ASSESSMENT OF LANDFILL LEACHATE BIODEGRADABILITY AND TREATABILITY BY MEANS OF ALLOCHTHONOUS AND AUTOCHTHONOUS BIOMASSES

Santo Fabio Corsino[a*], Marco Capodici[a], Daniele Di Trapani[a], Michele Torregrossa[a], Gaspare Viviani[a]

[a] Dipartimento di Ingegneria, Università degli Studi di Palermo, Viale delle Scienze, 90128 Palermo, Italy

*Corresponding author: tel: +39 09123896555; fax: +39 09123860810

E-mail address: santofabio.corsino@unipa.it (Santo Fabio Corsino)

Abstract

The biodegradability and treatability of a young (3 years old) municipal landfill leachate was evaluated by means of chemical oxygen demand (COD) fractionation tests, based on respirometric techniques. The tests were performed using two different biomasses: one cultivated from the raw leachate (autochthonous biomass) and the other collected from a conventional municipal wastewater treatment plant after its acclimation to leachate (allochthonous biomass). The long term performances of the two biomasses were also studied. The results demonstrated that the amount of biodegradable COD in the leachate was strictly dependent on the biomass that was used to perform the fractionation tests. Using the autochthonous biomass, the amount of biodegradable organic substrate resulted in approximately 75% of the total COD, whereas it was close to 40% in the case of the allochthonous biomass, indicating the capacity of the autochthonous biomass to degrade a higher amount of organic compounds present in the leachate. The autochthonous biomass was characterized by higher biological activity and heterotrophic active fraction (14% *vs* 7%), whereas the activity of the allochthonous biomass was significantly affected by inhibitory compounds in the leachate, resulting in a lower respiration rate (SOUR = 13 mg O_2 $gVSS^{-1}$ h^{-1} *vs* 37 mg O_2 $gVSS^{-1}$ h^{-1}). The long-term performance of the autochthonous and allochthonous biomasses indicated that the former was more suitable for the treatment of raw landfill leachate, ensuring higher removal performance towards the organic pollutants.

Keywords: Leachate biodegradability; Autochthonous biomass; Landfill leachate; Respirometry; SBR; COD fractionation

List of abbreviations

COD – Chemical Oxygen Demand

SOUR – Specific Oxygen Uptake Rate

SBR – Sequencing Batch Reactor

VSS – Volatile Suspended Solids

VFA - Volatile Fatty Acids

BOD – Biochemical Oxygen Demand

TSS – Total Suspended Solids

S_S – Soluble readily biodegradable

S_I – Soluble inert

Xs – Biodegradable and rapidly hydrolysable

X_I - Particulate Inert

Xa - Active biomass

Y_H – Maximum heterotrophic yield coefficient

f_{XH} – Active fraction of the ordinary heterotrophic biomass

b_H – Endogenous decay coefficient of the ordinary heterotrophic biomass

BCOD – Biodegradable fraction of Chemical Oxygen Demand

Introduction

Landfill leachates are complex aqueous effluents generated by rainwater percolation through wastes, the initial water content of wastes and biochemical processes involving wastes themselves within the landfill cells [1,2]. The quality of leachates is governed by several factors. Among these, the waste typology, regional climate conditions and especially the landfill age, are considered amongst the main factors affecting the composition of landfill leachates [3,4]. The leachates are known to contain biodegradable matter such as volatile fatty acids (VFAs), as well as toxic and non-biodegradable organic material [5]. The ratio between the biodegradable and non-biodegradable organic matter decreases with landfill age, because VFAs are gradually degraded or converted to biogas throughout the landfill service life [1,6].

Biological, chemical and physical procedures have been widely used in recent years to treat leachate [7]. Among these, chemical and physical methods are the most effective, although expensive, whereas biological treatments usually represent the less expensive degradation pathway for many biodegradable organic and nitrogen containing compounds [8,9]. However, the biological processes may not deal with refractory and hazardous compounds [10]. Consequently, the choice of biological based treatments depends on the amount of biodegradable compounds in the leachate [11].

The ratio of biological oxygen demand (BOD_5) to chemical oxygen demand (COD) is a commonly used indicator of leachate biodegradability [12]. Based on the landfill age, the BOD_5/COD ratio can vary from 0.4 in young (less than 5 years old), to < 0.2 in medium (5–10 years old), and 0.1 in old (>10 years old) leachates [13,14]. Thus, the BOD_5/COD is often used as a benchmark to establish the suitability of biological processes for leachate treatment. Indeed, such processes have been shown to be very effective in removing organic matter from leachates when the BOD_5/COD ratio has a high value (>0.3), whereas a lower value results in low removal performance insufficient to justify the application of a bio-based process [15]. In the case of landfill leachates, the BOD_5 measurement may be problematic, because the bacterial activity could be barely affected by the presence of toxic compounds, such as heavy metals, aromatic hydrocarbons, phenols, pesticides and inorganic salts [16]. Moreover, the oxygen consumed by bacteria during organic matter decomposition depends on the affinity of bacteria themselves towards the organic substances present in the leachate. Consequently, the use of acclimated bacteria rather than species non-acclimated to leachate characteristics could lead to very different results. Hence, the BOD_5 test can be considered as a relative measurement to assess the amount of biodegradable organic matter based on the bacteria that are used to degrade the organic substances.

The degradative ability of bacteria depends on their origins. Indeed, bacteria developing in the same environment in which the wastewater was produced (autochthonous) will be able to degrade the organic pollutants without an acclimation phase. Alternatively, if bacteria derive from another environment (allochthonous bacteria), biodegradation is possible after theyhave adapted to the new environment (acclimation). Several studies suggested that autochthonous microorganisms could be a valuable resource for bioremediation of the leachate [17,18]. Indeed, autochthonous microorganisms are necessarily tolerant of the high toxicity of the leachate and may take part in the degradation of recalcitrant molecules [19].

Based on the above considerations, the criteria for evaluating leachate biodegradability and the opportunity to apply biological processes for treatment should be reconsidered. The use of COD fractionation tests based on respirometric techniques [20] carried out with pre-cultivated autochthonous biomass could represent a novel and convenient tool for assessing actual leachate biodegradability.

The aim of the present study was to assess landfill leachate biodegradability by means of biomass cultivated from the raw leachate (autochthonous) and from a conventional municipal wastewater treatment plant gradually adapted to leachate (allochthonous). It reports the results of COD fractionation tests performed with the biomasses by means of respirometric techniques applied to the leachate collected from a municipal landfill. An experimental study was also carried out on two sequencing batch reactors (SBR) operating in parallel (one with the autochthonous and the other with the allochthonous biomass) and fed with a mixture of landfill leachate and a biodegradable co-substrate (sodium acetate). The COD removal efficiencies achieved are presented, highlighting the effect of the reduction of co-substrate dosage.

Materials and Methods

Leachate characterization

The landfill leachate was collected from the Municipal Landfill of Palermo (Bellolampo) during the period December 2017 to July 2018. It was collected from the drainage system of a landfill cell having an operating age of 3-4 years, and was thus considered as a young leachate. The samples were transferred to the laboratory and stored at 4°C before being used. The leachate features were characterized by significant variations according to the seasonal fluctuations of rainfall events. The leachate was collected five times (December, January, Mach, May and June). The average physico-chemical characteristics are reported in **Table 1**.

Table 1: Characteristic of the raw leachate in this study

Parameter	Units	Average value ± std
pH	-	7.49 ± 0.49
NH_4-N	mg L^{-1}	923 ± 41
NO_2-N	mg L^{-1}	n.a.*
NO_3-N	mg L^{-1}	11.1 ±3.6
TP	mg L^{-1}	3.2 ±1.3
COD	mg L^{-1}	11,137 ± 2,036
Conductivity	mS cm^{-1}	20.1 ± 0.3

*: below detection limit

Cultivation of the autochthonous and allochthonous biomasses

The cultivation of the biomasses was carried out in two SBRs designated R1 and R2 and had a duration of almost four months (110 days). R1 was seeded with raw leachate only, whereas R2 was inoculated with activated sludge collected at a conventional wastewater treatment plant (Acqua dei Corsari, Palermo, Italy). R1 was started up without inoculum of activated sludge with the aim of developing an autochthonous activated sludge constituted by bacteria naturally present in the leachate. This phase lasted for about 110 d, during which dispersed bacteria in the leachate started to form small aggregates that gradually evolved into mature activated sludge flocs.

The SBRs had an operating volume of 5 L and were operated according to a 24 h cycle, which included 30 min influent static feeding, 21 h aeration, 2 h settling followed by 30 min effluent discharge. At the end of each cycle, 1 L of effluent was discharged and was replaced with new raw leachate at the start of the next cycle. In order to speed up the start-up phase and avoid nutrient limitation, a solution containing a known amount of sodium acetate and potassium-hydrogen diphosphate (K_2PO_4) was added in both the SBRs at the beginning of each cycle, with a ratio equal to 1:1 v/v with the leachate. The amount of sodium acetate added provided a COD equal to 25% of the COD in the raw leachate (3000±41 mg L^{-1}), whereas the PO_4-P was dosed in order to achieve a C:N:P ratio of approximately 100:8:2.

Experimental campaign

R1 and R2 were monitored for approximately 175 days after the cultivation phase in order to evaluate the long-term COD removal efficiency of the biomasses. The experiments were divided into two periods. In Period 1 (80 d) the SBRs operated under the same conditions as the cultivation phase, whereas in Period 2 (100 d), the supply of sodium acetate was halved.

Analytical methods

The physico-chemical analyses, such as COD, NH_4-N, total suspended solids (TSS), pH and electrical conductivity were performed according to Standard Methods [21]. All the measurements were performed in triplicates and the results were averaged.

COD fractionation tests

To accurately examine the biodegradability of leachate, COD fractionation tests were performed by respirometric techniques [20]. The apparatus conmprised a flowing-gas/static-liquid respirometer (1.5 L of volume), connected to a thermostatic cryostat to maintain the sample temperature at 20 ± 0.1°C. COD fractions, classified as soluble readily biodegradable (Ss), soluble inert (S_I), biodegradable and rapidly hydrolysable (Xs), particulate inert (X_I) and active biomass (Xa), were evaluated according to [22]. The evaluation of the soluble rapidly biodegradable and the total biodegradable COD (BCOD), the latter including the slowly biodegradable and rapidly hydrolysable COD, was carried out using both biomasses. A known amount of biomass was withdrawn from R1 and R2 and each sample was aerated for 24 h to achieve endogenous respiration conditions. The TSS concentration in the respirometer was 3.0±0.1 g TSS L^{-1}, achieved by diluting, as necessary, the biomass samples with the effluent leachate. The COD fractionation tests were performed at the end of the cultivation phase.

Evaluation of biomass kinetics

The heterotrophic biomass kinetics in R1 and R2 were evaluated by using the same respirometric apparatus as above. The maximum heterotrophic growth rate (Y_H), the endogenous decay rate (b_H), the heterotrophic active fraction (f_{XH}) and the specific oxygen utilization rate (SOUR) were determined according to [20] using sodium acetate as organic substrate. Four kinetic tests were run at the end of the cultivation phase.

In order to evaluate the potential inhibitory effect of leachate on the biomasses, a specific batch test was performed at the end of the cultivation phase. This was intended to assess the oxygen utilization rate in response to supply of a rapidly biodegradable organic substrate and leachate, and was performed for both biomasses. The same amount of sodium acetate was added to two different batch reactors (the same as used for the respirometric tests), one containing the autochthonous biomass and the other the allochthonous at equal TSS concentration, in order to evaluate respiration rate in the absence of inhibiting factors. Thereafter, the same volume of leachate (0.3 L) was added to each batch reactor. The ratio between the OUR achieved after leachate addition and that of the acetate was used as an indicator to assess the leachate's inhibitory effect.

Results and Discussion

Cultivation phase

The cultivation phase lasted for 110 d. In R1, small bioaggragates with a size of approximately 10 µm started to appear after 35 d (**Figure 1**a). The TSS concentration in the mixed liquor gradually increased up to 6.5 g L^{-1} at the end of the cultivation phase, confirming the development of autochthonous biomass from the leachate. The activated sludge flocs in R1 were characterized by a regular and round shape and an average size of 80 µm (Figure 1b). In contrast, in R2, a significant deflocculation of the activated sludge was observed at the start of the cultivation phase (Figure 1c), resulting in a consistent loss of TSS in the mixed liquor from 3 g L^{-1} to less than 1.8 g L^{-1}. Gradually, the TSS concentration increased to 6 g L^{-1} and, accordingly, the size of the flocs increased to 70 µm (Figure 1d), suggesting that the activated sludge was successfully acclimated to the leachate.

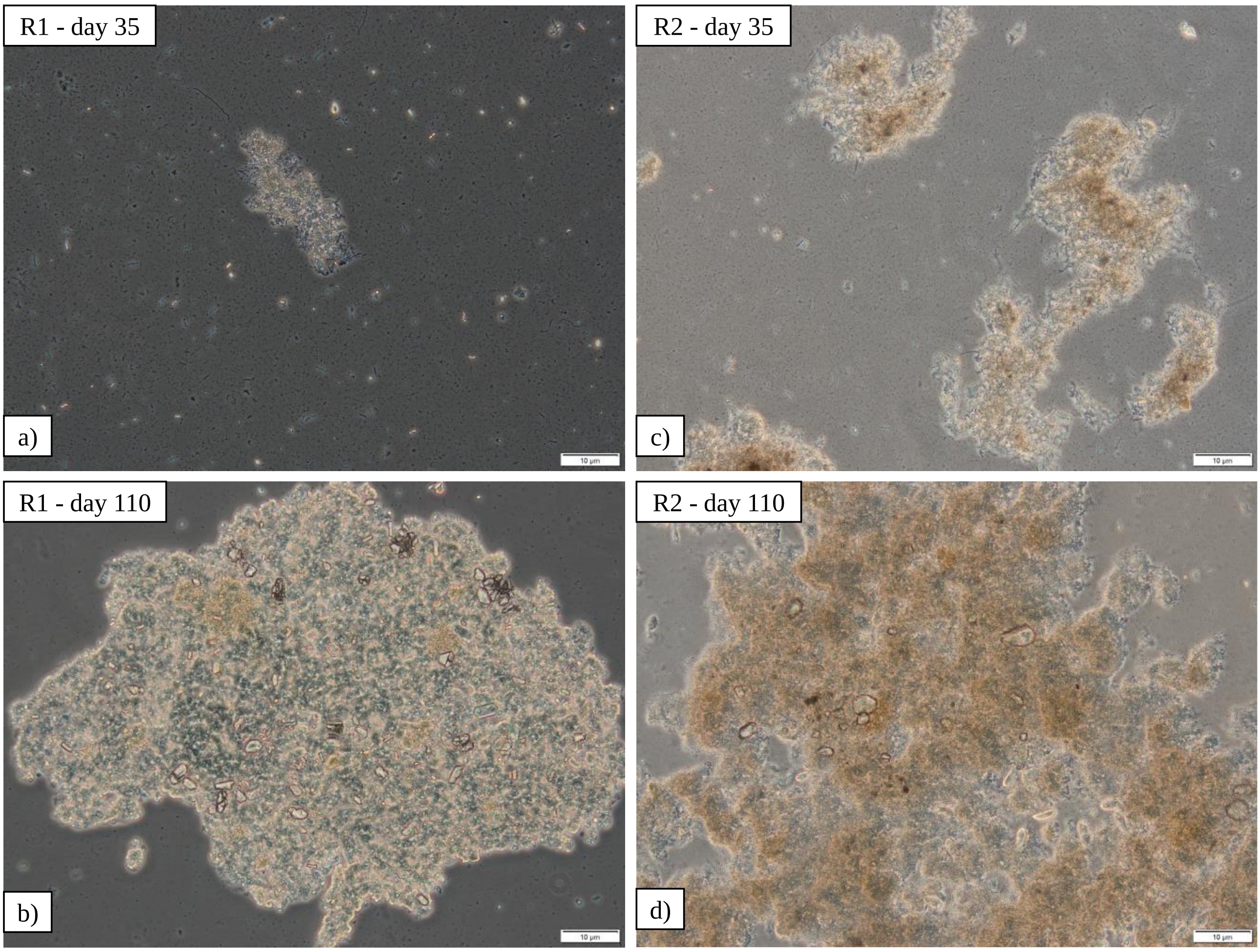

Fig. 1: Microscopic images (100x of magnification) of the activated sludge in R1 (a, b) and R2 (c, d) during the cultivation phase (bar size 10 μm)

Biodegradability of leachate

The biodegradability of the raw leachate was evaluated by means of COD fractionation tests performed with both the autochthonous and allochthonous biomasses at the end of the cultivation phase. The results are shown in **Figure 2.**

The total COD concentration of the raw leachate was 12,987±246 mg L^{-1}. The total biodegradable fraction, including the soluble readily biodegradable, the particulate slowly biodegradable and the rapidly hydrolysable, was 10,043±293 mg L^{-1} and 4,993±171 mg L^{-1} in the test carried out with the autochthonous and allochthonous biomasses respectively (Figure 2a). Similarly, the amount of soluble readily biodegradable COD was different in both tests, close to 2,000±97 mg L^{-1} and 730±56 mg L^{-1} for the autochthonous and allochthonous biomasses, respectively. Overall, the ratio of BCOD to total COD was 76±1.2 % with the autochthonous biomass, and approximately 39±2.4 % with the allochthonous biomass (Figure 2b). The fraction of readily biodegradable COD to the total biodegradable COD was likewise higher for the autochthonous biomass (19% *vs* 13%). For the rapidly biodegradable COD, it is speculated that both biomasses were able to degrade the simplest organic molecules (VFAs) comprising the soluble biodegradable fraction of the COD. The different results obtained in the two tests could likely be due to the ability of the autochthonous biomass to degrade an additional fraction of the soluble COD that the allochthonous biomass was unable to, thus representing an inert fraction.

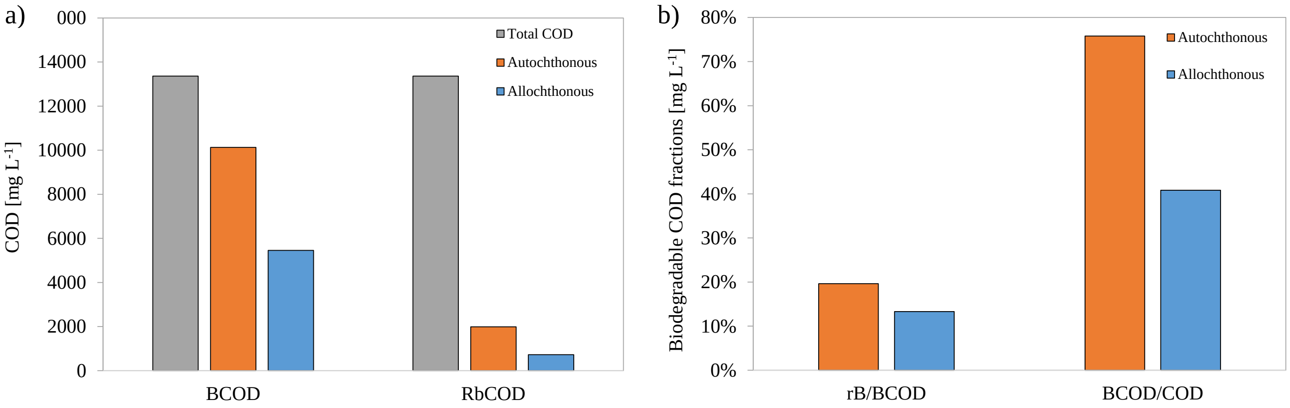

Fig. 2: Results of the COD fractionation tests carried out with autochthonous and allochthonous biomass (a); comparison between the fraction of readily and overall biodegradable COD (referred to the total COD) achievable with autochthonous and allochthonous biomasses (b).

The different values of the total biodegradable COD achieved with the two biomasses suggested that the autochthonous was able to degrade a higher amount of organic matter, which was recalcitrant for the allochthonous biomass. The ratio of BCOD to COD evaluated with the allochthonous biomass was approximately 40%, which was in agreement with those reported for a young leachate [23]. The ratio evaluated with the autochthonous biomass was approximately 76±1.9 %, which was much higher than the typical values observed in the literature for young leachates [8,24]. Thus, the results confirmed that the biodegradability of complex organic substrates is strictly related to the bacterial consortium operating the biodegradation. It may be speculated that the conventional BOD_5 test enables the establishment of only organic matter which is rapidly biodegradable and hydrolysable. Moreover, as reported in previous studies, autochthonous bacteria enabled a higher amount of organic substrate to be degraded and, in particular, thatwhich is slowly biodegradable [18,25]. Consequently, the application of bio-based process for the treatment of leachate could be more effective using autochthonous bacteria, since it allows removal of a larger amount of organic pollution by biological pathways.

Metabolic activity of the autochthonous and allochthonous biomasses

The main kinetic parameters of the two biomasses achieved at the end of the cultivation phase are reported in **Figure 3**. It can be noted that, the biomass kinetics were evaluated by using sodium acetate as organic substrate which allows assessment of the maximum values of the heterotrophic kinetic parameters [20,22].

The maximum heterotrophic growth yield, i.e. the biomass produced per unit of COD removed without limiting factors, was similar in both the SBRs. The Y_H was 0.58±0.03 mg VSS mg COD^{-1} in R1, and slightly lower in R2 (0.56±0.02 mg VSS mg COD^{-1}). Similarly, the endogenous decay rate was c. 0.15 d^{-1} in both systems. Conversely, the heterotrophic active fraction was significantly higher in the autochthonous biomass. At the end of the cultivation phase, the f_{XH} was 14±0.6 % of the volatile suspended solids in R1, whereas it was 6±0.3 % in R2. In agreement with the higher amount of active fraction, the highest SOUR was observed in the system with the autochthonous biomass (42±3.6 mg O_2 $gVSS^{-1}$ h^{-1}), and was slightly lower with the allochthonous biomass (37±1.2 mg O_2 $gVSS^{-1}$ h^{-1}).

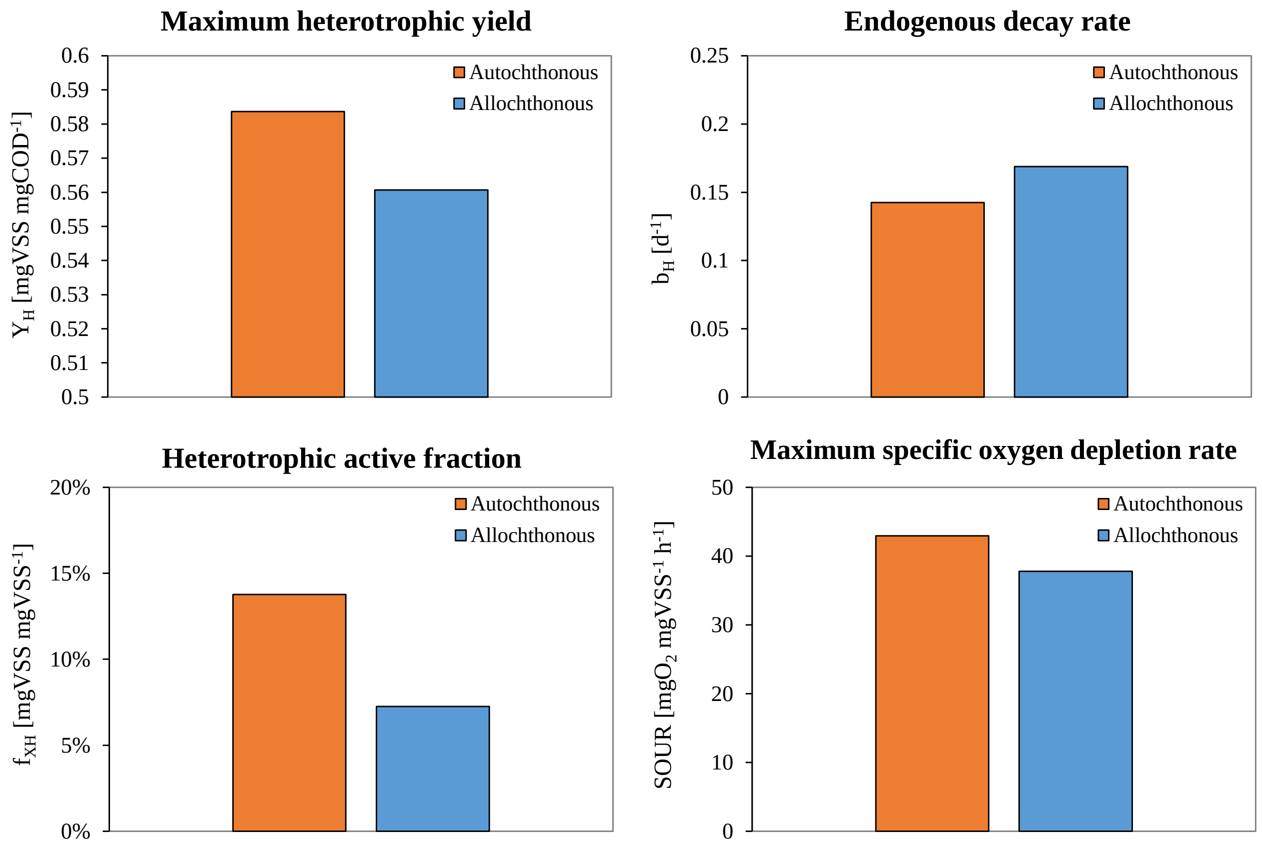

Fig. 3: Average values of the heterotrophic kinetic parameters, maximum yield coeffient - Y_H (a), endogenous decay rate - b_H (b), active fraction - f_{XH} (c) and specific oxygen uptake rate - SOUR (d) of the autochthonous and allochthonous biomasses during the experiment.

The above results indicated that the operating conditions during the cultivation phase did not hamper the growth of either biomass in the SBRs. Nevertheless, the higher amount of active fraction observed in R1 suggested that the conditions for biomass growth were more favorable than in R2. It is speculated that the higher substrate availability in R1, related to the greater capacity of the autochthonous biomass to degrade organic substrates that the allochthonous biomass was not able to use, resulted in a higher food-to-microorganisms ratio (0.33±0.05 kgBOD kgTSS^{-1} d^{-1} *vs* 0.17±0.03 kgBOD kgTSS^{-1} d^{-1}) that enhanced the growth of the autochthonous biomass. In contrast, the lower capacity of the allochthonous biomass to degrade the recalcitrant organic compounds in the leachate, determined the establishment of limiting or endogenous growth conditions that significantly reduced the amount of the heterotrophic active fraction in R2. However, it should be taken into consideration that other compounds in the leachate, such as heavy metals, inorganic compounds, etc., might have had inhibitory effects on the allochthonous biomass, causing a decrease in metabolic activity and reduction in the active fraction. In order to focus on this aspect, inhibitory tests were performed at the end of the cultivation phase. The results are shown in **Figure 4**.

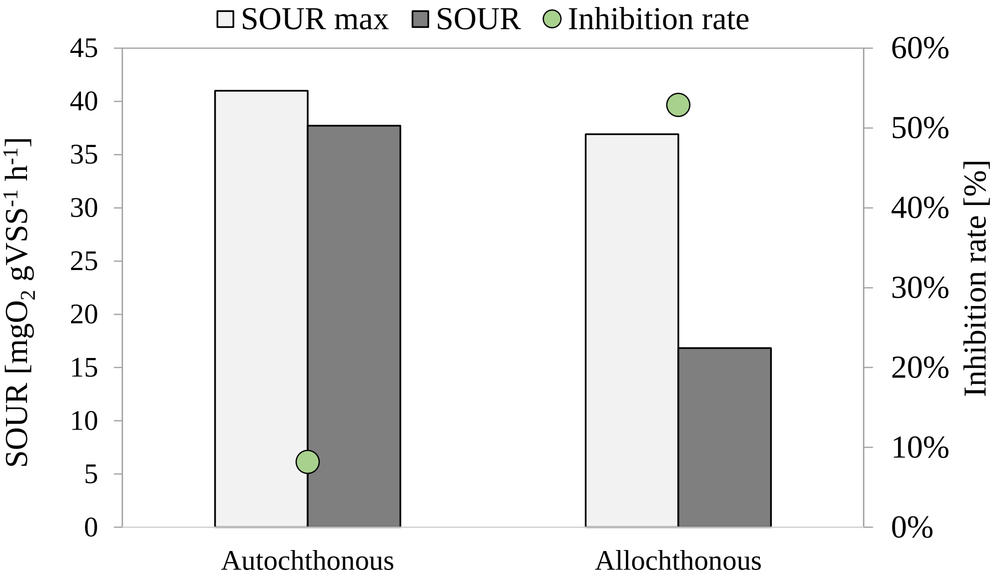

Fig. 4: Values of the SOUR and the inhibitory rates obtained during the inhibitory test performed on the autochthonous and allochthonous biomasses

SOUR values after the addition of the sodium acetate were 39±2.1 mg O_2 gVSS^{-1} h^{-1} and 36 mg O_2 gVSS^{-1} h^{-1} in the reactor with autochthonous and allochthonous biomass, respectively, indicating that both exhibited similar responses in terms of metabolic activity towards the readily biodegradable COD. In contrast, when the leachate was added, the respiration rate decreased by 9.5% in the autochthonous biomass system (SOUR 37±0.8 mg O_2 gVSS^{-1} h^{-1}), whereas the decrease was significantly higher for the allochthonous biomass (54%) (SOUR 16±0.4 mg O_2 gVSS^{-1} h^{-1}). The results demonstrated that leachate addition caused a significant decrease in biological activity of the allochthonous biomass, probably due to a partial inhibition of some bacterial strains. They confirmed that the autochthonous biomass was characterized by a higher biological activity in the presence of leachate because it did not suffer inhibitory effects of toxic compounds [26]. In contrast, the significant decrease of the respiration rate of the allochthonous biomass in presence of leachate, accounted for the lower amount of active fraction observed in R2. Thus, based on these observations, the autochthonous biomass is potentially more suitable than the allochthonous for the treatment of landfill leachate.

COD removal performance

R1 and R2 were monitored for 180 d to evaluate the long-term performance of the two biomasses. COD removal performances are shown in **Figure 5.**

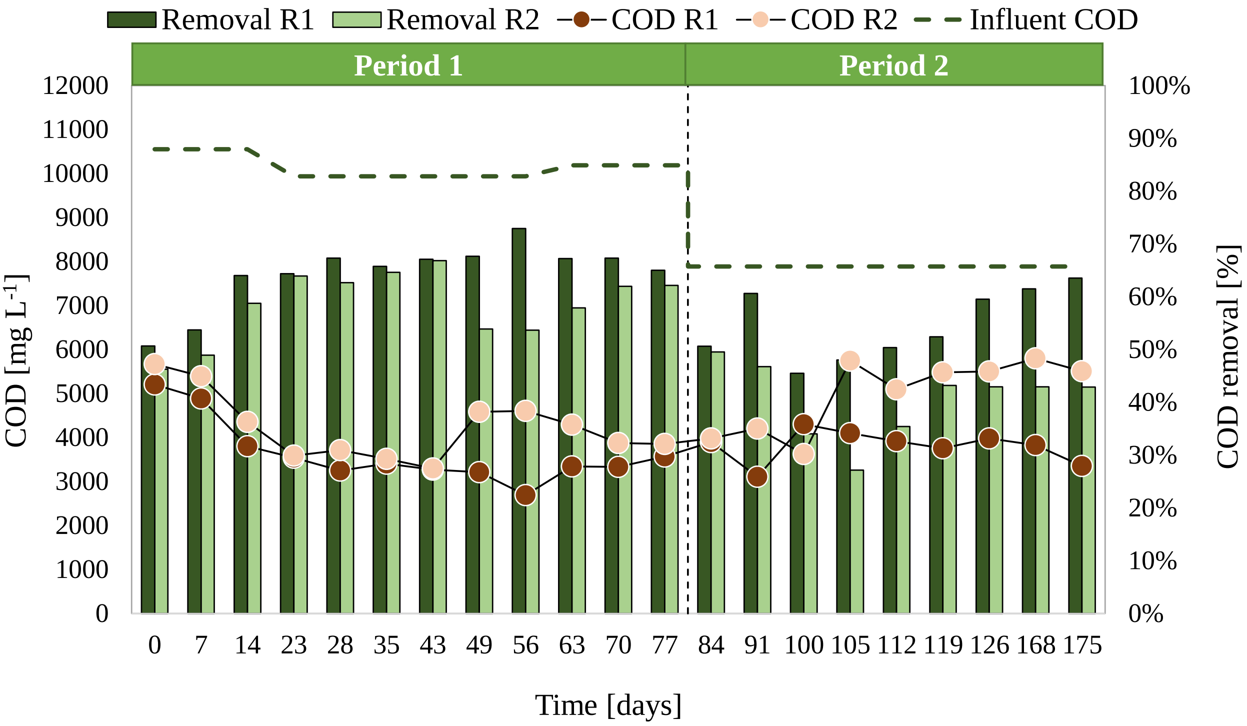

Fig. 5: COD influent and effluent concentrations in R1 and R2 and removal efficiencies during the experiment

In Period 1, when the readily biodegradable substrate was supplied in both the reactors amounting to 25% of the total COD of the leachate, the removal efficiencies were comparable in both the reactors. The effluent COD concentration was 3,480±49 mg L^{-1} in both R1 and R2 at steady state and the removal efficiency was 69±3%. Although the higher capacity of the autochthonous than the allochthonous bacteria in degrading organic compounds in the leachate, the comparable COD removal efficiencies suggested that the operating conditions favored a good organic pollution removal in both the reactors. In particular, the co-substrate may have played an important role in pollution removal as reported elsewhere [23,27]. Despite the lower biodegradation kinetics of the allochthonous biomass, the high hydraulic retention time in the SBRs (5 d) could have favored similar removal performances in both reactors [28].

In Period 2, the influent COD of the leachate decreased due to rainfall events and, simultaneously, the amount of co-substrate was halved, amounting to 12.5% of the total COD of the raw leachate. The COD removal efficiency slightly decreased in R1 during the first two weeks in Period 2, but rapidly increased reaching a steady value of 67±3 %, comparable with that observed in the previous period. In contrast, in R2 the COD removal efficiency decreased throughout Period 2, reaching a steady value of 40±2 % at the end of the experiments. Based on these results, the co-substrate addition favored COD removal with the allochthonous biomass, whereas its contribution was negligible for the autochthonous. As previously discussed, the inhibitory tests demonstrated that the metabolic activity of the allochthonous biomass significantly decreased when only raw leachate and no co-substrate was supplied. In Period 2 the lower co-substrate dosage caused a further decrease in the heterotrophic active fraction in R2 from 6% (Period 1) to 2.8% at the end of the period, whereas in R1 it was almost constant at a value close to that observed in Period 1 (14% *vs* 12.5%). This confirmed that the growth conditions for the autochthonous biomass were independent of the addition of co-substrate, due to its ability to metabolize organic compounds in the leachate that the allochthonous biomass is not able to degrade. In contrast, the supply of co-substrate enabled better growth conditions for the allochthonous biomass, ensuring a higher amount of active fraction and higher COD removal performance.

General considerations and potential applications

The metabolic activity and performances of the allochthonous biomass were affected by the supply of the organic co-substrate. Because of its low ability to degrade the organic compounds of the leachate, with the exception of the readily biodegradable organic fraction of the COD (VFAs) and a small portion of that slowly biodegradable, the scarcity of co-substrate limited its metabolic activity and growth kinetics, resulting in a very low heterotrophic active fraction (< 3%).

As far as we are aware, no studies in the literature report values of the BOD/COD ratio higher than 0.3 - 0.4 for young leachate [3,14]. In contrast, the amount of biodegradable COD achieved in this study with the autochthonous biomass was >70% whereas those with the allochthonous biomass were close to 40%, thus comparable with the results reported for young leachate [24]. These findings demonstrated that the same leachate has different biodegradability characteristics dependent on the biomass used to perform the BOD test. The autochthonous biomass enabled higher carbon removal performances than the allochthonous. Moreover, because of the higher amount of active fraction in the biomass, the kinetic parameters of the autochthonous biomass exceeded the allochthonous, indicating that the same removal efficiencies could be achieved in reactors characterized by lower hydraulic retention time.

The ability of the autochthonous biomass to degrade a higher amount of organic compounds offers different and new scenarios for the biological treatment of leachate. Medium and old leachate might also be biologically treated and, in particular, the leachate could be treated directly *insitu* in *adhoc* structures where the same biomass present in the leachate was previously cultivated. However, it should be stressed that biological treatments alone in many cases are unable to meet the quality standards required by current environmental laws. Nevertheless, the application of final cleaning processes would be targeted on the removal of a lower amount of pollutants (inorganic compounds), which would imply lower chemical and/or energy requirements.

Conclusions

This report has focused on the characterization and treatment of leachate from municipal solid waste landfills. In particular, the biodegradability of a young leachate was evaluated by means of autochthonous and allochthonous biomasses. It was found that the amount of biodegradable COD in the leachate was strictly dependent on the biomass used to perform the COD fractionation tests. The amount of biodegradable COD was 74% with the autochthonous biomass and 39% in the case of the allochthonous biomass, highlighting the capacity of the autochthonous biomass to degrade a higher amount of organic compounds in the leachate. Moreover, the autochthonous biomass was characterized by a higher biological activity and heterotrophic active fraction (14%), while not suffering from the inhibitory effects of toxic compounds present in the leachate. In contrast, the allochthonous biomass was significantly affected by inhibitory compounds, resulting in a very low active fraction (<3%) and low respiration rate (SOUR = 16 mg O_2 gVSS^{-1} h^{-1}). The significance of the present study is that it might be possible to achieve a more effective biological treatment of landfill leachate, even medium or old aged leachate, by enhancing the growth of the autochthonous biomass, cultivated in *adhoc* plants. Indeed, the autochthonous biomass does not need any co-substrate for its growth, since it is able to degrade the majority of the COD of the raw leachate. A reduced post-treatment (physico-chemical), with a low chemical demand, would likely enable meeting the limits for the final release into the environment.

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References

[1] Wang L, Zeng G, Yang Z, Luo L, Xu H, Huang J. Operation of partial nitrification to nitrite of landfill leachate and its performance with respect to different oxygen conditions. Biochem Eng J 2014;87:62–8. doi:10.1016/j.bej.2014.03.013.

[2] Castillo E, Vergara M, Moreno Y. Landfill leachate treatment using a rotating biological contactor and an upward-flow anaerobic sludge bed reactor. Waste Manag 2007; 27(5): 720-726. doi:10.1080/02783198809553172.

[3] Ferraz FM, Povinelli J, Pozzi E, Vieira EM, Trofino JC. Co-treatment of landfill leachate and domestic wastewater using a submerged aerobic biofilter. J Environ Manage 2014;141:9–15. doi:10.1016/j.jenvman.2014.03.022.

[4] Renou S, Givaudan JG, Poulain S, Dirassouyan F, Moulin P. Landfill leachate treatment: Review and opportunity. J Hazard Mater 2008;150:468–93. doi:10.1016/j.jhazmat.2007.09.077.

[5] Brennan RB, Clifford E, Devroedt C, Morrison L, Healy MG. Treatment of landfill leachate in municipal wastewater treatment plants and impacts on effluent ammonium concentrations. J Environ Manage 2017;188:64–72. doi:10.1016/j.jenvman.2016.11.055.

[6] Bernardo-Bricker AR, Singh SK, Trovó AG, Tang WZ, Tachiev G. Biodegradability Enhancement of Mature Landfill Leachate Using Fenton Process under Different COD Loading Factors. Environ Process 2014. doi:10.1007/s40710-014-0016-8.

[7] Naveen BP, Mahapatra DM, Sitharam TG, Sivapullaiah PV, Ramachandra TV. Physico-chemical and biological characterization of urban municipal landfill leachate. Environ Pollut 2017;220:1–12. doi:10.1016/j.envpol.2016.09.002.

[8] Azzouz L, Boudjema N, Aouichat F, Kherat M, Mameri N. Membrane bioreactor

performance in treating Algiers ' landfill leachate from using indigenous bacteria and inoculating with activated sludge. Waste Manag 2018. doi:10.1016/j.wasman.2018.02.003.

[9] Spina F, Tigini V, Romagnolo A, Varese G. Bioremediation of Landfill Leachate with Fungi: Autochthonous vs. Allochthonous Strains. Life 2018;8:27. doi:10.3390/life8030027.

[10] Jemli M, Karray F, Feki F, Loukil S, Mhiri N, Aloui F, et al. Biological treatment of fish processing wastewater: A case study from Sfax City (Southeastern Tunisia). J Environ Sci (China) 2015;30:102–12. doi:10.1016/j.jes.2014.11.002.

[11] de Albuquerque EM, Pozzi E, Sakamoto IK, Jurandyr P. Treatability of landfill leachate combined with sanitary sewage in an activated sludge system. J Water Process Eng 2018;23:119–28. doi:10.1016/j.jwpe.2018.03.011.

[12] Zhang Y, Liu J, Li R, Zeng X, Xue Y, Liu J, et al. Determining the Biodegradability of Leachate Through XAD-8 Adsorption. Procedia Environ Sci 2012;16:3–8. doi:10.1016/j.proenv.2012.10.002.

[13] Ahmed FN, Lan CQ. Treatment of landfill leachate using membrane bioreactors: A review. Desalination 2012. doi:10.1016/j.desal.2011.12.012.

[14] Lee AH, Nikraz H. BOD:COD Ratio as an Indicator for Pollutants Leaching from Landfill. J Clean Energy Technol 2014. doi:10.7763/JOCET.2014.V2.137.

[15] Renou S, Givaudan G, Poulain S, Dirassouyan F, Moulin P. Landfill leachate treatment: Review and opportunity. J Hazard Mater 2008;150:468–93. doi:10.1016/j.jhazmat.2007.09.077.

[16] Capodici M, Di Trapani D, Viviani G. Co-treatment of landfill leachate in laboratory-scale sequencing batch reactors: analysis of system performance and biomass activity by means of respirometric techniques. Water Sci Technol 2014;69:1267. doi:10.2166/wst.2014.005.

[17] Morris S, Garcia-Cabellos G, Enright D, Ryan D, Enright A-M. Bioremediation of Landfill

Leachate Using Isolated Bacterial Strains. Int J Environ Bioremediation Biodegrad 2018;6:26–35. doi:10.12691/ijebb-6-1-4.

[18] Spina F, Tigini V, Romagnolo A, Varese G. Bioremediation of Landfill Leachate with Fungi: Autochthonous vs. Allochthonous Strains. Life 2018;8:27. doi:10.3390/life8030027.

[19] Alijani Ardeshir R, Rastgar S, Peyravi M, Jahanshahi M, Shokuhi Rad A. A new route of bioaugmentation by allochthonous and autochthonous through biofilm bacteria for soluble chemical oxygen demand removal of old leachate. Environ Technol (United Kingdom) 2017;38:2447–55. doi:10.1080/09593330.2016.1264488.

[20] Capodici M, Fabio Corsino S, Di Pippo F, Di Trapani D, Torregrossa M. An innovative respirometric method to assess the autotrophic active fraction: Application to an alternate oxic–anoxic MBR pilot plant. Chem Eng J 2016;300:367–75. doi:10.1016/j.cej.2016.04.134.

[21] Apha. Standard Methods for the Examination of Water & Wastewater. 2005. Ed. Amer Public Health Assn. ISBN-10: 9780875530130

[22] Di Trapani D, Capodici M, Cosenza A, Di Bella G, Mannina G, Torregrossa M, et al. Evaluation of biomass activity and wastewater characterization in a UCT-MBR pilot plant by means of respirometric techniques. Desalination 2011;269:190–7. doi:10.1016/j.desal.2010.10.061.

[23] Bardi A, Yuan Q, Siracusa G, Chicca I, Islam M, Spennati F, et al. Bioresource Technology Effect of cellulose as co-substrate on old landfill leachate treatment using white-rot fungi. Bioresour Technol 2017;241:1067–76. doi:10.1016/j.biortech.2017.06.046.

[24] Ren Y, Ferraz F, Lashkarizadeh M, Yuan Q. Comparing young landfill leachate treatment efficiency and process stability using aerobic granular sludge and suspended growth activated sludge. J Water Process Eng 2017;17:161–7. doi:10.1016/j.jwpe.2017.04.006.

[25] Alijani Ardeshir R, Rastgar S, Peyravi M, Jahanshahi M, Shokuhi Rad A. A new route of

bioaugmentation by allochthonous and autochthonous through biofilm bacteria for soluble chemical oxygen demand removal of old leachate. Environ Technol 2017;38:2447–55. doi:10.1080/09593330.2016.1264488.

[26] Tigini V, Cristina G. Biosorption with autochthonous and allochthonous fungal biomasses for bioremediation and detoxification of landfill leachate. Environ Earth Sci 2018;77:1–10. doi:10.1007/s12665-018-7519-y.

[27] Bardi A, Id QY, Tigini V, Id FS, Varese GC, Id FS, et al. Recalcitrant Compounds Removal in Raw Leachate and Synthetic Effluents Using the White-Rot Fungus n.d.:1–14. doi:10.3390/w9110824.

[28] Yan F, Jiang J, Zhang H, Liu N, Zou Q. Biological denitri fi cation from mature land fi ll leachate using a food- waste-derived carbon source. J Environ Manage 2018;214:184–91. doi:10.1016/j.jenvman.2018.03.003.

Figure and Table Legends

Figure 1: Microscopic images (100x magnification) of the activated sludge in R1 (a, b) and R2 (c, d) during the cultivation phase (bar size 10 µm)

Figure 2: Results of the COD fractionation tests carried out with autochthonous and allochthonous biomass (a); comparison between the fraction of readily and overall biodegradable COD (referred to the total COD) achievable with autochthonous and allochthonous biomasses (b).

Figure 3: Average values of the heterotrophic kinetic parameters, maximum yield coefficient - Y_H (a), endogenous decay rate - b_H (b), active fraction - f_{XH} (c) and specific oxygen uptake rate - SOUR (d) of the autochthonous and allochthonous biomasses during the experiment.

Figure 4: Values of the SOUR and the inhibitory rates obtained during the inhibitory test performed on the autochthonous and allochthonous biomasses.

Figure 5: COD influent and effluent concentrations in R1 and R2 and removal efficiencies during the experiment

Table 1: Characteristics of the raw leachate in this study