated; high-pollution leachate (garbage pit); 10-100% load	9	5-40% [e]	5-35% [e]	n.a.	0-60% [e]	no growth above 30% load	n.a.	(Thongpinyochai and Ritchie 2014)
C. vulgaris	150-mL flask; batch	Untreated; low-pollution leachate (landfill base) 10-100% load	9	20-65% [e]	10-40% [e]	n.a.	18-65% [e]	highest growth in 30% load	n.a.	(Thongpinyochai and Ritchie 2014)
Nannochloropsis gaditana,Pavlova lutheri, Tetraselmis chuii and *Chetoceros muelleri*	2.5- and 12.5-L cylindrical PBR; batch	Filtered	10	n.a.	n.a.	n.a.	n.a.	up to $9 \cdot 10^6$ cells mL^{-1}	n.a.	(Richards and Mullins 2013)

n.a., not available.

[a] accumulation of.

[b] total nitrogen.

[c] total phosphorus.

[d] oxidized nitrogen.

[e] approximately as derived by the authors from graph.