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**A novel integrated treatment for mature landfill leachate via
active filtration and anaerobic digestion**

(Academic Discipline: ICAR/03 – Sanitary and Environmental Engineering)

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INTRODUCTION

According to the Lavoisier's law of conservation of mass, later amended by Einstein, total mass and energy remain constant in an isolated system despite chemical reactions or physical transformations. In other words, mass and energy cannot be created or destroyed but only subjected to transformation.

Whilst this basic principle has always been accepted and widely applied by scientists, engineers and ecologists in laboratory experiments, technological innovations and analyses of natural cycles, respectively, on the contrary, it has been almost completely neglected by the stakeholders of the economy model rising from the First Industrial Revolution. Linear Economy industry, based on the well-known "take-make-use-dispose" sequence, has exploited natural resources (ground and fresh water, minerals, metals, chemicals, fossil fuels, biomass and others) over the years with the aim of producing energy and/or goods and services to sell for making a profit and, concurrently, generating wealth. The other side of the coin was represented by outputs management. In fact, processes' by-products and emissions have been released into the environment without any treatment and end-of-life products have been disposed of as well. By this way, in accordance with the Einstein-Lavoisier's law, mass and energy extracted from the environment and employed in Linear Economy processes have been conserved and transformed into different substances, part of which turned out to be considerably harmful for the environment (e.g., greenhouse gases, pesticides, chemicals, landfill leachates, heavy metals and so on). With the Second and later Third Industrial Revolution, industrial productivity sharply increased and threats towards the environment were no longer limited in space and time but potentially global and irreversible. Neglecting the conservation of mass and energy's principle has led to environment's deterioration at a global scale in terms of biodiversity loss, water stress, waste accumulation, soil depletion, air pollution.

In this context, the adoption of a Circular Economy model is widely accepted as vital to promote a sustainable development. From a certain point in recent human history, environment has started to be considered no longer as both a resource reservoir and a depot for waste but rather as a closed system which supports human and non-human life and, therefore, needing protection. Moreover, it has appeared clear that by the application

of the conservation of mass and energy's law it is possible to link human development and environment's protection. Indeed, in the Circular Economy model, theoretically there is no waste production (in terms of both mass and energy) since the aforementioned outputs (i.e., processes' by-products and emissions and end-of-life items), instead of being disposed of, are subjected to further transformation into new substances which can be either recurrently employed in production processes as input resources (e.g., secondary raw materials, recovered energy) or safely discharged into the environment. For this reason, currently, the Circular Economy approach is heavily encouraged by national and international regulations.

Actually, since the recovery of various materials or components is yet technically and/or economically unfeasible, even in the Circular Economy model waste disposal is still necessary. Oftentimes, waste is disposed of in highly engineered landfills equipped with sophisticated pollution control and monitoring systems. Leachate, derived from the infiltration of water in waste, and gas (mostly methane), produced by the anaerobic degradation of biodegradable waste, are the two major emissions from landfills. Collected biogas is either used for energy production or flared on site but it partially escapes increasing global warming. Leachate has to be collected and treated prior to its discharge into the environment. However, since leachate is a solution made up of all the contaminants present in the discarded waste, its treatment is challenging. Particularly, treatment of leachate coming from mature landfill (i.e. > 10 years old) is even more complex due to the concentration of recalcitrant and toxic compounds.

Inspired by the law of conservation of mass and energy and in a perspective of Circular Economy, the purpose of this research is to realise an integrated mature landfill leachate treatment system wherein, eventually, virtually no waste is produced and treatment by-products can be used for energy generation and soil fertilisation. So far, the integrated treatment has been tested at a laboratory scale in order to study its effectiveness and optimise the parameters for a possible subsequent scaling-up. It consists of a physical-chemical pre-treatment of the mature landfill leachate with the aim of significantly reducing heavy metals and other contaminants refractory to biological treatment. Afterwards, the pre-treated aqueous matrix has been used as a nutrient solution useful for the metabolism of bacteria involved in the anaerobic digestion of a biodegradable organic substrate. Anaerobic digestion technology has become widely regarded as a

fundamental tool of the Circular Economy because of its final products: biogas and digestate. The former, made up of mostly methane and carbon dioxide, is normally employed in energy generation. This would allow to sustain the whole process from an energetic point of view. Instead, digestate, as final process residue, concentrates chemical compounds which typically makes it suitable for subsequent composting or for direct agricultural use.

The dissertation is divided into three main Chapters.

In the first one, the evolution of the economic model and its impacts on the environment are described first with the purpose of outlining the current necessity of pursuing the sustainability of human development, especially in terms of waste management. Secondly, landfilling technology is depicted as a still essential operation for waste disposal. Out of its two major emissions, leachate composition is exhaustively depicted. Finally, the crucial role played by the anaerobic digestion technology in the Circular Economy context is explained through the description and the technical application of the biological process.

The second Chapter reviews the state-of-the-art of mature landfill leachate treatments, with particular reference to biological and, especially, combined ones. Moreover, in this Chapter, issues related to the anaerobic digestion of food and market waste are examined in view of a co-treatment with landfill leachate.

Finally, Chapter 3 encompasses the theoretical experimental design and the results of its application at a laboratory scale. The Chapter starts with a brief description of the purposes of the experimental plan followed by the report of the materials and methods used and the analyses of the results obtained along with their discussion.

In conclusion of the dissertation, a summary of the entire experiment is provided.

CHAPTER 1

1.1. From Linear to Circular

Human pressure on the environment is expected to continue and increase as world population grows (10 billion people in 2057, Worldometer). Major environmental global concerns are pollution, global warming, waste disposal, biodiversity loss, pressure on water reserves and natural resource depletion. These issues are mostly a consequence of human activities and rapid industrialisation and urbanisation. The current economic context is the result of a thorough review of the life cycle of products (intended as both consumer goods and services) placed on the market. The need for a change in the global economy model has been driven over the years by a growing search for the sustainability of the production paradigm.

1.1.1. The Linear Economy model

Throughout the First and Second Industrial Revolutions (end of 18th century and beginning of 20th century), industrial productivity sharply increased in terms of quantity, variety and speed thanks to technical innovations. Particularly, mechanisation, development of new energy systems and materials and division of labour made manufacturing sector far more efficient. In this context, the growing demand of new products was easily satisfied and huge profits were generated (demand-supply-income cycle, Andrews, 2015). This economic model is considered as “Linear” since it is based on inputs (capital, machines and workers, raw materials, energy) which drive production processes and outputs (consumer goods and services) which make a profit. It is also known as the “take-make-use-dispose” model since it consists of a chain of subsequent operations: i) the extraction of raw materials from the environment (“take”), ii) their conversion into products by providing processes with energy (“make”), iii) the use of final products by consumers (“use”) and iv) the disposal of products when they no longer serve the user’s purpose (“dispose”) (Figure 1.1).

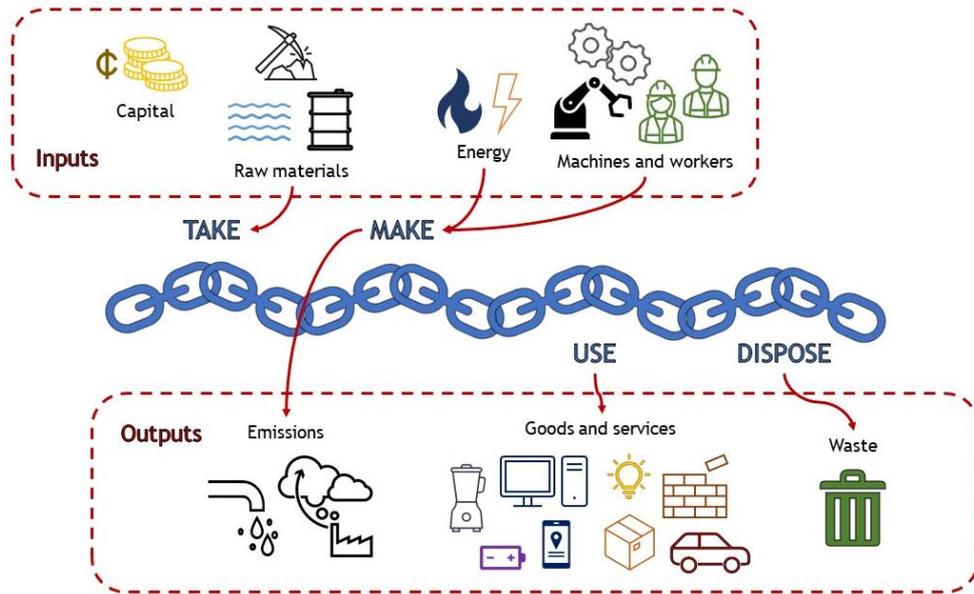


Figure 1.1. Linear Economy model's chain

In particular, the last ring (“dispose”) is necessary to complete and end the chain so that encouraging the sale of new products and sustaining the entire system. For this reason, when the demand-supply-income cycle was jeopardised by Wall Street and later global financial crash (1929), products were designed with even shorter usable lives (planned obsolescence) with the aim of stimulating replacement buying by consumers. By this way, products at end of their lives became waste with little or null material and energy recovery as, in 1960s, recovering of metals, paper, glass and textiles was often less economically attractive for companies than buying new (Andrews, 2015).

Soon after, negative consequences on environment of the Linear Economy were revealed. The first ring of the chain (“take”) brought to an intense global resources consumption over the years. In 1972, the report “The Limits to Growth”, published by the Club of Rome, highlighted the impossibility of sustaining exponential economic growth without causing natural resources depletion. In fact, the linear model depended on natural resources which are either non-renewable or consumed at a rate far faster than that necessary for their replenishment. Later, in the report “Our Common Future” (1987), drew up by the World Commission on Environment and Development, the concept of Sustainable Development was defined as the development which «meets the needs of the present without compromising the ability of future generations to meet their own needs». As a consequence, the Linear Economy model was clearly labelled as unsustainable since it

would not have ensured the availability of resources in the long run. However, natural resources depletion caused by their overexploitation was not the only critical point. In general, raw materials extracted from the environment are converted into complex products which are no longer suitable for being released in the environment again since discharging without any type of pretreatment unavoidably lead to environmental pollution. In the Linear Economy, being making a profit the main goal, either landfilling or incineration of end-of-life products were the most common and cheapest practices adopted. As a consequence, environment became severely damaged by the uncontrolled harmful compounds released by waste which threatened fresh and ground water, air and soil quality. Furthermore, especially land disposal meant that residual resources and energy embodied in waste were lost.

Currently, it is expected that global consumption of materials (such as biomass, fossil fuels, metals and minerals) doubles in the next forty years and annual waste generation increases by 70% by 2050 (OECD, 2019; Kaza et al., 2018). Moreover, resources extraction and processing are responsible for half of total greenhouse gas emissions and more than 90% of biodiversity loss and water stress (EC, 2019).

1.1.2. The Circular Economy model

For all these reasons, it appeared clear that global economy paradigm needs for a drastic change. In 1966, Boulding described the Earth as a closed and circular system in which «there are no inputs from outside and no outputs to the outside» (Boulding, 1966). Boulding claimed that this definition does not fit human experience since man is an open system with inputs (air, food, water) and outputs (exhalation, body waste). Therefore, Linear Economy has been developed on the basis of the human nature wherein environment was typically perceived as limitless. However, according to Boulding, economy needs to mirror natural cycles in order to maintain the sustainability of the life on Earth. Later, Stahel and Reday (1976) and Pearce and Turner (1990) conceptualised a Circular Economy in which waste would have become input resource for new production processes (defined as “cradle to cradle” system in contrast to the “cradle to grave” Linear Economy) (Figure 1.2). The reprocessing of goods and materials would have been driven by maximising products value at each point of their life rather than minimising their final

disposal's costs. By this way, resource consumption and waste production are reduced, energy is saved and even jobs are generated (Stahel, 2016). Finally, Ellen MacArthur Foundation provided the most renowned definition of Circular Economy, namely «an industrial economy that is restorative or regenerative by intention and design» (EMF, 2013).

1.1.2.1. Biorefinery

Biorefinery is one of the key strategies of the Circular Economy model to sustainably manage biodegradable waste and residues. Biorefinery concept exploits the potential of biomass to replace a large fraction of fossil resources as feedstocks for industrial productions. Indeed, it consists of separating biomass resources (wood, grasses, food and vegetables waste, organic fraction of municipal solid waste) into their building blocks (carbohydrates, proteins, triglycerides) which can be converted into biobased materials (value added products, chemicals) and bioenergy (biofuels). Biorefinery products are cost-competitive and marketable (Cherubini, 2010; IEA, 2014). Reducing the reliance on fossil fuels use and, as a consequence, the greenhouse gases footprint is one of the benefits of biorefinery. Furthermore, the valorisation of waste materials deriving from numerous sectors such as agro-industrial, fishery, domestic and so on, is consistent with the Circular Economy sustainability's principle.

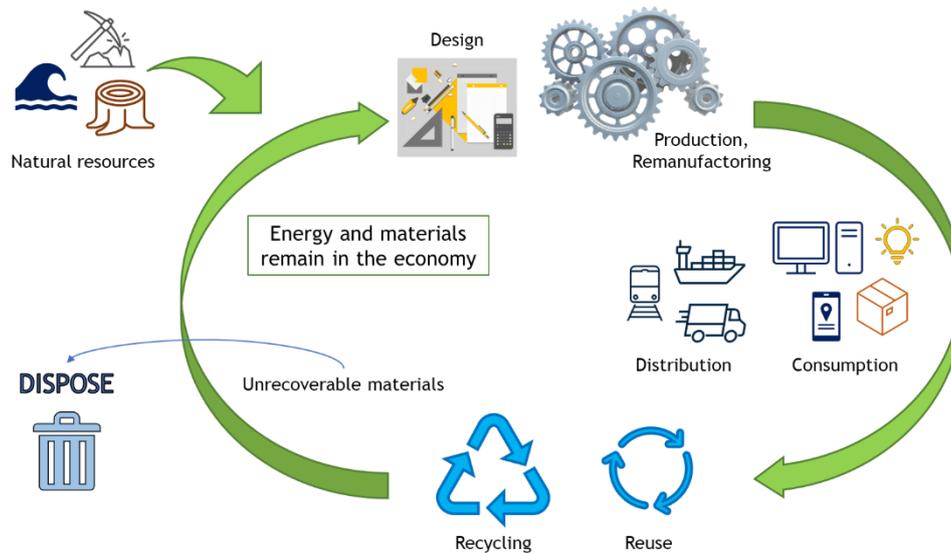


Figure 1.2. Circular Economy model

1.1.2.2. *The European Circular Economy Action Plan and the Directive 2008/98/EC on waste*

The Circular Economy concept has also gained importance with policymakers at a national and international level. For instance, the European Commission has adopted the newest Circular Economy Action Plan in March 2020, consisting of 35 legislative and non-legislative actions for 7 different areas to implement by 2022, with the aim of scaling up the Circular Economy by making it profitable for economic actors, consumers, citizens and civil society organisations. The ultimate purpose of the Plan is reducing human resources consumption footprint and doubling the circular material use rate in order to create and sustain an economic system which gives back to the planet more than it takes.

One of the Plan's strategies focuses on the entire life cycle of products, especially electronics, ICT, textiles, furniture and high impact intermediary products (steel, cement, chemicals), from their design to their recovery. Particularly, it identifies products' design as a crucial operation since up to 80% of products' environmental impacts are determined at this phase (EC, 2014). Designing products sustainably means improving their durability, reusability, repairability and recyclability and, as a consequence, facilitating the further recovery procedures.

Besides, referring to the waste issue, the Plan strategy is based on the Directive 2008/98/EC which lays down a waste management hierarchy (Figure 1.3) in order to turn waste into new resources and, thus, keep energy and materials in the economy for as longer as possible. Member States have to apply the waste hierarchy as a priority order in waste prevention and management legislation and policy: i) prevention, ii) preparing for re-use, iii) recycling, iv) other recovery (e.g., energy recovery) and v) disposal.



Figure 1.3. Waste management hierarchy

from: https://ec.europa.eu/environment/topics/waste-and-recycling/waste-framework-directive_en

As “prevention” the Directive means measures which have to be taken before that a product has become waste such as the extension of its lifespan or the reduction of harmful substances used in its production. Through “re-use”, products or components can be used again for the same purpose for which they were conceived after checking, cleaning or repairing operations. “Recycling” consists of waste reprocessing into secondary raw materials which can be used as a replacement of virgin raw materials in manufacturing processes. This stage constitutes another key element of the aforementioned Circular Economy Action Plan. Indeed, it aims at creating a well-functioning continental market in which secondary raw materials can compete with primary ones in safety, performance, availability and cost so that preventing both waste production and resources and energy consumption. “Recovery” is applied to materials when their recycling is inconvenient or impossible. It involves waste using as a fuel to generate energy with a specific process efficiency, composting or other biological transformation processes (i.e. anaerobic digestion) and others operations listed in the Annex II of the Directive. Lastly, according to the Directive, “disposal” means any operation which is not recovery even where the operation has as a secondary consequence the reclamation of substances or energy, such as landfill and incineration on land (Annex I).

To sum up, it can be stated that waste management hierarchy is a fundamental tool of the Circular Economy model. It describes a new pattern which is in stark contrast with the last chain of the Linear Economy (“dispose”) wherein, as discussed above, waste disposal was considered as the best choice due to its cheapness which allowed to economically sustain the entire system at the expense of the environment. However, even if in the Circular Economy context waste disposal becomes the least preferable option, it is to some extent still necessary due to the impossibility of a complete recovery of some materials with the current technical ability. For this reason, studying landfill technology, controlling its impacts on the environment and improving the management of its hazardous products (landfill leachate and gas) both in the short and long term, is still fundamental. In the next Paragraph, firstly, a brief description of landfill technology is provided, secondly, a more detailed overview on leachate characteristics is done.

1.2. Landfill Technology

Landfilling is still widely accepted and used due to its simplicity and economic advantages in many areas of the world (Robinson, 2005). According to the Eurostat, in 2018 the quantity of waste disposed of in landfill in the EU-28 was 885 million tonnes corresponding to about 37% of the total amount of managed waste in the same year (2383 million tonnes). Around the world, almost 40% of waste is disposed of in landfills and 33% of waste is openly dumped on roads, open land, or waterways. The former practice is prevalent in upper-middle-income countries whereas the latter is almost the only disposal method in low-income countries where 93% of waste is dumped or burned (Kaza et al., 2018).

Among European Member States, landfilling is regulated by Council Directive 1999/31/EC on the landfill of waste. According to the Directive, "landfill" means a waste disposal site for the deposit of the waste onto or into land (i.e. underground). By and large, "landfilling" can be defined as a long lasting accumulation of waste in facilities ("landfills") which follow specific technical and procedural criteria established by the law with the aim of minimising risks for the environment and the human health. As already mentioned in Paragraph 1.1.1.2., through landfilling any type of waste recovery is carried out.

Landfills are usually divided into different classes depending on the type of waste they can accept. Particularly, the Directive establishes three: i) landfills for hazardous waste (i.e. waste listed in the Annex I having hazardous constituents and properties according to Annexes II and III, respectively, of the Council Directive 91/689/EEC), ii) landfills for non-hazardous waste (mostly municipal solid waste), iii) landfills for inert waste (i.e. waste which does not undergo any significant physical, chemical or biological transformations). Only waste which has been subject to treatment can be landfilled except for inert one for which treatment is not technically feasible. Instead, liquid, explosive/high flammable, hospital waste are examples of waste not acceptable in landfills. Even biodegradable municipal waste will gradually be no longer accepted for landfill disposal.

Leachate and gas are the two principal emissions from landfills which threaten air, soil, surface and ground water. Leachate means «any liquid percolating through the deposited waste and emitted from or contained within a landfill» (Landfill Directive Article 2). Its origin and characterisation is exhaustively described in the next Paragraph. Landfill gas

is mostly made up of methane and carbon dioxide and is generated by the anaerobic degradation of waste. The theoretical and idealised sequence for the anaerobic degradation of a homogeneous volume of waste involves five distinct phases (Figure 1.4) (Christensen and Kjeldsen, 1989; Leary, 2004):

- in phase I, the easily biodegradable components in waste begin to undergo bacterial decomposition soon after they are placed in a landfill. This primary biological decomposition occurs under aerobic conditions at the expense of the air entrapped in the waste.
- in phase II anaerobic conditions start to develop. The activity of fermentative and acetogenic bacteria results in a rapid generation of volatile fatty acids, carbon dioxide and some hydrogen.
- Phase III is known as the acid phase since the bacterial activity accelerates with the production of significant amount of organic acids. Firstly, high molecular weight compounds (e.g., lipids, organic polymers and proteins) are converted by enzymes into simple organic compounds (e.g., amino acids, sugars) in the hydrolysis step. Then, the products of the hydrolysis step are transformed into organic acids by acid-forming bacteria (acidogenesis step). Slowly, the growth of methanogenic bacteria starts.
- Phase IV is the methane fermentation phase in which methane is generated as the product of the degradation of acetic acid and hydrogen carried out by methanogenic bacteria. In this phase, both methane and acid fermentation proceed simultaneously, although the rate of acid fermentation is considerably reduced.
- in phase V, the readily available biodegradable matter has been consumed and only refractory organic compounds remain in landfilled waste. The rate of landfill gas generation diminishes significantly along with its partial pressure consequently and, thus, nitrogen starts appearing in the landfill gas again due to diffusion from the atmosphere.

The duration of the individual phases varies depending on the distribution of the organic components in landfill, the availability of nutrients, the moisture content of waste and the degree of initial compaction.

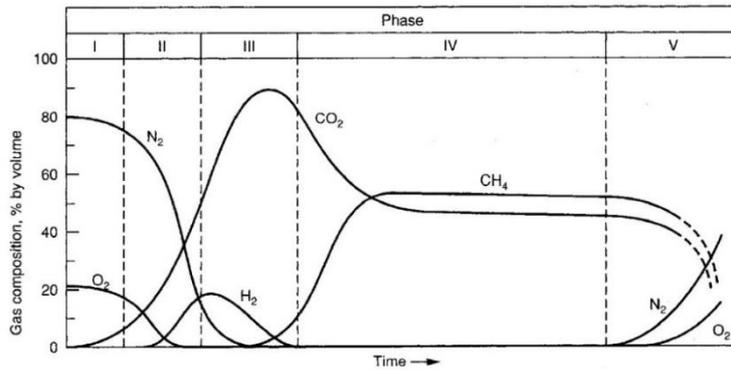


Figure 1.4. Generalized phases in the generation of landfill gases (Leary, 2004)

Historically, landfills have developed from open dumps to modern highly engineered facilities. Conventionally, their starting point is fixed in the 1950s when waste reflected the early industrialisation of society (Christensen et al., 2010b). As Cossu (1989) stated, the history of landfill technology evolution is largely a reactive history since concepts of design has been developed as a reaction to problems encountered at previous landfills.

Open and/or uncontrolled dumping refers to waste disposal into the environment without observing the basic legal standards (Butti et al., 2018). Open dumps are often clay and gravel pits or other low-value land filled with any type of waste (municipal or industrial, non-hazardous or hazardous) which has not been subjected to any pre-treatment (Christensen et al., 2010b). At a local level, open dumps impacts involves odour emissions, rodents presence and local fires. However, as urbanization develops and dumps become bigger, the lack of both leachate/gas collection and liners/cover laying allows surface/ground water contamination, soil depletion, release of greenhouse gases, loss of vegetation, serious fires, slope instability and so on (Sharma et al., 2018; Yadav et al., 2018). Currently, in many parts of the developing world, waste are still disposed of in this uncontrolled way with the aforementioned issues associated (O. Aderemi and C. Falade, 2012; Townsend et al., 2015a).

The first step in the evolution of modern landfill from uncontrolled dumps was the control of site access through fences which clearly defined landfill's boundaries. Moreover, landfill's volume was divided into distinct units (cells) and new disposal procedures were established; waste was usually placed into cells and, then, compacted. Occasionally, waste has been covered with soil in order to minimise fires, odours and disease vectors. The

implementation of these new measures brought to an improvement of the basic hygienic conditions. For this reason, facilities of this type were defined as sanitary landfills. Nevertheless, the two major pollutant emissions associated with landfilling (i.e., leachate and gas) were not still in focus and, in some large sanitary landfills, damages on soil and water and uncontrollable fires occurred because of leachate and gas accumulation (Christensen et al., 2010b; Leary, 2004; Townsend et al., 2015a).

Further improvements in landfill design have led to construction criteria and operative procedures currently adopted. According to the European Landfill Directive, the distance from landfill's boundaries to residential areas, water bodies and agricultural or urban sites has to be ensured as well as the geological and hydrogeological conditions in the area have to be taken into account. In the so-called controlled landfill, the protection of surface and ground water and soil have gained priority. As a consequence, landfills are expected to be equipped with a combination of a geological barrier and a bottom liner in both landfill's bottom and sides. The former consisting of a mineral layer which satisfies permeability and thickness requirements depending on the class of waste disposed of. In fact, in landfills for hazardous waste, permeability and thickness of the geological barrier must be at least 10^{-9} m/s and 5 m, respectively. In landfills for non-hazardous waste, the same permeability must be ensured but with a lower thickness (1 m). Finally, landfills for inert waste must have a geological barrier with permeability lower than 10^{-7} m/s and thickness of 1 m. If the geological barrier does not naturally meet the above conditions, it can be completed artificially and reinforced by other means (e.g., geosynthetic materials). Instead, bottom lining systems directly in contact with waste body can be composed of either single or double liners with each liner consisting of either a single element (clay, geomembrane, or geosynthetic clay liners) or of composite liners (typically a geomembrane directly over a clay layer) (Christensen et al., 2010a). In addition to that, a leachate collection and drainage system must be added. As the landfill bottom liner prevents the vertical movement of the leachate out of the landfill, the horizontal drainage of leachate can be ensured by the combination of natural granular materials, drainage geocomposites and drain perforated pipes. Subsequently, leachate is removed from the landfill by pumping and has to be properly treated prior to its release into the environment. In some cases, leachate recirculation in landfill body is carried out as a treatment option in order to keep waste sufficiently wet for accomplishing its degradation

(Knox et al., 2018). With regard to the gas issue, typically, wells are constructed within landfilled waste in order to collect produced methane. It is directed to a separate torch where it is safely flared or otherwise managed. Unfortunately, the efficiency of the collection systems is not absolute and, therefore, some of the produced biogas escapes in the atmosphere. Liners and collection systems failures and expensiveness of leachate treatments are some of possible shortcomings of the controlled landfill technology (Christensen et al., 2010b; Townsend et al., 2015a).

The so-called dry tomb is another type of landfill facility. It has been adopted as a reaction to the aforementioned shortcomings of the traditional technology. In a dry landfill the infiltration of water is virtually completely avoided by installing a top cover system. By this way, leachate generation is considerably reduced. However, the lack of moisture significantly delays both aerobic and anaerobic waste stabilisation with a resulting waste conservation which poses a serious risk to the environment in case of either top cover or bottom liner failure. For this reason, dry tomb is recommended only when a pre-treatment allows a preliminary waste stabilisation (Christensen et al., 2010b; Cossu, 2018). A completely different approach is adopted in the bioreactor landfills. This type of facility is based on leachate recirculation (or water addition) with the aim of controlling waste moisture content and, thus, creating the environment favourable to rapid microbial decomposition of the biodegradable solid waste. Leachate recirculation also provides distribution of nutrients and enzymes, pH buffering, dilution of inhibitory compounds and recycling and distribution of methanogens. By this way, according to bioreactor landfills' supporters, landfills become waste treatment systems rather than simply storage facilities (Reinhart and Townsend, 2018).

The European Landfill Directive prescribes that landfill's aftercare should continue for at least 30 years after its closure. During this period, monitoring and maintenance activities should be covered by a specific financial provision. The Directive designates the top cover as the main technical feature of landfill closure which aims at controlling water from precipitation entering into the waste body. Top cover generally consists of a mineral liner and a high density polyethylene membrane to be installed when the mechanical stabilisation of landfilled waste is completed. Being leachate the most lasting and harmful agent to surface and ground water, its monitoring starts with the site's water balance (including precipitation and evapotranspiration) according to the meteorological data

(volume of precipitation, evaporation, temperature and humidity). Moreover, leachate must be sampled and measured in terms of volumes and composition and surface and ground water must be monitored as well to evaluate whether leachate is building up in the landfill body or the site is leaking. In the next Paragraph, a detailed characterisation of landfill leachate generation and composition is provided.

1.3. Landfill Leachate

As mentioned above, among the potential environmental impacts arising from landfilling of waste, leachate represents a critical issue and its management is a major challenge (Gao et al., 2014). Leachate is the result of the infiltration of water from external sources, such as rainwater, uncontrolled runoff and irrigation water, through landfilled waste. It also includes water released from waste after compaction or biodegradation. When water percolates through solid wastes, both biological and chemical constituents are leached into solution (Leary, 2004). As a consequence, leachate is a complex matrix which is highly variable in terms of quantity and quality.

The former aspect is strongly related to local climate or season and it can be predicted by the study of landfill hydrology (Kjeldsen and Beaven, 2010). For instance, as Stegmann (2018) stated, leachate production rates are often between 15% and 25% of the annual precipitation under middle European climatic conditions. For this reason, landfill water balance is an essential tool to calculate the potential volume of leachate. Figure 1.5 illustrates the principal components involved in the water balance. Precipitation (P), surface water inflow (SI) and liquid contained in the waste or other managed waste inputs (MI) are the inputs of water to the landfill. Evapotranspiration from the surface (ET), surface run-off (SR), leachate (L), water vapour contained in landfill gas (G) and Water consumed by chemical or microbial processes (U) represents the outputs. In particular, leachate (L) stands for both the volume which is removed by the extraction systems and any leachate quantity which may leave the landfill through the bottom liner. Direct precipitation on the landfill surface (P) is probably the most important factor governing leachate generation in any climate except for dry or very dry one (e.g., desert). However, the fraction of this contribution which eventually enters the waste body is lower because of surface run-off (SR), namely water leaching out of landfill surface over the top cover, and evapotranspiration (ET) of the water from plants and soil. Surface water inflow (SI)

is the result of the site's topography which could direct surface water to the landfill (Kjeldsen and Beaven, 2010).

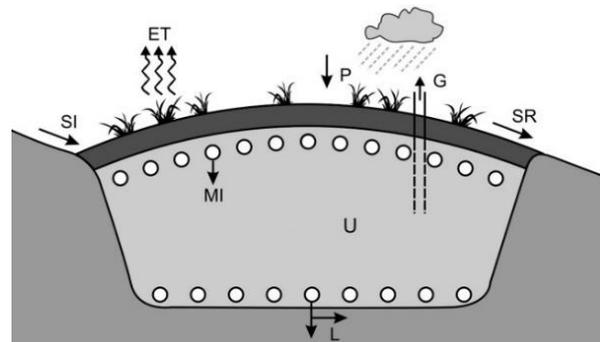


Figure 1.5. Landfill water balance (adapted from Kjeldsen and Beaven, 2010)

Instead, leachate quality is largely affected by landfill age and specific composition of waste disposed of. In general, leachate composition includes the following elements (Fan et al., 2006; Kjeldsen et al., 2002; Qasim and Chiang, 2017; Slack et al., 2005; Wiszniowski et al., 2006):

- dissolved organic matter (biodegradable and refractory);
- light metals (e.g., Al, K, Na, Mg);
- heavy metals and metalloids (e.g., As, Ca, Cd, Cu, Fe, Pb, Zn);
- anions (e.g., Cl^- , NO_2^- , NO_3^- , SO_4^{2-} , PO_4^{3-} , S_2^-);
- ammonia;
- chlorine compounds and dioxins.

In case of Municipal Solid Waste (MSW), small quantities of hazardous substances (e.g., batteries, medicines, detergents) do not significantly affect leachate quality (Ehrig and Stegmann, 2018) while, in absence of a proper control of items containing hazardous substances during waste collection, the amount of inorganic pollutants in leachate can increase (Calabrò and Satira, 2020). Moreover, most MSW contains high amount of biodegradable matter which results in a significant loading of organic substances and ammonia-nitrogen in the leachate. Industrial waste such as ash and slag, construction and demolition waste and others can significantly affect leachate quality (Barbosa Segundo et al., 2020; Sabbas et al., 2003). Therefore, in order to prevent environment pollution, leachate is collected and removed from the landfill through a drainage system (coarse sand, gravel or geocomposite, perforated pipes and mechanical pumps, Christensen et al., 2010; Townsend et al., 2015). After that, leachate is stored in lagoons or tanks on site

(Christensen et al., 2010) and, then, an on-site or off-site treatment method must be planned depending on its characteristics.

On account of landfill age, leachate can be defined as young (< 5 years), medium age (5 – 10 years) and mature (> 10 years) (Renou et al., 2008). This distinction is based on the five phases of the well-known idealized sequence of anaerobic waste degradation processes occurring over landfill lifespan (see Paragraph 1.2.). Leachate characteristics changing over the five stages can usually be represented by basic parameters such as pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and their ratio, nitrogen compounds and heavy metals contents (Figure 1.6) (Foo and Hameed, 2009; Gandhimathi et al., 2013; Renou et al., 2008).

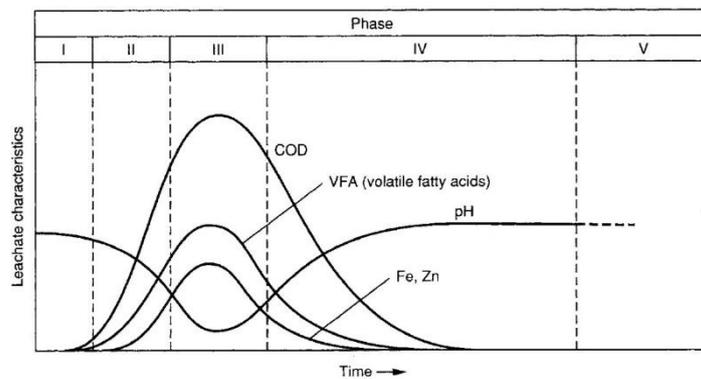


Figure 1.6. Leachate characteristics changing over waste anaerobic degradation (Leary, 2004)

In general, young leachates are collected from MSW landfills during the acid phase and have low pH (< 6.5) due to the presence of Volatile Fatty Acids (VFAs) originated by the initial degradation of organic matter in waste. They also have high BOD content owing to the presence of readily biodegradable compounds during the first years of landfill operation (Ehrig & Stegmann, 2018). As the methanogenic microorganisms develop in the waste, VFAs are converted into biogas so the organic fraction in the leachate becomes dominated by refractory compounds such as humic and fulvic acids (Renou et al., 2008). Indeed, BOD/COD ratio, which initially is higher than 0.5, in mature leachates is often in the range of 0.05 – 0.2 indicating the prevalence of slowly biodegradable or inert substances (Leary, 2004). In contrast to BOD, nitrogen does not decrease during phase changes (Ehrig & Stegmann, 2018). Ammonia-nitrogen, in fact, is the product of proteins

decomposition and it accumulates in leachate since it appears that no transformation mechanisms occur under methanogenic conditions (Burton and Watson-Craik, 1998). Therefore, ammonia concentration is proved not to decrease fast with time but it remains in the range of 500 – 2000 mg/L (Kjeldsen et al., 2002; Youcai, 2018). For this reason, it is considered the most significant harmful component of leachate in the long term (Christensen et al., 2010; Kjeldsen et al., 2002). Metals can be present in leachate as colloids, organic and inorganic complexes and free ions. The latter species is assumed as the most toxic for the environment but it constitutes typically less than 10% of the total metal concentration (Baun and Christensen, 2004). Heavy metals concentration in leachate is generally low because metals precipitate as sulphides or carbonates or co-precipitate with hydroxides (Ehrig & Stegmann, 2018). Depending on pH values, heavy metals concentrations are lower in mature leachates than in younger ones because most metals are less soluble at neutral pH values (Leary, 2004). Furthermore, metals can be absorbed on soils and organic matter present in landfill at neutral or alkaline pH values (Calabrò and Mancini, 2012; Christensen et al., 2010a; Kjeldsen et al., 2002).

As one of the landfill's aftercare requirements, leachate must be managed for at least 30 years after landfill closure as required by several international regulations (European Union, 1999; U.S. EPA, 1991). Taking into account that the aforementioned European Directive 2008/98/EC discourages landfilling use and that in many countries there are thousands of landfills which are already closed or will be in a short time (Laner et al., 2012), it would be reasonable to expect a growing necessity of treating mainly mature leachates in the near future.

In order to meet this demand, this research focused on an innovative way of treating growing volumes of mature landfill leachate according to the Circular Economy principles. The novel approach involved the use of a biological process, namely anaerobic digestion, even though biological treatments are usually considered unsuitable for mature landfill leachate because of its low biodegradability and high concentration of refractory compounds (Renou et al., 2008). Because of these characteristics, leachate needs to be pre-treated through a filtration with active granular materials. The significance of this study lies in the possibility of closing the cycle of mature landfill leachate treatment since methane produced in the anaerobic digestion can generate energy, which can partially compensate for the impacts of uncontrolled landfill gas emissions (Calabrò et al., 2015),

while digestate can replace fertilizers thanks to the presence of nitrogen and humic substances deriving from the pre-treated mature landfill leachate (De Melo et al., 2016; Riva et al., 2016).

The most common mature leachate treatments can be grouped in two categories: physical-chemical processes and a combination of physical-chemical and biological processes. The former are coagulation/flocculation, adsorption onto reactive granular filters and advanced oxidation processes (AOPs), while biological ones involves both aerobic and anaerobic processes (Abbas et al., 2009; Amokrane et al., 1997; Amor et al., 2015; Bove et al., 2015; Ehrig and Robinson, 2010; Torretta et al., 2017; Wu et al., 2021). In the second Chapter of this dissertation, a brief overview of these treatments is provided focusing in particular on the combination of adsorption treatment with further biological (anaerobic) process.

In the next Paragraph, anaerobic digestion technology is introduced. It plays a key role in the Circular Economy context since, roughly, its application allows to convert organic waste into by-products with virtually null waste production.

1.4. Anaerobic digestion

Anaerobic digestion is widely regarded as one of the most effective technologies in Circular Economy scenarios (Sawatdeenarunat et al., 2016). It is a biological process involving microorganisms which use a vast variety of organic waste and residues (food waste, municipal solid waste, lignocellulosic biomass, animal manure and so on) as feedstocks and generate biogas as final process product (Gunaseelan, 1997). In order to do that, a series of bio-chemical reactions (hydrolysis, acidogenesis and methanogenesis) occur simultaneously in an oxygen free environment (McCarty and Smith, 1986). Generated biogas is composed of methane (55 – 70%), carbon dioxide (30 – 45%) and other trace gases (H₂S, NH₃, H₂O) (Steinhauser and Deublein, 2011) and is extensively used as renewable energy source (Scarlat et al., 2018). Moreover, as solid-liquid by-product of the process, digestate is also produced (Schievano et al., 2009). Digestate contains macro and micro-nutrients which make it suitable for agricultural use as fertilizers replacement in some cases (Koszel and Lorencowicz, 2015; Makádi et al., 2012). For these reasons, anaerobic digestion can integrate biorefinery treatments of organic substrates by using their process residues in order to achieve a complete resource recovery.

1.4.1. Phases of the anaerobic digestion process

The conversion of the complex organic substrates into methane is carried out by distinct groups of microorganisms in syntrophic interrelation which differ both in substrates used and products of their metabolisms. The whole biodegradation process consists of the following four phases occurring concurrently and outlined in Figure 1.7.

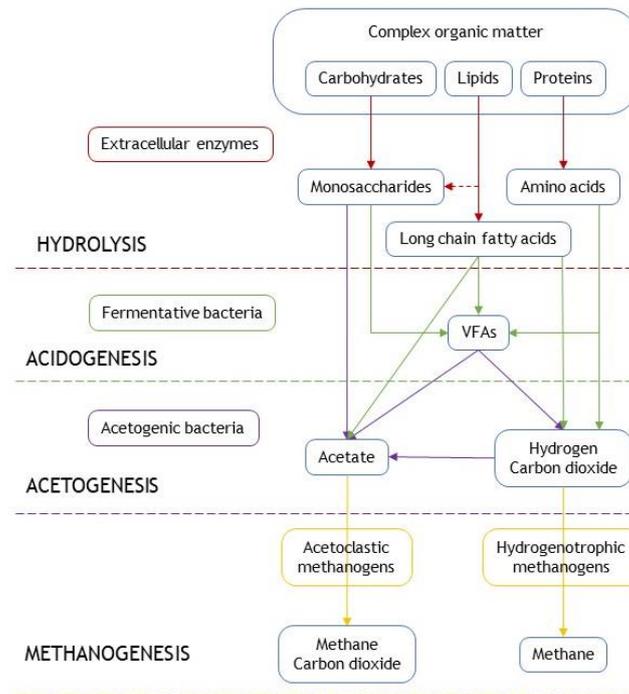


Figure 1.7. Anaerobic digestion process simplified scheme

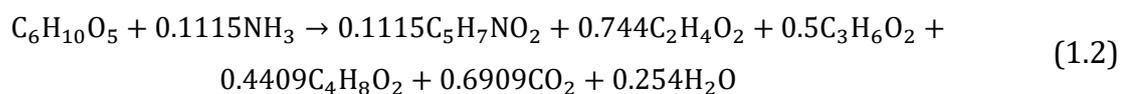
In the first phase of hydrolysis, insoluble organic materials and high molecular mass compounds are hydrolysed into simpler molecules by extracellular enzymes produced and secreted by a large number of hydrolytic microorganisms (*Clostridia*, *Micrococci*, *Bacteroides*, *Butyrivibrio*, *Fusobacterium*, *Selenomonas*, *Streptococcus*). Specifically, carbohydrates (such as cellulose, hemicellulose and starch), lipids and proteins are degraded to simple sugars, long chain fatty acids and amino acids respectively, by different hydrolysing enzymes such as cellulase, cellobiose, xylanase, amylase, protease, lipase (Merlin Christy et al., 2014). Generally, the hydrolysis of carbohydrates takes place within a few hours while the hydrolysis of proteins and lipids within a few days (Steinhauser and Deublein, 2011). Enzymes can be released by microorganisms into the liquid bulk and, then, they are absorbed onto a particle or react with a soluble substrate. Alternatively, microorganisms attached to a solid secrete enzymes in its vicinity and benefit from soluble products released by the enzymatic reaction (Vavilin et al., 2008). Bacteria colonise the particle surface, release enzymes and degrade it at a constant depth per unit of time (Merlin Christy et al., 2014). Eq. 1.1 shows an example of hydrolysis of cellulose ($C_6H_{12}O_6$)_n. Cellulose is an insoluble polymer made up of many soluble sugar units of glucose ($C_6H_{12}O_6$) joined together by chemical bonds. Its hydrolysis consists

of breaking the bonds between the units of glucose and is performed by the enzyme cellulase secreted by the hydrolytic bacterium *Cellulomonas*. As a result, soluble molecules of glucose are released (Gerardi, 2003).

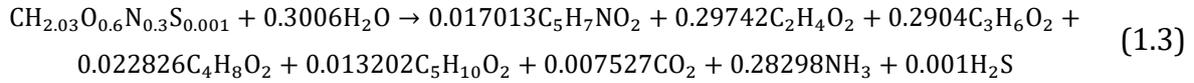


Oftentimes, hydrolysis constitutes the rate limiting step of the whole anaerobic digestion process, especially for lignocellulosic substrates because of the scarce accessibility of hydrolytic microorganisms due to the lignin presence. Lignocellulosic biomass has a complex chemical architecture characterised by three major constituents, namely cellulose (35 – 50%), hemicellulose (25 – 30%) and lignin (15 – 30%) (Paone et al., 2020). Whilst cellulose and hemicellulose have significant biomethane potentials, lignin is hardly anaerobically digested because of its complex chemical structure (Li et al., 2018). Indeed, lignin has a three-dimensional structure consisting of phenyl propane units which confers structural strength and rigidity to the plant tissue and fibres (Antunes et al., 2021). Moreover, being lignin chemically and physically connected to cellulose and hemicellulose in lignocellulosic substrates, it prevents the access of enzymes and microbes to the easily biodegradable cellulose (Khan and Ahring, 2019). For this reason, physical-chemical (and combined) pre-treatments are often necessary to break down lignin structure with the aim of making cellulose available to microorganisms. As a result, lignocellulosic substrates' biodegradability significantly improves (Sun et al., 2016).

In the second acidogenic phase, soluble compounds produced during the hydrolysis are degraded by fermentative bacteria to short chain organic acids (C₁ – C₅ molecules volatile fatty acids, VFAs, such as butyric acid, propionic acid, acetate, acetic acid), alcohols, nitrogen oxide, hydrogen sulphide, hydrogen, and carbon dioxide. In contrast to the hydrolysis phase, during acidogenesis some of the organic compounds are converted to new bacterial cells (anabolism mechanism) (Steinhauser and Deublein, 2011). In particular, the stoichiometry of simple carbohydrates conversion to acetate, propionate and butyrate can be described as Eq. 1.2:



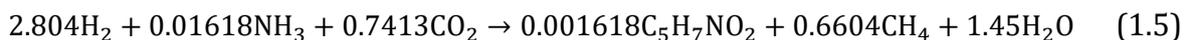
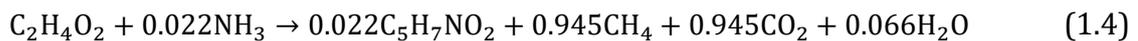
in which $C_6H_{10}O_5$, $C_5H_7NO_2$, $C_2H_4O_2$, $C_3H_6O_2$ and $C_4H_8O_2$ stand for simple carbohydrates, microbial cell formula, acetate, propionate and butyrate, respectively. The stoichiometry of amino acids ($CH_{2.03}O_{0.6}N_{0.3}S_{0.001}$) conversion to VFAs can be described as Eq. 1.3:



in which $C_5H_{10}O_2$ is valerate (Manchala et al., 2017).

Subsequently, VFAs produced during acidogenesis, as well as long chain fatty acids generated in the hydrolysis phase, are oxidized by slowly growing acetogenic bacteria into acetate, molecular hydrogen and carbon dioxide which are the precursors of methane formation (Manchala et al., 2017). Acetate formation is thermodynamically enabled only with low hydrogen partial pressures ($H_2 < 10^{-4}$ atm) (McCarty and Smith, 1986). Otherwise, acetogenic bacteria form predominantly butyric, capronic, propionic and valeric acids and ethanol in a relatively high hydrogen partial pressure environment. However, since methanogenic microorganisms can process only acetate, molecular hydrogen and carbon dioxide, a syntrophic correlation between acetogenic and methane-forming bacteria is established. Indeed, methanogens maintain a low hydrogen partial pressure in the environment by consuming hydrogen for methane formation. During this phase, also organic nitrogen and sulphur compounds are reduced to ammonia and hydrogen sulphide (Steinhauser and Deublein, 2011).

Methanogenesis represents the final phase. It is carried out by two groups of methanogenic bacteria: acetoclastic (or acetotrophic) and hydrogen-utilizing (or hydrogenotrophic) methanogens. The former splits acetate into methane and carbon dioxide whereas the latter uses hydrogen and carbon dioxide respectively as electron donor and acceptor to produce methane (McCarty and Smith, 1986). Stoichiometric equations of these two mechanisms are shown (Eqs. 1.4 and 1.5, Manchala et al., 2017):

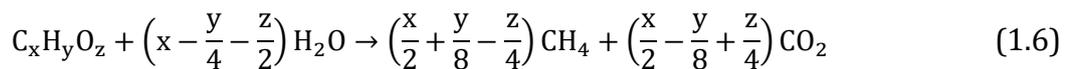


If methanogens' action is partially or totally inhibited, hydrogen partial pressure increases with the result of shifting acetogenic bacteria metabolism to acids production and acidifying the system. Acids, alcohols and organic nitrogen compounds which are not degraded by methanogens accumulate in the sludge. Moreover, ammonia formed at the

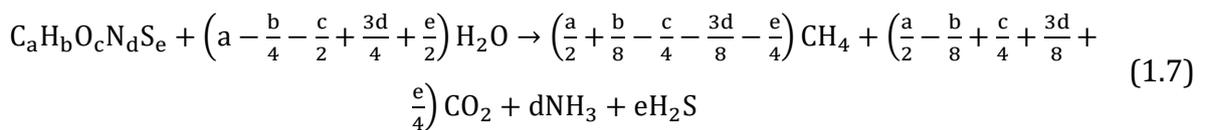
end of the proteins and amino acids degradation sequence reacts with carbon dioxide and water with the generation of ammonium carbonate which provides alkalinity to the sludge (Gerardi, 2003).

Biogas is the final gaseous product of the anaerobic digestion process. It consists of two main components: methane and carbon dioxide. Methane is insoluble and tends to pass from the liquid medium to gaseous phase. Typically, methane is the most abundant gas in the biogas mix (55 – 70%) and has the highest heating value ranging from 15 to 30 MJ/Nm³. Carbon dioxide reaches an equilibrium between liquid and gaseous phase. In the liquid medium, carbon dioxide participates in the formation of carbonic acid which contributes to increase the buffering capacity of the system. It represents the 30 – 45% of the total biogas volume and decreases the calorific value of the biogas. Other gases are present in biogas as impurities due to their usually negligible concentrations: nitrogen (0 – 15%), oxygen (0 – 3%), water (1 – 5%), hydrocarbons (0 – 200 mg m⁻³), hydrogen sulphide (0 – 10000 ppm_v), ammonia (0 – 100 ppm_v) and siloxanes (0 – 41 mg Si m⁻³). Biogas can be upgraded to bio-methane (95 – 99% of methane) prior to its utilisation or used directly. Upgrading consists of removing harmful and toxic compounds, such as H₂S, N₂, O₂, CO, NH₃, and reducing CO₂ content through several technologies (Awe et al., 2017).

Given the chemical composition of a substrate, the quantity of biogas produced during the anaerobic digestion can be predicted by the stoichiometric formula developed by Buswell and Mueller (1952) (Eq. 1.6):



Later, Boyle (1977) modified Eq. 1.6 introducing nitrogen and sulphur in substrate's chemical composition so that obtaining the fraction of ammonia and hydrogen sulphide in the produced biogas (Eq. 1.7):



(Achinas and Euverink, 2016).

1.4.2. Microbial growth and substrate utilisation kinetics

From a kinetic point of view, a microbiological system is characterized through two different processes: i) the net growth rate of the biomass and ii) the rate of substrate utilisation.

Typically, bacterial growth consists of five phases (Manchala et al., 2017): i) the lag phase with no increase in microbial growth rate but bacteria acclimatation occurs; ii) the acceleration growth phase wherein microbial growth rate monotonically increases followed by iii) the constant growth rate phase; iv) the decelerating growth phase (i.e., monotonic decrease of the growth rate) and, finally, v) the declining phase when the death rate of the old cells is higher than the growth rate of the new cells.

Microbial metabolism is the result of catabolism and anabolism mechanisms. In catabolism, microorganisms consume substrate and release energy in the form of adenosine triphosphate (ATP). The energy obtained from catabolic reactions is used for both synthesis of new cells and sustaining the old ones (anabolism). The ratio between the ATP mass produced [g/L] and the substrate mass consumed [g/L] is defined as ATP yield factor (Y_{ATP}). Likewise, the growth yield coefficient ($Y_{B/S}$) is the ratio between the amount of biomass generated (ΔB , [g/L]) and substrate consumed (ΔS , [g/L]), whereas, the product yield coefficient ($Y_{P/S}$) is the amount of product formed (ΔP , [g/L]) per substrate consumed (Eqs. 1.8 and 1.9, Gavala et al., 2003; Manchala et al., 2017):

$$Y_{B/S} = \frac{\Delta B}{\Delta S} \quad (1.8)$$

$$Y_{P/S} = \frac{\Delta P}{\Delta S} \quad (1.9)$$

Yield coefficients can be determined either experimentally or theoretically from the stoichiometry of the biochemical reactions. Usually, growth yield coefficient ranges from 0.05 to 0.2 g of biomass generated per g of substrate consumed (Gavala et al., 2003). Yield coefficients can be used to determine the correlation between the rates of microbial growth, product generation and substrate utilization (Eq. 1.10, Manchala et al., 2017):

$$-\frac{dS}{dt} = \frac{1}{Y_{P/S}} \frac{dP}{dt} \quad (1.10)$$

The bacterial growth rate (r_g , [g/L·d]) is defined according to the following Eq. 1.11:

$$r_g = \mu \cdot B \quad (1.11)$$

in which μ is the specific microbial growth rate [d^{-1}] and B is the microbial cell concentration [g/L]. μ is an essential model parameter defined by Monod equation (Eq. 1.12):

$$\mu = \mu_{\max} \frac{S}{K_s + S} \quad (1.12)$$

where μ_{\max} is the maximum specific growth rate [d^{-1}] achievable when $S \gg K_s$ and K_s stands for the half-saturation coefficient [g/L], namely the substrate concentration at which $\mu = 0.5 \mu_{\max}$. By combining Eqs. 1.11 and 1.12, the bacterial growth rate can be expressed as follows (Eq. 1.13):

$$r_g = \mu_{\max} \frac{S \cdot B}{K_s + S} \quad (1.13)$$

in which the growth rate of a microbial species (r_g , [g/L·d]) is related to the concentration of the substrate (S , [g/L]) and microbial cell mass (B , [g/L]). Moreover, considering Eqs. 1.8 and 1.9, it is also possible to express the substrate utilisation rate (r_s , [g/L·d]) as (Eq. 1.14):

$$r_s = \frac{1}{Y_{B/S}} \mu_{\max} \frac{S \cdot B}{K_s + S} = \frac{r_g}{Y_{B/S}} \quad (1.14)$$

Monod equation (Eq. 1.12) provides a basis for the development of many other microbial growth kinetics related equations. However, it is only applicable in absence of any type of inhibition of the system and it does not take into account other factors affecting the growth rate including permeation capacity of the substrate, microorganism adoption, enzyme activity, nature of the culture and growth phases of microorganisms. For these reasons, it has been modified by several authors adding corrective factors (Gavala et al., 2003; Manchala et al., 2017).

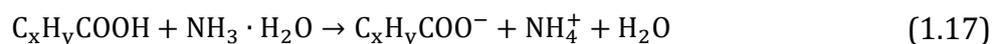
1.4.3. Factors affecting anaerobic digestion process

In this Paragraph, the main factors influencing the performance of the overall anaerobic digestion process are described. Methanogenic bacteria are widely considered as the most

sensitive microorganisms of the whole bacterial consortium involved in the process since they are characterised by a low growth rate.

1.4.3.1. pH

The optimum range of pH for methanogenic bacteria activity is 6.5 – 8.2 (Speece, 1996). Only *Methanosarcina* is able to tolerate lower pH values (even below 6.5) whereas metabolism of other bacteria is considerably suppressed at pH < 6.7 (Steinhauser and Deublein, 2011). Differently, for acidogenic and acetogenic bacteria, acid pH conditions are favourable (optimum pH between 5.5 and 6.5, Mao et al., 2015) even if fermentative microorganisms can develop in a wider range of pH between 4.0 and 8.5 (Appels et al., 2008). Two-stage anaerobic digestion processes separating the hydrolysis/acidification and acetogenesis/methanogenesis phases are sometimes carried out (Parawira et al., 2008; Srisowmeya et al., 2020). pH value in an anaerobic digestion process depends on carbon dioxide presence in the liquid medium and ammonia and VFAs concentration. According to the CO₂ – bicarbonate equilibrium (CO₂ + H₂O ↔ H₂CO₃ ↔ H⁺ + HCO₃⁻), for pH values higher than 6.5, carbon dioxide dissolved in the liquid medium forms carbonic acid which dissociates providing the system with hydrogen ions and bicarbonate. On the other hand, a decrease in the pH leads to carbon dioxide free molecules formation with hydrogen ions consumption by bicarbonate. Therefore, bicarbonate plays the role of buffering agent since it avoids strong acidification of the process. Similarly, ammonia provides buffering capacity to the system since, according to the ammonia – ammonium equilibrium, at pH below 9.3 ammonium ions are formed with release of hydroxyl ions (NH₃ + H₂O ↔ NH₄⁺ + OH⁻) and weak acidification is avoided due to reaction between ammonia and VFAs (represented by C_xH_yCOOH) as reported in the following Eqs. 1.15, 1.16 and 1.17 (Zhang et al., 2014):



However, when pH values rise above 8.5, ammonia begins to exert a toxic effect on methanogenic bacteria. VFAs produced during acidogenesis tend to reduce the pH of the system but, normally, this reduction is neutralised by the activity of the methanogenic

bacteria. However, in case of a rapid degradation of input substrate, VFAs accumulate in the anaerobic sludge and pH of the system may decrease below 6.5 causing methanogens inhibition. In order to tackle this occurrence, addition of neutralising compounds (e.g., $\text{Ca}(\text{OH})_2$, Na_2CO_3 , NaOH) and/or feeding suspension are advisable.

1.4.3.2. VFAs and FOS/TAC parameters

Hydrolytic and acidogenic bacteria produce short-chain fatty acids (mainly acetic, propionic, butyric, and valeric acids) as essential intermediates and metabolites during the anaerobic digestion process (see Paragraph 1.4.1.). The sum of all VFAs present in the digestate is represented by total VFAs parameter which could be determined by titration methods, colorimetric methods or through chromatography methods (Buchauer, 1998). Among these, titration is widely accepted as the cheapest, quickest and simplest method for biogas plant routine monitoring and control (Lahav and Morgan, 2004). However, a normal VFAs level is impossible to define since several concentrations of VFAs are found in literature in different reactor systems. Indeed, the optimal VFAs concentration in the digester depends on the composition of the substrates and the operating conditions (Ahring et al., 1995). In general, the stability of an anaerobic digestion is evaluated through the variation of VFAs content over process time rather than its absolute value. For instance, sudden increases in VFAs concentration indicate accumulation of acids and likely acidification of the system (APAT, 2005). Instead, considering the individual VFAs concentration, Wang et al. (2009) discovered that significant inhibition of methanogenic bacteria appears for propionic acid concentration of 900 mg/L while the degradative process proceeds in an optimum way for propionic acid concentrations lower than 200 mg/L (Steinhauser and Deublein, 2011). At $\text{pH} < 7$, the inhibiting threshold for acetic acid is reported to be 1000 mg/L (Steinhauser and Deublein, 2011).

In anaerobic digestion process, the pH value can be kept stable because of the buffering capacity of the system. The buffering capacity is mostly rendered as carbonate buffer (lime reserve) expressed in $\text{mg}_{\text{CaCO}_3}/\text{L}$. The ratio between the concentration of VFAs (in terms of acetic acid, $\text{mg}_{\text{HAC}}/\text{L}$) and the buffering capacity is the so-called FOS/TAC ratio where FOS stands for VFAs, while TAC stands for carbon buffering capacity. This parameter serves for a rapid evaluation of the state of a digester. Indeed, a process is

generally considered as stable if a FOS/TAC ratio of 0.15 to 0.45 is measured. Alkalosis conditions (rising pH) may be established for lower values. This occurrence outlines an inadequate feeding of the digester which suffers from lack of organic acids. On the other hand, a high FOS/TAC points out excessive accumulation of organic acids and pH decrease. FOS/TAC parameter is also easily determined by a titration method (Mézès et al., 2011).

1.4.3.3. Temperature

Microorganisms involved in anaerobic digestion process typically develop under three possible temperature regimes: thermophilic (48 – 55°C), mesophilic (32 – 42 °C) and psychrophilic (10 – 25 °C) with typical residence times of 14 – 16 days, 14 – 30 days and over 30 days, respectively. In general, in terms of microbial activity, mesophilic processes are the most stable while systems operating under psychrophilic conditions are very slow. Thermophilic condition is characterised by greater solubility of organic compounds and faster reactions rates than mesophilic one, especially in the hydrolysis and acidogenesis phases. For this reason, under high operating temperature acidification of the anaerobic system may occur so that inhibiting methane production. Moreover, thermophilic process may suffer from low diversity of microbial species because of high temperature and instability. In addition, a thermophilic anaerobic digestion needs larger investments for the higher energy input needed for heating. Besides all these issues, a well-functioning thermophilic process generally exhibits higher productivity compared with mesophilic one for certain types of substrates. For instance, anaerobic digestion of fat-rich materials carried out under thermophilic conditions results in a better microbial availability of the substrates and, thus, a higher biogas yield. Instead, mesophilic systems are more stable and rich in bacterial species. Most of the methanogenic bacteria thrive in mesophilic temperature regime whereas only a few species in thermophilic environment. Mesophilic methanogens can tolerate temperature's variations better than thermophilic ones but lose their activity irreversibly in the range 40 – 45°C (Steinhauser and Deublein, 2011). According to Mao et al. (2015), anaerobic digestion can be optimised by carrying out hydrolysis/acidogenesis phase under thermophilic conditions in order to improve substrates biodegradability and methanogenesis phase under mesophilic temperature range to ensure stable methane production.

1.4.3.4. *Nutrients*

Macro-nutrients (e.g., N, P, S) are indispensable constituents of biomass and also play a key role as buffering agents. Normally, they are required in relatively large quantities by bacteria. Micro-nutrients, especially metals (e.g., Fe, Ni, Zn, Mo, Co, Se), take part in numerous enzymatic reactions involved in the biochemistry of the anaerobic digestion process. They are required in relatively small quantities. However, excessive concentrations of both types of nutrients may lead to the inhibition of the anaerobic digestion process (Gerardi, 2003; Romero-Güiza et al., 2016). Since nutrients' presence is so essential for optimal function of anaerobic microorganisms, several authors suggest to integrate digesters with external solutions in order to avoid any deficiencies (Angelidaki et al., 2009; Holliger et al., 2016). In particular, the Italian norm UNI/TS 11703:2018 prescribes the use of three different nutrients solutions containing KH_2PO_4 , $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, NH_4Cl (solution A), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (solution B) and $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, H_3BO_3 , ZnCl_2 , CuCl_2 , $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, Na_2SeO_3 (solution C) according specific dosages. The optimum ratio of all nutrients and trace minerals results from the elemental composition of the cell biology.

In the following, the focus of interest is on the roles played by nitrogen and heavy metals in the anaerobic digestion since they are present in the pre-treated leachate which is tested as nutrient solution for optimising anaerobic digestion.

Nitrogen

Nitrogen is an essential element for bacterial growth. Taking into account the empirical formula $\text{C}_5\text{H}_7\text{O}_2\text{N}$ commonly used to represent bacteria (see Eq. 1.2), it is noteworthy that nitrogen represents approximately 12% of the cell mass. Nitrogen is involved in the synthesis of proteins, enzymes, ribonucleic acid (RNA) and deoxyribonucleic (DNA) (Parkin and Owen, 1986). It is available to anaerobic bacteria as ammoniacal-nitrogen ($\text{NH}_4^+\text{-N}$) even though some methane-forming bacteria can obtain nitrogen either from its molecular form (N_2) or by using the amino acid alanine ($\text{CH}_3\text{CHNH}_2\text{COOH}$) (Gerardi, 2003). According to the aforementioned Italian norm, nitrogen is supplied to digesters in form of ammonium chloride (NH_4Cl) dissolved in distilled water (5.3 g in 0.5 L).

Biological degradation of nitrogenous matter (i.e., proteins and urea) in the anaerobic process leads to ammonia generation. The two main forms of inorganic ammonia nitrogen in aqueous solution are ammonium ion (NH_4^+) and free ammonia (NH_3). According to the ammonia – ammonium equilibrium (see Paragraph 1.1.1.3.), under the usual pH conditions of an anaerobic digestion process (i.e., around neutral), ammonium ion is prevalent while ammonia exerts its toxic effects, especially on methanogens, under alkaline conditions. Although it is widely accepted that ammoniacal nitrogen concentrations below 200 mg/L are beneficial to anaerobic process, free ammonia is known to be the main cause of inhibition (already at concentration of 150 mg/L according to McCarty and McKinney, 1961) since it may diffuse passively into the cell causing proton imbalance and/or potassium deficiency. Other proposed mechanisms for ammonia inhibition are change in the intracellular pH, increase of maintenance energy requirement and inhibition of specific enzymes reactions (Chen et al., 2008). As Chen et al. (2008) reported after reviewing several studies, a total ammonia nitrogen (TAN) concentration ranging from 1.7 to 14 g/L caused a 50% reduction in methane production. Yenigün and Demirel (2013) were more accurate in reporting a more narrow range of TAN concentration (i.e., 1.7 – 1.8 g/L) which leads to reactor failure. Actually, nitrogen inhibition depends on various factors and operational parameters (e.g., pH, OLR, HRT, temperature) and, therefore, it is hardly predictable. For instance, an already adapted inoculum can tolerate a possible ammonia accumulation occurring during the digestion. Yenigün and Demirel (2013) reported some studies proving anaerobic bacteria acclimation to TAN. No signs of process slow down or failure were recorded as a result of controlled ammonium addition to high rate digesters up to 2700 mg/l (Melbinger et al., 1971) and adaptation of methanogenic bacteria was observed for TAN concentrations up to 5000 mg/l and 3075 mg/l in the digestion of sewage sludge and piggery manure, respectively (Van Velsen, 1979). What emerges is that the use of an already acclimated inoculum is essential in case of anaerobic digestion of substrates with high initial TAN and protein concentrations such as sea-food industry waste, manure and wastewater among others. Alternatively, substrates pre-treatment (e.g., air stripping and chemical precipitation) or dilution with carbonaceous materials are advisable. Also, addition to the system of adsorbents as zeolite allows to selectively remove ammonia. Light metals ions (such as Na^+ , Ca^{2+} and Mg^{2+}) if present in the inoculum/substrate are known to interfere with NH_3 inhibitory action due to their antagonistic effect (Chen et al., 2008). However, as

Yenigün and Demirel (2013) stated, an anaerobic digestion process inhibited by ammonia accumulation could be recovered. For instance, Parkin et al. (1983) investigated the reversibility of mesophilic anaerobic process exposed to TAN concentrations of 4000 mg/L, 8000 mg/L and 24000 mg/L. Authors observed that only removing ammonium in the supernatant allowed system to rapidly recover to full biogas production. Similarly, Omil et al. (1995) reversed in 10 days the inhibition of anaerobic digestion of sea food processing wastewater by decreasing the protein content in the influent. Alternatively, it is suggested that lowering pH leads to free ammonia concentration decrease (Wu et al., 2009).

Heavy metals

Heavy metals are not biodegradable elements and, thus, can accumulate in the anaerobic sludge up to potentially toxic concentrations for bacteria. Their toxicity is greater if they are present in the form of free ions which compete with other molecules for enzymatic binding sites. Because of metals ions – enzymes interaction, inhibition of enzymatic functions may occur (Oleszkiewicz and Sharma, 1990). However, trace amounts of metals are beneficial for i) the activity of some enzymes and ii) stimulating methanogens' growth and activity. Indeed, many heavy metals are part of the essential enzymes which drive numerous anaerobic reactions. Heavy metal composition of the cell follows the order $Fe \gg Zn \geq Ni > Co = Mo > Cu$ (Oleszkiewicz and Sharma, 1990). For instance, Cu^{2+} , Ni^{2+} and Fe^{2+} have been observed to promote both cellulase action and methanogens' activity up to specific concentrations in anaerobic sludge. Specifically, according to Guo et al.'s study (2019), Cu, Ni, Fe, Cd and Zn promote (through different mechanisms) biogas production if present in anaerobic system in concentrations 0- 100 mg/L, 0.8 – 50 mg/L, 50 – 4000 mg/L, 0.1 – 0.3 mg/L, 5 mg/L, respectively. On the other hand, they act as inhibitory agents at concentrations of 500 mg/L, 100 mg/L, 20000 mg/L, 1.2 mg/L and 50 mg/L, respectively. However, some mechanisms of metal detoxification can be triggered. Firstly, acclimation of anaerobic bacteria is possible by gradually increasing the concentration of metals in continuous flow systems. In this way, bacteria can tolerate metals' concentrations even higher than those found to be toxic. Moreover, some metals' ions act as antagonists to each other, namely the effect of one metal which alleviates the toxic effect of another metal. In addition, detoxification of the system can occur by

immobilisation of heavy metals by using absorbent agents (sorption) or inducing chemical precipitation.

1.4.4. Parameters governing the anaerobic digestion process

In this Paragraph, some parameters which control the anaerobic digestion process are discussed. They have to be set prior to the beginning of the process in order to maximise process stability and biogas yield.

- Carbon to Nitrogen ratio (C/N) reflects substrate's composition specifically on the basis of the balance between nutrients and carbon sources. A substrate suitable for anaerobic digestion should have a C/N in the range 16:1 – 30:1 with a ratio of 25 being the most commonly used one (Mao et al., 2015; Steinhäuser and Deublein, 2011). Substrates with excessively high C/N values do not meet the nutritional requirements for the fundamental activities of microbial metabolism (synthesis of proteins, enzymes, RNA, and DNA, see Paragraph 1.1.1.6.). Furthermore, rapid degradation of biodegradable components of the substrate into VFAs occurs during initial stage of digestion and leads to excessive acidification of the system (Kumar and Samadder, 2020). This is the case of fruit and vegetable market waste which is rich in simple sugars which are converted into VFAs and their accumulation causes inhibition of the process (Wang et al., 2014). On the other hand, substrates characterised by very low levels of C/N (e.g., fish waste, manure and slaughterhouse waste) are often relatively poor in carbon source and rich in nitrogen compounds. Due to their low biodegradable matter content, these substrates requires large input quantities to reach an adequate organic loading rate (see below). However, the higher the input the larger the conversion of nitrogen to ammonia which can lead to process inhibition (Jeung et al., 2019). Co-digestion of two or more substrates is widely accepted and recommend as the most appropriate solution to reach the optimum C/N of the feeding. By this way, both carbon- and nitrogen-rich substrates concurrently provide microorganisms with biodegradable matter and nutrients required for their metabolism, respectively (Hagos et al., 2017).

- Organic Loading Rate (OLR) represents the amount of volatile solids fed into a digester per day under continuous feeding [gVS/L·d]. Theoretically, the higher OLR is set the more biogas is produced. However, adding a too large volume of input material daily (i.e., overloading the reactor) may result in bacterial activity inhibition during the early stages of fermentation. Indeed, high OLRs lead to an hydrolytic/acidogenic phase quicker than the methanogenic one so that causing VFAs accumulation and, eventually, acidification of the system (Mao et al., 2015). In general, there is not an optimum OLR as it strongly depends on chemical characteristics of substrate. Usually, optimum OLR of a specific anaerobic digestion process is preliminary determined by long-term bench-scale studies (Labatut and Pronto, 2018). In conventional anaerobic digestion of organic waste (e.g., food waste, dairy manure, sewage sludge), OLRs of 1 – 4 gVS/L·d are commonly used (Liu et al., 2017).
- Hydraulic Retention Time (HRT) is the time required to complete the degradation of organic matter and is the result of the ratio between reactor volume (V) and influent flow rate in time (Q). It depends on microbial growth rate, process temperature, OLR and type of substrate. Since HRT must be greater than anaerobic bacterial doubling time, an HRT of 15 – 30 days is typically required for a mesophilic process of sewage sludge and livestock waste. Instead, HRT ranges from 2 to 7 days in case of rapidly biodegradable organic substrates or it can be set even at about 50 days for lignocellulosic materials. Usually, decreases in HRT lead to VFAs accumulation, whereas too long HRTs imply carbon and nutrients demands (Mao et al., 2015).

1.4.5. Direct Interspecies Electron Transfer (DIET)

A stable and efficient anaerobic digestion process demands optimal conditions for bacteria survival and specific interactions among microorganisms. Parameters such as alkalinity, pH, temperature and oxidation reduction potential directly influence microbial and enzymatic activity as well as presence of macro and micro-nutrients (Xu et al., 2019). Furthermore, as discussed above, syntrophic interactions between microbial species involved in the system are crucial for process balance to avoid accumulation of

intermediates. For instance, the syntrophic association between hydrogenotrophic methanogens and acidogenic bacteria ensures the maintenance of hydrogen partial pressure in the system below the concentration level of 10^{-4} atm so that making the process thermodynamically feasible (Parkin and Owen, 1986). Similarly, acetoclastic methanogens consume acetate to produce carbon dioxide and methane and doing that they allow to overcome the internal energy barrier for acetate production from VFAs performed by fermentative bacteria (Wu et al., 2020). Since both of these interactions consist of oxidation and reduction reactions, electrons exchange among syntrophic microorganisms is carried out. In particular, interspecies electron transfer (IET) via hydrogen and formate as electron carriers is known as the major pathway. However, recent studies (Baek et al., 2018; Dubé and Guiot, 2015; Gu et al., 2019) discovered that electrons can flow from one microbial cell to another without being shuttled by molecules but through direct contacts between cells. This mechanism makes methane production faster and thermodynamically more efficient. It is known as direct interspecies electron transfer (DIET) and has been observed in several defined bacterial co-cultures (Kato et al., 2012; Rotaru et al., 2014; Summers et al., 2010). DIET mechanism is carried out in three possible ways involving electrically conductive pili, electron transport proteins on cell surfaces and electrically conductive materials (Lovley, 2017) (Figure 1.8).

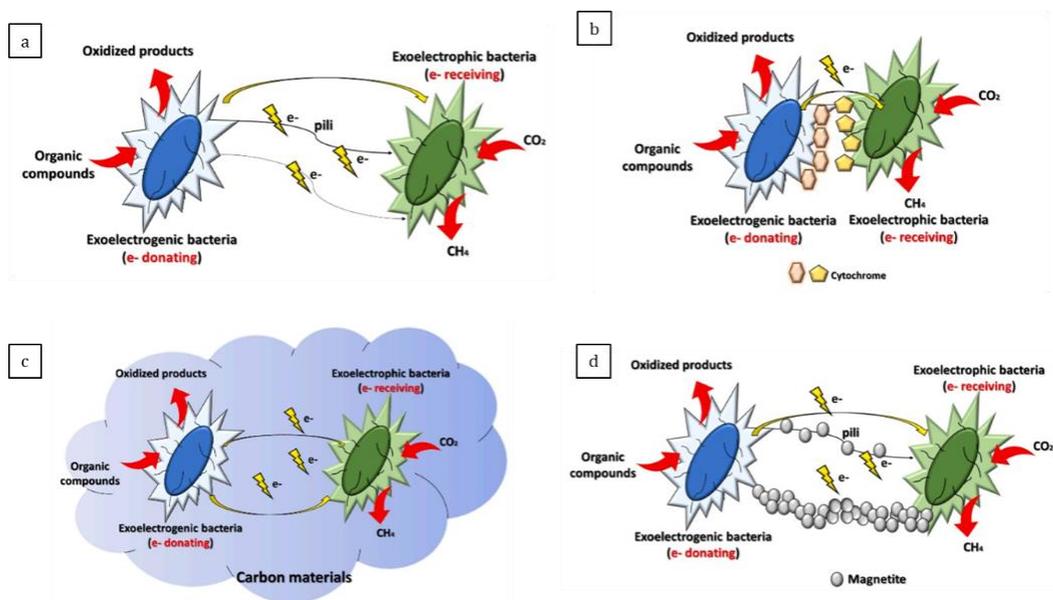


Figure 1.8. DIET mechanism via a) pili, b) cytochrome, c) carbon materials and d) magnetite (Wang and Lee, 2021)

Pili (Figure 1.8, a), collectively referred to as microbial nanowires, are protein filaments produced by microorganisms which are conductive *in vivo* under physiologically relevant conditions (Malvankar and Lovley, 2012). They have been observed in many studies by various microscopy technologies (Eaktasang et al., 2016; Summers et al., 2010). Electrons flow through pili in a relatively long-range transfer from one cell to another. Two mechanisms for pili electrical conductivity have been proposed (Figure 1.9). One mechanism involves cytochromes present on pili surface and consists of electrons moving by hopping and tunneling from cytochrome to cytochrome (Figure 1.9, a). The other mechanism is based on the metal-like conductivity of pili. In this case, charges are spread across the entire filament (Malvankar and Lovley, 2014) (Figure 1.9, b).

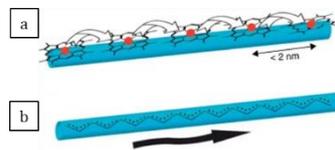


Figure 1.9. Mechanisms of electron flow along microbial nanowires: a) electrons hopping and tunneling, b) metal-like conductivity (adapted from Malvankar and Lovley, 2014)

Cytochrome *c* is a heme protein which acts as an electron carrier (redox protein) in the mitochondrial electron transfer chain leading to ATP production (Hough et al., 2014). Many exoelectrogenic microorganisms, such as *Geobacter* and *Shewanella*, are evidenced to be able to transport electrons through a chain of cytochrome *c* toward extracellular electron acceptors (Rotaru et al., 2013) (Figure 1.8, b).

Some conductive materials are widely known to promote DIET in anaerobic digestion process (Wu et al., 2020). Carbon based materials, such as granular activated carbon, carbon cloth and biochar, thanks to their high surface area and electrical conductivity, provide sites where bacteria can attach to and proliferate and develop the aforementioned electrical connections among microbial species (i.e., pili and cytochrome electron transfer) (Lovley, 2017; Barua & Dhar, 2017) (Figure 1.8, c). Furthermore, carbon materials are commonly used in anaerobic digestion bioreactors owing to their micro-porous structure that allows to adsorb possible toxic organic compounds inhibiting methane production (Calabrò et al., 2019a).

Among metal based materials, iron in its different morphologies and valence states is widely used in anaerobic digestion processes. Iron has been shown to facilitate electron transport, stimulate bacterial growth and promote enzymes' activity as most enzymes contain metallic elements, such as Fe and Ni. In particular, zero valent iron (ZVI) is extensively used as an additive in anaerobic digestion processes (Zhang et al., 2020). The reason lies in ZVI corrosion pathways in aqueous solution under anaerobic condition (Calabrò et al., 2019b). According to equations listed below, ZVI reacts with water (Eq. 1.18) resulting in hydrogen production. Moreover, electrochemical corrosion, normally caused by SO_4^{2-} released by sulphate reducing bacteria, may occur (Eq. 1.19).



Iron hydroxides and oxides are the products of ZVI corrosion. Particularly, hydroxide is not stable in anaerobic environments and is liable to be transformed to magnetite (Eq. 1.20) (Wei et al., 2018).



Magnetite (Fe_3O_4) produced via iron(II) hydroxide transformation plays an important role in DIET mechanism. Indeed, according to the studies cited by Lovley (2017), magnetite particles take part in electrons transfer among microbial species by sticking onto pili for enhanced electron transfer rate (Figure 1.8, d). Otherwise, since the size of magnetite's particles is much smaller than microorganisms, aggregation of its nanoparticles can occur in order to directly link the syntrophic cells so that conducting electrons (Wang and Lee, 2021) (Figure 1.8, d).

Furthermore, zero valent iron addition can indirectly enhance methane production since hydrogen formed through its corrosion serves as substrate for hydrogenotrophic methanogens in the last phase of anaerobic digestion process, as discussed before. Also, consumption of H^+ ions during iron corrosion improves the buffering capacity of the system maintaining favourable pH conditions as a consequence. Finally, according to Eq. 1.19, iron enrichment improves biogas quality owing to desulphurization which prevents hydrogen sulphide formation.

1.4.6. Digestate

Besides biogas, digestate is the other product of anaerobic digestion processes. Digestate is a mixture of microbial biomass and undigested material. Usually, digestate is mechanically separated into a liquid and a solid fractions (Monlau et al., 2015). Depending on technology and reactor design, digestate could be a semi-solid or a liquid material on the basis of its dry matter content. Particularly, when dry matter content is lower than 15%, digestate is defined as liquid, otherwise it is considered as a semi-solid material (Makádi et al., 2012). Because of the lack of homogeneous information about digestate composition, agronomic properties and potential environmental impacts connected to agronomic reuse or disposal, in some countries (such as Italy) legislation on digestate use is still deficient compared to that dealing with compost and biosolids (Schievano et al., 2009). However, digestate application in agriculture is widely accepted as fertilisers or soil amendment replacements (Koszel and Lorencowicz, 2015).

First of all, digestate contains considerable amount of mineral elements (nitrogen, phosphorus, potassium) which makes it suitable for being employed as fertiliser. Indeed, since macro- and micro-nutrient total content only slightly decreases during anaerobic digestion, nutrients elements tend to be mineralised and concentrated in the anaerobic sludge. For instance, mineral nitrogen is present in digestate in form of ammonium which is known to be readily available for plants absorption (Makádi et al., 2012; Schievano et al., 2009)

Secondly, considering that anaerobic digestion process leads to large reduction in easily degradable organic matter, residual organic compounds in digestate show relatively high biological stability. These recalcitrant molecules (mostly humic substances) are possible humus precursors. This feature gives digestate good amendment properties (Schievano et al., 2009). Generally, digestate quality as soil amendment depends on digester retention time as the longer HRT the lower the organic matter content in digestate because of the more effective methanogenesis (Makádi et al., 2012).

Makádi et al. (2012) summarised the effects of digestate on soil and crops claimed that digestate agriculture application (either in solid or liquid form) could result in significant improvements of soil fertility, microbiological activity and food quantity and quality. Furthermore, in a perspective of circular economy, digestate use as a fertiliser or

amendment replacement allows to close the loop of organic waste and residues recovery through anaerobic digestion with virtually no further waste production.

However, digestate use for land application has to deal with some drawbacks. Firstly, being digestate continuously produced over the year, it needs to be stored as it cannot always be used immediately on farm lands. Indeed, digestate application on lands is limited according to regulations on nutrient loading per hectare. Moreover, soil fertilisation has to follow specific growth stages of crops. As a consequence, possible transportation far from the production site and storage of large volumes of produced digestate may represent expensive steps which can adversely affect the economic value of digestate. Secondly, digestate reuse can be hindered by the possible presence of environmental pollutants (e.g., pathogens, heavy metals, pesticides, steroid hormones, toxic organic compounds) and volatile solids which can be converted into gaseous species (e.g., CH₄, CO₂, NH₃, N₂O) released into the atmosphere during storage and/or land use. In these cases, digestate treatment for nutrients (i.e., N, P, K, S) recovery or digestate pre-treatment (via air stripping or membranes separation among others) prior to land spreading may be applied (Monlau et al., 2015).

As already mentioned, the next Chapter provides an overview of conventional and innovative treatments specifically for mature landfill leachate.

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CHAPTER 2

2.1. Mature Landfill Treatments

As discussed in Paragraph 1.3., leachate is the liquid effluent emitted from landfill which requires appropriate treatment prior to any discharge into the environment. It is characterised by an extremely variable chemical composition greatly depending on landfill type and age. The complexity of its composition results in problematic management. In the past, the most important feature of leachate treatment was the reduction of biologically treatable components (i.e., BOD and nitrogen) in the simplest ways possible. However, the necessity of removing also non-biodegradable organic substances and inorganics (e.g., metals, metalloids) gradually gained relevance. As a consequence, over the years, leachate treatment has become increasingly sophisticated (Ehrig and Robinson, 2010).

Typically, leachate treatments can be grouped into two major categories: i) biological processes and ii) physical and chemical processes (Luo et al., 2020). The former basically consists of aerobic and anaerobic treatments while the latter includes membrane filtration, chemical precipitation, coagulation and flocculation, chemical oxidation, air stripping and adsorption (Bilardi et al., 2018). However, oftentimes leachate treatment requires the combination of both biological and physical-chemical processes for the concurrent removal of organic and inorganic compounds in order to meet the strict quality standards for leachate final discharge. Generally, leachate can be: i) directly treated and discharged on-site, ii) pre-treated on-site prior to further off-site treatments and iii) sent to off-site treatment facilities (Townsend et al., 2015). Although the first solution appears to be the most cost-effective, however the choice of the most appropriate treatment mainly depends on leachate characteristics, facility and site specific conditions and regulatory requirements.

With regard to leachate characteristics, it is widely accepted that performances of typical leachate treatments are largely influenced especially by landfill age (Renou et al., 2008) as summarised in Table 2.1.

Table 2.1. Performances of some typical leachate treatments on the basis of landfill age (adapted from Amokrane et al., 1997, Luo et al., 2020, Yadav and Dikshit, 2016)

Leachate treatment	Landfill age (years)		
	Young (< 5)	Intermediate (5-10)	Mature (>10)
Leachate recirculation	Good	Fair	Poor-Fair
Micro and Ultrafiltration	Poor	Good	Good
Nanofiltration	Good	Good	Good
Reverse osmosis	Fair-Good	Good	Good
Chemical precipitation	Poor-Fair	Fair	Fair
Coagulation/Flocculation	Fair	Fair-Good	Fair-Good
Chemical oxidation	Poor-Fair	Fair	Fair
Air stripping	Poor	Fair	Fair
Carbon adsorption	Poor-Fair	Fair	Good
Biological treatment	Good	Fair	Poor

The focus of this dissertation is on the management of mature landfill leachate (henceforth MLL). This type of leachate is mainly characterised by very low biodegradability (BOD/COD < 0.1 – 0.2 given by the presence of recalcitrant organic compounds), considerable ammoniacal nitrogen and chloride contents and low or negligible amount of phosphorus (Christensen et al., 1989). Accordingly, Table 2.1 shows that the treatment of MLL only through biological processes turns out to be scarcely effective as it is discussed later in the Chapter. On the contrary, some physical and chemical (e.g., adsorption, reverse osmosis) processes appears to be more suitable. For this reason, the complete treatment of MLL is often achieved through combinations of biological and physical-chemical processes (not necessarily in that order) as explored in the following Paragraphs.

In this Chapter, an overview of studies found in literature on technologies applied exclusively for MLL treatment is provided. The Chapter starts with the description of the leachate recirculation practice which represents a form of treatment in some cases. Then, it continues with the theoretical description of specific processes in two major sections arranged according to the aforementioned leachate treatment categories (i.e., biological and physical-chemical processes). Particular attention is given to the anaerobic treatment of MLL (either with or without pre- or post-treatments) as it is the key process of this thesis.

2.2. Leachate Recirculation

Recirculation of leachate back to the landfill body has been largely carried out in the past decades since it was one of the cheapest and easiest means of disposal (Renou et al., 2008). Leachate recirculation system involves two main elements: leachate collection and storage infrastructures (already present in modern landfills) and reinjection equipment (Beaven and Knox, 2018). Originally, leachate was pumped into the landfill with the aim of being absorbed by dry solid waste. Later, the adoption of stricter regulations discouraged the addition of any liquid to landfill in favour of the “dry tomb” approach (see the Council Directive 1999/31/EC). Indeed, as briefly reported in Paragraph 1.2., after landfill closure, waste is suggested to be isolated through a top impervious cover. As a consequence, leachate and gas production during post closure care (i.e., 30 years at least for European member states) is dramatically reduced. However, in this way, waste cannot complete its biological stabilisation because of insufficient moisture available in the landfill body. Thus, this approach is advantageous for landfill owners and operators but it poses risks to human health and environment in the long run. In fact, in case of failure of the cover, two scenarios could occur: i) mechanically unstable waste is released into the environment, ii) moisture enters the landfill body and waste degradation process starts again with consequent leachate and/or gas emissions (Scharff, 2010). For this reason, the concept of bioreactor landfill based on leachate recirculation has been developed (Reinhart et al., 2002). Rather than merely containing waste, bioreactor landfill aims at enhancing waste biological degradation through moisture addition with the purpose of shortening the aftercare. The direct correlation between moisture content and gas generation rates is witnessed by numerous studies (Knox et al., 2018). Leachate plays a key role in this technology since it is used as an additive which provides moisture, allows nutrients and microorganisms distribution, buffers pH and dilutes inhibitory compounds. Concurrently, leachate is treated mainly in terms of quality improvement (e.g., reduction of recalcitrant compounds and ammoniacal nitrogen contents) and volume reduction. On the other hand, leachate recirculation practice poses some technical problems. First of all, leachate injection is well known to increase pore pressure inside the landfill with adverse effects on slope stability. Risk of slope failure increases with leachate injection pressure (Beaven and Knox, 2018; Feng et al., 2018). Secondly, volumes and rates of inflow leachate have to be accurately calculated during the design of a leachate recirculation system.

Indeed, recirculation of excessively large volumes of leachate may lead to saturation and ponding and too high flow rates may result in methanogens removal so that interrupting waste biological stabilisation (Şan and Onay, 2001). To overcome these issues, leachate storage (1 – 2 days at least) in tanks or lagoons can be foreseen for flow balancing (Beaven and Knox, 2018). Lastly, long-term recirculation of leachate may eventually increase the concentration inside the landfill of persistent organic and inorganic substances such as slowly or non-biodegradable organic matter, chloride, ammoniacal nitrogen and metals (Heyer et al., 2001). For all these reasons, leachate recirculation should be performed only for a limited period of time.

Several applications of mature leachate recirculation at laboratory, pilot and full-scale can be found in literature. Some relevant studies are discussed below.

Calabrò and Mancini (2012) performed a laboratory-scale experiment in which a synthetic leachate, representative of a methanogenic phase, was pumped into a column filled with stabilised organic fraction of municipal solid waste simulating a landfill. Authors observed an improvement in effluent leachate quality. In fact, biodegradable organic matter was biologically degraded and metals (copper and zinc) were trapped in waste due to a combination of physical (adsorption), biological (bacterial sulphate reduction) and chemical (precipitation of metal sulphides) sorption mechanisms. On the other hand, ammoniacal nitrogen and chloride were essentially unaffected by the treatment. Especially for nitrogen, authors suggested that leachate pre-nitrification could be an option to achieve better results in terms of ammonia removal after the recirculation.

Suna Erses and Onay (2003) simulated at laboratory-scale (by using two 96-litre PVC reactors) a system in which leachate produced by an old landfill was recirculated into a young one. Authors proved that the recycled mature leachate introduced into the reactor simulating a young landfill provided acclimated anaerobic microorganisms and nutrients which accelerated waste stabilisation. As a result of leachate addition, they observed a decrease in COD content of the effluent (i.e., improvement of leachate quality) and an increase in daily gas volume. The highest degree of waste stabilisation was obtained with a leachate recirculation rate of three times per week combined with buffer addition (100 g/L Na₂CO₃ solution). Chugh et al. (1999) performed a similar experiment (involving 200 L, 316 stainless steel reactors and 42 L leachate collection tanks) in which a leachate coming from a stabilised-waste reactor was fed to a fresh-waste one. The results outlined

i) the buffer capacity of the mature leachate which flushed out excess VFAs in the fresh-waste reactor and ii) the acceleration of waste decomposition which implies enhanced biogas generation.

Morris et al. (2003) presented findings from their long-term studies on two full-scale MSW landfills located in Delaware, USA, equipped with leachate recirculation. The relevance of recirculation apparatus emerged from this study. Indeed, while in one landfill the positive effects (i.e., faster waste degradation) of leachate recirculation have been observed, in the other one differences with traditional “dry tomb” approach were not as dramatic as anticipated likely due to a low efficiency of the recirculation system at distributing leachate throughout waste. In terms of effluent leachate quality, after 7 years of efficient leachate recirculation, concentrations of heavy metals, chlorinated volatile organic compounds (VOCs) and aromatic compounds (BTEX) reduced below drinking water standards within only 5 years of landfill closure in contrast to ammoniacal nitrogen content which did not reduce over monitoring time. The potential of leachate recirculation in terms of accelerating waste decomposition has been confirmed by similar studies on full-scale landfills in the short and long term (Benson et al., 2007; Demir et al., 2004). In Mehta et al. (2002), landfill cells irrigated with recirculated leachate showed higher methane yields and better waste settlements compared to control cells. However, also in this case, authors noticed that waste was not uniformly wet and, as a consequence, not completely decomposed because of possible nonuniform distribution of recycled leachate.

To sum up, on the one hand, it can be inferred that leachate recirculation potentially represents a simple method to store and treat leachate often on the same site of its production. Indeed, what emerges from literature studies is that leachate recirculation may lead to: i) shorten waste stabilisation time by providing waste with essential moisture, ii) increase biogas production rates so that limiting landfill gas emission during the post closure period, iii) accelerate landfill settlement before final closure, iv) partially improve leachate quality and reduce its volume (Schiopu and Gavrilescu, 2010). On the other hand, especially for MLLs (see Table 2.1), leachate recirculation is often not effective in reducing pollutants contents but it tends to concentrate harmful substances inside the landfill in the long-term. Besides, the final effluent collected after recirculation does not directly meet the discharge standards but further treatments are necessary anyhow.

Secondly, as outlined by Mehta et al. (2002) and Morris et al. (2003), practical operations of leachate recirculation can be problematic for example in terms of homogeneous distribution of leachate throughout the landfill body which is hardly achieved. Moreover, the entire recirculation system involves a series of infrastructures for leachate injection and monitoring which further complicates landfill management (Knox et al., 2018). Finally, the application of leachate recirculation does not always provide solid results in terms of leachate treatment, while the technical issues related to this practice are definite and well known.

2.3. Physical and Chemical Processes

As shown in Table 2.1, physical and chemical processes are widely accepted as the most appropriate methods to treat MLLs since they are far less sensitive to the presence of recalcitrant organic compounds (e.g., humic substances) and toxic elements in contrast to biological processes. A physical or chemical process may be applied for the proper management of this type of leachate alone or in combination with other technologies. In the following Paragraphs, the most common processes used for MLL treatment are presented.

2.3.1. Membrane Filtration

Microfiltration, ultrafiltration, nanofiltration and reverse osmosis are the main membrane processes applied in landfill leachate treatment (Renou et al., 2008). Generally speaking, membrane filtration operates a physical separation of pollutants from the influent when it passes through the membrane. As a result of the filtration, two different streams are distinguished: the permeate, which is the treated effluent, and the concentrate, which is the residual liquid stream where the pollutants are concentrated. While the former may meet discharge requirements right after the filtration, the latter always requires additional treatment or proper disposal.

Figure 2.1 roughly shows the range of pore sizes for the different membrane technologies (listed at the bottom) and the range of particle sizes for different compounds and microorganisms present in leachate (in the upper part). Microfiltration is frequently used in wastewater treatment for its capacity to capture microorganisms, suspended matter, emulsion droplets and large colloidal. It may be recommended as pre-treatment prior to another membrane processes. Ultrafiltration is commonly applied to eliminate microorganisms from effluents of biological processes utilising pressures up to 10 bar. Furthermore, being also effective in removing large macromolecules from leachate, it can be used to prevent blocking (fouling) of further reverse osmosis application. However, only a limited reduction of COD can be achieved by this method of filtration. Nanofiltration is able to remove recalcitrant organic compounds, sulphate ions and heavy metals from landfill leachate but smaller molecules (such as chlorides, ammonium or sodium ions) can

pass through these membranes. Nanofiltration membranes are characterised by charged surfaces which can interact with charged solutes even smaller than membrane pores and reject them along with bigger neutral solutes and salts. Reverse osmosis is the other membrane filtration technique able to operate over wide temperature and pH range with considerable removal efficiencies (88 – 99% rejection for dissolved solids and metals). It is widely used for the removal of heavy metals, suspended/colloidal materials and dissolved solids from landfill leachate (Cossu et al., 2018; Gao et al., 2014; Renou et al., 2008).

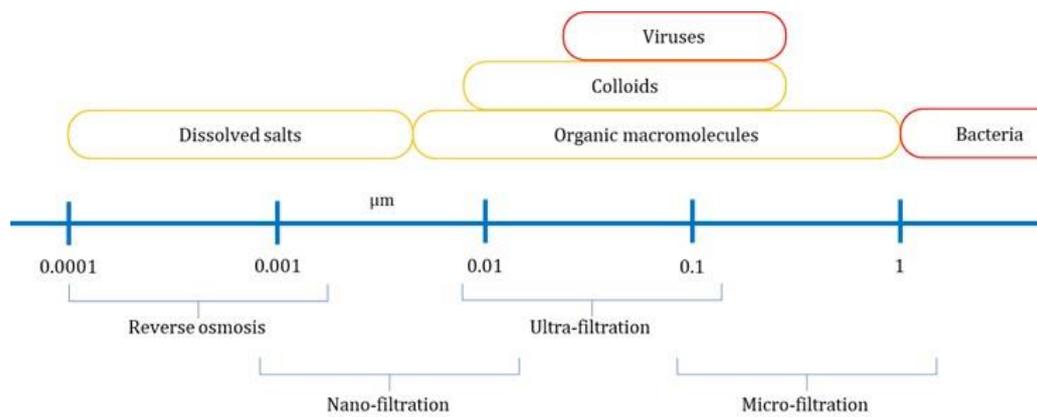


Figure 2.1. Approximate ranges of: pore sizes of the different membrane technologies (bottom) and particle sizes of some leachate components (top) (adapted from Cossu et al., 2018)

The natural tendency of water is flowing through a semipermeable membrane from the one side with low concentration of solutes to the other high concentrated one until solutes' concentrations on both sides become identical (osmosis mechanism). In reverse osmosis systems, water is forced by an external pressure to do the opposite so that concentrating pollutants on one side only. The more external pressure is applied (at the expense of more energy, though), the more water permeates through the membrane and the higher the concentration of pollutants in the other side of the membrane. Typically, on the basis of different factors (e.g., leachate composition, membrane type, system configuration, operational pressure), the fraction of incoming leachate which can be recovered as permeate after reverse osmosis treatment (i.e., permeate recovery rate) is 75 – 95%, while the flux rates ranged from 10 L/m²·h to 44 L/m²·h (Cossu et al., 2018). From a technical point of view, when inflow direction is vertical to membrane surface, fouling of the membrane often occurs and the filter needs to be periodically back-washed. To limit this issue, cross-flow membrane systems (with inflow direction parallel to

membrane surface) are preferred (Cossu et al., 2018). Due to its high removal efficiencies towards the vast majority of pollutants in landfill leachate, reverse osmosis is employed either as an individual treatment or as the main step of a leachate treatment chain. However, as a consequence of high operational pressures (30 – 200 bar), energy consumption is high (Ehrig and Robinson, 2010; Gao et al., 2014). Furthermore, the treatment of the residual concentrate is challenging. It can be recirculated into the landfill (Calabrò et al., 2018) or stabilised into solid material usually after an evaporation (or drying) phase (Hendrych et al., 2019).

Typical technologies for reverse osmosis applications are tubular modules, spiral wound modules, hollow fibre modules and disk tube modules while membranes can be made up of synthetic polymers (polyethylene, polytetrafluoroethylene, polypropylene, cellulose acetate, poly vinyl alcohol, polyimide, polysulfone, polyethersulfone) or inorganic materials (ceramics such as Al_2O_3 , TiO_2 , ZrO_2 , and zeolites) (Ehrig and Robinson, 2010; Lee et al., 2016).

Literature on the use of membranes in combination with other biological or physical-chemical processes for the treatment of MLL is extensive (Bohdziewicz et al., 2001; Chen et al., 2020; Dolar et al., 2016; Kulikowska et al., 2019; Primo et al., 2008; Singh et al., 2014; Thörneby et al., 2003; Zielińska et al., 2020). However, there are some instances in which successful results of MLL treatment are achieved by using membrane processes as an individual treatment. In the following, some of those studies are presented.

The effects of landfill leachate ageing on the ultrafiltration with titania-zirconia (ZrO_2 - TiO_2) tubular membranes have been studied (Collado et al., 2020). What emerged from the study was that the permeability of the membranes was twofold higher for mature leachates respect to young ones. Authors suggested that this was likely due to higher concentrations of proteins and carbohydrates in young leachates.

Xu et al. (2006) investigated at laboratory-scale the possibility of recycling humic substances, which can be used as a liquid fertilizer, from a MLL collected from a landfill site in Beijing, China, through a combination of two-stage ultrafiltration and evaporation. They observed that, after ultrafiltration, the concentrate stream contained relatively high amount of dissolved organic matter (91% of humic substances) and a negligible concentration of heavy metals. This stream was further concentrated by evaporation so

that meeting the main requirements as a fertilizer product. The capacity of recovering humic substances through ultra and nanofiltration was further confirmed (Xu et al., 2017; W. Ye et al., 2019).

Urase et al. (1997) tested the treatment of a leachate containing high concentrations of heavy metals through nanofiltration. Results confirmed that chloride ions are not removed by nanofiltration (rejection of zero) in contrast to heavy metal removal efficiency which was more than 93% for Cr, Cu and Pb. Trebouet et al. (2001) used two different organic membranes of nanofiltration (made up of polyester support with polyacrylonitrile and polypropylene support with polysulfone, respectively) for the treatment of an old leachate at pilot-scale. In both cases, total dissolved solids and COD were reduced by more than 99% and 70 – 80%, respectively. Permeates were clear and colourless and met requirements for discharge for these two parameters. Authors also observed that some pre-treatments (pH modification, pre-filtration and coagulation with FeCl_3) did not enhance membranes' performances.

Šír et al. (2012) performed a two-stage reverse osmosis (spiral wound module) treatment on hazardous waste landfill leachate in batch mode. They achieved a conversion rate of the input stream into the permeate of 94% with removal efficiencies of 94%, 99%, 97% (on average) for ammoniacal nitrogen, total dissolved solids and total organic carbon, respectively. However, authors claimed that the presence of humic acids may adversely affect the separation efficiency of other leachate components. Wu and Li (2021) evaluated the performance of a disk tube reverse osmosis emergency system for on-site MLL treatment (in Southwest China). The treatment achieved a water recovery rate higher than 83%, effectively removed refractory organic matter and chloride and reduced carbonaceous and nitrogenous pollutants to a level suitable for discharge.

A summary of the performances of the different membrane processes on removal of pollutants from leachate is provided in Table 2.2.

Table 2.2. Average removal efficiencies of the different membrane processes towards some pollutants present in leachate (adapted from Gao et al., 2014 and Renou et al., 2008)

Membrane process	Average removal (%)				
	BOD	COD	Total Nitrogen	Suspended Solids	Turbidity
Ultrafiltration	-	50	60 – 80	> 99	> 99
Nanofiltration	80	60 – 80	60 – 80	> 99	> 99
Reverse osmosis	> 90	> 90	> 90	> 99	> 99

In conclusion, it can be stated that membrane processes, especially reverse osmosis, can be successfully applied in the field of leachate treatment as an individual treatment or in combination with other technologies. However, some drawbacks have to be taken into account. First of all, as a consequence of high operational pressures, energy demand is often very high. Secondly, since membrane processes merely concentrate contaminants in the residual concentrate wastewater, their applicability strongly depends on the further concentrate treatment. In case of reverse osmosis, the almost complete removal of contaminants expectedly results in very highly polluted concentrate. The recirculation of the concentrate back to the landfill has been carried out owing to its cost-effectiveness and simplicity (Calabrò et al., 2018; Calabrò and Mancini, 2012). However, this practice appears to be not sustainable as it does not represent a definitive disposal but rather a never-ending recirculation of leachate pollution. Indeed, in this way, inorganic content inside the landfill increases (i.e., ammonia and chloride) and contaminants can accumulate in the leachate up to high concentrations in the long-term. Natural evaporation is another method for concentrate treatment but it requires a lot of space and time. Alternatively, external thermal energy can be applied but the process becomes very expensive. However, the cost of this operation can be balanced by the possibility of recycling the products of the evaporation (e.g., humic substances) as proved by Xu et al. (2006). Other conventional concentrate treatment are solidification and stabilisation using Portland cement, coagulation, adsorption and advanced oxidation processes. Anyway, Keyikoglu et al. (2021) in their review outlines the complexity of the treatment

of membrane concentrate claiming that stand-alone treatment can hardly achieve good process performance.

2.3.2. Precipitation, Coagulation and Flocculation

Precipitation, coagulation and flocculation are chemical processes widely and successfully employed for the removal of suspended solids, organic compounds (including non-biodegradable ones such as fulvic and humic substances), ammoniacal nitrogen and heavy metals from MLL. They can be used as an individual treatment or prior to other biological or physical-chemical processes or as a final polishing treatment step for the removal of the residual non-biodegradable organic matter (Kurniawan et al., 2006; Renou et al., 2008).

Precipitation occurs when chemical compounds dissolved in a solution are transformed into insoluble solid substances. The addition of chemical reagents (precipitants) triggers this mechanism. The formed precipitates can be further separated by sedimentation or filtration. Coagulation process involves the destabilisation of colloidal particles of a solution in order to enhance their agglomeration into larger particles. Destabilisation occurs through the addition of coagulants with the aim of neutralizing the electrical charge present in colloidal particles' surfaces. Then, to facilitate settlement, coagulation is usually followed by flocculation of the unstable particles into single bulky flocs through the addition of flocculating agents (flocculants) (Cossu et al., 2018; Kurniawan et al., 2006).

Typically, ammonium and heavy metals represents the target pollutants of the chemical precipitation. The former is selectively removed by magnesium, ammonium and phosphate (MAP) addition for the precipitation of struvite (MgNH_4PO_4) in form of crystals (Eq. 2.1). However, this mechanism needs for external sources of magnesium and phosphate because of the significant deficiency in magnesium and phosphate of landfill leachate which does not allow to reach the necessary molar ratio (i.e., at least 1:1:1 of magnesium, ammonium and phosphate, respectively). Moreover, the process may require pH adjustment since it occurs in a pH range of 7.5 – 9.0. During MAP precipitation, COD of the solution is not significantly reduced (Cossu et al., 2018; Gao et al., 2014; Torretta et al., 2017). Due to the presence of nutrients like Mg, ammonia and P, struvite produced

during the precipitation, if properly collected and not contaminated by undesired substances, could be used as a fertiliser.



Metal precipitation is induced by either aluminium/iron salts or lime. Metal precipitates are in form of hydroxides (Kurniawan et al., 2006). Chemical precipitation performs well in heavy metal removal when metal ions' concentration in the solution is considerable. Otherwise, it is ineffective (Torretta et al., 2017). Alum and iron salts (i.e., aluminium sulphate, $\text{Al}_2(\text{SO}_4)_3$, ferrous sulphate, FeSO_4 , ferric chloride, FeCl_3) are the most common used coagulants in view of their ability to overcome the repulsive forces between particles. Precipitation and coagulation include pH adjustment to acidic conditions. Particularly, pH ranges are 4.5 – 5.0 for iron and 5.0 – 5.5 for aluminium. In contrast to young leachates which are naturally characterised by acidic pH, MLL is slightly alkaline (see Paragraph 1.3.) and, as a consequence, its pH must be necessarily adjusted. However, the more chemicals are added, the more sludge is produced as a residue of the process. Moreover, the addition of iron or aluminium solutions in combination with acids increases the salt content of treated leachate (i.e., chloride or sulphate) (Ehrig and Robinson, 2010). To improve COD removal, lime is also frequently employed as a coagulant (Gao et al., 2014; Kurniawan et al., 2006). Lime can also act as a flocculating agent as well as activated silica or clay (inorganic flocculants) and polymers (organic flocculants) (Cossu et al., 2018).

These techniques require high energy inputs over a very short period of time for intensive mixing of reagents in the first place. Later additional slow mixing with low energy input must be applied to allow floc settlement. If the intensive mixing phase exceeds the optimum time, new electrical charges can create with consequent destruction of produced flocs. Besides the simplicity of process operations, the high capital costs corresponding to considerable chemicals' consumption, the sensitivity of the processes to pH and the generation of residual sludge which concentrates pollutants must be taken into account (Cossu et al., 2018; Gao et al., 2014; Kurniawan et al., 2006).

(Li et al., 1999) investigated at laboratory-scale MAP precipitation with the aim of reducing the significant ammonium content of a leachate generated by a MLL located in Hong Kong. The performance of the process was close to 100% of removal after 15 min of

treatment with magnesium, ammonium, phosphate at the optimal molar ratio of 1:1:1 and pH value between 8.5 and 9.0. Li et al. (2012) sought to optimise process parameters of MAP precipitation carried out on leachate samples taken from Ah Suwei landfill site (over 15 years old) located in Beijing (experimental tests involving 250 mL beakers). Under the optimal conditions of 1.4:1.0:0.8 of molar ratio (related to Mg, N and P, respectively), 60 min of reaction time, original pH of leachate (i.e., 7.8 – 8.1) and 30 min of setting time, it was observed a significant ammonium removal efficiency higher than 85% (residual concentration in the effluent of 400 mg/L) and a limited COD removal efficiency of 10 – 20%. As a consequence, authors stated that effluents from MAP precipitation should generally require proper subsequent biological treatment. Authors also focused on the effects of calcium ions and carbonate ions present in the leachate: it emerged that their presence could adversely affect MAP precipitation efficiency. Finally, they discovered that residues of MAP could be reused for a further stage of ammoniacal nitrogen removal (up to 90% of total removal) if they are decomposed by heating (90°C for 2 h) under alkaline conditions. Almost complete nitrogen recovery from a leachate collected from a mature municipal landfill (in Apulia, Italy) was obtained by Di Iaconi et al. (2010). In their study, authors performed MAP precipitation at laboratory-scale evidencing a considerable ammonium removal efficiency (95%) by using a molar ratio of 2:1:1. The struvite produced in the experiment was characterised by an heavy metal content lower than that of typical raw soils so that allowing its possible use as a fertiliser. The total cost of the treatment was calculated to be 9.6 €/kg NH₄-N_{removed} (phosphate addition accounts for 50%) which is consistent with other studies for other kinds of wastewater but two-fold higher than that of nitrification/denitrification biological processes using external carbon sources (see Paragraph 2.4.1.). However, authors claimed that MAP precipitation can economically compete with biological processes if the value of the produced struvite is considered in the analysis.

From a comparative study at laboratory-scale on leachate samples collected from the sanitary landfill site of Thessaloniki Greater Area (Northern Greece) (Tatsi et al., 2003) emerged that old leachate is more suited for coagulation processes. In fact, the addition of ferric or aluminium coagulants to leachate samples collected from the landfill section where fresh solid wastes were deposited resulted in a 25 – 38% COD reduction, while higher COD removal capacities (about 80%) were obtained through the addition of FeCl₃

to leachate samples collected at the lowest point of the landfill having the characteristics of a MLL. Gotvajn et al. (2009) investigated at laboratory-scale different possible treatments (i.e., aerobic process, air stripping, adsorption to activated carbon, coagulation/flocculation and advanced oxidation process with $\text{Fe}^{2+}/\text{H}_2\text{O}_2$) for a MLL coming from an industrial (tannery) landfill located in Slovenia. Coagulation was performed with FeCl_3 and $\text{Al}_2(\text{SO}_4)_3$ used as the coagulants. COD was removed by 34% and 25% by the former (at pH 6) and the latter, respectively, both at a concentration of 1000 mg/L. The addition of a flocculant (anionic polyelectrolyte) at a dose of 10 mg/L improved the performance of FeCl_3 (up to 40% of COD removal efficiency). Amokrane et al. (1997) used the coagulation/flocculation process on a MLL (landfill located in France) as a pre-treatment for further reverse osmosis with the aim of reducing fouling problems. The experiment was carried out at laboratory-scale. Coagulation phase involved the mixing (for 3 min at 200 rpm) of either $\text{Al}_2(\text{SO}_4)_3$ or FeCl_3 (at different doses) with 500 mL of raw leachate (starting COD of 4100 mg/L). Flocculation was induced by adding non-ionic, anionic or cationic polymers (at different doses) mixed for 15 min at 40 rpm. FeCl_3 showed better results in terms of reduction of both turbidity and COD (95% and 55%, respectively compared to 87% and 42%, respectively, in case of $\text{Al}_2(\text{SO}_4)_3$ addition) with flocculation improvement given by non-ionic polymer addition (40 mg/L) and optimal pH of 5. Equal equipment was used by Hamidi et al. (2007) for testing the effects of three types of coagulants (i.e., $\text{Al}_2(\text{SO}_4)_3$, FeCl_3 and FeSO_4) on the treatment of a MLL (BOD/COD of 0.15). Under specific operational conditions (300 rpm of rapid mixing, 50 rpm of slow mixing and 60 min settling time), FeCl_3 (at the dose of 600 mg/L) was found to be superior to other tested coagulants with removal efficiencies for suspended solids, colour and COD of 95%, 90% and 43%, respectively, at pH 4. Better removals were achieved at higher temperature. Comstock et al. (2010) investigated coagulation performance on leachate samples collected from old landfill sites in Florida, USA, characterised by low BOD/COD ratio (< 0.1), slightly alkaline pH and high concentrations of total nitrogen (TN). Leachate samples (1 L per beaker) were coagulated by using FeCl_3 , FeSO_4 and $\text{Al}_2(\text{SO}_4)_3$ (rapid mix at 200 rpm for 5 min, slow mix at 20 rpm for 20 min and settling for 60 min). FeSO_4 addition showed the best coagulation performance followed by $\text{Al}_2(\text{SO}_4)_3$ and FeCl_3 albeit levels of dissolved organic matter removal were relatively low. It was also observed that better performances were achieved for both salts (i.e., ferric and aluminium sulphate) when pH was adjusted to 5. A similar study was conducted by Liu et al. (2012) who treated

a MLL investigating the use of three iron-based coagulants: polyferric sulphate (PFS), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{Fe}_2(\text{SO}_4)_3 \cdot 7\text{H}_2\text{O}$. The obtained removal efficiencies for COD, colour, turbidity and humic acids (HAs) were, respectively, 56.4%, 63.4%, 89.8%, 70.4% for PFS, 68.7%, 93.3%, 98.9%, 80.2% for $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 55.9%, 74.7%, 94.1%, 53.6% for $\text{Fe}_2(\text{SO}_4)_3 \cdot 7\text{H}_2\text{O}$. In particular, results on the removal of recalcitrant compounds (i.e., COD and HAs) confirmed the suitability of coagulation and flocculation as a leachate pre-treatment prior to biological processes.

Several studies show good results in terms of MLL treatment by the combination of coagulation/flocculation process with other physical and/or chemical treatment technologies (Alejandro et al., 2011; Amor et al., 2015; Ishak et al., 2017; Papastavrou et al., 2009; Rivas et al., 2004; Vedrenne et al., 2012).

Taking the presented literature results into consideration, it can be inferred that:

- MAP precipitation is an effective method for ammonium removal from leachates (removal efficiencies close to 100% under optimum operational conditions). As the product of the process, struvite can be recovered and used as a fertiliser. However, since especially MLL is lacking in magnesium and phosphate, the external addition of these compounds to reach the optimum molar ratio significantly contributes to increase the overall cost of the process (see Di Iaconi et al., 2010). On the other hand, other chemical additions for pH adjustment to 7.5 – 9.0 can be avoided for MLL which is naturally characterised by slightly alkaline pH. Anyway, according to Cossu et al. (2018), MAP precipitation is currently too expensive to warrant widespread application. Lastly, COD removal has been shown not to be significantly affected by MAP precipitation so that treated leachate may require further steps of treatment.
- Coagulation and flocculation are suitable methods for leachate treatment since they have been proved to reduce concentrations of several contaminants (suspended solids, biodegradable and non-biodegradable organic compounds, ammoniacal nitrogen and heavy metals). However, removal efficiencies are insufficient for direct discharge (e.g., COD removal efficiency commonly ranges from 40% to 60%, Renou et al., 2008) and further treatments have to be applied to the effluent to meet discharge limits. Conversely, coagulation and flocculation are very effective in removing suspended solids and, therefore, they can be successfully applied prior to filtration processes to prevent membrane fouling. High capital costs related to

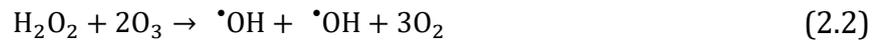
chemicals' additions (i.e., coagulants, flocculants and especially pH adjusting agents necessary for MLL treatment) and energy demand for highly intensive mixing, sensitivity of the process to pH and generation of residual sludge are the major drawbacks. In particular, the residual sludge concentrating removed pollutants should be treated prior to its disposal (Aguilar et al., 2002).

2.3.3. Chemical Oxidation (Advanced Oxidation Processes, AOPs)

Contaminants present in leachate (e.g., soluble organic, non-biodegradable and/or toxic substances) can be oxidised into carbon dioxide, water and inorganics or less harmful compounds through the treatment with oxidant agents such as chlorine, ozone, potassium permanganate and calcium hydrochloride (Torretta et al., 2017; Wiszniowski et al., 2006). With regard to organic compounds, only a limited part of them is broken down into the optimal end-products (i.e., water and carbon dioxide) after oxidation. Rather, organic residues can be either biodegradable or non-biodegradable compounds. Indeed, in many cases, chemical oxidation leads to COD reduction and concurrent BOD increase. For this reason, oxidation is frequently used as a pre-treatment to improve leachate biodegradability prior to further biological treatments (Cossu et al., 2018).

Many oxidation processes are based on the direct reaction of the oxidant with the contaminants. However, a different approach in which reactive oxidant species, namely •OH radicals, are generated directly in the wastewater has been developed (Torretta et al., 2017). The hydroxyl radical is known to be a strong oxidant. It is able to oxidise almost all reduced material present in wastewater. Only fluorine has a better oxidising power. The hydroxyl radical reacts with the dissolved compounds and, after a series of oxidation reactions, causes their complete mineralisation (Tchobanoglous et al., 2002). Processes which generates •OH radicals and exploits its oxidising power to treat wastewater are known as Advanced Oxidation Processes (AOPs). They involve the use of ozone (O₃) and hydrogen peroxide (H₂O₂) possibly along with ultra-violet radiation (UV), ultrasound (US) or a catalyst (e.g., Fe²⁺, Fe³⁺ and TiO₂) (Luo et al., 2020). The three most applied AOPs techniques are O₃/H₂O₂, H₂O₂/UV and O₃/UV (Torretta et al., 2017). The overall reaction

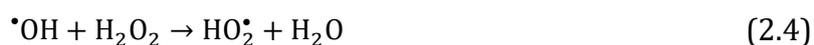
for the production of •OH radicals using O₃ and H₂O₂ is as follows (Eq. 2.2, Tchobanoglous et al., 2002):



Ozone can be generated from air by electric discharge. However, currently, the use of pure oxygen is preferred because of its cheapness and easier handling. Being a toxic gas, ozone should be used in closed tanks and removed before the discharge of exhaust air to environment (Ehrig and Robinson, 2010).

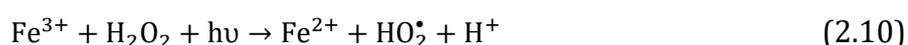
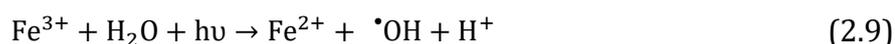
Tizaoui et al. (2007) compared the use of ozone alone and O₃/H₂O₂ for the treatment at laboratory-scale (150 mL glass reactor) of a leachate characterised by high COD and low biodegradability coming from a landfill facility located in Tunis (Tunisia). They observed that the presence of hydrogen peroxide (up to 2 g/L) enhanced COD removal in comparison with ozone alone (48% versus 27% after 1 h) and also the biodegradability (effluent BOD/COD of 0.7 starting from 0.1). Different conclusions were reached by Wang et al. (2010). Indeed, authors stated that, for the treatment of a MLL (Alberta, Canada), the effect of the addition of H₂O₂ on COD and colour reduction as well as increase in biodegradability improvement were lower and a dose of 2.6 g/L of ozone only was suggested. Another comparative study conducted by Wu et al. (2004) found that O₃/UV after coagulation was the most effective approach among the ozone-based AOPs for the enhancement of leachate biodegradability (BOD/COD from 0.1 to 0.5 at the applied ozone dosage of 1.2 g/L).

AOPs also include Fenton and photo-Fenton processes which have been proved to be the best AOPs applications according to the Zhou et al.'s review (2010) on AOPs for organic matter removal from leachate. Indeed, Fenton process is effective (49 – 89% of COD removal with COD ranging from 837 to 8894 mg/L), cost-effective (reagents are cheap), technologically simple (no sophisticated equipment) and environmentally safe (use of non-toxic chemicals) (Ameta et al., 2018; Zhou et al., 2010). It is named after H. J. Fenton (1894) and it consists of enhancing the oxidative potential of H₂O₂ through iron used as a catalyst under acidic conditions (2.0 – 4.5 for leachate treatment, Cossu et al., 2018). The reactions involved in Fenton process are (Eqs. 2.3, 2.4, 2.5, 2.6, 2.7 and 2.8, Ameta et al., 2018):



•OH radicals are responsible for oxidation. Then, coagulation occurs due to the formation of ferric-hydroxo complexes. The optimal molar ratio of H₂O₂ to Fe²⁺ appears to be from 1.5 to 3.0 according to research (Zhou et al., 2010).

Also, photo-Fenton process implies the combination of H₂O₂ and UV with a Fe²⁺ or Fe³⁺ ion which produces more hydroxyl radicals so that increasing the degradation rate of organic pollutants (Eqs. 2.9 and 2.10, Ameta et al., 2018):



Several restriction during hydrogen peroxide storage and transport (pumping) must be considered in order to prevent its breakdown before the usage (Ehrig and Robinson, 2010).

Lopez et al. (2004) carried out the Fenton process on a real MLL with the aim of improving its biodegradability prior to a subsequent biological treatment. The highest COD removal efficiency (starting from a concentration of 10540 mg/L) was 60% reached by using high reagent dosages (i.e., 10000 mg/L and 800 mg/L of H₂O₂ and Fe²⁺, respectively), while the maximum biodegradability increase (BOD/COD up to 0.5) was obtained at pH = 3.0 (adjusted with H₂SO₄), H₂O₂ and Fe²⁺ concentration of 3300 mg/L and 275 mg/L, respectively, after 2 h of reaction time. Finally, treated leachate was subjected to coagulation (by adding Ca(OH)₂ and a cationic polyelectrolyte) to remove residual ferric ions which could have disturbed the subsequent biological process. Two AOPs (i.e., photo-Fenton Fe²⁺/H₂O₂/UV and H₂O₂/UV) were used also by De Morais and Zamora (2005) for the same purpose of improving MLL biodegradability. Regardless of the treatment, BOD/COD ratio reached the value of about 0.4, from an initial value of 0.1. As a consequence, authors confirmed the possibility of the application of a photochemical-

biological integrated system for MLL treatment. Similar results were achieved by other studies (Cortez et al., 2011).

Zazouli et al. (2012) compared the application of two different Fenton processes ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ as the reagents, respectively) and photo-Fenton for the treatment at laboratory-scale of a leachate collected from the old sanitary landfill in Qaem-Shahr (Iran). Results showed that optimum concentrations of H_2O_2 were 5 g/L and 3 g/L when $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were used as the reagents, respectively. Regardless of the process, 8:1 was the optimum ratio of $\text{H}_2\text{O}_2:\text{Fe}^{2+}$ or Fe^{3+} anyhow. For $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ Fenton and photo-Fenton: i) COD removal efficiency was 69.6%, 65.9% and 83.2%, respectively, ii) optimum pH value was 3, 5 and 3, respectively and iii) optimum contact time was 150, 90 and 120 min, respectively. Also, biodegradability (previously being of 0.012, BOD/COD) was increased after all processes with the highest value measured in the photo-Fenton one (BOD/COD of 0.173). Fenton's oxidation was the most effective treatment among those investigated in Gotvajn et al.' study (2009) (see Paragraph 2.3.2) in which COD content in a mature industrial leachate was reduced by 86% for a $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ ratio of 1/10, 30 min and 40°C.

According to Abu Amr and Aziz (2012) the combination of ozonation and Fenton process ($\text{O}_3/\text{H}_2\text{O}_2/\text{Fe}^{2+}$) improve COD removal from MLL. Authors reached a removal efficiency of 65%, 98%, and 12% of COD (starting from a concentration of 2180 mg/L), colour and ammoniacal nitrogen, respectively, after 90 min of ozonation and by dosing 1700 mg/L of H_2O_2 and 2800 mg/L of Fe^{2+} at pH = 7. Moreover, this study confirmed that a systematic and stable oxidation of ammoniacal nitrogen through AOPs is frequently difficult to achieve (Cossu et al., 2018).

In summary, AOPs has been reported to be able to degrade a wide variety of refractory compounds in landfill leachate. Highly reactive hydroxyl radical ($\bullet\text{OH}$) plays a key role in AOPs; it can be produced through O_3 -based or Fenton oxidation systems. On the one hand, AOPs are suitable especially for MLL treatment prior to further biological processes as their application results in considerable biodegradability improvement. In fact, non-biodegradable or refractory organic matter is broken down and transformed to more biodegradable compounds. On the other hand, as in case of coagulation and flocculation

(Paragraph 2.3.2.), reaching the acidic condition necessary for Fenton reactions (i.e., pH of 2.0 – 4.5) requires far more chemicals' addition in MLLs than in young ones.

Although removal efficiencies of biodegradable and non-biodegradable organic compounds are considerable (up to 89% of COD removal, Zhou et al., 2010), AOPs are hardly able to remove ammoniacal nitrogen as studies confirmed. For this reason, further physical-chemical or biological treatments are necessary to meet nitrogen discharge limits.

The excessive presence in the treated leachate of ions added as either catalysts or pH adjusting agents (e.g., Fe^{2+} , Fe^{3+} , SO_4^{2-} , Cl^-), even if environmentally safe, is likely to disturb the subsequent biological processes. For instance, Fe^{2+} exceeding specific concentrations (i.e., 20 g/L) can significantly inhibit anaerobic digestion process (Guo et al., 2019). Chloride, as well, may cause strong inhibition of the anaerobic process if present in concentrations ranging from 4 to 9 g/L (Viana et al., 2012). For this reason, coagulation is recommended to be applied to treated leachate (see Lopez et al., 2004) with the aim of reducing residual ions prior to further biological treatments.

From an economical point of view, the high demand of oxidant (O_3 or H_2O_2) and electrical energy (UV lamps) may represent a drawback of AOPs technology. However, considering that O_3 -based and Fenton oxidations has been proved to considerably increase leachate biodegradability, using AOPs as a leachate pre-treatment prior to anaerobic digestion can improve biological process performance in terms of biogas generation (i.e., energy production).

2.3.4. Air Stripping

Air stripping treatment consists of aerating leachate with air. Through the stripping process, dissolved gaseous or volatile compounds are transferred from the water to treat to the pumped air. They firstly move from the bulk liquid solution to the water-air interface and, subsequently, to air through molecular diffusion. The process is slow but the transfer rate may be increased by turbulent conditions and continuous surface exchange between water and air. Air stripping is mainly carried out in aerated tanks or

stripping towers. The exhaust gas containing the removed compounds may be collected and treated before release (Cossu et al., 2018).

Generally, stripping is employed to remove volatile organic compounds (VOCs) and ammonia from wastewater. However, since VOCs are usually present in landfill gas, their concentration in leachate is considerably low. For this reason, ammonium (NH_4^+) is the main target pollutant of air stripping. As shown in Paragraph 1.1.1.3., ammoniacal nitrogen content is the result of a chemical equilibrium dependent on temperature and pH conditions. To implement an efficient air stripping treatment, ammonium should first be converted into gaseous ammonia (NH_3) by means temperature and pH adjustment. Accordingly, ammonia removal rate due to air stripping increases with increasing pH value or air temperature. In most cases, the removed ammonia can be recovered through sulphuric acid sorption: when air outcoming from the stripping tower comes into contact with sulphuric acid, ammonia reacts to produce ammonium sulphate (according to the Eq. 2.11) which can be directly used as a fertilizer or in the chemical industry (Cossu et al., 2018; Ehrig and Robinson, 2010).



Ferraz et al. (2013) conducted a study at pilot-scale on the ammonia removal and recovery from a MLL collected from a sanitary landfill located in Sao Paulo, Brazil. The first step of pH adjustment to 11 by $\text{Ca}(\text{OH})_2$ addition resulted in a preliminary coagulation and removal from leachate of colour and heavy metals (i.e., Zn, Fe and Mn) (removal efficiencies of 82% and 70 – 90%, respectively). The initial ammonia content of about 1200 mg/L was then reduced to 10 mg/L using an air flow rate of 4500 L/h for 24 h on 12 L of leachate sample. In this operational conditions, no ammonia recovery from the effluent was possible due to an excessive turbulence induced on the limited volume of sample. Treating a larger volume of sample (100 L), the air stripping tower removed 88% of ammoniacal nitrogen after 72 h of aeration with the same air flow rate and, in this case, 87% of the stripped ammonia was recovered in absorption units filled with tap water. Gotvajn et al. (2009) performed air stripping (air flow of 120 L/h) on a mature industrial leachate. At pH 11, COD and ammonia were reduced by 33% and 84%, respectively.

Abood et al. (2014) used air stripping in combination with coagulation as leachate pre-treatment steps with the aim of reducing both ammonia content and recalcitrant organic

matter prior to further biological processes. Ammonia removal efficiency was 96% when leachate sample was subjected firstly to aeration (air flow rate 10 L/min at pH 11 for 3 h) and then agitation (2 h at pH 11.5 in 150 s⁻¹ gradient velocity). As a coagulant, PFS was added to the effluent of stripping process at a dosage of 1200 mg/L at pH 5. Overall, the combined stripping and coagulation system allowed to reduce NH₃-N, COD, BOD and turbidity by 96%, 71%, 56% and 92%, respectively, after 50 h of treatment.

Air stripping process is widely accepted as one of the most promising methods to remove ammonium from leachate (removal efficiency > 80% under optimum operational conditions). Its application is technically simple but expensive at the same time. First of all, to implement an efficient air stripping treatment, a chemical pre-treatment of the influent is necessary to promote the shift of ammonium to gaseous ammonia. In fact, pH must be adjusted to highly alkaline values (~ 11) by adding soda or lime. Actually, being MLLs already characterised by high initial pH values, this preliminary operation does not significantly affect the overall process cost. Secondly, to improve removal efficiencies, air temperature should be increased at the expense of energy consumption. The cost of temperature control can account for up to 50% of the total process cost (Liu et al., 2015). Finally, according to theoretical calculations, exceedingly high amount of compressed air is required and cost rises as a consequence. For instance, at pH and temperature of 11 and 15°C, respectively, approximately 21000 m³ and 230000 m³ of air is required to reach ammonium removal efficiencies of 90% and 99%, respectively (Cossu et al., 2018). Nevertheless, it is noteworthy that stripping processes are increasingly cost-effective in presence of very high concentrations of ammonium in leachate. On the other hand, being ammonium basically the only target pollutant is another drawback of air stripping process as further treatments must be applied to the outcoming leachate to reduce the residual organic and inorganic toxic compounds. For these reasons, the use of this technology for leachate treatment can be economically advisable especially in three cases: i) to treat large volumes of leachate with high ammonium concentrations (>3.000 mg/L), ii) prior to biological treatment to limit the toxicity of ammonia for microorganisms and iii) if ammonium (e.g., as a salt) is recovered from exhaust air and used in agricultural or industrial sector (Cossu et al., 2018).

2.3.5. Adsorption

Adsorption implies the transfer of organic or inorganic substances from a liquid phase onto the surface of a solid phase. Adsorption is a widely employed technique for removal of recalcitrant organic compounds and heavy metals from landfill leachate (Luo et al., 2020). Generally, adsorption materials are characterized by large specific surface area.

In the field of wastewater treatment, activated carbon (AC) is the most frequently used material due to its large specific surface area (800 – 1200 m²/g), microporous structure and high degree of surface reactivity which make it able to adsorb about 0.2 g of total organic carbon (TOC) per gram of AC (Bansal and Goyal, 2005; Luo et al., 2020). AC has showed to be suitable for the removal of organic and inorganic pollutants. According to Kurniawan et al.'s review (2006), AC is effective in the removal of non-biodegradable compounds from leachate (more than 90% COD removed with initial concentrations ranging from 940 to 7000 mg/L) and, moreover, Foo and Hameed (2009) stated that in some cases AC has also revealed the capacity of reducing ammonia nitrogen (Abdul Aziz et al., 2004) even if Cossu et al. (2018) report a relatively low adsorption capacity for ammonium due to the AC's nonpolar surface.

AC derives from various carbon sources (coal, wood, peat etc.) and it is the result of their pyrolysis. AC preparation consists of two steps: carbonisation and activation. The former is carried out on the carbonaceous raw material at temperature lower than 800°C in an inert atmosphere. Most of the non-carbon elements such as oxygen, hydrogen, and nitrogen are converted to volatile gaseous species and eliminated. Later, activation process occurs at 800 – 900°C in an atmosphere of air, CO₂ or steam with the aim of forming randomly distributed pores of various sizes and shapes in the internal surface of carbonised material. All carbonaceous materials can be transformed into AC, although the properties of the final product are different on the basis of the type of raw material used and the operational conditions of both carbonisation and activation steps (Bansal and Goyal, 2005). AC typically is used in form of either pulverized activated carbon (PAC) or granulated activated carbon (GAC). The former is characterised by particle sizes of less than 150 µm with a main fraction between 4 and 70 µm. For this reason, PAC is dosed into the wastewater as a suspension in a mixing and reaction tank. Instead, GAC has a particle size of 0.2 – 5 mm and it is used as a filter where leachate flows through. GAC carbon can be thermally reactivated by means of the oxidation of the adsorbed organic

substances which are subsequently incinerated. For each reactivation, less than 10 – 20% of AC mass is loss. PAC is cheaper than GAC but it cannot be reactivated. Thus, at the end of the treatment stage, PAC should be incinerated (Cossu et al., 2018).

Numerous studies over the years have testified the efficiency of AC in pollutants reduction from MLLs. A previously biologically treated leachate having the characteristics of a MLL, collected from the Goslar landfill, Germany, was treated using GAC illustrating a COD removal of 91% (initial concentration of 940 mg/L) (Morawe et al., 1995). Likewise, PAC was also showed to be suitable for COD removal (95%, initial concentration of 5690 mg/L) from a leachate collected after recirculation (Diamadopoulos, 1994). Wasay et al. (1999) demonstrated the effectiveness of GAC adsorption towards heavy metals: for pH ranging from 6.0 to 7.7, removal efficiencies of 80 – 96% for several heavy metals (Cd^(II), Cu^(II), Cr^(III), Mn^(II), Pb^(II) and Zn^(II)) at initial concentration of 184 mg/L were determined. Gotvajn et al. (2009) improved the biodegradability of an old industrial leachate (see Paragraph 2.3.2.) from 0.18 to 0.56 by removing 40% of COD through GAC addition at a dose of 10 g/L. Modin et al. (2011) demonstrated at laboratory-scale that GAC removed from a real landfill leachate more than 90% of Co, Cr, Cu, Fe, Mn and Ni while other metals (i.e., Ca, Pb, Sr and Zn) to a lesser extent.

AC adsorption was also used in combination with other physical-chemical treatments. Li et al. (2010) combined coagulation/flocculation process with PAC adsorption (at a dose of 10 g/L) for a MLL treatment achieving removal efficiencies of 86%, 97.6% and 99.7% for COD, Pb and Fe, respectively. Eljaiek-Urzola et al. (2017) used carbon adsorption (GAC) to improve COD removal from a real MLL after an oxidation (H₂O₂) treatment step achieving an overall 97% of COD removal. Similar results were obtained by a previous study on an analogous integrated treatment of MLL (Kurniawan and Lo, 2009). From the combination of AC adsorption and membrane processes (MF, UF, fine-UF), Zielińska et al. (2020) achieved about 90% of COD and colour removal efficiency.

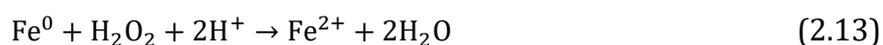
Besides AC, a series of materials can be used in the adsorption process: zeolite, activated alumina, lignite coke, bentonite, limestone among others (Cossu et al., 2018). Aziz et al. (2004) carried out on site a filtration of a MLL through columns filled with limestone to attenuate total iron content. As a result, 90% of Fe was removed at a retention time of 57.8 min and surface loading of 12.2 m³/m²·d. In a comparative study, Halim et al. (2010) tested the adsorption capacity of zeolite, GAC and of a composite material (made up of

45.9% zeolite, 15.3% limestone, 4.4% AC and rice husk carbon and 30% of Portland cement used as a binder) towards ammoniacal nitrogen and COD present in a real MLL (starting concentration of 1891 mg/L and 2338 mg/L, respectively). It emerged that the composite medium and GAC were the most effective materials in terms of ammonia and COD adsorption capacity. The former showed removal rates of 24.4 mg/g and 23.0 mg/g towards ammonia and COD, respectively, while the latter absorbed 6.1 mg of ammonia per g of GAC and 37.9 mg of COD per g of GAC. In the aforementioned Modin et al.'s study (2011), bone meal and iron fines showed their capacity of adsorption of heavy metals: the former removed more than 90% of Sr and Mn and approximately 80% or more of Cr, Fe and Hg; the latter removed only Ca by over 90% and other metals were removed to some extent. Zeolite at concentration of 1 g/L was used in order to integrate the treatment of a leachate (collected from a landfill located in Coquimbo, Chile) with AOPs (Poblete et al., 2019). As a result of 2 h of stirring at 150 rpm, adsorption used as a post-treatment led to total reductions in COD, ammonium, and chloride of 36%, 99%, and 18%, respectively.

Recently, the use of Permeable Reactive Barriers (PRBs) have been extensively studied as a remediation method for groundwater pollution. This technology involves the emplacement in a contaminated soil of a barrier which is permeable to the polluted groundwater (plume) flowing through it. The barrier contains reactive materials which intercept and treat the contaminants of the plume. The filtration through the barrier is mainly due to the hydraulic gradient and therefore this technique does not require any energy for its operation (passive technique). The effectiveness of PRBs for the removal of a wide range of contaminants has been demonstrated by numerous literature studies (Moraci et al., 2015; Obiri-Nyarko et al., 2014). Granular materials such as GAC, Zero Valent Iron (ZVI) and zeolite are the most frequently employed reactive media in PRBs applications. In the context of this thesis, PRBs literature has been used to design an active filtration system for MLL pre-treatment.

ZVI is widely used as a reactive medium for PRBs because it is able to treat a wide range of pollutants, both organic and inorganic (e.g., toxic organic compounds and heavy metals) (Obiri-Nyarko et al., 2014). ZVI is non-toxic, abundant, cheap, easy to produce, and its reduction process requires little maintenance. ZVI is a reactive metal (- 0.44 V of standard redox potential) and thus it acts as an effective reductant. In aqueous solutions under oxic conditions, ZVI corrosion occurs through an electrochemical mechanism which consists

of ZVI dissolution (anodic process) and oxygen reduction (cathodic process) with release of Fe²⁺ (Eq. X) which plays a key role in the formation of iron hydroxides/oxides. This process is facilitated by acidic conditions. ZVI is expected to remove contaminants from contaminated solutions through mechanisms of reduction, adsorption onto its surface or onto its corrosion products and co-precipitation with its precipitating oxides (Fu et al., 2014). Reduction involves the transfer of electrons from ZVI to contaminants which results in their transformation into non-toxic or less toxic species. Adsorption implies the accumulation of contaminant molecules either on ZVI surface or on produced iron hydroxides/oxides. However, in contrast to AC, ZVI has a lower specific surface area and, indeed, simple adsorption is not the main removal mechanism. Co-precipitation refers to an unspecific removal mechanism wherein contaminants present in the solution are progressively incorporated in the matrix of precipitating iron compounds (i.e., iron hydroxides/oxides). On the basis of redox conditions and ZVI dissolution yields, a wide variety of porous and highly adsorptive iron hydroxides (e.g., Fe(OH)₂, Fe(OH)₃, ferrihydrites) may be present in the aqueous solution. However, they are further transformed into various less porous and less adsorptive oxides (e.g., FeO, Fe₃O₄, Fe₂O₃). In laboratory-scale experiments, co-precipitation mechanism of contaminant removal has been proved to dominate simple adsorption (Noubactep, 2007). Furthermore, a series of organic compounds can be oxidised and degraded by ZVI in presence of dissolved oxygen. Indeed, as shown in Eqs. 2.12, 2.13 and 2.14, ZVI transfers two electrons to O₂ to produce H₂O₂ which can be reduced to water by another two-electron transfer from ZVI. Equally to Fenton reactions (see Eqs. 2.3, 2.4, 2.5, 2.6, 2.7 and 2.8), the combination of H₂O₂ and Fe²⁺ can generate hydroxyl radicals which has strong oxidising capability towards a variety of organic compounds. Reactions cause the increase of pH due to the H⁺ consumption and OH⁻ generation (Fu et al., 2014; Jun et al., 2009).



The major limitations of ZVI include: i) low surface reactivity due to an intrinsic passive layer generated in ZVI manufacturing process and low specific surface area, ii) reactivity loss with time due to growing surface passivation caused by precipitation of metal hydroxides and metal carbonates, iii) slower ZVI corrosion with consequent lower

effectiveness of removal mechanisms under alkaline conditions (as in case of MLL used as contaminated solution) even if this can imply the functionality of the filter for a longer period of time. In order to overcome this issues, various countermeasures can be taken (e.g., ZVI pre-treatment, coupling ZVI with other adsorptive materials and recovery of exhausted ZVI) (Guan et al., 2015).

Different authors have evaluated the application of PRBs either for remediation of landfill leachate-contaminated groundwater (Jun et al., 2009; Komnitsas et al., 2013; Zhou et al., 2014) or in landfill leachate treatment system as it represents a cost-effective method for in-situ leachate treatment. The vast majority of these studies have been carried out on young acidic leachates, especially using activated filter as a pre-treatment prior to further biological processes (Bartzas et al., 2006; Bilardi et al., 2020, 2019, 2018). Conversely, to date, few studies on their application for treatment of intermediate or MLLs have not been conducted. In the following, some instances are provided.

Van Nooten et al. (2008) simulated at laboratory-scale the use of a system involving different sequencing PBRs for the removal of ammonium, adsorbable organic halogens (AOX) and COD (initial concentrations of 200 – 400 mg/L, 0.70 mg/L and 395 mg/L, respectively) from leachate originating from a 40-year-old Belgian landfill. Ammonium was almost completely removed by the first compartment in which the biological process of nitrification-denitrification occurred (see Paragraph X). As expected, AOX and COD were unaffected by biological process and therefore a second barrier filled with GAC was used. After 54 days of operation, leachate concentrations of AOX and COD were respectively reduced to 0.24 mg/L and 203 mg/L meeting the regulatory discharge limits. Jun et al. (2009) showed the capacity of a laboratory-scale PBR filled with mixture of ZVI and zeolite of removing Zn, Mn, Mg, Cd, Sr, and NH_4^+ from a landfill leachate (taken from Shibailing Landfill in Changchun, China) by 97.2%, 99.6%, 95.9%, 90.5% and 97.4%, respectively. Moreover, authors observed a rise in BOD/COD (from 0.32 to 0.60) of the leachate after the treatment which can be attributed to ZVI capacity of oxidising recalcitrant organic substances to simpler biodegradable organic compounds (see Fenton reactions). Zhou et al. (2014) demonstrated the capacity of a granular filter made up of ZVI, AC and zeolite of removing organic and inorganic compounds which are peculiar in landfill leachate composition. Authors conducted the tests at laboratory-scale employing plexiglass columns filled with granular materials. Leachate coming from a closed landfill

in Guangdong, China, was pumped at a rate of 0.5 mL/min and tests were carried out at room temperature (20°C). At the optimum weight ratio of 5:1:4 (ZVI:zeolite:AC), average reductions in COD, TN, ammonium, Ni, Pb and 16 polycyclic aromatic hydrocarbons (PAHs) were 55.8%, 70.8%, 89.2%, 70.7%, 92.7% and 94.2%, respectively. Under these operational conditions, tested filter maintained adequate hydraulic conductivity. Authors stated that ZVI and AC were the main contributors to the removal of both organic and inorganic contaminants.

The main drawback of PBR application lies in its limited longevity. Indeed, it is extensively reported that PBR performance declines with time as the surface reactivity of the granular activated filters decreases. This is principally due to the accumulation of precipitates (in case of ZVI PBR, mainly iron hydroxides, other iron corrosion products, and salts such as carbonates). These compounds clog the pore of the PBR in the long term affecting the performance of the treatment in terms of both contaminant removal efficiency and hydraulic permeability of the barrier. As a consequence, contaminated solution may flow along preferential higher-permeability paths bypassing the barrier itself (Moraci and Calabrò, 2010). In order to overcome this issue, mixtures of different granular materials can be used. Recently, it is investigating the behaviour of PBR filled with conventional granular materials (i.e., ZVI, GAC, zeolite) along with non-expansive and abundant aggregates (e.g., sand, gravel, pumice and lapillus) as admixing agents. The addition of aggregates not only would preserve PBR reactivity and permeability in the long term but also would partially replace ZVI or GAC with consequent cost saving (Calabrò et al., 2021).

To sum up, besides the evident benefits of PBR technology in terms of organic and inorganic contaminant removal, prior to any PBR full-scale application, it is essential to calculate how reactivity and permeability change over operational time. Typically, preliminary batch experiments are carried out in order to study the suitability of potential granular activated materials for removal of specific contaminants. Subsequently, chosen materials are tested in laboratory-scale columns simulating full-scale PBR under real operational conditions (e.g., inflow rate, residence time, temperature). In this stage, the durability of the barrier is evaluated (Calabrò et al., 2021).

2.4. Biological Processes

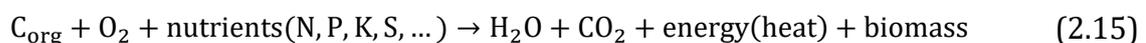
As already mentioned, biological processes are usually not the most appropriate treatments for MLL because of its peculiar characteristics which include low biodegradability (BOD/COD < 0.1 – 0.2), presence of recalcitrant organic compounds, heavy metals and ammonia. For this reason, additional chemical and/or physical technologies are often necessary either as pre-treatments to reduce organic and inorganic substances toxic for biological processes or post-treatments to meet the discharge values. Irregular generation of leachate over time is another feature which could influence biological processes' efficiencies since most biological treatments generally operate better and more reliably at relatively constant input rates.

Basically, biological processes operates under two main different conditions: aerobic and anaerobic.

2.4.1. Aerobic processes

In aerobic treatments, microbes consume organic materials as their energy source in presence of oxygen (Kurniawan et al., 2010). Aerobic processes require high energy input to provide oxygen while the heat produced during the process (microbial reactions are exothermic) cannot be used. As residue of the process, excess sludge is generated and it must be properly treated. In the following, some instances found in literature of aerobic treatments of MLL are reported.

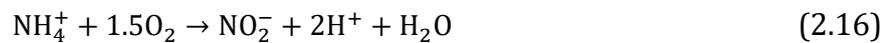
Given the generalised equation of aerobic biological degradation of organic carbon (Eq. 2.15, Ehrig et al., 2018):



the limits of aerobic process application for the treatment of MLL are evident. First of all, MLL is lacking in both easily biodegradable matter (C_{org}) which is converted to biomass (from 20% to 50% of the initial C_{org}) and phosphorous which is required for the production of new biomass (about 0.3 – 0.5% of degraded COD). Secondly, leachate is often characterised by high concentrations of compounds potentially inhibitory for biomass (e.g., humic substances, toxic organic substances, ammonia, heavy metals, chloride). Nevertheless, aerobic processes are known to be occasionally capable of

tolerating the presence of toxic compounds (Christensen et al., 1989). Finally, nitrogen reduction is also limited as organic nitrogen in stabilised leachate may be partly incorporated into the bio-refractory humic matrix.

Instead, the removal of ammonium (inorganic nitrogen) can be achieved with a two-step biological treatment process: nitrification and denitrification (Canziani et al., 2006; Ehrig et al., 2018). The first step consists in the oxidation of ammonia to nitrite (NO_2^-) by ammonia oxidising bacteria (AOB, *Nitrosomonas* species) (Eq. 2.16) followed by an oxidation of nitrite to nitrate (NO_3^-) by nitrite oxidising bacteria (NOB, *Nitrobacter* species) (Eq. 2.17).



pH of the process should be above 6.5 but not higher than 7.8 – 8.0 (risk of ammonia inhibition). The stoichiometry of the reactions highlights that 2 mol of oxygen is required for the complete oxidation of 1 mol of ammonia to nitrate. Instead, the so-called partial nitrification occurs when ammonia is oxidised to nitrite (first reaction) and no further transformations take place. Compared to complete nitrification, approximately 25% oxygen demand is saved as 1.5 mol of oxygen per mol of ammonia is required in this case.

The second step (i.e., denitrification) represents the reduction of nitrate to gaseous nitrogen (N_2) in presence of a readily biodegradable carbon source (methanol, CH_3OH , in the Eq. 2.18):

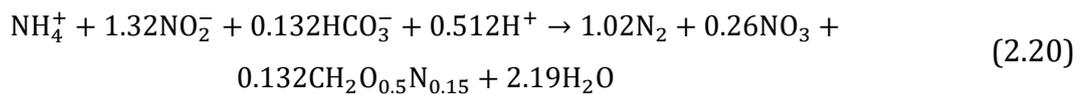


Since denitrification is basically the anoxic degradation of organic carbon in which oxygen is substituted by NO_3^- , in this case, dissolved oxygen (> 1 – 2 mg/L) could inhibit the process. Approximately 4.5 kg of biodegradable COD is required per kg of N denitrification ($\pm 20 - 30\%$). In this regard, being long-term methanogenic leachate poor in organic degradable carbon, external organic carbon source has to be supplied. As a result, process becomes cost intensive. Alternatively, denitrification can also occur in presence of nitrite (Eq. 2.19):



Nitrite reduction to nitrogen gas requires less organic substrate (40% in case of methanol) and it is usually 1.5 – 2 times faster than reaction with nitrate. Thus, the combination of partial nitrification and denitrification at the expense of nitrite may be attractive. This condition can be achieved by favouring the growth of AOB over NOB by manipulating operational conditions such as pH, temperature, free ammonia or dissolved oxygen concentration (Canziani et al., 2006; Jianlong and Ning, 2004).

To further reduce oxygen demand (and amount of energy for aeration, as a consequence), the so-called anammox process (i.e., anaerobic ammonium oxidation) has been successfully developed. It consists of a complete conversion of ammonium to nitrogen gas without the addition of organic matter carried out by autotrophic microorganisms (Eq. 2.20). By this way, 100% biodegradable organic carbon and at least 50% oxygen are saved compared to conventional nitrification/denitrification process (Sri Shalini and Joseph, 2012).



Anammox process occurs under anoxic conditions in which nitrite is the preferred electron acceptor but nitrate, iron III, sulphate and bicarbonate may be as well. Good anammox activity was observed in processes running under 30 – 37°C in a pH range of 6.7 – 8.5. The optimum wastewater C/N ratio and HRT are below 1 and 1 day, respectively (Sri Shalini and Joseph, 2012). The practical application of anammox process for leachate treatment has to deal with some hindrances: i) high concentration of organic matter may severely inhibit anammox bacteria, ii) excessive presence of nitrogen may lead to the release of free ammonia which also act as an inhibitor to anammox bacteria and iii) the maximum theoretical TN removal efficiency is about 90% thus further treatments are possibly required to meet the discharge standards. For all these reason, several studies on the optimisation of anammox process for leachate treatment are in progress (Sri Shalini and Joseph, 2012; J. Ye et al., 2019).

In the following, some leachate treatment technologies lying in the aforementioned biological processes are discussed on the basis of different reactors' configurations.

2.4.1.1. *Activated Sludge*

The process is carried out in an aeration tank where the influent undergoes aerobic treatment in combination with a sludge separation unit (clarifier) in which produced sludge is separated: a part (produced biomass) is recirculated to the aeration tank, the other is destined to treatment (excess sludge) while clarified water is released as the effluent of the process. This procedure allows to uncouple the Solid Retention Time (SRT) from the Hydraulic Retention Time (HRT) (Ehrig et al., 2018). Activated sludge represents the key process of the Wastewater Treatment Plants (WWTPs).

In general, landfill leachate is commonly co-treated with domestic sewage at conventional WWTPs, especially in Italy (Calabrò et al., 2018). Leachate transport represents one of the major issues of this method since oftentimes the distance between the landfill facility and the treatment plant is considerably long. For this reason, leachate transport cannot be accomplished by sewers but rather by tanker trucks. This contributes to increase the overall cost of the treatment which is around 50-100 €/m³ of treated leachate (in Italy) (Calabrò et al., 2018). Moreover, as already mentioned, especially MLL is rich of recalcitrant organic matter and inorganic pollutants as ammonia, heavy metals and chloride. These features may make it unsuitable for co-treatment with domestic sewage since the usual biological activated sludge process could suffer from partial or severe inhibition. Actually, since leachate quantities should be small compared to influent volumes for a WWTP, dilution of difficult-to-treat substances can occur (Townsend et al., 2015). Nevertheless, by this way, recalcitrant compounds may concentrate in the excess sludge potentially preventing its further recovery as fertiliser. In this context, leachate pre-treatment on-site may be necessary (Townsend et al., 2015). An argument in favour of leachate co-treatment with domestic sewage at WWTPs is the supply of two elements essential to the activated sludge process: nitrogen (brought by leachate) and phosphorus (brought by sewage) (Gao et al., 2014; Renou et al., 2008).

Del Borghi et al. (2003) aerobically treated by activated sludge process at pilot-scale two leachates with different biodegradability mixed with municipal wastewater. The used leachates were collected from two different areas of a landfill operating in Genoa (Italy): the leachate from the older part was characterised by low biodegradability in contrast to that coming from the younger section. The mixture employed for tests contained leachates in the ratio 9:1 (old:young). Both wastewater and sludge used for the aerobic

treatment came from a WWTP located in Genoa. Preliminary batch tests suggested to dilute leachates' mixture into wastewater according to a ratio of 1/10 in accordance with other studies (Cecen et al., 2001; Diamadopoulou et al., 1997). The best results in terms of both COD and ammonium removal rates (126 mg/L·h and 51 mg/L·h, respectively) were obtained using a previously acclimated sludge at a hydraulic residence time of 4 h. As a result, authors claimed that old leachate, diluted with young one, can be effectively treated by activated sludge process. Pilot-scale activated sludge reactors were also implemented by Ferraz et al. (2016) for the co-treatment of MLL and domestic wastewater. The leachate was collected from a Brazilian sanitary landfill and was pre-treated through air stripping to reduce ammoniacal nitrogen. Among different leachate – wastewater mixtures, the best removal efficiencies were obtained for a volumetric ratio of leachate of 2%. Under a continuous-flow regime (HRT of 24 hours and SRT of 20 days), the slowly biodegradable organic matter present in leachate was reduced by 51% primarily due to partial biodegradation rather than the dilution or absorption of organics on the sludge as spectroscopic analysis confirmed. In a previous study, Campos et al. (2014) achieved the same conclusions with a similar experiment. Chen et al. (2016) performed a pilot-scale activated sludge process with the aim of removing TN from a MLL coming from a municipal landfill plant closed in 2004 in South China. The entire process was carried out in a single tank composed of four equal chambers. Firstly, a stable partial nitrification occurred with high ammonium removal (> 95% from an initial average concentration of 1425) and nitrite accumulation due to *Nitrosomonas* species detected by microbial community analysis. Partial nitrification was realised under low dissolved oxygen levels (0.1 – 0.5 mg/L), low influent NH₄⁺-N loading (influent flow rate of 60 L/d) and HRT of 22.7 days. Then, denitrification led to TN removal efficiency of 66% in spite of low carbon source. However, authors stated that extra dosage of carbon to improve nitrogen removal is necessary.

What emerges from the aforementioned studies is that activated sludge process represents a feasible and effective treatment for MLL along with municipal wastewater. However, it appears to be possible only if leachate is diluted with wastewater at very low ratios (e.g., 1/10 in Del Borghi et al., 2003, 2% v/v in Ferraz et al., 2016). Moreover, the application of activated sludge typically requires the addition of readily biodegradable carbon source to complete denitrification.

2.4.1.2. *Membrane Bio-Reactor (MBR)*

MBR is an alternative to the conventional activated sludge plant which combines membrane separation technology (see Paragraph 2.3.1.) with biological process. Basically, MBR consists of an aeration tank equipped with submerged membrane modules which allows the separation of solids from the effluent in replacement of the clarifier unit (Ehrig et al., 2018). In this system, organisms such as nitrifiers or organisms which are able to degrade slowly biodegradable substances are not washed out, contrary to conventional biological processes, but they remains in the tank thanks to membrane separation. MBRs have been widely applied at full-scale on industrial wastewater treatment and some plants have been adapted to leachate treatment (Renou et al., 2008).

Canziani et al. (2006) employed a MBR (500 L oxidation tank equipped with an UF ceramic membrane) for the partial nitrification of a real leachate (collected from an old Italian landfill). The second step of denitrification was performed in a moving-bed biofilm reactor (MBBR), namely a tank filled with floating plastic media used as supports for biofilm growth. Likewise Chen et al. (2016) (see activated sludge section), authors stated that it was possible to oxidize more than 95% of influent TN to nitrite with a dissolved oxygen concentration lower than 0.5 mg/L. The ammonium concentration at the effluent was observed to be always lower than 50 mg/L. The addition of an adsorbent (i.e., PAC) in MBR resulted in a mean removal value of TN and ammoniacal nitrogen around 91% and 88%, respectively, from a MLL in Peyravi et al.'s study (2016). Moreover, COD removal efficiency reached 75% and it was improved up to 94 % by integrating a post-treatment NF process. Authors claimed that PAC addition increased biological process performance by both reducing the toxic effects of COD on microorganisms and acting as a reaction site for substrates and microorganisms. Yan et al. (2018) investigated the possibility of achieving biological nitrogen removal from MLL (low C/N ratio, < 3) in a laboratory-scale aerobic/anoxic MBR. In order to do this, the addition of two kinds of acidogenic liquids (i.e., fermented from oil-removed food waste and oil-added food waste) as external carbon sources was tested separately. At the optimum conditions of C/N > 7, 25°C and HRT 7 days and by using acidogenic liquid fermented from oil-removed food waste, the overall process allowed to meet the local (China) discharge standards of NH₄⁺-N and TN. Also, COD concentration was well reduced to 50 – 55 mg/L allowing effluent discharge without further physical-chemical treatment.

Especially this latter study provides a solution to solve the issue of the extra dosage of carbon source which is peculiar of denitrification, regardless of the type of technology adopted for biological treatment of leachate. The use of biodegradable waste as the external readily biodegradable carbon source can make overall process economically and environmentally sustainable as different waste materials are finally disposed of.

2.4.1.3. Sequencing Batch Reactor (SBR)

SBR system involves different processes taken place in sequence in a single aeration tank or in two parallel reactors. A possible cycle of leachate treatment includes the following phases (Ehrig et al., 2018):

- Aeration for nitrification
- Mixing with addition of external carbon source for denitrification
- Post-aeration to remove remaining external carbon
- Sedimentation
- Discharge of treated effluent
- Discharge of excess sludge

Commonly, for leachate treatment, cycle periods of 24 or 12 hours are used. The volume of a SBR tank is close to that of a tank of a conventional activated sludge plant. Aeration systems and mixing devices have to ensure sufficient turbulence and uniform and fast oxygen supply. For this reason, process may require high energy demand. However, aeration and mixing systems do not operate continuously as, during certain periods, sludge sedimentation is planned. Occasionally, solid concentrations in the effluent may be too high as discharge phases are too short. In these cases, ultrafiltration step may be necessary. Moreover, during settling phases, precipitated inorganic substances may accumulate and compact at the bottom of the reactor.

Robinson et al. (2003) investigated the possibility of aerobically treating a real landfill leachate typical of methanogenic conditions. A preliminary pilot-scale test was conducted through a SBR which treated about 16 L of leachate each day at a controlled temperature of 10°C. The air was provided by diffusers at the base of the reactor with a flow rate ranged between 10 and 35 L/min (dissolved oxygen levels above 3.0 mg/L). Satisfactory results

were obtained in terms of ammoniacal nitrogen removal (< 99%) while COD concentration remained higher than discharge limits (about 300 mg/L versus 100 mg/L). Afterwards, effluent was treated by pilot-scale ozonation. The full-scale biological treatment process was subsequently designed. It comprised twin SBRs, 2000 m² of reed bed and ozonation unit. Also in this case, complete nitrification was routinely achieved but COD content hardly was below 250 – 350 mg/L. Spagni and Marsili-Libelli (2009) treated a leachate generated in an old municipal landfill of northern Italy in a SBR at laboratory-scale (maximum working volume of about 24 L, at 20 ± 1°C for almost 300 days). The nitrification step was optimised by adjusting the duration of the process. As a result, nitrite accumulated because of NOB inhibition (see Paragraph X). Denitrification was accomplished by adding external COD (i.e., a concentrated solution of acetate). The overall nitrogen removal efficiency was of 95% while COD was reduced only by 20 – 30% due to the low biodegradability of organic matter in the leachate. Capodici et al. (2014) used SBR for the co-treatment of MLL and synthetic wastewater at laboratory-scale. They tested two different mixtures of leachate-wastewater (i.e., 10% and 50% v/v of leachate, respectively). In both tests, high COD removal efficiencies were achieved, with average values respectively equal to 92% and 89%, suggesting a good acclimation of the biomass to the leachate. It was also possible to maintain an ammoniacal nitrogen removal efficiency higher than 60% thanks to the amount of readily biodegradable matter provided by the wastewater. Similarly, Fudala-Ksiazek et al. (2014) and Ranjan et al. (2016) investigated the effects of MLL concentration on the co-treatment with municipal wastewater in SBR at laboratory-scale. In the former study, the maximum COD removal efficiency was of 73% at 10% leachate concentration and it decreased with the increase in leachate concentration. On the other hand, nitrification efficiency did not considerably decline with the increased leachate concentration and an high ammonia removal efficiency (90%) was achieved at 25% leachate concentration. For denitrification step, sodium acetate was added as the external carbon source with the maximum nitrate reduction (83%) occurred at 25% leachate concentration. However, denitrification efficiency dropped considerably at 40% leachate concentration. Finally, phosphate was removed by 90% at 10% and 15% leachate concentrations. Fudala-Ksiazek et al. (2014) sought to establish conditions for a partial nitrification by feeding an increasing quantity (from 1 to 10% v/v) of MLL in SBR previously treating municipal wastewater (start-up period of 9 weeks). As a result, partial nitrification occurred since both low dissolved

oxygen concentration (from 0.5 to 1.5 mg/dm³) and free ammonia supplied by added leachate led to NOB inhibition. However, denitrification was not complete likely due to lack of assimilable organic carbon although the occurrence of nitrite pathway in denitrification should have reduced the organic carbon required by 35 – 40%. The average reduction of TN in the SBR system was 81%, 80%, 66% and 59% for 1%, 2%, 5% and 10% leachate concentration, respectively. While, the COD efficiency dropped from 90% measured during the start-up to approximately 60% in correspondence of 10% leachate concentration. For both pollutants, the larger volume of leachate concentration in the feeding mixture the less removal efficiencies. Zhang et al. (2019) performed a partial nitrification in a SBR fed with MLL and, then, the effluent of this process was pumped into an anoxic reactor along with waste activated sludge to undergo integrated fermentation and denitrification. The nitrogen removal efficiency of the entire process was 95% and an average nitrogen removal rate of 0.63 kg/m³·d during the last operational phase. On the other hand, COD concentration in the effluent was 1865.9 mg/L versus an influent concentration of 2324.5 mg/L (removal efficiency of 20%). Notably, denitrification step did not require any external carbon integration.

From a technical point of view, one of the advantages of the SBR is that the complete treatment process takes place in one or two reactors, at most. As mentioned above, energy demand is very variable depending on the phases of the cycle. For instance, during aeration, aerators and mixing devices operate with high energy input to provide adequate turbulence and oxygen supply. However, another advantage of SBR technology is that this energy demanding phase is usually short. Moreover, SBR application has been shown to effectively perform partial nitrification and denitrification via nitrite pathway with consequent oxygen and external biodegradable carbon savings. Particularly, in Fudala-Ksiazek et al.' study (2014), it was demonstrated that MLL can be used as NOB inhibitory agent as it provides the system with low dissolved oxygen concentration and high free ammonia (i.e., conditions which promote partial nitrification). However, also in SBR experiments, oftentimes an extra dosage of readily biodegradable carbon source is necessary. Likewise activated sludge application, leachate can be effectively co-treated with municipal wastewater by SBR only at specific low dilution ratios.

2.4.2. Anaerobic processes

Anaerobic biological processes involve the biological decomposition (in absence of oxygen) of organic or inorganic matter and are effective in the treatment of concentrated organic wastewater (Mojiri et al., 2021). The anaerobic digestion (AD) process consists of different degradation steps (see Chapter 1.4.) and can be summarized as follows (Eq. 2.21, Ehrig et al., 2018):



In general, anaerobic process could represent a way to achieve a complete recovery of the leachate in terms of both energy generation (biogas) and soil fertiliser (digestate). However, it is widely accepted that fresh young leachates characterised by high biodegradability ($BOD/COD > 0.5$) are more suitable for successful AD, as several studies testified (Luo et al., 2015), compared to MLL. Overall AD process, operational conditions and main parameters have been extensively described and discussed in Chapter 1. In light of those considerations, in the following, advantages and drawbacks of the application of AD technologies for leachate treatment are explored.

As previously mentioned, MLL is widely known to be problematic for biological treatment because it mostly contains organic matter recalcitrant to biodegradation such as humic substances (HSs) which have been shown to be inhibitory during AD, specifically for hydrolysis and methanogenesis (Azman et al., 2015; Li et al., 2019). As the major form of HSs, humic acids (HAs) have very complicated chemical structures with presence of many active functional groups (carboxylic acid, phenolic, alcoholic hydroxyls, quinone and ketone groups). Particularly, carboxylic and phenolic groups are responsible for the inhibition of the action of key hydrolytic enzymes during the hydrolysis of organic substances. Moreover, HAs can also inhibit methanogenesis (either hydrogenotrophic or acetoclastic) likely due to their negative charge on surface and/or the capacity as terminal electron acceptor. On the other hand, this feature makes HAs able to shuttle electron and favour the electron transfer during the acidogenesis (Li et al., 2019). However, HSs presence in the residual anaerobic sludge (digestate) could be beneficial for agriculture as HSs are well documented to i) promote plant growth (biostimulation), ii) sustain microorganisms life, iii) regulate both soil carbon and nitrogen cycling, iv) provide the

stabilisation of soil structure, v) create complexes with metals (Canellas et al., 2015; De Melo et al., 2016).

Ammonia-nitrogen in leachate can be either beneficial or harmful to anaerobic bacteria. On the one hand, since nitrogen is an essential nutrient element for anaerobic microorganisms, ammonia concentration in anaerobic sludge below 200 mg/L boosts the process. In addition, nitrogen from leachate can be helpful to reach the optimal Carbon to Nitrogen ratio (C/N) of 16:1 – 25:1 (Steinhauser and Deublein, 2011) necessary for the AD process, especially for carbon-rich feedstocks (e.g., lignocellulosic materials). On the other hand, higher concentrations lead to process failure since ammonia molecules diffuse into cells causing proton imbalance and/or potassium deficiency (Chen et al., 2008). In fact, it is widely accepted that Total Ammoniacal Nitrogen (TAN) concentration higher than about 1.7 g/L is responsible for process failure in unacclimated inocula (Chen et al., 2008; Yenigün and Demirel, 2013). Eventually, likewise HSs, ammonia presence in digestate suggests its potential use as replacement for mineral N fertilizers (e.g., urea) (Riva et al., 2016).

Furthermore, chloride can be another potential inhibitory element for AD after leachate addition. Indeed, it is stated that chloride cause strong inhibition of methanogenesis if present in anaerobic environment in concentrations typically ranging from 4 to 9 g/L (Viana et al., 2012). However, inhibitory threshold is well above usual chloride concentration in MLL.

Lastly, leachate addition in AD process can be beneficial to compensate possible substrates deficiencies in heavy metals which are known to be stimulatory for anaerobic bacteria up to certain concentrations (Alkalay et al., 1998; Guo et al., 2019; Liu et al., 2021; Oleszkiewicz and Sharma, 1990). In particular, as summarised in Guo et al. (2019), Cu, Ni and Zn exert promoting effects on anaerobic process, especially on methanogenic activity, if present in concentrations up to 100, 50 and 5 mg/L, respectively. Conversely, they can inhibit the process above 500, 100 and 50 mg/L, respectively. Although heavy metals content in leachate is by far lower than inhibitory thresholds, in order to avoid problems linked to the subsequent digestate agricultural use, Bilardi et al. (2018) suggested a leachate pre-treatment preliminary to any biological treatment.

Anaerobic treatment of leachate can follow two approaches: the use of anaerobic technology as an actual treatment for leachate with the aim of reducing pollutants to meet discharge limits or the addition of leachate as an amendment for the optimisation of the AD of organic waste or residues. Anyway, physical-chemical treatments are recommended to be applied on raw leachate before or after the anaerobic process so that, respectively, preventing the biological process from inhibition (due to the presence of toxic compounds) or refining the anaerobically stabilised leachate. Nevertheless, the integration of physical-chemical pre- or post-treatments and AD specifically for MLL treatment has not been sufficiently investigated yet. Some instances of individual AD and combined processes are shown in the following.

Kettunen et al. (1999) investigated at pilot-scale the on-site treatment of a municipal landfill leachate (COD 1.5 – 3.2 g/L) in a Up Flow Anaerobic Sludge Blanket (UASB) reactor at low temperatures (13 – 23°C) for 226 days. Despite the used leachate was not completely stabilised and its quality changed over time, 65 – 75% COD removal was achieved with organic loading rates (OLRs) of 2 – 4 kgCOD/m³·d at 18 – 23°C. COD removal efficiency dropped to 50 – 55% with lower temperatures and OLRs. Methane yield averaged 320 mL/gCOD.

Several studies investigated the anaerobic co-digestion of MLL and synthetic wastewater or sewage sludge in order to determine the optimal concentration of leachate in the influent and process parameters. In general, the higher the leachate proportion in the influent the lower the COD removal and methane production due to inhibition of microbiological activity. Bohdziewicz et al. (2008) used a laboratory-scale anaerobic MBR for the treatment of a MLL obtained from the Sobuczyna plant in southern Poland. Treatment efficiency was studied under different leachate dilutions in the range of 5 – 75% (v/v) with a synthetic wastewater. For leachate addition of 10% and 20%, over 95% COD removal was observed while a significant decrease of treatment efficiency occurred for leachate concentration in the influent above 30%. The highest value of biogas yield (0.45 dm³/gCOD·d) was achieved at HRT of 2 days for 20% leachate in the influent. Similarly, Kawai et al. (2012) varied MLL proportion in the mixture with synthetic wastewater from 0% to 100% for the feeding of an UASB reactor at laboratory-scale. COD removal efficiency decreased from 93% to 24% as the proportion of the leachate in the influent was increased from 0% to 100%. Moreover, for 100% leachate influent, methane

production was suppressed. When synthetic wastewater was reintroduced into the influent, the COD removal efficiency rapidly increased up to steady-state value of 68%. Authors concluded that MLL may be treated in UASB reactor by alternately conducting mono-digestion of refractory leachate and co-digestion with labile materials. Montusiewicz et al. (2018) performed a co-digestion in semi-flow mode digesters of sewage sludge and MLL (at 5% v/V) pre-treated by cavitation with the purpose of degrading recalcitrant and toxic compounds. For an optimal hydraulic retention time (HRT) of 20 days, both biogas yield and volatile solids removals increased by 30% and 50%, respectively, compared to the co-digestion of sewage sludge and raw MLL under the same operational conditions. In Berenjkar et al. (2019), MLL was co-digested with sewage sludge at different dosages (0%, 5%, 10%, 20%, and 40% v/v). They found that increasing the leachate dose in the treatments made the influent more digestible than the control (i.e., 0% leachate). The highest methane yield (1.01 m³/kgVS) was obtained from the treatment with 10% leachate. In this study, MLL appeared not to have a substantial influence on digestibility up to a dose of 20%.

Some of the limits of the application of AD on leachate treatment can be overcome by using additives. Lei et al. (2018) performed an AD of a leachate coming from a MSW incineration plant in Beijing, China, containing high concentrations of complex organics (COD > 40000 mg/L) through a laboratory-scale UASB reactor amended with magnetite (10 g/L). Compared to a control reactor, the addition of magnetite improved overall process performance by enhancing COD removal efficiency from 79% to 90% and methane production rates from 3.7 m³/m³·d to 4.8 m³/m³·d at an OLR of 18.2 kg of COD per m³·d. Magnetite was found to stimulate DIET mechanism and adsorb toxic compounds.

Yadav and Dikshit (2016) investigated at laboratory-scale the effect of coagulation before anaerobic treatment on MLL collected from India's largest and oldest landfill Deonar, Mumbai. The pre-treatment was carried out by using poly-aluminium chloride and FeCl₃ as the coagulants. The further biological step involved two anaerobic batch reactors: one fed with raw leachate, the other fed with pre-treated leachate. 35% and 38% of TOC and COD, respectively, was removed by anaerobic SBR on raw leachate while removal efficiencies of 70% for TOC and 72% for COD were obtained in the integrated system of coagulation-AD process. Coagulation/flocculation can be used as both pre- or post-treatment to AD of MLL (Mojiri et al., 2021). In the former case, the treatment is used to

improve biodegradability, reduce COD and metal content in the leachate; in the latter, the treatment allows the removal of residual refractory compounds.

A combined system consisting of a MBR equipped with ultrafiltration and electro-chemical oxidation process was used by Zolfaghari et al. (2016) to treat highly polluted raw or pre-treated MLL. The optimal condition for COD (63%), TOC (35%), ammonium (98%) and phosphorous (52%) removal efficiencies was found for 1.2 gCOD/L·d of OLR and 80 days of sludge retention time.

Cirik and Gocer (2020) used air stripping as a pre-treatment prior to an anaerobic MBR for the treatment of MLL. Under the operational condition of pH 8 and air flow rate of 1.4 L/s, air stripping process was able to remove about 90% of ammonium and 26% of COD thus reducing the ammonia toxicity effect and increasing the methane yield up to 350 mL/gCOD and the COD removal rate up to 5 gCOD/L·d. The addition of lime at 24 g/L for pH adjustment was observed to increase the fouling rate of membranes due to possible inorganic precipitates. Also, 85% of ammonia removal and 26% of COD reduction were obtained by Smaoui et al. (2018). In their study, air stripping was found to better improve biogas production (over 550 mL/gCOD) compared to coagulation/flocculation and Fenton oxidation.

Since oxidizing radicals generated in high power ultrasound treatment degrade organic matter (see Paragraph 2.3.3.), generally, AOP processes are considered suitable pre-treatments for AD. Indeed, they enhance the biodegradability of recalcitrant contaminants (He et al., 2020). Sonication was used as a pre-treatment of low biodegradable landfill leachate thus facilitating its degradation prior to AD. It was observed that high power input reduced soluble COD concentration resulting in low biogas production. On the contrary, low to moderate sonication power input did not cause degradation of biosolids but disintegration and solubilization instead, resulting in higher soluble COD value and biogas yield (Nazimudheen et al., 2018). Oz & Yarimtepe (2014) fed anaerobic batch reactors with ultrasonically pre-treated leachate obtaining increases of 40% and 18% in biogas production and methane content in biogas, respectively, compared to control reactor (raw leachate). Authors attributed these improvements to the solubilisation of organic matter achieved with ultrasonic pre-treatment. The AOP of photo-electro Fenton was used prior to an anaerobic MBR to effectively treat highly contaminated landfill wastewater (Nivya and Minimol Pieus, 2016). The photo-electro Fenton treatment

allowed about 72% of BOD and 85% of COD removal. Lower COD and BOD reductions (about 50% and 30%, respectively) were obtained by Fenton oxidation treatment in Smaoui et al. (2018). These removal efficiencies increased (over 90% and 96% for BOD and COD, respectively) when photo-electro Fenton was combined with AD (Nivya and Minimol Pieus, 2016).

Combinations of different biological metabolisms are also used as treatment systems. The anaerobic phase is often used before the aerobic step using a combined and sequential anaerobic-aerobic system. The first anaerobic phase allows the conversion of the COD to biomethane, while the subsequent aerobic phase reduces by 50% the ammonia nitrogen produced during the anaerobic step (Azad Pashaki et al., 2021). This system also allowed a BOD removal efficiency of about 97% (Azad Pashaki et al., 2021) and, depending on the leachate quality, the final effluent could fulfil the official requirements (Kettunen et al., 1999).

Recently, anaerobic co-digestion of food waste with municipal solid waste leachate is gaining increasing attention. The advantage lies in the ultimate disposal of two types of waste which are commonly considered as challenging to manage with virtually no further waste production but energy (biogas) and mass (digestate) recovery. In this last section of the Chapter, well-known issues related to the AD of food waste are reported as it represents the key process of the integrated MLL treatment of this thesis.

In general, anaerobic process is widely accepted as an appropriate method for treatment of the organic fraction of municipal solid waste (OFMSW). Indeed, AD would allow the recovery of the organic matter in form of biogas which could be directly used for energy production and/or upgraded to obtain high quality methane (fuel in the transport or energy production sectors). Typically, the OFMSW is composed of two different organic waste streams: i) green waste is the residue of pruning derived from the maintenance of private or public gardens, ii) food waste is the separately-collected organic fraction derived from household and other commercial activities (e.g., market, canteens, restaurants). In particular, market waste fraction consists of residues of fruit and vegetable, such as fruit skins, potatoes, onions and other rotten vegetables (Ranade et al., 1987). It is produced in huge amount and it can be easily source separated even in countries where a simplified MSW management is adopted. Food waste is considered as a suitable source for biogas production as it is characterised by a high level of

carbohydrates and moisture (Swamy et al., 2021). However, AD of food waste still faces several technical and economic challenges such as VFAs inhibition, lack of micro-nutrients (e.g., trace metals), high C/N, low buffer capacity, foaming and high cost of transportation and operation (Lv et al., 2021; Xu et al., 2018). Highly degradable sugars present in food waste are readily converted to VFAs at an early stage of the digestion process resulting in possible rapid acidification of the system if no sufficient buffering capacity is present. When VFAs consumption rate is lower than VFAs production one, VFAs accumulate and cause inhibition of the methanogenic activity (Wang et al., 2014). To prevent process failure for VFAs accumulation, AD of food waste is often carried out at low OLRs (below of 3 gVS/L·d) (Mata-Alvarez et al., 1992) and co-digestion with other substrates such as animal manure or sewage sludge which can provide alkalinity is a common practice (Xu et al., 2018). As mentioned in Paragraph 1.1.1.6., trace metals are essential micronutrients for anaerobic microorganisms. Their absence may affect the stability of the biological process in the long term. For instance, some specific metals (Fe, Co, Mo, and Ni) are required to synthesize some key coenzymes related to methanogenesis process. Besides, iron is fundamental as it also serves as both electron acceptor and electron donor in oxidative-reductive reactions which take place. Thus, lacking of trace metals may adversely affect microbe activity with low efficiency in methane production as a consequence (Lv et al., 2021; Romero-Güiza et al., 2016; Xu et al., 2018).

In this context, landfill leachate has been found to improve buffering capacity and trace metals supplementation of the system while, concurrently, AD process allows refractory organics conversion to biogas to some extent (Lv et al., 2021). Moreover, leachate may serve as a source of nitrogen to balance the low Carbon to Nitrogen (C/N) ratio of the food waste so that stabilising the biological process and enhancing the methane yield. Finally, landfill leachate can effectively dilute the suspended solid content of the food waste (Ma et al., 2018). In Liao et al. (2014), a real MLL (from Wuhan, China) played the role of nutrient solution in the AD of food waste integrating the nitrogen necessary for bacterial growth. Moreover, total ammonia nitrogen present in the MLL (2000 – 3000 mg/L) formed a buffer system with VFAs preventing system acidification. At an organic loading rate of 41.8 gVS/L, the digestion process without leachate addition failed. Conversely, dilutions with different amount of MLL led to methane yields ranged from 369 mL/gVS to 466 mL/gVS.

In conclusion, it can be stated that although AD is usually not appropriate to treat MLL especially because of its low biodegradability, however, several studies showed good results in terms of pollutants' removal through AD application. Oftentimes, physical and/or chemical pre-treatments are required to improve leachate biodegradability and/or reduce organic and inorganic compounds toxic for anaerobic microorganisms. On the one hand, this makes the overall process more expensive and technologically complex. On the other hand, in this way, the performance of AD process is usually improved. It implies biogas/methane yield increase with consequent possibility of energy generation which can compensate the extra energy demand. Furthermore, as already mentioned, residual digestate can be used as soil fertiliser, possibly after proper treatment. Besides process operational conditions (i.e. pH, temperature, OLR, HRT) and inoculum source, digestate composition is greatly dependent upon substrates and additives fed to anaerobic reactors. In this regard, it should be considered that substances unaffected by the biological treatment (e.g., metals, ammonia, mineral elements or hardly biodegradable compounds) unavoidably concentrate in the residual digestate. While some elements (such as nitrogen, phosphorous, potassium, sulphur, humic substances) may be beneficial in view of a further use of digestate as a fertiliser or soil amendment (see Paragraph 1.4.6.), metals and toxic organic compounds may be present in concentrations such that not meeting regulation standards on digestate use in agriculture. Heavy metals exceeding tolerable trace concentrations are known to adversely affect plants' growth both directly and indirectly. Heavy metals present in soil in soluble (or easily solubilised) forms can be available for plant uptake by means of roots. As metals cannot be degraded, they accumulate inside plants inhibiting cytoplasmic enzymes and damaging cell structures. Furthermore, excessive metals' concentration in soil affect number, diversity, and activities of autochthonous microorganisms. As a consequence, organic matter decomposition and availability of soil nutrients decrease (Chibuiké and Obiora, 2014). As well, the possible presence of organic pollutants in digestate can compromise its agriculture use. Persistent organic pollutants (e.g., polycyclic aromatic hydrocarbons, polychlorinated dibenzo-p-dioxins or -furans, polychlorinated biphenyls, brominated flame retardants, perfluorinated alkyl substances and chlorinated paraffins) are widely accepted to interfere with organisms because of their peculiar properties (i.e., toxicity, persistence, carcinogenic and/or endocrine disrupting effects) (Brändli et al., 2007). To sum up, it is inferred that the choice of using AD process as MLL treatment strongly

depends on leachate initial composition. Indeed, both substances which may inhibit anaerobic microorganisms and refractory compounds which can concentrate in final digestate hampers process performance in terms of biogas/methane generation and high-quality digestate production, respectively.

Taking all these considerations into account, with the aim of researching a possible sustainable solution for MLL treatment, in this thesis it has been decided to combine active filtration technology and AD process. The former is considered as the pre-treatment necessary to make MLL suitable for the further biological treatment step. It has been simulated at laboratory-scale by using column tests under real operational conditions. The latter has been chosen as it allows to perform an almost complete recovery of energy and material. Three different laboratory-tests are carried out in order to optimise process performance. In the next Chapter, the description of the integrated treatment of MLL is provided in more detail.

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CHAPTER 3

3.1. Experimental design

The proposed integrated system for the mature landfill leachate (henceforth MLL) treatment at a laboratory scale consisted of two main phases which were carried out separately, one after the other.

The first phase represented the pre-treatment of the MLL and consisted of a physical-chemical filtration by an active material with the purpose of reducing the content of refractory and toxic compounds, such as heavy metals. On the basis of previous research, two different granular reactive filters were tested: one was made up of Zero Valent Iron and Lapillus (ZVI/Lap 20:80 w/w) and the other of Zero Valent Iron and Granular Activated Carbon (ZVI/GAC 40:60 w/w). Each granular filter completely filled a plexiglass column (50 cm of height and 5 cm of internal diameter) used during the experiment. A synthetic leachate, representative of a mature landfill one, was prepared in laboratory according to the typical composition found in literature and was fed to the two columns by a peristaltic pump.

The second phase of the integrated treatment system aimed at testing the suitability of the pre-treated MLL both as a supplement of macro (nitrogen) and micro (trace metals) nutrients for the anaerobic bacteria metabolism and as a source of nitrogen with the purpose of balancing the high carbon content of mainly carbonaceous substrates (e.g. lignocellulosic materials). In order to do that, three different cycles of semi-continuous anaerobic digestion (henceforth AD) tests were carried out. The pre-treatment columns experiments lasted 42 days and pre-treated leachates were weekly collected during this period. For each column, a composite sample of pre-treated leachate was arranged and used for the AD experiments. In both the I and the II cycle, semi-continuous reactors were fed with pure cellulose which is considered as a model organic substrate because of its predictable methane production. Instead, in the III cycle, a more realistic waste, made up of fruits and vegetables residues (market waste, henceforth MW), was used. The volumes of the pre-treated leachates added to the reactors were calculated in order to reach different Carbon to Nitrogen ratios.

In the I cycle, four semi-continuous reactors were used. In two of them, the pre-treated leachate coming from the ZVI/Lap column was added in two different dosages up to C/N (in terms of COD/NH₄-N) of 50 and 25 (acceptable and low ratio, respectively). In the other two reactors, the leachate pre-treated by the column filled with the ZVI/GAC mixture was added in order to reach the same ratios.

In the II cycle of semi-continuous AD tests, only the pre-treated leachate coming from the ZVI/Lap column was tested as a nutrient solution since it had given the best result in terms of methane production in the previous cycle. Indeed, cellulose and pre-treated leachate were fed to all of the reactors employed in the II cycle of tests and a COD/NH₄-N of 50 was set. The purpose of this cycle was improving the methane production and the stability of the processes through the addition of materials which can promote the interspecies electrons transfer (DIET mechanism). In this regard, two materials were tested separately: GAC and biomass fly ash. Since the tests were carried out in duplicate (i.e. two reactors used for each configuration), six reactors in total were employed (2 Blanks + 2 GAC + 2 Biomass Fly Ash).

Finally, the III cycle consisted of the semi-continuous AD of a realistic waste (MW) with a high carbon content which was balanced by the addition of both pre-treated leachates (coming from ZVI/Lap and ZVI/GAC column, respectively) up to a C/N of 25. Moreover, both the stimulation of the DIET mechanism and the possible adsorption of humic substances present in the leachates were tested through the addition of GAC. Thus, reactors were fed with the substrate and leachate pre-treated by the ZVI/Lap and the ZVI/GAC column, respectively. In two reactors, in addition to leachate and substrate, GAC was also added.

The entire above described experimental design is depicted in Figure 3.1.

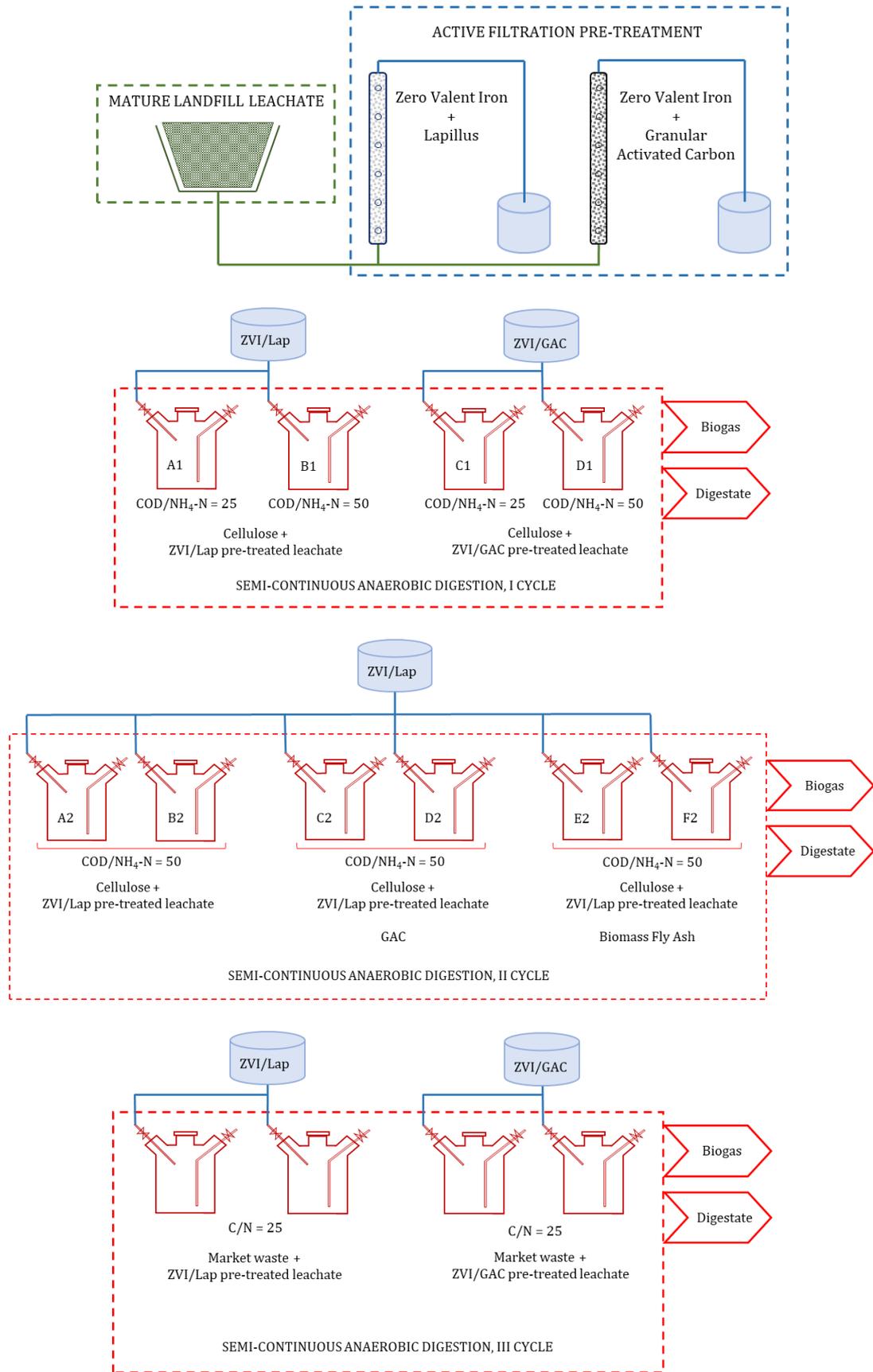


Figure 3.1. Experimental design

3.2. Materials and Methods

In this Chapter, materials, laboratory equipment, test procedures and measurement methods employed in the research are listed and described in detail. It starts with the description of the material used for the research (the synthetic MLL) and continues with the illustration of the technologies representing the preliminary (column tests) and final (semi-continuous AD tests) treatments of leachate. At the same time, the methods adopted for measuring the key parameters are reported.

3.2.1. Synthetic leachate

Considering the novelty of the investigated MLL treatment, it was decided to employ a synthetic landfill leachate as much similar as possible to a real one in order to ensure the necessary repeatability of the entire experiment and its possible scaling-up. The synthetic leachate used in this experiment was representative of a mature landfill one and was prepared in laboratory according to the typical composition found in literature (Kjeldsen et al., 2002; Qasim and Chiang, 2017; Renou et al., 2008). Specific quantities of the selected reagents were dissolved in distilled water in order to reach their set concentrations (C) (Figure 3.2 and Table 3.1). After that, some drops of hydrochloric acid (Sigma-Aldrich, 37%) were added to the solution to adjust the pH to the value of 8 (alkaline leachate).



Figure 3.210. Chemical reagents used for the preparation of the synthetic MLL

Table 3.1. Synthetic MLL chemical composition

Element	Reagent	C [mg/L]
Cu	CuCl ₂ , Sigma-Aldrich (purity ≥ 99.99%)	2
Ni	NiCl ₂ ·6H ₂ O, Alfa Aesar (purity = 99.95%)	2
Zn	ZnCl ₂ , Fluka (purity > 98%)	5
NH ₄ ⁺	NH ₄ Cl, Acros Organics (purity > 99%)	900
Cl ⁻	see the reagents above	3100
COD	90% of humic acids, Haifa Italia	3500
	10% of acetic acid, Fisher Chemical (purity ≥ 99.7%)	

3.2.2. Pre-treatment tests

Column tests are commonly carried out in order to simulate the behaviour of filter materials at laboratory scale in the field of wastewater, groundwater and leachate remediation (Moraci and Calabrò, 2010). Therefore, in this research, column tests represented the physical-chemical pre-treatment phase of the integrated system for the MLL treatment.

3.2.2.1. Column tests

Two columns made up of polymethyl methacrylate (PMMA – Plexiglas) with internal diameter and height of 5 ± 0.1 and 50 ± 0.1 cm, respectively, were used in the tests with the purpose of investigating the leachate treatment capacity of two different granular active filters. Indeed, one column was completely filled with a granular mixture of Zero Valent Iron (ZVI) and lapillus at a weight ratio of 20:80 (designed as ZVI/Lap column), whereas the other, similarly completely filled, contained a granular mixture of ZVI and Granular Activated Carbon (GAC) at a weight ratio of 40:60 (designed as ZVI/GAC column) (Figure 3.5) (Bilardi et al., 2020, 2019, 2018a). In both of them, ZVI mass was kept equal to 300 g (Table 3.2).

Table 3.2. Amount of the granular reactive mixtures tested in the column tests

Column	Weight ratio of the mixture	ZVI mass [g]	Lapillus, GAC mass [g]
ZVI/Lap	20:80	300	1200
ZVI/GAC	40:60	300	450

3.2.2.2. Granular reactive materials

The ZVI used in the tests was of the type FERBLAST RI 850/3.5 distributed by Pometon S.p.A. (Mestre, Italy) with an iron content higher than 99.74%. Before of this experiment, a wet gradation was carried out (Figure 3.3, top). The granular lapillus, distributed by SEM s.r.l. (Viterbo, Italy), mainly consisted of silica (47%) and oxides of various elements: $\text{Al}_2\text{O}_3 = 15\%$, $\text{K}_2\text{O} = 8\%$, $\text{Na}_2\text{O} = 1\%$, $\text{Fe}_2\text{O}_3\text{-FeO} = 7\text{--}8\%$, $\text{MnO} = 0.15\%$, $\text{MgO} = 5.5\%$ and $\text{CaO} = 11\%$. Before the column tests, lapillus was sieved in order to obtain a particle size distribution similar to that of the ZVI. To do that, lapillus grains both retained on sieve No. 20 (>0.84 mm) and passing through sieve No. 200 (<0.074 mm) were removed. The result of the wet gradation carried out on the resulting lapillus sample is depicted in Figure 3.3 (middle). Finally, GAC (type CARBOSORB 2040) was provided by Comelt s.r.l. (Milan, Italy). Also the size of GAC particles was similar to that of ZVI (0.85 – 0.425 mm) as deduced by a previous wet gradation (Figure 3.3, bottom).

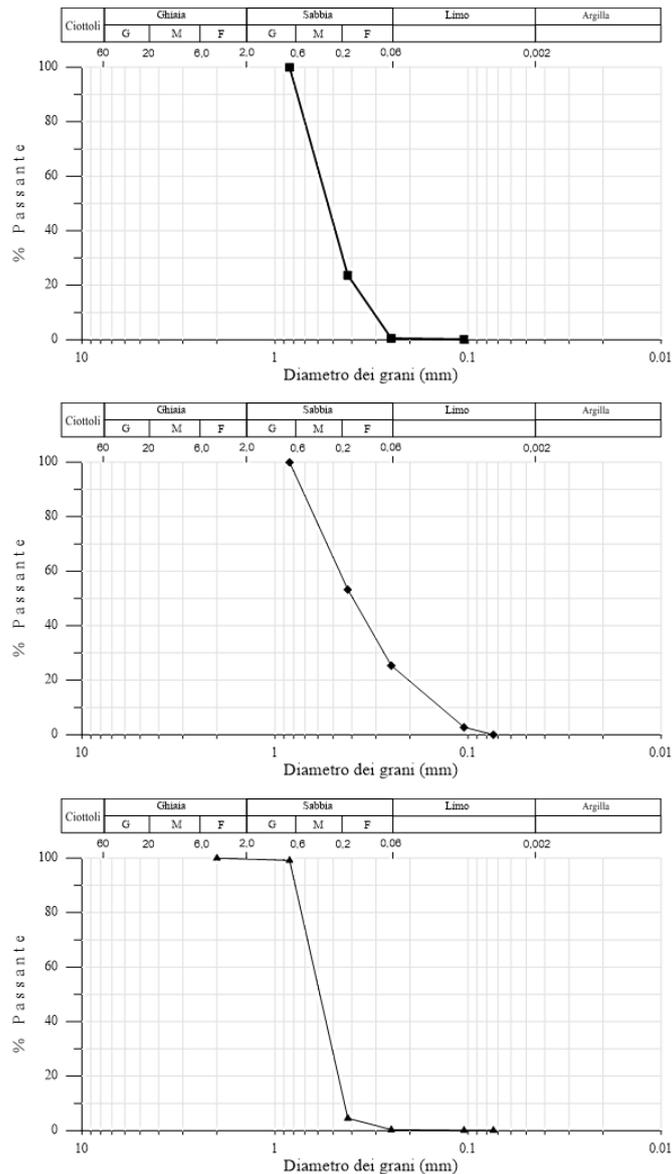


Figure 3.3. Grain size distribution of the ZVI (top), lapillus (in the middle) and GAC (bottom).

In Table 3.3, coefficient of uniformity ($U = d_{60}/d_{10}$, i.e., the ratio between diameters corresponding to 60% and 10% finer grain size distribution, respectively), mean grain size (d_{50} , i.e., diameters corresponding to 50% finer grain size distribution) and particle density (ρ) of the reactive granular materials are summarised. The porosity of both granular mixtures was 0.42.

Table 3.3. Coefficient of uniformity, mean grain size and particle density of the granular reactive materials

Parameter	ZVI	Lapillus	GAC
U	2.0	3.3	1.45
d_{50} [mm]	0.5	0.4	0.6
ρ [g/cm ³]	7.87	2.2	-



Figure 3.4. ZVI (on the left), lapillus (in the middle) and GAC (on the right) used in the experiment

3.2.2.3. Tests conditions and samples analyses

The synthetic MLL was fed to both columns by a peristaltic pump (Watson Marlow 205S) under a constant upward flow of 0.68 mL/min which is equivalent to 0.5 m³/d of leachate per m² of granular filter. This condition led to an Empty Bed Contact Time (EBCT, i.e., the time during which a water to be treated is in contact with the treatment medium) of about 10 hours calculated by dividing the empty bed volume by the flow rate. Along the columns, sampling ports and pressure transducers are located: the former for the periodic withdrawal of contaminated solution samples aiming at evaluating the performance of the process; the latter with the purpose of evaluating a possible permeability reduction over test time. Samples were collected by a 10 mL syringe through the ports located at 5, 28 and 50 cm (outlet) from the column inlet, respectively. The column tests were stopped after 38 days of continuous operation corresponding to about 90 treated pore volumes. The duration of the filtration tests was set to a minimal removal efficiency of about 40%.

Leachate samples pH was measured by a digital pH-meter (XS instruments), ammonium, chloride and COD contents were analysed by specific pre-dosed cuvettes (Ammonium Cell Test 114559, Chloride Cell Test 114730 and COD Cell Test 114555, respectively) using a photometer (WTW Photolab S12) and Cu, Ni and Zn contents which were evaluated by Inductively Coupled Plasma Emission Spectroscopy (ICP-OES, Perkin Elmer OPTIMA 8000). All the analyses were performed on the liquid fractions of the leachate samples obtained after centrifugation (3 min at 6000 rpm) and filtration (1.2 µm). The removal efficiency (R_E) of contaminants was calculated through Eq. 3.1 where C_{in} and C_{out} stand

for, respectively, the concentration (in mg/L) of the contaminant in input and output from the column:

$$R_E = \frac{C_{in} - C_{out}}{C_{in}} \cdot 100 \quad (3.1)$$

The removal capacity of the granular mixtures for each pollutant (R_C) was also determined (Eq. 3.2) which M_{in} and M_{out} standing for the mass (in mg) of contaminants in input and output from the column, respectively, and M_{filter} the mass of the filter medium (in Kg):

$$R_C = \frac{M_{in} - M_{out}}{M_{filter}} \quad (3.2)$$

For each column, every week, the quantity of pre-treated leachate collected at the outlet was stored in 2 L plastic bottles at 4°C.

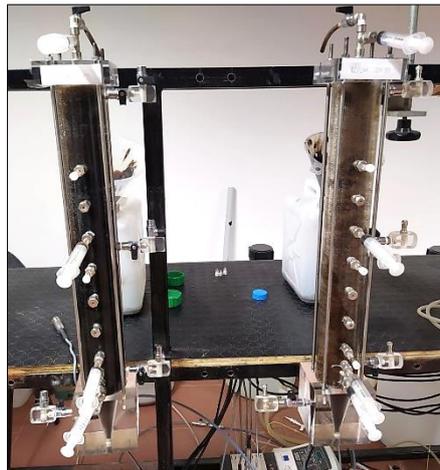


Figure 3.5. Plexiglass columns used in the tests (ZVI/GAC on the left and ZVI/Lap on the right) with syringes in the sampling ports

3.2.3. Semi-continuous anaerobic digestion tests

Semi-continuous AD tests, in general, involve reactors loaded with an anaerobic inoculum which is periodically fed with an external organic substrate while excess digestate is withdrawn. These tests have the main purpose of evaluating the feasibility of the anaerobic process in terms of biogas/methane production and the stability of the process in the long term. Commonly, an AD process is considered stable if it does not show any significant slowdown or inhibition of the methanogenesis for at least three times the set

Hydraulic Retention Time (HRT). In contrast to the Biochemical Methane Potential (BMP) tests, where substrates and supplements are fed only at the beginning (batch mode), the periodic feeding of the semi-continuous reactors provides the possibility to observe the effects of the eventual accumulation of toxic substances. Therefore, semi-continuous tests simulate the process in a more realistic way.

In this research, the semi-continuous AD tests represented the final phase of the integrated system for the MLL treatment in which the pre-treated leachate was used as a supplement for macro and micro nutrients and especially as source of nitrogen to balance the Carbon to Nitrogen ratio (C/N). As already mentioned, three different tests conditions were tested separately in three different experimental cycles (see Paragraph 3.1.).

At the beginning of the tests, reactors were loaded with specific volumes of inoculum and distilled water. Moreover, in both the II and the III cycle, conductive materials (GAC and biomass fly ash and GAC only, respectively) were also added. Then, a mixture of pre-treated leachate, water and substrate was fed to reactors two or three times per week. Only in the III cycle, GAC was also included in the feeding mixture.

3.2.3.1. Inocula

Inoculum plays a key role in the AD tests since it represents the source of the specific microbial community which runs the digestion process. Inocula used in the I and the II cycle of the semi-continuous AD tests were both collected from a full-scale AD plant located in Reggio Calabria ("Coop. Fattoria della Piana Soc. Agr.", Contrada Sovereto, 89020, Candidoni, RC, Italy). They were the liquid digestates remaining after the digestion of manure and agro-industry waste. After the collection, inocula were sieved in order to remove undigested materials (e.g. straw) (Figure 3.6) and, then, stored at 35°C for a few days until the beginning of the corresponding semi-continuous AD cycle. Inoculum used in the III cycle of the semi-continuous AD tests, instead, was a residual digestate coming from previous BMP tests carried out in our laboratory. Also in this case, inoculum was sieved and stored at 35°C for about a week in order to reduce the endogenous ("non-specific", i.e., not due to substrate) biogas production.



Figure 3.6. Inoculum sieving and storage

Inocula were characterised in terms of pH, Total Solids (TS) and Volatile Solids (VS, complementary to the Fixed Solids) (Figure 3.7) according to standard methods (APAT, IRSA-CNR, 2003; APHA et al., 2012) before the beginning of the corresponding semi-continuous AD cycles (Table 3.4). TS are the residue after humidity evaporation at a defined temperature. In this research, samples of about 100 g were prepared and dried to constant weight in an oven (Binder ED 115) set at 70°C. VS are determined as percentage of TS and roughly represent the biodegradable fraction of the material. VS are determined igniting TS to constant weight at 550°C: the remaining solids are defined as Fixed Solids while those lost on ignition are VS.



Figure 3.7. Inocula Total (on the left) and Fixed (on the right) Solids

Table 3.4. Inocula characterisation

Parameter	Inoculum		
	I cycle	II cycle	III cycle
pH	7.65	7.54	7.93
Total Solids, TS [%]	6.1%	5.6%	2.8%
Volatile Solids, VS [%TS]	76.4%	78.9%	64.9%

3.2.3.2. Substrates

The bacterial consortium involved in the AD degrades, through a series of bio-chemical reactions, the organic matter provided by an external organic substrate. In both the I and the II cycle of the semi-continuous AD tests, the organic substrate was α -cellulose (CAS

9004-34-6) distributed in powder by Sigma-Aldrich (Figure 3.8). Cellulose is often employed as standard substrate in AD tests since it is an easy biodegradable material with a simple and well-defined chemical composition (only glucose as the monomer) which makes AD process stable and methane production predictable (Holliger et al., 2016). In this way, it is easier to determine how the presence of the pre-treated leachates affects the digestion process.



Figure 3.8. α -cellulose (CAS 9004-34-6, Sigma-Aldrich) used as substrate

MW was the substrate used in the III cycle of the semi-continuous AD tests. It was chosen in order to investigate the influence of the addition of the pre-treated leachates on the AD of a more realistic substrate. Moreover, being a carbonaceous material (high content of carbon) it allows to reach an ideal value of the C/N of the feeding mixture along with the pre-treated leachate. MW was made in laboratory by mixing and milling apples, potatoes and carrots (44.4%, 49.0% and 6.0%, w/w, respectively) (Figure 3.9).



Figure 3.9. Market waste

Both substrates were characterised in terms of Total and Volatile Solids (APAT, IRSA-CNR, 2003; APHA et al., 2012). Furthermore, COD content of cellulose was estimated from stoichiometry while pH and C/N of MW were calculated through a digital pH-meter (XS instruments) and an elemental analyser TOC-LCSH (Shimadzu, Kyoto - Japan), respectively. The results are summarised in Table 3.5.

Table 3.5. Substrates characterisation

Parameter	Cellulose	Synthetic market waste
pH	-	5.26
Total Solids, TS [%]	94.9%	19.4%
Volatile Solids, VS [%TS]	100.0%	93.3%
COD [mg/g]	1185	-
C/N	-	36.3

3.2.3.3. Conductive materials

In both the II and the III cycle of the semi-continuous AD tests, the effect on the digestion process of the addition of conductive materials was investigated. Materials were expected to stimulate the DIET mechanism (see Paragraph 1.4.5.) and, thus, to improve the performance of the whole process. In the II cycle, GAC and biomass fly ash were used while, in the III one, only GAC was added. GAC used in the AD tests was the same material adopted as granular reactive filter in the column tests (see 3.1.1.2.). Biomass fly ash, in form of dark grey powder, derived from the biomass combustion carried out in a waste treatment plant located in Reggio Calabria province (“Ecopiana Company srl”). Its physical and chemical proprieties were previously determined (Porcino et al., 2020). A scanning electron microscope (SEM) analysis showed a well graded grain size distribution and an irregular morphology while Ca, Si, Al, K, S and C were recognised as the main elements arranged in form of calcium, aluminum and silicates crystalline phases. The release of heavy metals in water was also investigated through batch leaching tests (with a solid sample to water ratio of 1:10). It was found that iron, zinc and copper were released (about 3 mg/L, 2 mg/L and 0.2 mg/L, respectively) under neutral conditions.



Figure 3.1011. GAC and biomass fly ash used in the experiment

3.2.3.4. Pre-treated leachates

Before the beginning of the semi-continuous AD tests, for each column, a composite sample of pre-treated leachate, representative of the entire pre-treatment tests (38 days of operation), was prepared by mixing weekly samples coming from the respective column outputs (Figure 3.11). In a perspective of scaling-up of the integrated system for the MLL treatment, this procedure would mirror the collection in tanks on site of the leachate pre-treated by a series of filters in different stages of operation of the active material (new to exhausted). Later, the pre-treated leachate would be pumped to the AD plant in order to be used as nutrient solution according to specific dosages.



Figure 3.11. Pre-treated leachates storage and composite samples generation

Both pre-treated leachates were characterised by the same parameters measured in the leachate samples periodically collected during the column tests (see 3.1.1.3.). The chemical compositions of the two pre-treated leachates used in the semi-continuous AD tests are summarised in Table 3.6. Regarding nitrogen content, in both pre-treated leachates nitrogen was in ammoniacal form as expected and as proved by the fact that similar results were obtained from ammonium and total nitrogen analyses (Ammonium Cell Test 114559 and Nitrogen (total) Cell Test 14537, respectively).

Table 3.6. Chemical compositions of the pre-treated leachates used in the semi-continuous AD tests

Parameter	Pre-treated leachate	
	ZVI/Lap	ZVI/GAC
pH	7.5	7.1
COD [mg/L]	2363	1533
N _{tot} [mg/L]	860	820
NH ₄ -N [mg/L]	839	827
Cl ⁻ [mg/L]	3067	3013
Cu [mg/L]	0.30	0.41
Ni [mg/L]	0.52	0.55
Zn [mg/L]	1.64	0.97

As already mentioned, the purpose of this phase of the experiment was to assess the use of the pre-treated leachates in the AD process. Since macro and micro-nutrients are necessary for the optimal function of anaerobic microorganisms (Speece, 1983), several authors suggest to provide anaerobic digesters with opportune solutions (Angelidaki et al., 2009; Holliger et al., 2016). In particular, the Italian norm on BMP tests UNI/TS 11703:2018 states the use of three different solutions defined as A, B and C containing several elements (N, P, Mg, Ca, K, Fe, Bo, Zn, Cu, Mn, Mo, Co, Ni and Se) in different concentrations. In this research, it was decided to replace those solutions with the pre-treated leachates owing to the presence of metals and ammonia. The formers can be stimulatory to anaerobic microorganisms under particular conditions (Oleszkiewicz and Sharma, 1990). The residual evaluated presence of Cu, Ni and Zn (Table 3.6) made pre-treated leachates similar to the Solution C prepared with CuCl₂, NiCl₂·6H₂O, ZnCl₂ and other metal compounds. Likewise, total ammonia nitrogen, which could be toxic or beneficial to anaerobic microorganisms depending on its concentration (Chen et al., 2008), is found in both Solution A of the UNI/TS norm (in the form of NH₄Cl) and pre-treated leachates in tolerable concentrations (see Table 3.6).

3.2.3.5. Semi-continuous anaerobic digestion tests equipment

Regardless of the cycle, semi-continuous anaerobic tests were carried out in reactors which were 1.1 L sealed glass bottles (WTW-Germany) with three necks (Figure 3.12). The central neck was used to load the reactors at the beginning of the tests and, then, it

was closed by a stopper. Instead, the two lateral ones were equipped with valves and flexible tubes so that periodically allowing, in order, biogas collection, digestate withdrawal (discharging) and feeding by using a syringe (Figure 3.12). All the aforementioned operations were performed in the same days, two or three times per week.

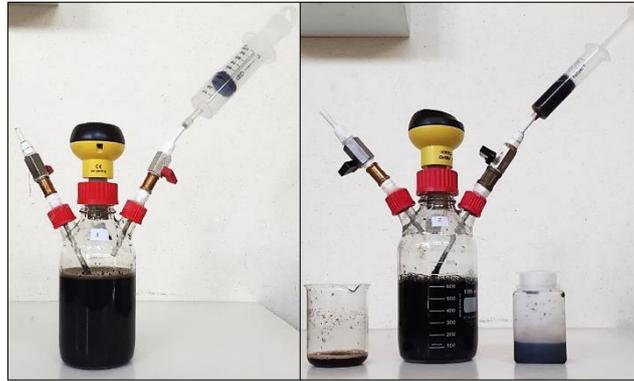


Figure 3.12. Biogas collection (on the left) and discharging/feeding (on the right) phases

Since the AD tests were performed in mesophilic conditions, reactors were placed in a thermostatic cabinet at $35 \pm 0.5^\circ\text{C}$ under continuous mixing throughout tests periods by a magnetic stirrer. Two or three times per week, biogas generated by the digestion was sampled and measured by using a 100 mL syringe. Furthermore, in order to evaluate the volume of methane in biogas samples, biogas was slowly transferred from the syringe into an alkaline trap containing 0.8 L of a 3 M NaOH solution (Figure 3.13). In this way, carbon dioxide present in the biogas was absorbed into the alkaline solution while methane provoked a pressure increase in the trap inducing, finally, the displacement of an equivalent volume of solution measured in a graduated volumetric cylinder (Calabrò et al., 2019). Since the volume of the displaced alkaline solution was considered equal to that of methane present in the biogas, the volume of carbon dioxide resulted by the difference of the methane volume from the total biogas one.



Figure 3.13. Methane content in biogas evaluation through alkaline trap

3.2.4. Semi-continuous anaerobic digestion tests setups

3.2.4.1. *I cycle of digestion tests*

In the I cycle of the semi-continuous AD tests, four reactors designed as A1, B1, C1 and D1 (“1” stands for “I cycle”) were used. In this cycle, pre-treated leachates were added to reactors in two different dosages in order to reach two fixed values of C/N of the feeding mixture which was expressed in terms of COD/NH₄-N on the basis of cellulose characterisation. Accordingly, for the feeding of each reactor, the ratio was calculated considering the COD contents of both cellulose (Table 3.5) and the respective pre-treated leachate divided by the total ammoniacal nitrogen of the pre-treated leachate (Table 3.6). The desired values of COD/NH₄-N were 50 and 25, acceptable and slightly low ratio, respectively, as 50:1 is a recommended value according to Ammary (2004).

Each reactor had a working volume of 600 mL. Initially, in all reactors, quantities of inoculum and distilled water were mixed up to a TS content of the mixture of 4.1%. Cellulose, along with volumes of pre-treated leachate and dilution water, was fed two times per week at an Organic Loading Rate (OLR) of 1.0 gVS/L·d (1.25 gCOD/L·d) for the whole duration of the tests. Pure cellulose is a highly degradable substrate and in batch tests biomethane production is mostly completed in 10 days. For this reason, the Hydraulic Residence Time (HRT) was set at 20 days for the first week (bacteria acclimatation period) and, then, at 10 days for the remaining part of the tests (regime phase) with daily feeding volumes of 30 and 60 mL per day, respectively. The AD tests

were stopped on day 38 (about six weeks). In Table 3.7, reactors types and pre-treated leachates and water daily dosages are reported.

Table 3.7. I cycle of semi-continuous reactors programme

Reactor	Pre-treated leachate	COD/NH ₄ -N	Week I (Acclimation)		Week II-VI (Regime phase)	
			Pre-treated leachate [mL/d]	Dilution water [mL/d]	Pre-treated leachate [mL/d]	Dilution water [mL/d]
A1	ZVI/Lap	25	35.8	0.0	35.8	24.2
B1	ZVI/Lap	50	17.9	12.1	17.9	42.1
C1	ZVI/GAC	25	36.3	0.0	36.3	23.7
D1	ZVI/GAC	50	18.1	11.9	18.1	41.9

3.2.4.2. II cycle of digestion tests

In the II cycle of the semi-continuous AD tests, six reactors designed as A2, B2, C2, D2, E2 and F2 (“2” stands for “II cycle”) were used. In this cycle, only the pre-treated leachate coming from the ZVI/Lap column was used in the AD process and COD/NH₄-N was kept equal to 50 in the reactors. The tests were carried out in duplicate in order to validate results. Reactors A2 and B2 repeated the process of the I cycle (i.e., cellulose and pre-treated leachate digestion). The reactors C2 – D2 and E2 – F2 were supplemented with GAC and biomass fly ash, respectively, in concentrations of 10 g/L.

Also in this cycle, the total operational volume per reactor was 600 mL while the desired TS content of the mixture was set at 3.7% even if the presence of the conductive materials in reactors C2, D2, E2 and F2 led it to 4.7%. As in the I cycle, the OLR was 1.0 gVS/L·d (1.25 gCOD/L·d) for the whole duration of the tests and the HRT was set to 20 days for the first two weeks and, then, 10 days until the end of the tests. The tests were stopped after 57 days of operation (about eight weeks). This implied feeding volumes of 30 mL per day (17.2 and 12.8 mL of pre-treated leachate and water, respectively) in the first two weeks and of 60 mL per day (17.2 and 42.8 mL of pre-treated leachate and water, respectively) from the third week onwards.

3.2.4.3. III cycle of digestion tests

In the III cycle of the semi-continuous AD tests, four reactors designed as A3, B3, C3 and D3 (“3” stands for “III cycle”) were used. In this cycle of tests, both pre-treated leachates were used as supplements and it was possible to calculate the C/N of the feeding (set at 25) thanks to the knowledge of the elemental composition, in terms of carbon and nitrogen, of the MW. Contrary to the previous cycles, in this case, the carbon content of the leachates coming mainly from humic acids was neglected due to their very limited biodegradability.

Each reactor had a total operational volume of 600 mL and an initial TS content of the mixture of 1.8% which reached 2.3% in reactors B3 and D3 where GAC was added in concentration equal to 5 g/L. This concentration was kept constant in those reactors during the tests by adding a quantity of GAC in the feeding mixtures equal to the amount of GAC lost with digestate withdrawals (0.25 g/d).

The OLR and the HRT were set at 1.0 gVS/L·d (1.25 gCOD/L·d) and 20 days, respectively, for the whole duration of the tests. Contrary to the previous cycles, no acclimation period had been planned in this cycle. The tests were stopped after 80 days of operation (about eleven weeks). In Table 3.8, reactors types and pre-treated leachates, water and GAC daily dosages are reported.

Table 3.8. III cycle of semi-continuous reactors programme

Reactor	Pre-treated leachate	GAC addition	Pre-treated leachate [mL/d]	Dilution water [mL/d]	GAC [g/d]
A3	ZVI/Lap	×	20.3	9.7	-
B3	ZVI/Lap	✓	20.3	9.7	0.25
C3	ZVI/GAC	×	21.3	8.7	-
D3	ZVI/GAC	✓	21.3	8.7	0.25

3.2.5. Digestates analyses

For each reactor employed in the semi-continuous AD tests, digestate was withdrawn before every feeding procedure (see 3.1.1.8.). Except for pH, which was measured on daily digestate samples, in each cycle of tests, average weekly samples of digestates were

prepared and analysed in terms of TS, VS, COD, NH₄-N (in the I and the II cycle), N_{tot} (only in the III cycle), total Volatile Fatty Acids (VFAs) and the Volatile Organic Acids/Buffering Capacity ratio (FOS/TAC) (in the II and the III cycle). pH, TS and VS were determined on raw digestates whereas COD, NH₄-N, N_{tot}, total VFAs and FOS/TAC analyses were carried out on the liquid fractions resulting from digestates centrifugation (10000 rpm for 10 min). All the parameters were determined as 3.1.1.3. and 3.1.1.4. except total VFAs and FOS/TAC which were determined through a four-point titration method (Liebetrau et al., 2016) consisting of titrating 20 mL of centrifuged digestate up to pH values of 5.0, 4.4, 4.3 and 4.0 with 0.1 N sulphuric acid solution. The parameters were calculated by using Eqs. 3.2 and 3.3 (Liebetrau et al., 2016; Mézes et al., 2011):

$$\text{Total VFAs [mg/L]} = \left[131140 \cdot (V_{\text{pH}_{4.0}} - V_{\text{pH}_{5.0}}) \cdot \frac{N_{\text{H}_2\text{SO}_4}}{V_{\text{sample}}} \right] - \left[3.08 \cdot V_{\text{pH}_{4.3}} \cdot \frac{N_{\text{H}_2\text{SO}_4}}{V_{\text{sample}}} \cdot 1000 \right] - 10.9 \quad (3.2)$$

$$\text{FOS/TAC} = \frac{[(V_{\text{pH}_{4.4}} \cdot 1.66) - 0.15] \cdot 500}{V_{\text{pH}_{5.0}} \cdot 250} \quad (3.3)$$

where: $V_{\text{pH}_{4.0}}$, $V_{\text{pH}_{4.4}}$, $V_{\text{pH}_{4.3}}$, $V_{\text{pH}_{5.0}}$ stand for volumes (in mL) of added solution until a pH of 4.0, 4.4, 4.3 and 5.0, respectively, was reached; V_{sample} stands for volumes (in mL) of sample (20 mL); $N_{\text{H}_2\text{SO}_4}$ is the normality of the acid solution (0.1). VFAs and FOS are rendered as acetic acid equivalent (mg_{HAC}/L) and TAC as lime equivalent (mg_{CaCO₃}/L).

3.2.6. Results' discussion

The performance of each semi-continuous AD reactor is mainly evaluated on the basis of the corresponding methane production. In particular, this is expressed in terms of methane yield and cumulative methane production. The former represents the total volume of methane produced by the digestion process per mass of VS added in the reactor until the day considered. When the process runs stably, it exhibits a constant trend which witnesses that biodegradable matter is thoroughly consumed by bacteria for methane generation. The latter represents the total volume of methane generated by the reactor over test duration.

Alongside these two parameters, for each cycle of the semi-continuous AD tests, digestate analyses (pH, VFAs, FOS/TAC, COD, NH₄-N and N_{tot}) are also presented in order to complete the assessment of the process and better understand its evolution over time.

As explained in Paragraph 3.1. (i.e., Experimental design), the semi-continuous AD tests consisted of three different cycles. Both the I and the II cycles involved the addition of the pre-treated leachates to the AD of a model organic substrate (i.e., pure cellulose) with the purposes of preliminary proving the feasibility of this novel process design and finding out the optimum conditions. For this reason, in the corresponding discussions, only the comparison with the ideal cellulose anaerobic biodegradation is made. Conversely, a more realistic AD process is carried out in the III cycle of AD tests by supplying the pre-treated leachates to the AD of MW. In this case, a comparison with similar experiments (especially on the basis of biogas and methane productions and process stability) can be useful to investigate the actual advantages of the proposed system.

3.3. Results and Discussion

In this Paragraph, results obtained from both preliminary and final phases of the integrated system for the MLL treatment are reported and discussed. It starts with the column tests results in terms of pollutants concentrations in the effluent (pre-treated leachate). On the basis of them, a discussion about the efficiency of the pre-treatment takes place. Afterwards, semi-continuous AD tests are interpreted mainly on the basis of methane production and process stability.

3.3.1. Column tests

3.3.1.1. pH

pH was measured in the effluents withdrawn from three sampling ports (5, 28 and 50 cm) on day 3 and 7 (Table 3.9).

Table 3.9. pH values in leachate samples withdrawn from column tests

Day	ZVI/Lap column			ZV/GAC column		
	5 cm	28 cm	50 cm	5 cm	28 cm	50 cm
3	8.13	8.32	8.26	8.33	7.52	6.10
7	8.00	7.92	7.96	8.15	8.40	8.39

According to these results, it seems clear that, in both columns, pH stabilised on the influent value of about 8 (alkaline leachate) for the entire duration of the tests.

3.3.1.2. Ammonium, Chloride and COD

Ammonium, chloride and COD concentrations were measured in pre-treated leachates samples withdrawn from the columns outlets during the tests. The results, reported as averages of two replicates with respective standard deviations, are summarised in Table 3.10 for both tested columns.

Table 3.10. Ammonium, chloride and COD concentrations in the effluents coming from ZVI/Lap and ZVI/GAC columns

Day	ZVI/Lap column			ZVI/GAC column		
	Ammonium [mg/L]	Chloride [mg/L]	COD [mg/L]	Ammonium [mg/L]	Chloride [mg/L]	COD [mg/L]
Input leachate	911 ± 1	3060 ± 20	3535 ± 35	912 ± 14	3120 ± 40	3450 ± 30
2	395 ± 3	2880 ± 40	1455 ± 35	158 ± 14	3220 ± 20	1220 ± 60
8	898 ± 14	3100 ± 20	3475 ± 15	923 ± 13	3100 ± 20	2200 ± 10
14	897 ± 11	3220 ± 20	3335 ± 5	934 ± 26	3180 ± 20	2520 ± 10
23	900 ± 0	3080 ± 0	3355 ± 5	924 ± 30	3140 ± 20	2835 ± 25
38	887 ± 1	3100 ± 20	3435 ± 5	897 ± 5	3120 ± 0	2785 ± 25

In general, ammonium, chloride and COD removal was basically negligible for both granular media with the only exception of COD in the ZVI/GAC column.

In the first days of operation, ammonium content in both pre-treated leachates decreased of 57% (ZVI/Lap column) and 83% (ZVI/GAC column) compared to the concentration in

the influent leachate. Instead, from day 8 onwards, ammonium amount in the pre-treated leachates remained close to influent concentration regardless of the column. GAC was not expected to significantly take part in ammonium removal (Bilardi et al., 2018a; Halim et al., 2010) due to its nonpolar surface, which results in poor interactions with polar adsorbates (Park and Kim, 2005). Nevertheless, it is noteworthy that the column filled with the mixture of ZVI and GAC showed a better performance in ammonium removal compared to the ZVI/Lap one, even if only for a really short period of tests time.

Similarly, chloride was basically unaffected by the pre-treatment. Its content in both pre-treated leachates slightly decreased only in the effluent from the ZVI/Lap column on day 2 (6%).

COD concentrations measured after 2 days witnessed a decrease in both tests (R_E of 59% and 65% for ZVI/Lap and ZVI/GAC column, respectively) whereas, during the tests, COD was slightly removed only by the ZVI/GAC granular mixture due to the key role played by the presence of GAC. Indeed, GAC is known as an effective adsorbent for organic matter (Bansal and Goyal, 2005), and its application is common in leachate treatment research (Bilardi et al., 2020; Mohammad-pajooch et al., 2018; Morawe et al., 1995; Zhou et al., 2014). In general, as Kurniawan et al. (2006) asserted, the application of activated carbon adsorption is effective for the removal of organic compounds from MLLs but not for ammonia nitrogen. ZVI was also showed to be effective in COD removal from leachate through oxidation–reduction reactions between the ZVI and the organic matter or by entrapment in the matrix of iron corrosion products (Bilardi et al., 2018a; Zhou et al., 2014).

On the contrary, lapillus was found to not be a suitable material for ammonium, chloride, and COD removal. This proves that lapillus has no interactions with these contaminants, although they were not the specific targets of the pre-treatment.

3.3.1.3. *Heavy metals*

Likewise ammonium, chloride and COD, heavy metals concentrations were measured in pre-treated leachates samples withdrawn from the columns outlets during the tests and their trends over the tests time are depicted in graphs of Figure 3.14 while, in histograms, the corresponding removal efficiencies are reported.

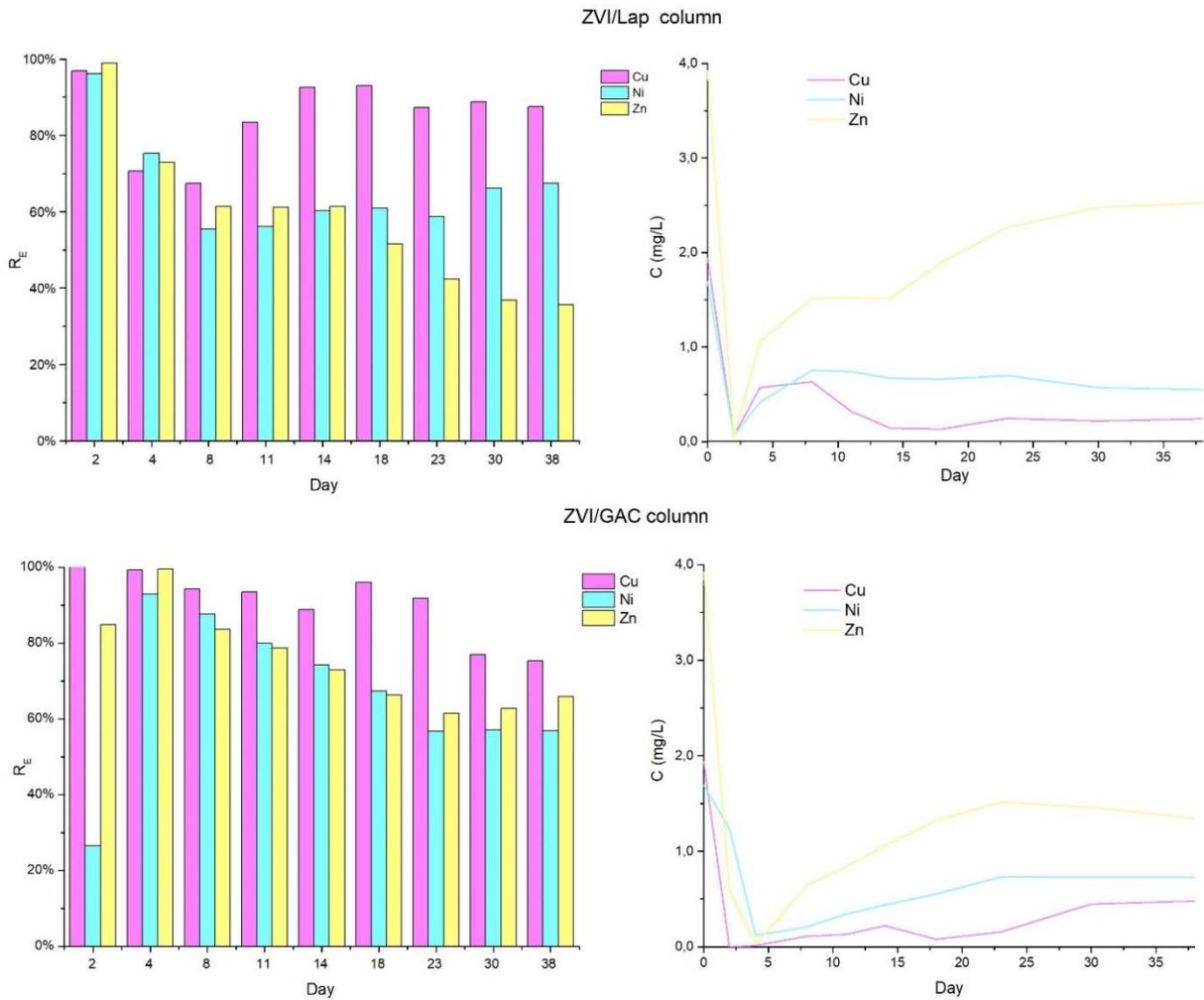


Figure 3.14. Heavy metals removal efficiencies and concentrations in the effluents coming from ZVI/Lap and ZVI/GAC columns

After 2 days of operation, metals removal in the ZVI/Lap filter was almost complete with an efficiency close to 100% for the three metals. Afterwards, the removal efficiency toward Cu reduced down to 67% on day 8 and, then, assumed values between 84% and 93% until the end of the test. The removal of both Ni and Zn was less considerable: it was in the range 55–67% for Ni while it gradually decreased over the test from 61% on day 8 to 36% on day 38 for the Zn.

Referring to the column filled with the ZVI/GAC mixture, Cu was completely removed at the beginning of the test (R_E of about 100%). Its removal efficiency decreased only in the last days of the test assuming the lowest value of 75% on day 38. Ni removal was scarce until day 2 ($R_E = 27%$) but significant on day 4 ($R_E = 93%$). From this point, the removal efficiency toward Ni progressively decreased to 57% on day 38. Lastly, Zn removal

efficiency increased to almost 100% until day 4 and, then, gradually decreased from 84% on day 8 to 66% on day 38.

In general, results show that both granular filters were effective in removing the three contaminants according to the following sequences for ZVI/Lap and ZVI/GAC granular mixtures, respectively: Cu > Ni > Zn and Cu > Zn > Ni.

ZVI was expected to remove heavy metals through mechanisms of reduction, adsorption onto its corrosion products, and precipitation and coprecipitation with its precipitating oxides (Madaffari et al., 2017; Wu et al., 2020). In particular, Cu was removed via cementation process, while Ni and Zn removal relied on adsorption onto ZVI corrosion products and coprecipitation with ZVI oxides. These processes are facilitated by low pH values. For this reason, since in these tests an alkaline solution was used, it can be supposed that ZVI corrosion was slowed down. However, this could imply the functionality of the filter for a longer period of time.

The role of GAC in heavy metals removal from leachate is well known (Foo and Hameed, 2009). For instance, its removal efficiency was tested by Wasay et al. (1999) for solutions with pH ranging from 6.0 to 7.7 with a removal efficiency for several heavy metals (Cd^(II), Cu^(II), Cr^(III), Mn^(II), Pb^(II), and Zn^(II)) in the range of 80–96% from an initial concentration of 184 mg/L. GAC can remove heavy metals through sorption on its porous surface as well as through both surface and pore precipitation (Chen and Wang, 2000; Reed, 1995). Moreover, sorption can also occur with organic matter because heavy metals in landfill leachate are supposed to form complexes with it (Modin et al., 2011).

Similarly, lapillus is expected to remove heavy metals ions from aqueous solution via adsorption as demonstrated in previous studies (Bilardi et al., 2019; Bilardi et al., 2018; Madaffari et al., 2014).

Cu was, as expected, the most easily removed metal by both filters (Figure 3.14). The ZVI/GAC filter performed slightly better than the ZVI/Lap one with R_E values of 91% and 85% on average, respectively. Similar results in terms of removal efficiency were reported by Bilardi et al. (2020) for a ZVI/GAC mixture (weight ratio 30:70 and 225 g of ZVI) but considering an acidic leachate (pH = 5) and a longer residence time (flow rate of 0.1 mL/min).

Ni removal had an almost identical efficiency (66% and 67% on average) for both filters. However, the ZVI/Lap column removed Ni with a more regular trend compared to the ZVI/GAC filter (Figure 3.14). Ni removal was far more efficient in Bilardi et al.'s study (2020) where a ZVI/GAC granular mixture was tested toward an acidic leachate (removal efficiency ranged from 93% to 80%). This behaviour can be attributed to the aforementioned more favourable pH conditions for iron corrosion and the higher residence time (upflow rate of 0.1 mL/min).

Lastly, the main difference between the two columns was exhibited on account of Zn removal. In fact, the ZVI/GAC granular mixture removed Zn better than the ZVI/Lap one with efficiencies of 75% and 58% on average, respectively. As in the case of Ni, Zn removal performance was considerably better for the ZVI/GAC filter studied by Bilardi et al. (2020) (R_E ranged from 98% to 95% using an acidic leachate) confirming the better aptitude of the filter for the treatment of acidic solutions due to enhanced iron corrosion.

Nevertheless, the metal removal sequence of this test (Cu > Ni > Zn) was in accordance with that of Bilardi et al. (2020).

In Table 3.11, the removal capacities of the columns for each pollutant are reported.

Table 3.11. Heavy metals removal capacities of each column

Day	Rc [mg/Kg _{filter}] ZVI/Lap column			Rc [mg/Kg _{filter}] ZVI/GAC column		
	Cu	Ni	Zn	Cu	Ni	Zn
2	2.5	2.1	5.1	5.1	1.2	8.7
4	4.6	4.0	9.5	10.1	3.8	18.1
8	8.1	6.9	16.3	19.9	11.8	36.8
11	10.9	8.7	21.0	27.1	17.3	49.3
14	14.3	10.7	25.7	34.0	22.4	60.9
18	19.0	13.3	31.5	43.3	28.7	75.2
23	24.7	16.6	37.5	55.2	35.5	91.5
30	32.5	21.5	44.6	70.2	44.3	113.8
38	41.4	27.4	52.0	85.6	54.3	140.1

The ZVI/GAC filter exhibited a specific removal capacity towards each metal about double than that of the ZVI/Lap granular mixture over tests time. This result is in accordance with the better removal efficiency observed for the ZVI/GAC filter, compared to that of the

ZVI/Lap one (see Figure 3.14), related to a lower amount of granular mixture necessary to fill the column (i.e., 750 g of ZVI and GAC versus 1500 g of ZVI and Lap, see Table 3.2).

All results considered, it can be stated that the ZVI/GAC granular filter showed a better metal removal performance than the ZVI/Lap granular mixture for the MLL treatment. The aptitude of GAC for effective metal removal in alkaline conditions supported the ZVI which normally performs better with acidic leachate. On the contrary, lapillus seemed less efficient in sufficiently supporting ZVI with metal removal. However, it is far cheaper since it is widely available and often produced as a by-product of pumice extraction, which is a big advantage for its practical use.

3.3.2. Semi-continuous anaerobic digestion tests

3.3.2.1. Pre-treated leachates

As shown in Table 3.6, the composite samples of the MLL pre-treated by ZVI/Lap and ZVI/GAC mixtures had very similar compositions in terms of pH as well as ammonium, chloride, Cu, and Ni concentrations. Conversely, COD and Zn concentrations were lower in the pre-treated leachate derived from the ZVI/GAC column.

The concentrations of heavy metals and chloride in the feeding volumes were preliminary calculated for each reactor of each cycle on the basis of the added volumes of pre-treated leachates and water (Table 3.12). Under the reasonable hypothesis that both heavy metals and chloride were conserved during the digestion tests (i.e., unaffected by the biological process) and taking into account that before the feeding phase an equal volume of digestate was discharged, these concentrations were considered as the maximum ones potentially present in the anaerobic systems over tests duration.

Table 3.12. Maximum concentrations of heavy metals and chloride in anaerobic reactors

	Reactor	C_{\max} [mg/L]			
		Cu	Ni	Zn	Cl ⁻
I cycle	A1	0.18	0.31	0.98	1828
	B1	0.09	0.16	0.49	914
	C1	0.25	0.33	0.59	1821
	D1	0.12	0.17	0.29	911
II cycle	A2	0.09	0.15	0.47	880
	B2	0.09	0.15	0.47	880
	C2	0.09	0.15	0.47	880
	D2	0.09	0.15	0.47	880
	E2	0.09	0.15	0.47	880
	F2	0.09	0.15	0.47	880
III cycle	A3	0.20	0.35	1.11	2077
	B3	0.20	0.35	1.11	2077
	C3	0.29	0.39	0.69	2140
	D3	0.29	0.39	0.69	2140

In case of heavy metals and chloride, concentrations in the AD tests were far lower than the inhibitory thresholds which were found to be 500, 100, and 50 mg/L for Cu, Ni, and Zn, respectively, (Guo et al., 2019) and 4–9 g/L for chloride (Viana et al., 2012). Similarly, it can be stated that the low amount of pre-treated leachates added as well as the dilution with water and inoculum would significantly reduce the concentration in the AD reactors of other persistent contaminants possibly present in real leachates (e.g., pharmaceuticals, pesticides, hydrocarbons and microplastics).

3.3.2.2. I cycle

The I cycle of the semi-continuous AD tests aimed at testing the response of the process (AD of pure cellulose) to the addition of pre-treated leachate used as nutrient solution. Both leachates coming from the column tests were employed and two different values of COD/NH₄-N (50 and 25) were set (see the I cycle of semi-continuous reactors programme in Table 3.7).

Reactor B1, loaded with cellulose and leachate pre-treated by the ZVI/Lap column, was the best-performing reactor after 38 days of test probably due to its high COD/NH₄-N of 50 which resulted in a lower amount of leachate in the feeding mixture. Nevertheless, a good performance was recorded just for the first two weeks of the test. The other reactor fed with ZVI/Lap pre-treated leachate (i.e, A1) but with a COD/NH₄-N set at 25, so with a higher volume of leachate in the feeding mixture, was abruptly inhibited after about half the test time most probably because of the accumulation of humic substances present in leachate. Reactor C1, fed with a volume of ZVI/GAC pre-treated leachate so that reaching a COD/NH₄-N of 25, was the second-best performing reactor in terms of methane production with a starting good performance followed by a decline probably for the same reason of reactor A1 failure. Finally, reactor D1 was the most stable but with the lowest methane production. In this case, the addition of low volumes of ZVI/GAC pre-treated leachate, in order to reach a COD/NH₄-N of 50, slowed down the digestion however without causing a complete inhibition of the process.

As mentioned in Paragraph 3.2.6., the performance of the semi-continuous reactors is evaluated in terms of methane production. Thus, methane yields and cumulated methane productions for each reactor are depicted in Figures 3.15 and 3.16, respectively.

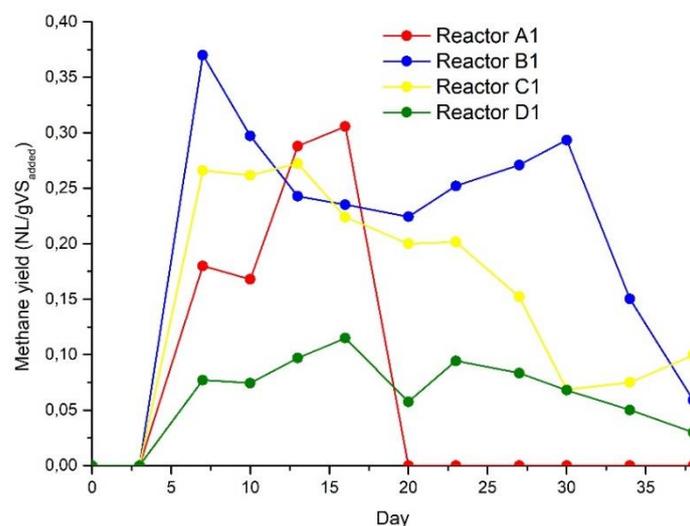


Figure 3.15. I cycle semi-continuous anaerobic reactors methane yields

Reactor A1 exhibited a gradually growing methane production up to 0.3 NL/gVS_{added} (days 16 – 20) but, then, methane production abruptly stopped. Reactor B1 reached the highest

methane yield (0.37 NL of methane per grams of VS added) after the first week of operation. From days 10 to 30, methane yield was fairly stable remaining between 0.22 and 0.30 NL/gVS_{added} (0.26 NL/gVS_{added} on average). During the last week of operation, methane production sharply decreased to 0.06 NL/gVS_{added} on the last day of the test. Reactor C1 showed a trend similar to reactor B1 but with lower methane yield values. The maximum (0.27 NL/gVS_{added}) was measured on day 13, before that its production gradually decreased. In reactor D1, no considerable methane yield variations were measured. Indeed, methane yield remained close to 0.1 NL/gVS_{added} for almost the entire test duration.

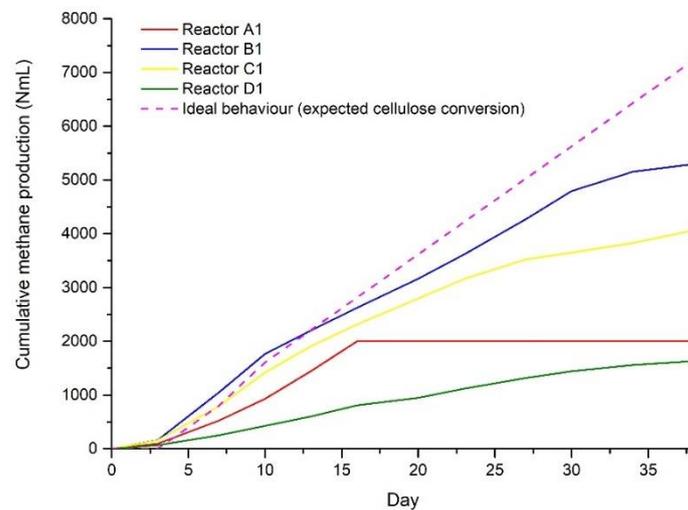


Figure 3.16. I cycle semi-continuous anaerobic reactors cumulative methane productions

Cumulative methane productions of the four reactors were compared to that of the ideal AD of pure cellulose. The latter was determined by accounting for the cellulose a biomethanation potential of 335 NmL/gVS reported on the basis of the aforementioned UNI/TS 11703:2018 norm. After 38 days, the complete digestion of pure cellulose would have generated 7.2 NL of methane on average. However, this expected cellulose conversion is based on the strong hypothesis that all the cellulose is completely and immediately consumed in the biological process (“ideal” AD process). For this reason, the comparison between expected cellulose degradation and results coming from semi-continuous tests allows to evaluate how tested AD processes are far from their ideal behaviours. In other words, it serves to preliminary comprehend the effects of the pre-treated leachate addition to the AD processes. During the first two weeks of operation, the

methane amount produced by reactor B1 process was consistent with the expected production from the complete conversion of cellulose, whereas at the end of the test, the final cumulative methane production was about 5.3 NL. Reactor C1 was the second-best process in terms of cumulative methane volume with a final value of about 4.1 NL and a production close to that of reactor B1 for the first four weeks. Reactor A1 reached a final methane production of 2.0 NL on day 16, as, from that point onward, methane generation was null. Conversely, reactor D1 generated methane throughout the experiment, despite maintaining low yields, achieving 1.6 NL of total methane production.

pHs of the semi-continuous anaerobic systems were periodically recorded. In Figure 3.17, pH trends of reactors involved in the I cycle of AD tests are depicted.

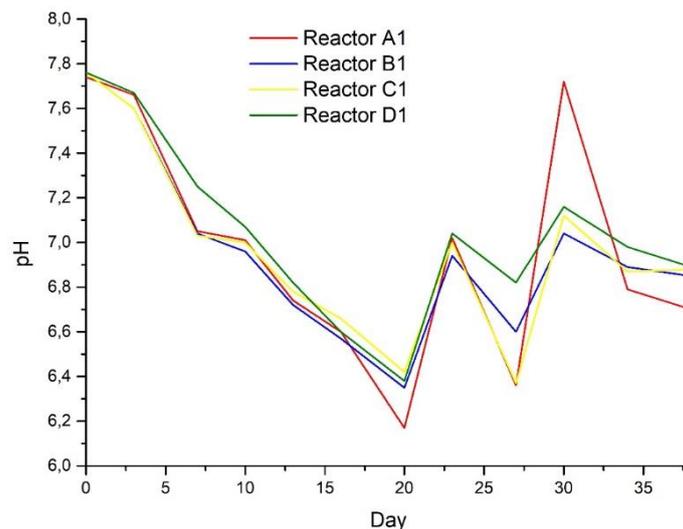


Figure 3.17. I cycle semi-continuous anaerobic reactors pH trends

During the semi-continuous AD tests, pH of all reactors decreased from an initial value of around 7.8 showing similar trends. The lowest value (6.2) was reached in reactor A1 on day 20 probably due to an accumulation of VFAs (see below). On days 20 and 27, 4 g of NaHCO_3 were added to all reactors in order to buffer the pH. As a result, a peak of 7.7 was measured on day 30 in reactor A1. At the end of the tests, all reactors exhibited a pH of 6.9 except for reactor A1 (6.7). Despite the decreasing trends, the pH did not reach inhibitory values for methanogenic bacteria. However, the very irregular pH trend in reactor A could possibly hint at process imbalance.

The analysis of the average weekly digestates collected from the four reactors is summarised in Tables 3.13 and 3.14 (Total and Volatile Solids contents, respectively) and Figures 3.18, 3.19 and 3.20 (VFAs, COD and NH₄-N concentrations, respectively).

Table 3.13. TS contents in weekly digestates of the I cycle of semi-continuous reactors

Week	TS (%)			
	A1	B1	C1	D1
I	3.6%	3.6%	3.6%	3.7%
II	2.5%	2.4%	2.5%	2.5%
III	1.2%	1.5%	1.4%	1.1%
IV	1.6%	1.3%	1.2%	1.2%
V	1.4%	1.0%	1.1%	1.0%
VI	0.8%	0.6%	0.6%	0.6%

Table 3.14. VS contents in weekly digestates of the I cycle of semi-continuous reactors

Week	VS (%TS)			
	A	B	C	D
I	71.0%	74.7%	74.5%	74.8%
II	73.2%	76.0%	77.2%	77.4%
III	75.0%	81.5%	75.9%	73.4%
IV	59.3%	60.8%	55.3%	59.2%
V	45.0%	47.1%	38.0%	43.9%
VI	45.2%	52.5%	30.6%	49.6%

Both TS and VS trends were similar among digestates and decreased during the tests in each reactor. Considering the initial TS value of each reactor mixture (4%), solid matter was scarcely consumed by the microbial community during the first week of operation whereas the solid content decreased from the second week onward. At the end of the tests, TS reached values of 0.6–0.8%. VS remained almost constant for the first three weeks of tests in all reactors and, then, their presence sharply decreased. The abrupt and abnormal reduction in VS from week IV onward hints at a reduction in microbial activity in the reactors as discussed below.

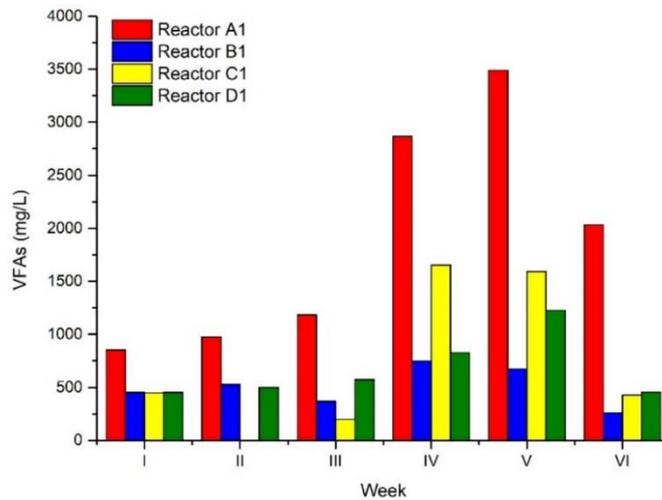


Figure 3.18. VFAs contents in weekly digestates of the I cycle of semi-continuous reactors

During the first two weeks of the tests, the highest contents in VFAs (852 and 975 mg/L, respectively) were recorded in reactor A1 while those in reactors B1, C1, and D1 were similar (data for reactor C1 from the second week were not recorded because of a sampling problem). From week III onward, a continuous increase in VFAs was recorded in reactor A1 again suggesting a probable imbalance of the process. The VFAs concentration reached inhibitory levels from week IV onward in this reactor. VFAs also increased to a lesser extent in reactors C1 and D1 in weeks IV and V. The drop in the level of VFAs in week VI, when methane production was already scarce, even in the best-performing reactor (i.e., B1), could have been due to a possible inhibition of hydrolysis.

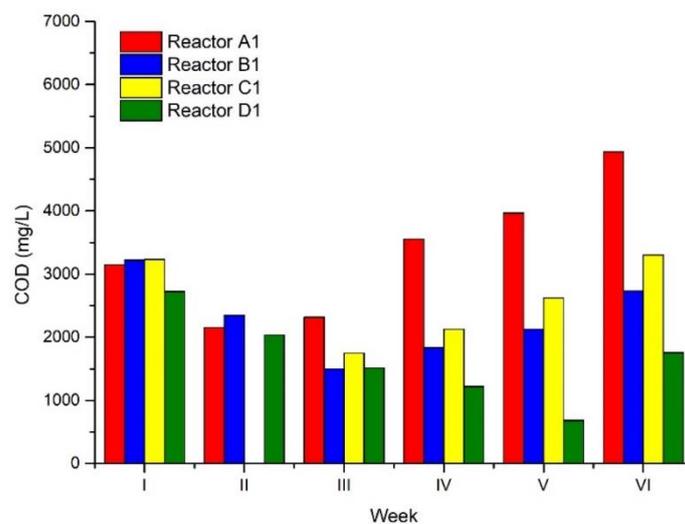


Figure 3.19. COD contents in weekly digestates of the I cycle of semi-continuous reactors

In the centrifuged composite digestate samples from the first week, a COD of about 3200 mg/L was measured in each reactor. Besides the aforementioned sampling problem for reactor C1, in the second week, slight decreases in COD content were recorded for all reactors. From the third week onward, COD concentration in reactor A1 started to increase again reaching a peak of 4935 mg/L at the end of the experiment. The COD concentration in reactors B1 and C1 increased from the fourth week until the end of the tests but with lower values compared to reactor A1 behaviour. Conversely, the COD concentration in reactor D1 smoothly decreased during the digestion, with the lowest value of 680 mg/L recorded in the fifth week, and increased only in the last week up to 1755 mg/L.

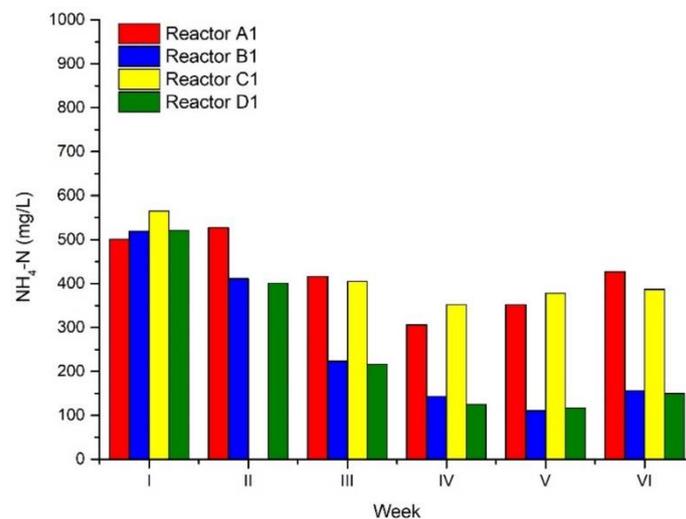


Figure 3.20. $\text{NH}_4\text{-N}$ contents in weekly digestates of the I cycle of semi-continuous reactors

The highest $\text{NH}_4\text{-N}$ concentrations were recorded in the first week of tests for reactors B1, C1, and D1 (519, 565, and 521 mg/L, respectively) and in the second week for reactor A1 (527 mg/L). In reactor A1, ammonium content decreased to 306 mg/L in the fourth week, before increasing until the end of the experiment. In other reactors, analogous trends were recorded but with $\text{NH}_4\text{-N}$ concentration values which were lower in reactors B1 and D1 and similar in reactor C1 compared to reactor A1. As with heavy metals and chloride (Table 3.12), the $\text{NH}_4\text{-N}$ content in the AD reactors was lower than the inhibitory threshold (1.7 g/L) found in the literature (Chen et al., 2008; Yenigün and Demirel, 2013).

The graphs of methane yields and cumulative methane productions in Figures 3.15 and 3.16 clearly show that, with the exception of reactor D1, methane production from all reactors was not steady. This occurrence is probably due to both operating conditions of the tests and inhibitory effects of the pre-treated leachates. Normally, cellulose digestion is not expected to be problematic because of cellulose biodegradability. However, the choice of a relatively low HRT (i.e., 10 days) coupled with a non-regular feeding (i.e., two times per week) could have severely affected the performance of the reactors especially in presence of possible inhibitory agents present in the pre-treated leachate. Also, the addition of the pre-treated leachates perturbed anaerobic systems to some extent. Indeed, after an initial methane production quite close to the ideal one expected for cellulose, especially from reactors B1 and C1, then, all processes showed clear signs of inhibition regardless of the type of pre-treated leachate added or COD/NH₄-N value. Although tests were stopped after three times the set HRT (i.e., 30 days without counting the first acclimatation week), however, shortly before that time anaerobic processes in reactors did not run stably and efficiently.

In fact, inhibition was early and abrupt for reactor A1, where after day 16, methane production totally stopped, whereas it was more delayed for reactor B1 (i.e., the best-performing system) where it was significant and quite steep from day 30 onward. On the other hand, inhibition was more gradual in reactor C1, with a steady and slow decline beginning on day 13. Reactor D1 exhibited a very peculiar behaviour whereby the methane production was very slight but regular throughout the test, equal to, approximately, 25% of the ideal production expected for pure cellulose. The analysis of trends for pH, VFAs, and NH₄-N (Figures 3.17, 3.18 and 3.20) of reactor D1 did not justify this observation. Indeed, even though pH and VFAs values are consistent with those measured for reactors B1 and C1, its methane production was far lower compared to those of the just mentioned reactors. This behaviour could be classified as an “inhibited steady state”, namely, a condition in which the process runs stably but with methane yield lower than expected due to limited bacterial activity (Chen et al., 2008).

The identified inhibition of methanogenesis is most probably not attributable to an excess of metals or chloride (Table 3.12) or NH₄-N (Figure 3.20) since, as already shown, the concentrations of those parameters were well below their respective inhibition thresholds. Li et al. (2019) reported the inhibition of anaerobic processes (particularly

hydrolysis and methanogenesis) due to the presence, as in this case, of noticeable amount of humic substances. In addition, they also stated that the inhibition was irreversible and exacerbated by an increase in the humic substances/VS ratio and that humic substances interfere with hydrolytic and methanogenic enzymes. In this test, since the biomass could not adapt to the presence of humic substances, the abundance of microorganisms decreased, which is in agreement with the reduction in VS measured for all reactors. It is interesting to underline that the two reactors where methane production was more noticeable (reactor B1) or more regular albeit quite scarce (reactor D1) were those with the highest set value of COD/NH₄-N (50) which corresponded to the lowest volume of pre-treated leachates added and, thus, of humic substances.

To sum up, according to the results of the I cycle of tests, the addition of pre-treated MLL to the AD of pure cellulose did not boost the process but, conversely, caused its inhibition principally due to humic substances presence. Nevertheless, it was observed that the reactor B1 supplemented with leachate pre-treated by the ZVI/Lap column (COD/NH₄-N of 50) exhibited a promising behaviour at least in the first half of the test.

3.3.2.3. *II cycle*

In the II cycle of the semi-continuous AD tests, it was decided to test the same operational conditions of the best-performing reactor of the I cycle (i.e., B1) in order to both validate its results (in reactors A2 and B2) and investigate two possible solutions to the inhibition issue in the long term (couple of reactors C2 – D2 and E2 – F2) (see the II cycle of semi-continuous reactors programme in Paragraph 3.1.1.10.).

The addition of GAC to reactors C2 and D2 was one of the adopted solutions. GAC was expected to stimulate DIET and adsorb humic substances which were found to be toxic for microorganisms involved in the previous tests of semi-continuous AD. However, GAC did not completely succeed in enhancing the AD of the pure cellulose. In fact, even though C2 was the second best-performing reactor of this cycle, it performed well just in the first part of the test before that its methane production slowed down and, eventually, stopped. Moreover, the other reactor loaded with GAC (i.e., D2) did not achieve the same level of performance. The second solution tested in order to overcome reactors inhibition in the long term was the addition to reactors E2 and F2 of biomass fly ash with the same

expectations of GAC addition. In this case, no positive effects were recorded but, conversely, it appeared clear that biomass fly ash severely perturbed the system since reactors E2 and F2 were the worst-performing ones of the cycle. Finally, reactors A2 and B2 replicated the process of reactor B1 of the previous cycle. A2 was the best-performing reactor of the cycle producing methane for a longer time than reactor B1. However, likewise reactor B1, its process suffered from inhibition in the long term probably for the accumulation of humic substances. On the other hand, process of reactor B2 actually started only after ten days of operation and was never able to reach an acceptable level of performance.

Methane yields and cumulated methane productions for the reactors of the II cycle are depicted in Figure 3.21 and 3.22, respectively.

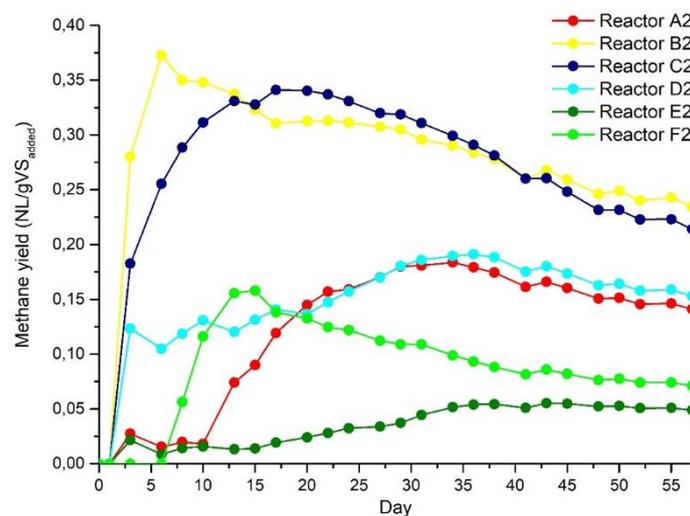


Figure 3.21. II cycle semi-continuous anaerobic reactors methane yields

The two best-performing reactors of this cycle (i.e. B2 and C2) showed similar methane yield trends. Reactor B2 reached the highest level of methane yield (almost 0.4 NL/gVS_{added}) on day 6 whereas in reactor C2 methane yield curve increased more gradually and reached its maximum (0.3 NL/gVS_{added}) on day 17. After their respective peaks, methane yield trends of reactors B2 and C2 were continuously decreasing over test time however with higher values compared to those measured in the other reactors. When test was stopped, reactors had a methane yield of 0.23 and 0.21 NL/gVS_{added}, respectively. The corresponding replicates of reactors B2 and C2 (i.e., reactors A2 and D2,

respectively) behaved in different ways. The anaerobic microorganisms in the reactor A2 took time (about ten days) before starting to convert organic matter and produce methane appropriately. Afterwards, the methane yield increased up to 0.18 NL/gVS_{added} on day 34 and, then, it slightly decreased until the end of the experiment (0.14 NL/gVS_{added} on day 57). Reactor D2 exhibited a basically identical methane yield trend from day 10 onwards however, in contrast to reactor A2, its process immediately started as testified by the peak of its methane yield on day 3 (0.12 NL/gVS_{added}). Reactors E2 and F2 also had different behaviours. The former was the worst-performing reactor of the cycle; its methane yield maintained almost constant over test time but with very low values. The latter had a methane yield comparable to reactors A2 and D2 in the first twenty days but, then, considerably reduced until almost reaching methane yield values of reactor E2.

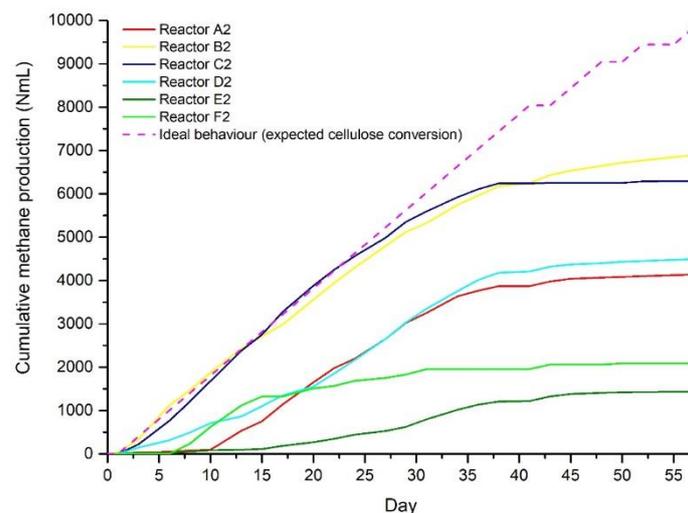


Figure 3.22. II cycle semi-continuous anaerobic reactors cumulative methane productions

In this cycle, the expected cellulose conversion would have guaranteed a cumulative volume of methane over test time of 9.8 NL. Three times from day 41 onwards, feeding in all reactors was suspended in order to prevent inhibition since process slowdowns had been observed. This resulted in level lines in methane production from ideal cellulose conversion. Unfortunately, processes in all reactors were already jeopardised to different extents. Indeed, cumulative methane production trends levelled off from day 38. Cumulative methane production from reactor B2 followed the ideal cellulose conversion for thirteen days before gradually withdrawing from it until the end of the test.

Nevertheless, reactor B2 produced the highest volume of methane of the test after 57 days (6.9 NL). Reactor C2 performed a little better until the inhibition on day 38 where it achieved its maximum volume of cumulative methane (6.2 NL). In contrast, cellulose conversion into methane in the other reactors was far slower and lower than the ideal one. Reactors A2 and D2 produced similar volumes of methane over test time (4.1 and 4.5 NL, respectively) whereas reactors E2 and F2 hardly reached 2.0 NL after 57 days.

In the next figure (Figure 3.23), pH trends of all reactors are depicted.

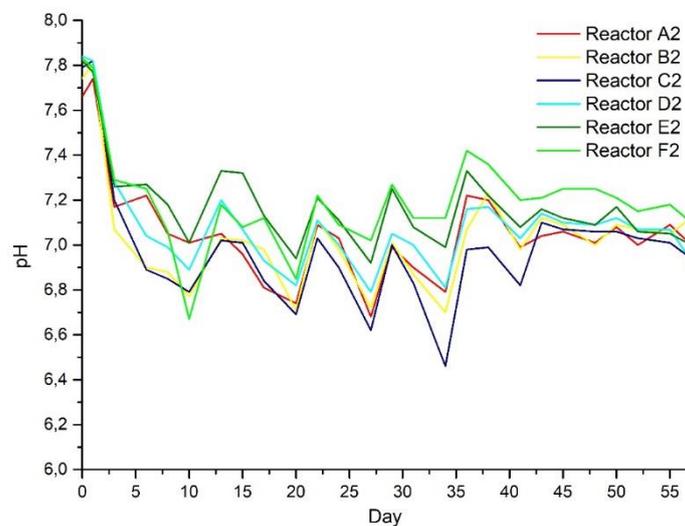


Figure 3.2312. II cycle semi-continuous anaerobic reactors pH trends

pH values of all reactors fell from their initial value of about 7.8 as soon as digestion process started. In order to prevent severe pH declines, four times (on days 10, 20, 27 and 34) 4 g of NaHCO_3 were added to all reactors. As a consequence, pH trends peaked in the next days. The lowest pH value of the cycle was measured in reactor C2 (6.5 on day 34) just before that its methane production stopped. On the contrary, it can be noticed that reactors with limited methanogenesis (i.e., E2 and F2 both supplemented with biomass fly ash) had slightly higher pH than the others. At the end of the test, pH of all reactors was in the range 6.9 – 7.1. Also in this cycle of AD, apart from reactor C2, pH did not reach inhibitory values for methanogenic bacteria even though its irregular trend is a symptom of process deficiencies.

The characterisation of digestates withdrawn from reactors is reported in terms of TS and VS (Tables 3.15 and 3.16, respectively), VFAs and FOS/TAC (Figures 3.24 and 3.25, respectively) and COD and NH₄-N (Figures 3.26 and 3.27, respectively).

Table 3.15. TS contents in weekly digestates of the II cycle of semi-continuous reactors

Week	TS (%)					
	A2	B2	C2	D2	E2	F2
I	3.1%	3.1%	3.7%	3.4%	4.1%	4.3%
II	2.8%	2.7%	3.1%	2.6%	3.4%	3.4%
III	1.8%	2.0%	2.1%	1.8%	2.7%	2.6%
IV	1.3%	1.3%	1.6%	1.4%	1.4%	1.5%
V	1.3%	1.2%	1.5%	1.4%	1.3%	1.6%
VI	1.6%	1.1%	1.3%	1.2%	1.4%	1.4%
VII	1.3%	1.3%	1.2%	1.0%	1.3%	1.6%
VIII	1.9%	1.2%	0.6%	0.6%	0.5%	1.1%

Solid matter started to reduce in all processes since the first week of operation from the initial TS content of 3.7% (reactors A2 and B2) and 4.7% (others). The downward trajectory of the TS content is evident in all reactors over test time with the lowest values of solid contents at the end of the test. This peculiar behaviour points out that the solids washed out and those degraded by anaerobic bacteria were higher than the quantity periodically added with feeding. In particular, reactors C2 and D2 supplemented with GAC exhibited the largest solid matter consumption from their starting values. Very low TS concentrations on last weeks can be attributable to the feeding suspensions on days 41, 48 and 52 which allowed a further reduction of solid matter already present.

Table 3.16. VS contents in weekly digestates of the II cycle of semi-continuous reactors

Week	VS (%TS)					
	A2	B2	C2	D2	E2	F2
I	77.2%	77.2%	78.6%	79.5%	62.8%	61.9%
II	73.9%	72.7%	72.5%	70.6%	65.3%	64.7%
III	63.7%	66.9%	68.1%	60.4%	66.7%	64.1%
IV	49.2%	48.3%	57.4%	49.6%	59.0%	51.0%
V	49.5%	47.5%	55.7%	51.1%	46.3%	56.1%
VI	60.1%	44.2%	52.3%	46.8%	58.2%	59.7%
VII	66.5%	68.2%	61.2%	57.0%	68.0%	74.9%
VIII	87.8%	78.8%	51.6%	50.3%	48.6%	72.1%

VS concentrations of all reactors followed the decreasing trend of TS contents until the fifth/sixth week confirming that the variation of solids during the experiments was mainly attributable to the degradation of the volatile fraction. Conversely, from those weeks onwards, VS contents increased. In particular, in reactor A2 volatile organic matter represented almost the 90% of the total solid content of the system. Notwithstanding feeding was interrupted on days 41, 48 and 52, in reactors A2, B2 and F2 organic matter did not consumed in the last weeks of operation indicating processes sufferings.

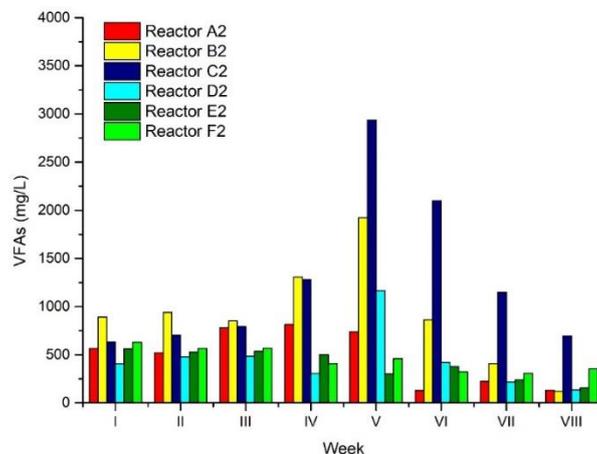


Figure 3.24. VFAs contents in weekly digestates of the II cycle of semi-continuous reactors

VFAs concentrations were stable in all reactors for the first three week of test. VFAs started to accumulate in digestates collected from reactors B2 and C2 from the fourth week in contrast to the other reactors wherein they slightly decreased. VFAs content in reactor C2 exceeded the optimal concentration in anaerobic systems on week V while, in reactor B2, they remained below the alarm threshold. It is noteworthy that B2 and C2 were the best-performing reactors of this cycle in terms of methane production. In the last two weeks, considerably low concentrations of VFAs were recorded in all reactors as evidence of the inhibition of the anaerobic microorganisms activity.

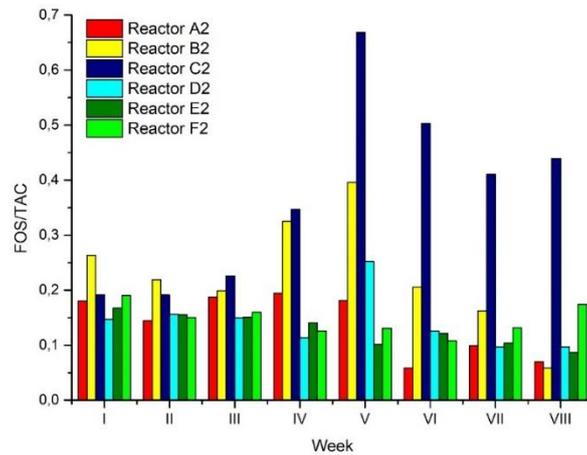


Figure 3.25. FOS/TAC in weekly digestates of the II cycle of semi-continuous reactors

FOS/TAC parameter allows the evaluation of an AD process stability. In general, a FOS/TAC ratio ranging from 0.15 to 0.45 is assumed as a general reference value for a stable process. A FOS/TAC ratio lower than 0.15 leads to a rise in pH values and decreasing contents of the organic acids (alkalosis) while values higher than 0.45 entail an excessive accumulation of organic acids (acidosis) (Weichgrebe, 2009). In this cycle, FOS/TAC can be considered as optimum for all processes in the first three weeks of operation. On week V, the highest FOS/TAC value (0.67) was measured in reactor C2. This confirms the accumulation of VFAs observed in Figure 3.24. With regard to reactor B2, FOS/TAC ratio also peaked on week V but remaining within the optimal range. In the last week of the test, apparently all reactors suffered from lack of organic matter (FOS/TAC less than 0.15) with the exception of reactor C2 (FOS/TAC ratio of 0.44).

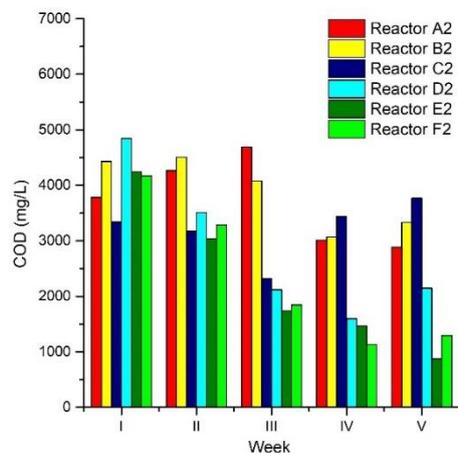


Figure 3.26. COD contents in weekly digestates of the II cycle of semi-continuous reactors

In the first week of the test, reactor D2 exhibited the highest COD content (4843 mg/L) followed by reactors B2, E2, F2, A2 and C2 (reactor with the lowest COD content of the first week, 3342 mg/L). This order changed in the second and third week wherein reactors A2 and B2 (namely the ones not supplemented with any material) had the highest COD contents compared to the other reactors in which the porous materials added to the systems (i.e., GAC and biomass fly ash) played their role of organic matter adsorption. From week IV onwards, COD of reactor C2 gradually rose up probably because the adsorption of the organic acids produced (Figure 3.24) led to an exhaustion of the GAC's adsorption sites. COD content of the couple of reactors A2 and B2 remained high ranging from 2500 and 3500 mg/L in contrast to COD of the other reactor which sharply decreased until the end of the test. From the fifth week onwards, COD contents in digestates were not determined because of technical problems.

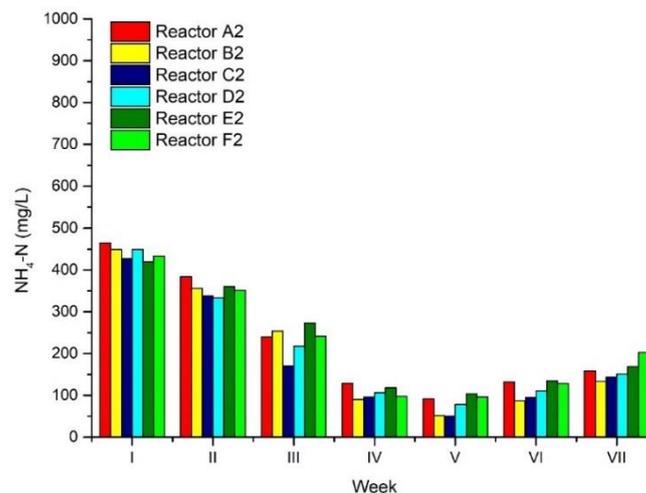


Figure 3.27. NH₄-N contents in weekly digestates of the II cycle of semi-continuous reactors

As already observed in the previous cycle, also in this case ammonia nitrogen contents did not reach the inhibitory threshold in any reactor over test time. In each week, NH₄-N contents in the six reactors were similar to each other. In particular, in the first week, the average NH₄-N content was 440 ± 15.2 mg/L. Ammonia nitrogen contents gradually fell until week V before to sharply climb up in the last two weeks. At the end of the test, reactors exhibited an average NH₄-N content of 160 ± 21.9 mg/L.

In general, according to data analyses it can be stated that none of the reactors showed an efficient and stable AD process over test time. Indeed, likewise in the I cycle, processes were clearly disturbed by leachate presence especially for its humic substances content. As discussed for the previous cycle of semi-continuous AD tests, the adaptation of anaerobic microorganisms to humic substances turns out to be problematic. Likely, this represents the reason why duplicates of the same designed reactors (A2 – B2, C2 – D2, E2 – F2) behaved so differently. Digestion process could be improved by increasing the HRT (set here at 10 days) in order to reduce the wash-off of the active biomass.

Even reactors with valid methane productions (i.e., B2 and C2) suffered from some form of inhibition from a certain point. Particularly, for reactor C2 the severe pH drop on day 34 (Figure 3.23) was a symptom of oncoming process failure. Indeed, the accumulation of VFAs produced during acidogenesis phase (see the peak in VFAs concentration on week V in Figure 3.24) led to acidosis condition. In order to avoid process failure, pH was adjusted four times (days 10, 20, 27 and 34) with 4 g of NaHCO₃ and feeding was suspended on days 41, 48 and 52 to allow methanogenic bacteria to digest the extra quantity of organic acids. Nevertheless, methane generation from reactor C2 stopped (Figure 3.21) indicating a serious inhibition of the methanogenesis phase. This occurrence shows how humic substances affect particularly methanogenic microorganisms whilst acidogens seem to better tolerate their presence as stated in Li et al. (2019). The supplementation of GAC did not exert the expected beneficial effects on the AD (adsorption of toxic compounds, improvement of methane production and increase of buffering capacity (Fagbohunbe et al., 2017; Zhang et al., 2018)) probably because of the addition of GAC only once at the beginning of the test. This led to a quick exhaustion of its adsorption capacity as observed in other studies (Calabrò et al., 2021).

Reactors E2 and F2 supplemented with biomass fly ash showed the worst performances in terms of methane generation. The additive was supposed to increase the alkalinity of the system, supply micronutrients and immobilise microorganisms so that promoting direct interspecies electron transfer (Romero-Güiza et al., 2016). In terms of pH regulation, fly ash played its role effectively since pH values in reactors E2 and F2 were observed to be the highest in the experiment (see Figure 3.23). On the other hand, it appears evident how all bacterial species involved in the AD process suffered from fly ash presence. Indeed, both the modest and irregular methane generation (Figure 3.21) and

the steadily low contents of VFAs over test time (Figure 3.24) could indicate a flawed performance of methanogens and acidogens, respectively. So far, few studies investigated the effect of adding biomass fly ash to the AD systems. Promising results in terms of pH control and methane production efficiency have been achieved by Novais et al. (2018) and Sailer et al. (2020). However, according to Alavi-Borazjani et al. (2020), distinctive potential impacts can be expected from the use of this type of material, as occurred in this study, considering the high diversity of biomass-derived ashes and their associated properties and compositions. Furthermore, it seems possible that the leaching into the aqueous solution of the heavy metals present in the fly ash (as proved by the chemical characterisation of the material, see Paragraph 3.1.1.6.) would inhibit the AD process.

With regard to the couple of reactors A2 – B2, which replicated the process of the best-performing reactor of the previous cycle (i.e., B1), they performed differently from each other. In fact, whilst methane was rapidly generated from reactor B2 (see the peak in the methane yield on day 6 in Figure 3.21), in contrast, reactor A2 showed a much longer lag phase. Furthermore, the overall methane production of reactor B2 was far higher (Figure 3.22). However, methane yield in reactor B2 gradually slowed down from day 29 onwards, namely when humic substances accumulated in the system. A similar behaviour has been observed in the reactor B1 of the previous cycle of semi-continuous AD tests wherein methane production severely decreased from day 30.

3.3.2.4. *III cycle*

In the last cycle of the semi-continuous anaerobic tests, digestion was carried out in four reactors (A3 – D3) and performed under a more realistic condition by employing MW as substrate instead of pure cellulose. Again in this cycle, both pre-treated leachates were tested as nutrient solutions and mixed with MW in order to reach the optimal C/N of 25 (see the III cycle of semi-continuous reactors programme in Paragraph 3.1.1.11.). Contrary to the other cycles, in this one, HRT was set at 20 days for the entire duration of the tests. Furthermore, the peculiarity of this cycle was that GAC was added to the respective reactors (i.e., B3 and D3) not only once, at the beginning of the tests like in the II cycle, but along with the feeding mixture in order to replace the quantity of GAC lost during digestate withdrawal and so provide the system with new sites for the adsorption

of humic substances. This way was expected to make GAC action more effective and, thus, overcome the long term inhibition observed in the reactor C2 of the II cycle. Indeed, the aim was that of making a realistic AD process stable and reliable in spite of leachate addition.

In general, regardless of the type of pre-treated leachate used, all of the four processes ran stably and efficiently for the entire duration of the tests (80 days, namely three times the set HRT without considering the first HRT of acclimatation). This seems to point out how the AD process suffered far less if pre-treated leachate is coupled with MW rather than pure cellulose. Particularly, reactors B3 and D3, supplied with GAC and with ZVI/Lap and ZVI/GAC pre-treated leachate, respectively, had methane yields and cumulative productions equal to those achieved by the expected conversion into methane of MW. Instead, reactors A3 and C3 performed slightly worse and this divergence would confirm the positive effect of the GAC addition to the systems.

Methane yields and cumulated methane productions of reactors A3 – D3 are depicted in Figure 3.28 and 3.29, respectively.

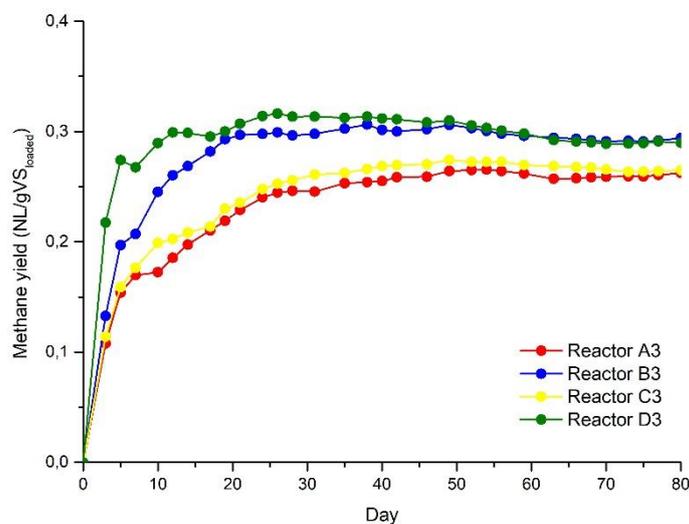


Figure 3.28. III cycle semi-continuous anaerobic reactors methane yields

After the first HRT of acclimatation (i.e., about 20 days), methane yield trends of all reactors remained level until the end of the tests. Reactors B3 and D3, supplied with GAC, reached the highest values of methane yield. In particular, from day 20 onwards, their methane yields were, on average, 0.297 and 0.302 NL/gVS_{added}, respectively, with

negligible standard deviations (0.005 and 0.010 NL/gVS_{added}, respectively). In the same range of time, 0.256 and 0.264 NL/gVS_{added} (on average) were calculated for reactors A3 and C3, respectively. Looking at the trends, it can be stated that all the processes ran stably and efficiently over tests time and that reactors supplied with GAC (i.e., B3 and D3) performed about 14% and 13%, respectively, better than reactors A3 and C3. All the processes performed better from day 21 to 56 (about the first two HRT of regime operation), while, in the last HRT of the tests, processes curves slightly slowed down even if signs of further rising are present in the last days (see also Figure 3.29).

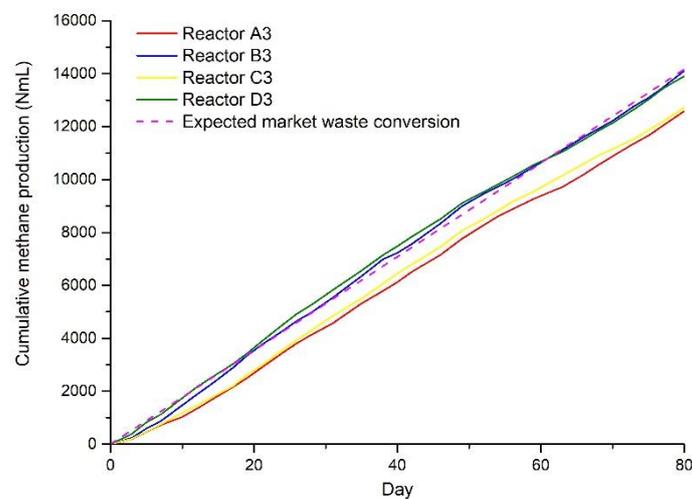


Figure 3.29. III cycle semi-continuous anaerobic reactors cumulative methane productions

The expected MW conversion into methane through a semi-continuous AD process was calculated on the basis of the MW BMP. It was assessed by a previous batch test carried out on the same substrate used in this cycle of the semi-continuous AD tests (data not shown). MW BMP was determined as average of three batch replicates and was found to be 422 ± 14.7 NmL/gVS. The expected cumulative methane production from the conversion of MW through a semi-continuous AD process (dashed line in Figure 3.29) was calculated reducing by 30% batch tests production (295 NmL/gVS) since this value is consistent with literature on evaluating the reduction of production in continuous processes respect to batch test (Browne et al., 2014; Moeller et al., 2018; Ruffino et al., 2015; Zhang et al., 2013). Figure 3.29 highlights how methanogenesis proceeded steadily in all processes over tests time. Cumulative methane productions of reactors B3 and D3

clearly followed the estimated methane production from MW semi-continuous AD. At the end of the tests, overall, reactors A3, B3, C3 and D3 produced 13.0, 14.5, 13.2 and 14.3 NL of methane, respectively, versus the expected cumulative methane production of 14.5 NL. Also from this data, it is evident how the supplementation of GAC enhanced process performance.

In Figure 3.30, pH values of the four reactors measured over tests time are depicted.

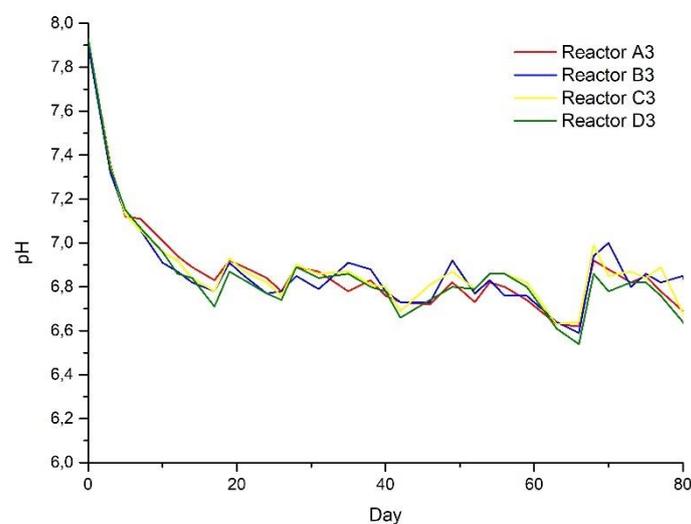


Figure 3.30. III cycle semi-continuous anaerobic reactors pH trends

At the beginning of the tests, a pH of 7.9 was measured in all reactors. In the first days of digestion, all pH trends were continuously decreasing. Shortly before completing the first twenty days of acclimatation (day 17), 2 grams of NaHCO_3 were necessary to stop pH decrease. As a result, a peak in pH trends was recorded in all reactors on day 21. For about two HRT (from day 21 to 59), pH fluctuated around the desirable value of 6.8. Within this range of time, four times (days 26, 31, 42 and 46) 2 grams of NaHCO_3 needed to be added to the systems. At the beginning of the last HRT, all reactors seemed to severely suffer from acidification (pH of 6.6 on day 66). This could have affected methanogenic bacteria since methane generation in all reactors slowed down correspondingly, as observed in Figures 3.28 and 3.29. For this reason, 4 grams of NaHCO_3 were added to the systems implying a pH increase up to about 7.0. After that, systems did not require further pH

adjustments until the end of the tests on day 80 (i.e., three HRT). Since according to Steihauser and Deublein (2011) the metabolism of most of methane-forming microorganisms is considerably suppressed at $\text{pH} < 6.7$, it can be confirmed that bacteria involved in the processes did not suffer from acidic environmental conditions.

The analyses carried out on weekly digestates withdrawn from the respective reactors are reported in the following Tables 3.17 and 3.18 (TS and VS contents, respectively) and Figures 3.31, 3.32, 3.33 and 3.34 (VFAs, FOS/TAC, COD and N_{tot} contents, respectively).

Table 3.17. TS contents in weekly digestates of the III cycle of semi-continuous reactors

Week	TS (%)			
	A3	B3	C3	D3
I	1.8%	1.9%	1.7%	2.5%
II	1.3%	1.5%	1.4%	1.9%
III	1.2%	1.4%	1.2%	1.8%
IV	1.1%	1.2%	1.1%	1.6%
V	1.2%	1.2%	1.2%	1.5%
VI	1.0%	1.0%	0.9%	1.0%
VII	1.1%	1.3%	0.9%	1.4%
VIII	1.0%	1.0%	0.9%	0.8%
IX	0.8%	1.0%	0.8%	1.2%
X	0.9%	1.3%	0.9%	1.6%
XI	0.8%	1.0%	0.8%	1.4%

In general, TS content trends of all reactors were slowly decreasing over tests time indicating that the degradation of organic solid matter was higher than the amount of solids added with feeding. TS content reduced by about one point of percentage after eighty days of digestion in all reactors. Starting from an initial TS fixed value of 1.8% for reactors A3 and C3 and 2.3% for reactors B3 and D3 (larger due to GAC presence), after the first week of operation, basically only in reactor B3 solid matter was consumed. Instead, in the other reactors, TS consumption started to be observed from the second week of the tests. Whilst TS contents of reactors A3, B3 and C3 seemed to remain almost level until the end of the tests, solid matter of reactor D3 showed large variations over test time probably due to some sampling problems. Indeed, it firstly decreased down to 1.0%

on week VI, then increased and, finally, reached its lowest value (0.8%) on the eighth week before to increase again.

Table 3.18. VS contents in weekly digestates of the III cycle of semi-continuous reactors

Week	VS (%TS)			
	A3	B3	C3	D3
I	64.2%	65.9%	65.4%	68.7%
II	61.1%	61.4%	64.0%	67.6%
III	56.6%	60.5%	57.4%	67.9%
IV	53.9%	55.7%	52.8%	63.3%
V	49.9%	51.9%	48.1%	61.6%
VI	48.7%	54.5%	43.8%	51.8%
VII	45.4%	55.1%	41.1%	57.1%
VIII	42.9%	47.8%	42.8%	39.7%
IX	41.5%	49.9%	39.5%	59.7%
X	40.7%	57.2%	39.9%	63.7%
XI	41.7%	52.8%	41.2%	62.1%

To different extents, VS contents of all reactors were decreasing in the first five weeks. From this point, VS amount of reactor B3 increased for the next two weeks (i.e., VI and VII) but, then, it reached its lowest value (47.8% of TS on week VIII) before to increase again. Similarly, VS of reactor D3 had a clear downward trend for the first six weeks with a significant VS consumption (almost ten percentage points) after the fifth week. Afterwards, VS increased only for one week (VII) before reaching their lowest value of 39.7% of TS on week VIII and, then, significantly increasing again of twenty points of percentage. On the other hand, in reactors A3 and C3, VS were consumed for all the duration of the tests with the only exception, in reactor C3, of weeks VIII and XI when little rises in VS content were measured. The parallelism in TS and VS behaviours, especially for reactor D3, confirms that the variation of solids during this test was mainly attributable to the degradation of the volatile fraction. Likewise TS, by analysing VS trends it can be stated that the rate of conversion of biodegradable matter in all AD processes was so efficient and regular that no accumulation of organic matter occurred.

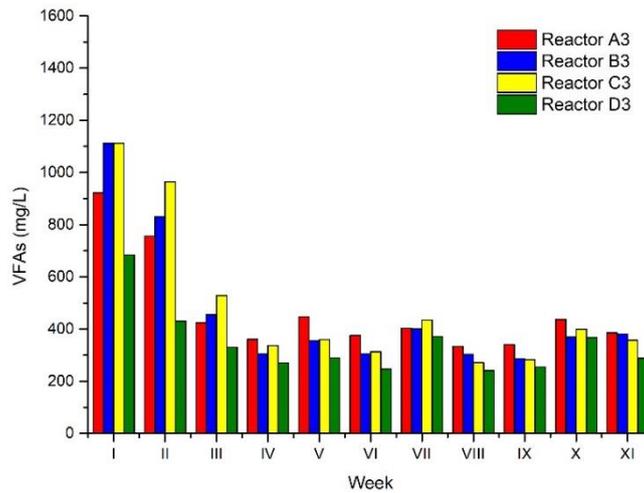


Figure 3.31. VFAs contents in weekly digestates of the III cycle of semi-continuous reactors

Besides the first two weeks in which reactors B3 and, especially, C3 had relatively high concentrations of total VFAs (about 1100 mg/L on the first week), for the rest of the tests time the amount of volatile fatty acids was kept under control in all reactors. Indeed, VFAs values of all reactors ranged from 241 mg/L (reactor D3 on week VIII) to 528 mg/L (reactor C3 on week III). Since no accumulation of acids occurred, it can be stated that all the processes proceeded in a stable way under this point of view. In contrast to the other cycles, in this case an HRT of 20 days allowed anaerobic microorganisms to take the right time to convert the incoming biodegradable matter into acids in the first place and, then, easily consume VFAs in order to produce biogas. For this reason, differences in VFAs trends of reactors of the three cycles (Figures 3.18, 3.24 and 3.31) are evident.

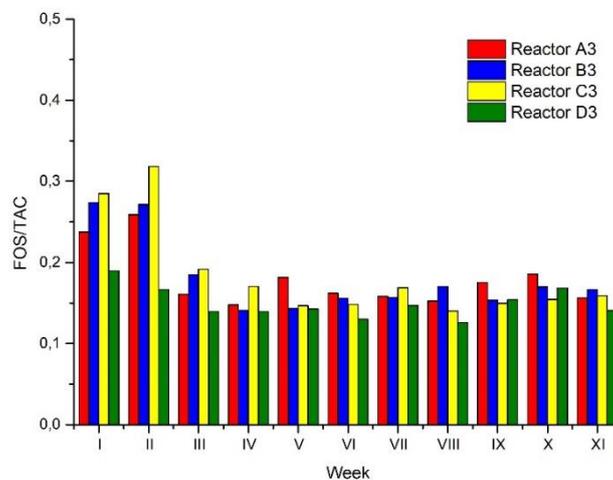


Figure 3.32. FOS/TAC in weekly digestates of the III cycle of semi-continuous reactors

As explained in Paragraph 1.1.1.4., a FOS/TAC ratio lower than 0.15 leads to alkalosis conditions wherein pH of the system rises due to lack of organic acids essential to the digestion process. In contrast, FOS/TAC values higher than 0.45 entail an excessive accumulation of organic acids (acidosis) (Weichgrebe, 2009). At the beginning of the tests of this cycle, FOS/TAC of reactors A3, B3 and C3 were relatively high in the first two weeks compared to the rest of the tests. In particular, in reactor C3, FOS/TAC exceeded the value of 0.3 in the week II. Nevertheless, FOS/TAC values of all reactors levelled off from the third week onwards around the value of 0.15 until the end of the tests. According to Mézes et al. (2011), when FOS/TAC is around 0.2, the biomass input is considered as low for the system and, therefore, it is advisable to increase the organic load of the feeding. For this reason, it is possible to assume that, even though methane production of all reactors was stable, it could have been enhanced by increasing the OLR.

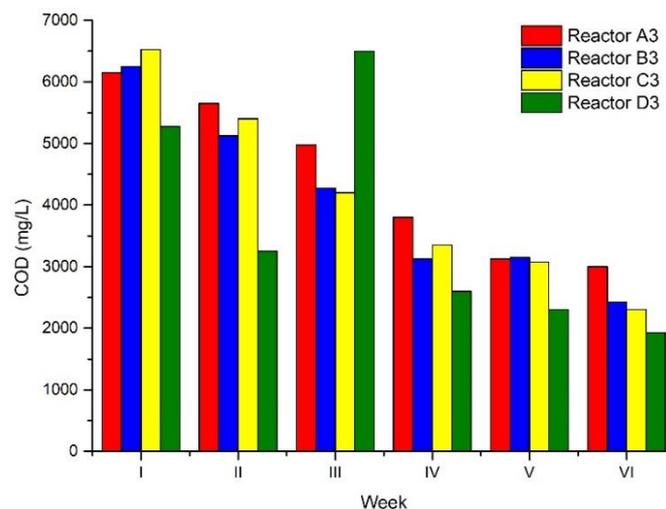


Figure 3.3313. COD contents in weekly digestates of the III cycle of semi-continuous reactors

At the beginning of the tests, in reactors A3, B3 and C3 a COD higher than 6000 mg/L was measured while reactor D3 had a COD content just over 5000 mg/L. In the second week, COD concentration of reactor A3, B3 and C3 reduced by 8%, 18% and 17%, respectively, while in reactor D3 it fell down to 3250 mg/L. On the third week, COD concentrations continued to slowly decline in all reactors except for D3 one in which COD shotted up to 6500 mg/L. This value can be reasonably considered as an outlier since no anomalies were observed in the examination of the other parameters on the same week (Tables 3.16

and 3.17 and Figures 3.30, 3.31 and 3.32) and, moreover, COD content of reactor D3 shows a clear downward trend overall. Besides the aforementioned outlier value, from week II onwards, reactor A3 exhibited the highest values of COD content throughout weeks with the exception of the fifth week in which COD concentrations in reactors A3, B3 and C3 were basically identical (about 3100 mg/L). From the seventh week onwards, COD contents in digestates were not determined because of technical problems.

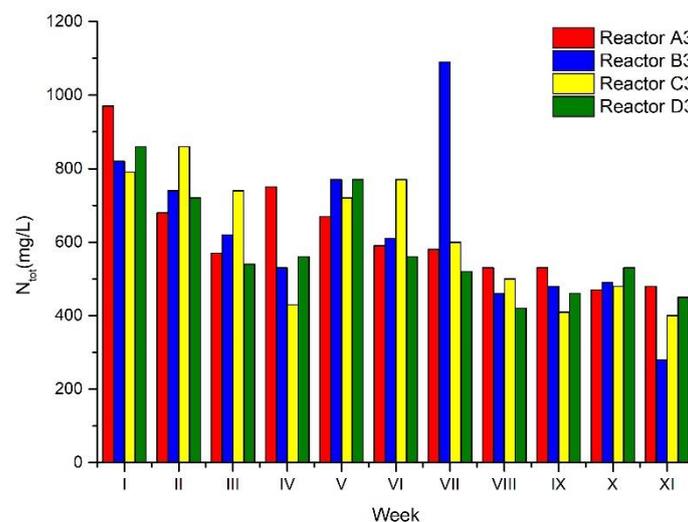


Figure 3.34. N_{tot} contents in weekly digestates of the III cycle of semi-continuous reactors

Total nitrogen content in reactor A3 was close to 1000 mg/L in the first week of operation and, then, it reduced down to 570 mg/L on week III. On the fourth week, the concentration of nitrogen rose up to 750 mg/L before decreasing until the end of the test (480 mg/L on week XI). N_{tot} content in reactor B3 ranged from the initial value of 820 mg/L to 530 mg/L on the fourth week before increasing up to 770 mg/L. Besides the peak on the seventh week, probably an outlier like the COD in reactor D3 on week III (Figure 3.33), from the fifth week onwards, N_{tot} content followed a downward trend and reached the lowest value on the last week (280 mg/L). In reactor C3, total nitrogen content, after an early increase (860 mg/L on week II), declined down to 430 mg/L on week IV. Later, it climbed up to 770 mg/L on week VI but, then, its trend was continuously decreasing until the end of the test (400 mg/L). Finally, reactor D3 exhibited the same behaviour in terms of N_{tot} concentration of reactor B3. As in the I cycle, also in this case nitrogen concentrations are well below the inhibitory threshold found in the literature (Yenigün and Demirel, 2013).

All results considered, it can be speculated that the use of a more complex matrix as substrate made the III cycle of the semi-continuous AD tests more valid respect to the others. Indeed, feeding to anaerobic reactors a more realistic substrate allowed to trigger the synergic/antagonistic interactions that occur among chemicals (trace metals, anions, organic compounds) even if their influent content is low. These may not show up in the case of ideal substrate like pure cellulose. In this realistic scenario, the type of pre-treated leachate did not affect the processes as evidenced by the fact that reactors couples A3 – C3 and B3 – D3, fed with different types of pre-treated leachate, performed almost equally. Furthermore, the choice of a larger HRT (20 days versus 10 days of the previous cycles) enhanced the performance of both hydrolysis and methanogenesis steps. In fact, hydrolytic microorganisms had more time to break down the complex organic polymers and methanogens took the adequate time to grow and thrive despite their low specific growth rate. Under this condition of HRT, all processes were able to run stably.

In general, methane yields from AD of fruit and vegetables waste (FVW) are highly variable depending on waste composition and reactor design. A range from 0.16 to 0.4 L/gVS was reported by Bouallagui et al. (2005) while other authors reported methane productions up to 0.5 NL/gVS under optimum conditions (see below). Thus, results found in literature are in accordance with those obtained in this experiment. Previously, Liao et al. (2014) performed a stable single-batch AD of food waste (methane yield of 0.369 – 0.466 L/gVS) at high OLR (41.8 gVS/L) by adding raw leachate which was found to be able to prevent acidic inhibition of the process. In fact, according to the authors, the introduction into the system of the total ammonia nitrogen present in leachate buffered VFAs accumulation as well as providing bacteria with nitrogen necessary for their growth. These mechanisms could have been triggered in this experiment since both VFAs and nitrogen remained almost level over tests time (Figures 3.31 and 3.34) and well below the inhibitory thresholds found in the literature (Steinhauser and Deublein, 2011; Yenigün and Demirel, 2013). Also, Guven et al. (2018) recorded good process performances in the co-digestion of different mixtures of organic fraction of MSW and leachate. In this case, methane yields increased as leachate contribution decreased in waste mixtures. Whilst about the use of landfill leachate as nitrogen supplement in AD processes literature is still scarce, co-digestion of cellulosic waste from agriculture with low-cost nitrogen-rich substrates is widely performed. This practice is well-known to make digestion process

more efficient due to the positive synergistic effects of the mixed materials with complementary characteristics and the supply of missing nutrients by the co-substrate (Garcia-Peña et al., 2011; Zia et al., 2020). The addition of high nitrogen content co-substrates to adjust the nutrient content of FVW was evaluated by Bouallagui et al. (2009) in laboratory-scale anaerobic sequencing batch reactors. As in this experiment, authors stated that the combination of FVW with other substrate (i.e., slaughterhouse wastewater and waste activated sludge) resulted in a highly buffered system due to ammonia supply. Mixing FVW with slaughterhouse wastewater or waste activated sludge (at a ratio of 30:70 on volume basis corresponding to C/N ratios of 25 and 28, respectively) resulted in methane yields ranged from 0.3 to 0.35 L/gVS. Similarly, Alvarez and Lidén (2008) obtained a methane yield of 0.3 L/gVS from a semi-continuous co-digestion of FVW and cattle and pig manure mixture (which played the role of nutrient source and buffering agent). Results from reactors B3 and D3 of this experiment are comparable with the aforementioned studies. This points out that the use of pre-treated leachate (along with positive effects of GAC presence) could represent a valid alternative to the conventional co-digestion practice for optimising the AD of MW.

On the other hand, what emerges from the analyses of some digestates parameters is that reactors seemed to be underloaded. Particularly, downward trends of TS and VS (Tables 3.16 and 3.17) suggest that processes consumed more organic solid matter than added with feeding. This observation is confirmed by the relatively low FOS/TAC values during the regime phase (Figure 3.32) which points out the low biomass input in the systems. Also, VFAs concentrations (Figure 3.31) turned out to be well below the inhibitory thresholds found in literature (Wang et al., 2009). Being AD of food and MW potentially problematic because of low methane yields and fast acidification due to its high simple sugars content (Xu et al., 2018), the maximum OLR for the process was proven to be below 3 gVS/L·d (Mata-Alvarez et al., 1992) and several authors reported satisfactory methane productions (on average 0.5 NL/gVS) for a HRT of 20 days or more (Mata-Alvarez et al., 1992; Ranade et al., 1987; Scano et al., 2014). For these reasons, in further research, OLR could be increased in order to optimize the process.

It was demonstrated that GAC supplied in reactors B3 and D3 made digestion processes more efficient in terms of methane generation compared to reactors A3 and C3 (controls) where no GAC was added. GAC beneficial action can be attributable to both DIET

mechanism stimulation and recalcitrant organic compounds adsorption. The former consisted of the promotion of electron transfer among bacterial species involved in the AD thanks to the colonisation of microbes of the pores present in the GAC surface, whereas the latter allowed to remove humic substances from the system by entrapping them on GAC microporous bulk. As observed in Figure 3.28, in these tests GAC presence enhanced methane yield by 13 – 14%. This result is consistent with Peng et al.'s study (2018) in which methane yield from anaerobic sludge digestion was increased by 13.1% through the GAC addition. Furthermore, since carbon materials in AD processes are found to reduce wastewater toxicity (Zhu et al., 2018), it can be reasonably supposed that GAC presence prevented reactors B3 and D3 from the inhibitory effect of humic substances. As stated in Zhu et al. (2018), toxic and refractory compounds could severely affect the electron transfer between syntrophic bacteria and methanogenic archaea therefore DIET mechanism allowed to overcome this issue. With regard to COD removal, effluents from reactors B3 and D3 had lower COD concentrations compared to control reactors (i.e., A3 and C3), albeit differences were subtle, as observed, for instance, in Li et al. (2015) and Lee et al. (2016) with the addition to their AD tests of carbon nanotubes and GAC, respectively. Instead, the improvement in the methane content in biogas did not occur in this study (on average, roughly 64% of methane in biogas was recorded in all reactors over regime phase) although carbon materials are known to raise CH₄ content (Zhang et al., 2018). According to previous studies, the establishment of DIET mechanism can also shorten the lag time for start-up of AD by 6%–65% (Wang et al., 2021). In this experiment, reactors B3 and D3 supplied with GAC reached the regime phase after 19 days. Instead, methane yields of control reactors A3 and C3 continued to smoothly rise until stabilising their values on day 40 as testified by Figure 3.28.

Finally, concentrations of heavy metals in weekly collected digestates were calculated, under the hypothesis that the biological process does not affect the amount of heavy metals, by dividing expected regime concentrations of heavy metals in anaerobic reactors (Table 3.12) by total solid contents of weekly digestates (Table 3.16). Results are reported in Table 3.19 only for the worst case possible (lowest amount of TS in digestates) which corresponds to the maximum expected heavy metals concentrations.

Table 3.19. Maximum expected heavy metals concentrations in digestate

Reactor	Cu [mg/kgTS]	Ni [mg/kgTS]	Zn [mg/kgTS]
A3	24.1	41.7	131.7
B3	20.2	35.1	110.6
C3	35.9	48.2	85.0
D3	35.1	47.1	83.1

Values reported in Table 3.19 amply fulfil the limits set by the Italian Standard for waste admission to compost production (i.e., 1000 mg/kg_{TS}, 300 mg/kg_{TS}, 2500 mg/kg_{TS} for Cu, Ni and Zn, respectively). This implies that heavy metals addition to AD processes did not affect the quality of effluents which can be employed as material for compost production so that closing the loop of leachate and MW treatment.

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CONCLUSION

The adoption of the Circular Economy model on a worldwide scale is vital to lower the human pressure on the environment (expected to increase as World population grows) so that reaching within 2030 the Sustainable Development Goals set by the United Nations.

Waste management is one of the most environmentally impactful sectors. The previous Linear Economy model led to the exploitation of raw materials (e.g., water, wood, natural gas, coal, oil, metals) which were non-renewable or consumed at a rate far faster than that necessary for their replenishment. Besides pollutant emissions during manufacturing, especially in terms of wastewater and greenhouse gases, the uncontrolled disposal of end-of-life products caused waste accumulation over the years with consequent soil, water and air pollution. Currently, according to the Circular Economy approach, waste is considered as a source of new materials and energy to be recovered by proper treatments.

In this context, landfilling (i.e., long lasting waste accumulation in facilities called landfills without any type of recovery) is intended as the least preferable operation for waste management according to the waste management hierarchy laid down by Directive 2008/98/EC. However, since some materials cannot be cost-effectively recovered with the current technical ability, landfilling is still necessary. Landfill gas and leachate are widely accepted as the two major emissions from landfill. Particularly, landfill leachate is the result of the water (either infiltrated in the landfill body from external sources or released from waste after compaction or biodegradation) percolating through landfilled waste which concentrates biological and chemical constituents of waste. Leachate must be collected during landfill operation (and even after landfill closure) and treated before its discharge in the environment. Dissolved organic matter (biodegradable and refractory), toxic and persistent organic compounds (e.g., polycyclic aromatic hydrocarbons), ammonium, heavy metals and metalloids, chlorine compounds, dioxins and emerging contaminants (e.g., perfluorinated alkyl substances) are the main pollutants present in leachate. Leachate quality strongly depends on landfill age. Leachate coming from old landfills (i.e., > 10 years old) has slightly alkaline pH and it is rich in hardly biodegradable or non-biodegradable organic matter (e.g., humic substances, HSs). This peculiarity makes mature landfill leachate (MLL) treatment challenging.

This thesis investigated an innovative MLL treatment at a laboratory-scale. The reason why the focus of interest was on leachate coming from mature landfills lies in the fact that Directive 2008/98/EC discourages landfilling use and in many countries thousands of landfills are already closed or will be in a short time. Accordingly, it is reasonable to expect a growing necessity of treating mainly MLL in the near future. Previously, MLL treatment aimed mainly at removing pollutants in order to meet discharge limits and little attention was paid on recovering useful substances from leachate. In a perspective of Circular Economy, in this study, a novel approach was explored with the purpose of maximising the recovery of potential useful compounds present in MLL. Considering the novelty of the experiment, it was decided to use a synthetic MLL prepared in laboratory according to the typical composition found in literature. The entire process consisted of two distinct phases: a pre-treatment using an active granular filter and anaerobic co-digestion of the pre-treated leachate. The former was necessary for the preliminary removal of constituents (e.g., heavy metals and refractory organic compounds) which are well-known to disturb or inhibit anaerobic microorganisms' activity. The latter allowed to use pre-treated MLL as a supplement of macro (nitrogen) and micro (trace metals) nutrients for the anaerobic bacteria metabolism with the purpose of balancing the high carbon content of mainly carbonaceous substrates (e.g. lignocellulosic materials).

In the active granular filtration phase, two different granular filters were tested: a mixture of Zero Valent Iron (ZVI) and Lapillus (Lap) (20:80 w/w) and a mixture of ZVI and Granular Activated Carbon (GAC) (40:60 w/w). Towards ammonium, chloride and COD, both filters demonstrated negligible removal capacity with the only exception of COD in the ZVI/GAC column as expected by GAC attitude to absorb organic matter. In particular:

- Ammonium was removed only in the first days of operation (57% and 83% for ZVI/Lap and ZVI/GAC, respectively).
- Chloride was basically unaffected by the pre-treatment.
- COD was removed by 59% and 65% by ZVI/Lap and ZVI/GAC, respectively, after the first 2 days of operation. For the rest of the tests time, only ZVI/GAC column continued to remove COD to a lesser extent.

Heavy metals involved in this experiment were Cu, Ni and Zn. They were chosen for their respective ability to activate the different removal mechanisms associated with the use of

ZVI. In general, results showed that both granular filters were effective in removing the three contaminants according to the following sequences for ZVI/Lap and ZVI/GAC granular mixtures, respectively: Cu > Ni > Zn and Cu > Zn > Ni. In particular:

- Cu was the most easily removed metal. ZVI/Lap and ZVI/GAC showed an average removal efficiency of 85% and 91%, respectively.
- Ni removal had an almost identical efficiency (66% and 67% on average) for both filters.
- ZVI/GAC granular mixture removed Zn better than the ZVI/Lap one with average removal efficiencies of 75% and 58%, respectively.

Both filters showed positive results in terms of metals removal. Nevertheless, from a comparison with similar studies, it emerges that this type of treatment is more effective for younger leachates due to their acidic pH values (4 – 5) which facilitate iron corrosion and the associated removal mechanisms (i.e., reduction, adsorption onto iron corrosion products and coprecipitation with iron oxides). However, using the alkaline MLL slowed down iron corrosion but this could imply the functionality of the filter for a longer period of time. Especially GAC well supported ZVI thanks to its aptitude of removing organic matter and heavy metals also in alkaline conditions through adsorption on its porous surface. On the contrary, lapillus seemed less efficient in sufficiently supporting ZVI with metals' removal but its practical use is far cheaper.

The second phase implied the addition of pre-treated MLLs (i.e., coming from the two different filters) to semi-continuous anaerobic digestion (AD) process. AD tests were carried out in three different cycles. The I cycle aimed at proving the feasibility of periodic pre-treated MLLs addition to the AD of pure cellulose (easily biodegradable substrate) according to different values of COD/NH₄-N ratios (i.e., 25 and 50). It was observed that both pre-treated MLLs adversely affected AD processes. The best performing reactor (i.e., B1 loaded with leachate pre-treated by the ZVI/Lap column, COD/NH₄-N of 50) generated methane (average methane yield of 0.26 NL/gVS_{added}) for 30 days before to stop. The inhibition of methanogenesis is most probably attributable to HSs which are well-known to interfere with hydrolytic and methanogenic enzymes if present in noticeable amount as in this case. In reactor B1, the lowest volume of pre-treated MLL (and HSs, as a consequence) was added.

In the II cycle, the same operational conditions of reactor B1 were tested in order to both validate results and investigate DIET mechanism as a possible solution to the inhibition issue in the long term. To do that, two different materials were respectively added to reactors: GAC and biomass fly ash. Tests of this cycle were performed in duplicate. Also in this case, inhibition caused by leachate is evident. First of all, it was observed that duplicates of the same designed reactors (A2 – B2, C2 – D2, E2 – F2) behaved very differently. Reactors A2 and B2 showed average methane yields of 0.13 NL/gVS_{added} and 0.29 NL/gVS_{added}, respectively. GAC did not completely succeed in enhancing process performance. Reactor C2 supplied with GAC reached its maximum methane yield (0.3 NL/gVS_{added}) after 17 days but, then, it continuously decreased until 0.21 NL/gVS_{added} when test was stopped. Biomass fly ash severely perturbed the system (average methane yields of reactors E2 and F2 of 0.04 NL/gVS_{added} and 0.09 NL/gVS_{added}, respectively) albeit its positive effect of pH control.

Finally, the III cycle consisted of the semi-continuous AD of a realistic waste (market waste) with a high carbon content which was balanced by the addition of both pre-treated MLLs up to a C/N of 25. Also, in this cycle, the Hydraulic Retention Time (HRT) was increased to 20 days (previously it was 10 days in the I and II cycle) in order to reduce the wash-off of the active biomass. GAC was added to some reactors not only once, at the beginning of the tests like in the II cycle, but periodically along with the feeding mixture. In contrast to the I and II cycle, reactors processes ran stably and efficiently for the entire duration of the tests with average methane yields of 0.256 NL/gVS_{added} and 0.264 NL/gVS_{added} for reactors loaded with market waste and pre-treated MLLs (i.e., A3 and C3, respectively) and 0.297 and 0.302 NL/gVS_{added} for reactors supplied with GAC (i.e., B3 and D3, respectively). The use of a more realistic substrate seemed to trigger the beneficial effects of leachate on the AD process. First of all, synergic/antagonistic interactions among chemicals (trace metals, anions, organic compounds) could have alleviated the toxicity of HSs towards anaerobic microorganisms. Secondly, total ammonia nitrogen present in leachate buffered volatile fatty acids (VFAs) accumulation typical of market waste AD. Also GAC exerted its positive effect of stimulating DIET mechanism as it is proved by the improvement of the process performance in terms of methane production (+ 13 – 14%). In order to verify the possible use of residual digestate as a fertiliser, calculation of heavy metals' concentrations in digestate was made. Values amply fulfil the

limits set by the Italian Standard for waste admission to compost production for Cu, Ni and Zn.

In conclusion, it can be stated that the feasibility of the integrated MLL treatment has been proved at a laboratory-scale. The significance of this study lies in the possibility of closing the cycle of MLL treatment following a Circular Economy approach. Indeed, at the end of the overall integrated MLL treatment, methane and digestate are produced during AD with a limited waste production principally constituted by the amount of exhausted filtration material then recycled to recover iron or disposed of in a landfill. From a technical and economic point of view, a full-scale application of the integrated MLL treatment would lead to remarkably saving costs associated with leachate management. The first filtration step can be performed on-site: preliminary, suspended solids and colloids present in the leachate can be easily removed by a settling tank or a microsieving in order to avoid bed clogging. Subsequently, the leachate is pre-treated through a battery of gravity/pressure filters (similar to those used in potabilization plants) with different degrees of exhaustion of the reactive media. As this dissertation demonstrated, the average effluent of the filters after 38 days of operation is compatible with the use in an anaerobic digestion process. However, in a full-scale application, in order to optimise the filtration step, an earlier substitution of the filter material has to be taken into account. To this regard, the use of a low cost admixing agent (i.e., lapillus) represents an attempt to both reduce the overall cost of the process and make the substitution of the filter material more feasible. After the first filtration step on-site, the pre-treated leachate can be temporary stored in tank prior to be transferred to the anaerobic digester. This represent a vital step for the sustainability of the process as transportation costs are well-known to be relevant. For this reason, the distance between the landfill site where leachate is generated and the anaerobic digestion plant needs to be limited. According to the presented result (especially of the III cycle of tests), it can be stated that the use of the pre-treated leachate as nutrient solution can make the anaerobic digestion process of the market waste stable and efficient. As a result, methane generated by the process can be used for energy generation so that partially compensating the impacts of uncontrolled landfill gas emissions and the energy used in the previous steps of the leachate treatment. Furthermore, residual digestate, if not contaminated by hazardous pollutants originally

present in leachate, can replace fertilisers due to the presence of nitrogen and HSs deriving from the pre-treated MLL.

Obviously, further research needs to be carried out. First of all, the same experiment can be replicated using a real MLL to consider all the possible interactions among solution constituents and active granular filters. Also, negative effects of HSs on anaerobic microorganisms could be alleviated or exacerbated by antagonistic or synergic interactions, respectively, with chemical compounds present in anaerobic sludge and substrate. Secondly, both active filtration and AD could be optimised by changing operational parameters. For instance, in the former, the proportions of materials in the mixture or the inflow rate can be varied. In the latter, the beneficial effects of the increase of HRT (from 10 to 20 days) were already evident. Also, the Organic Loading Rate (OLR) could be gradually increased even up to 3 gVS/L·d (the current maximum OLR for successful AD of fruit and vegetables waste tested in literature) since, from the analyses of weekly digestates, reactors of the III cycle seemed to be underloaded. Lastly, larger scales experiments and life cycle analyses of the overall MLL treatment would be necessary prior to possible full-scale application.

