






Article

Water Resource Recovery Facilities (WRRFs): The Case Study of Palermo University (Italy)

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Abstract: The wastewater sector paradigm is shifting from wastewater treatment to resource recovery. In addition, concerns regarding sustainability during the operation have increased. In this sense, there is a need to break barriers (i.e., social, economic, technological, legal, etc.) for moving forward towards water resource recovery facilities and demonstration case studies can be very effective and insightful. This paper presents a new water resource recovery case study which is part of the Horizon 2020 EU Project “Achieving wider uptake of water-smart solutions—Wider Uptake”. The final aim is to demonstrate the importance of a resource recovery system based on the circular economy concept. The recovery facilities at Palermo University (Italy) are first presented. Afterwards, the resource recovery pilot plants are described. Preliminary results have underlined the great potential of the wastewater treatment plant in terms of resources recovery and the central role of the University in fostering the transition towards circular economy. The fermentation batch test highlighted a volatile fatty acids (VFAs) accumulation suitable for polyhydroxyalkanoates (PHAs) production. The results of static adsorption and desorption tests showed that the highest amount of adsorbed NH_4^+ was recorded for untreated and HCl-Na treated clinoptilolite.

Keywords: circular economy; wastewater treatment; water resource; water smart solutions

1. Introduction

In recent years, the exploitation of freshwater resources and the constant increase in the sewage sludge production from wastewater treatment processes have been a major environmental concern and a challenge in the context of sustainable development [1]. Solving this issue coincides with the objectives of the circular economy. The circular economy approach has been proposed as a response to the conventional and unsustainable linear economy model, which involves the exploitation of incoming non-renewable resources and outgoing waste production. In contrast, the concept of circular economy entails the reuse of waste by creating a new market, thus exploiting the full potential of the recovered resources [2]. The main objective is to restore and regenerate material cycles, i.e., to maintain the value of materials at every stage of a product's life, minimise waste generation and eventually close the material cycle through high-value recycling [3]. The broader goal lies in simultaneously creating environmental quality, economic prosperity and social equity [4].

To promote the transition from a linear to a circular economy model, a system must be created in order to minimize waste production and the use of energy and natural resources [5]. This is achieved by using models to manage material and energy flows in a regenerative way and following the principles of reduction, reuse and recycling [6]. Circular business refers to solutions (including products and services) and models aimed at improving the circular economy and responding to resource scarcity, while minimising environmental impacts and producing short and long-term economic benefits [7]. In the transition to a sustainable socio-technical system, intermediary actors have been identified as key catalysts that speed up the process [8].

Wastewater treatment plants (WWTPs) represent excellent facilities to recovery resources: water, energy, sludge and nutrients. Modern wastewater treatment plants are effective in removing organic contaminants but involve great economic, energy and material costs. Therefore, systems with low operating costs, direct water reuse and nutrient recovery capabilities should be implemented in order to achieve sustainable wastewater treatment [9]. Moreover, sustainable nutrient recovery falls within a vision that aims at circular economy and industrial symbiosis [1], which are two goals towards which the European Union pushes. However, despite the need to perceive wastewater as a resource, most water management services in Europe still focus on wastewater collection and treatment rather than resource recovery [10]. Recent scientific production has increasingly focused on technological solutions to establish a water sector based on the circular economy, implementing large-scale resource recovery technologies in the wastewater sector [10]. However, the global economy is currently estimated to be less than 10% circular [11]. Implementing resource-oriented processes can be difficult because modifying the current wastewater treatment system is costly and resource consuming [12]. Due to the increasing number of available resource recovery technologies, WWTP design is no longer a simple technical problem but a complex problem that requires an integrated approach to make effective decisions [13]. The pivotal question is which of the growing range of available technical options should be selected for implementation in a full-scale plant [14].

In addition to the technical uncertainties that apply to many emerging resource recovery technologies, various non-technological bottlenecks may hinder the successful implementation of such technologies in wastewater treatment processes [15]. The water sector has been poorly equipped to address factors outside its traditional engineering scope. Institutional compartmentalisation within the sector hinders integrated water resources management and needs to be resolved to make progress in developing resource-oriented wastewater management strategies [16]. Consequently, it is necessary for water utilities to plan strategically the transition from wastewater treatment to resource recovery. The transfer of scientific knowledge to decision-makers in water utilities is an important requirement for this planning process. Resource recovery technologies can be implemented and their potential exploited only if decision-makers in water utilities have a clear understanding of available and emerging technologies [10].

The existence of social, technical, economic and regulatory barriers will therefore hamper the widespread implementation of wastewater and sewage sludge reuse. As far as wastewater reuse is concerned, the main barrier is public acceptance [10]. The strongest objections concern scenarios in which humans may come into close contact with recycled water [17]. Therefore, it is necessary to increase public awareness to avoid this kind of barrier. Furthermore, from a regulatory point of view, a main problem that should be addressed to reduce health and environmental risks related to wastewater reuse is that standards and guidelines in different Member States can be significantly different, even at the regional level. Therefore, there is a need for unified European regulation. Moreover, regarding sewage sludge reuse, the main barriers are related to legislation and public acceptance. One of the main options for sludge reuse is its use in agriculture. However, the main limitation concerning the application of sludge on soils is the adoption by some EU countries of limit values for contaminants that are stricter compared to the Sewage Sludge Directive (86/278/CEE) [18]. In addition, sludge composting is limited by the

requirements for organic fertilisers and the acceptance of this matrix by end users. It is therefore necessary not only to provide the appropriate knowledge to the end users of the resource, but it is also of paramount importance to educate new technician classes to adequately manage the new technologies for resource recovery. In this light, sludge management is becoming a challenging issue, since the production of sewage sludge has increased significantly in the last years, with about 13 million tons of dry matter produced in EU in 2020, which is about 34% more than that produced in 2015 [19,20].

This paper presents the case study of Palermo University (IT) which is aimed at demonstrating the effectiveness of a resources recovery system from wastewater treatment. The activities presented are part of the H2020 European Project “Achieving wider uptake of water-smart solutions—Wider Uptake” [1]. In particular, the core of the case study is represented by the pilot plants for wastewater treatment. Specifically, three pilot plant lines have been built: (i) water reuse and minimization of sludge production, (ii) biopolymer production as polyhydroxyalcanoates (PHA) and (iii) column system for nitrogen (N) and phosphorus (P) recovery. The station and pipeline pumping the wastewater from the University Residence and canteen to the resource recovery laboratory (to feed pilot plants) are also presented. The reuse system of treated water for the irrigation of the green areas of the university campus and for the agricultural experimental area are elucidated. Moreover, the composting process of the sewage sludge coming from the wastewater treatment and the application of compost in the experimental field as fertilizer are illustrated. Finally, the paper presents an in-depth microbiological analysis through metagenomics techniques which has been carried out in the different activities, in order to enhance the performance, including the reduction of greenhouse gas (GHG) emissions from the pilot plants.

As far as authors are aware, the present case study is one of the first examples in the literature of a Water Resource Recovery Facility (WRRF) within a University Campus where innovative water smart solutions are applied to the wastewater sector. The idea behind this project realized at Palermo University Campus is to involve the new generations in overcoming the existing barriers (i.e., social, economic, administrative, technological, etc.) that hamper the transition and enhancing the principle of environmental sustainability.

2. The Case Study of Palermo University

The system for the treatment and resource recovery from wastewater of Palermo University aims to provide a demonstration of the application of the circularity concept in the resources exploitation. Inside the University Campus, there are eight points of interest concerning the case study as shown in Figure 1. The wastewater from the university residence and the canteen is collected by the pumping station (point 1, Figure 1) and sent via a pipeline (point 2, Figure 1) that crosses a part of the campus to the resource recovery laboratory (point 3, Figure 1) of the Engineering Department. The laboratory produces treated water which through a pipeline (point 4, Figure 1) reaches the storage tanks (point 5, Figure 1). The treated water is used for the irrigation of some green areas and an experimental field (points 6, Figure 1). The sewage sludge from the laboratory is subjected to composting in a specific area (point 7, Figure 1). The sludge and nutrients also extracted from wastewater are tested in a greenhouse and in the experimental field of the Department of Agricultural, Food and Forestry Sciences (point 8, Figure 1) as conditioner and fertilizer for the soils. In addition, in order to optimize the water purification process and resource recovery, metagenomic analyses—carried out at the Department of Biological, Chemical and Pharmaceutical Sciences and Technologies—are used to relate the operational parameters and microbial community structure residing in the activated sludge and to provide information on metabolic capabilities thereof.

All the points of interest present inside the university campus are described in detail below.

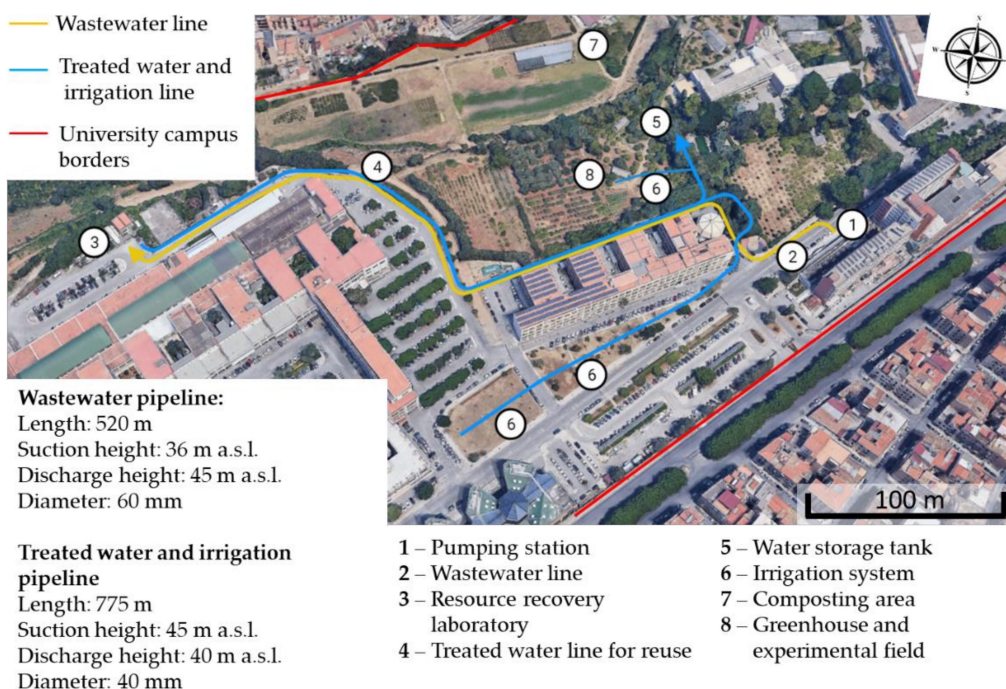


Figure 1. Satellite photo of the University Campus with an indication of the points of interest and the specifications for the wastewater and treated water transport pipelines.

2.1. The Pumping Station

The pumping station (Figure 2) is located under the roadway of *Viale delle Scienze*, the main road of the University Campus, in correspondence with the wastewater collection pit of the University canteen, which also acts as a collection of wastewaters from an area of the university residence.

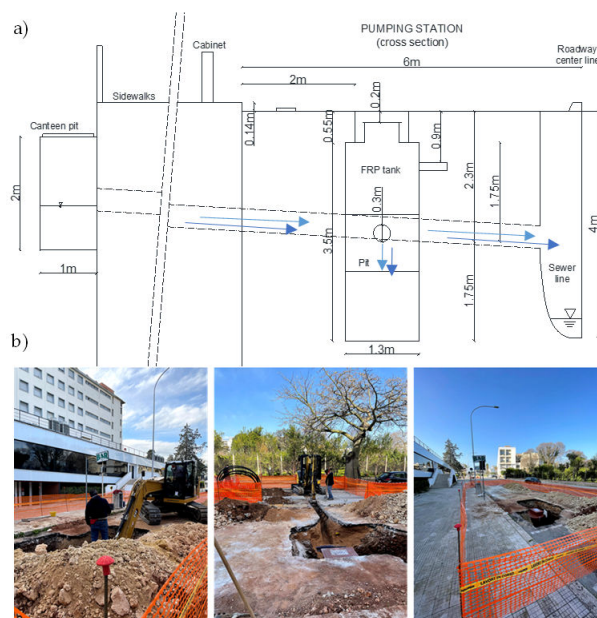


Figure 2. (a) Scheme of the pumping station; (b) photos of the excavation and pumping station construction.

The station consists of a cylindrical glass fiber reinforced polymer (GFRP) wastewater collection tank (“Xylem MAXISUB 1300”— $d = 1.3$ m, $h = 3.5$ m, $V = 4650$ L). Inside there are two submersible electric pumps (“Xylem Flyght MP 3069.170 HT”— $Q = 2.49$ L/s) each

having a flushing valve (“Xylem Flygt 4910”) with the automatic cleaning function of the pumping station to avoid accumulation of sludge. The reservoir was fed by making a deviation from a pit that intercepts the wastewater discharge pipeline from the canteen pit to the main sewer located at the middle of the road. The pumping station is directly controlled from the resource recovery laboratory.

To transport the wastewater to the resource recovery laboratory located about 450 m away and 9 m higher, there is a 520 m long high-density polyethylene (HDPE) pipeline ($d = 60$ mm) which conveys the wastewater to a storage tank located outside the laboratory.

2.2. Resource Recovery Laboratory

The resource recovery laboratory represents the heart of the system inside the university. Located in a building of the Engineering Department, it houses the pilot plant for the treatment of wastewater conceived for water reuse and minimization of sludge production, the pilot plant for biopolymer production as polyhydroxyalcanoates (PHA) and the system for nitrogen (N) and phosphorus (P) recovery through sorption on biochar and zeolite columns.

The wastewater from the pumping station is collected in a storage tank with a capacity of 1500 L and equipped with a mechanical stirrer, located outside the building. A solenoid valve system controlled by water level sensors regulates the feeding of wastewater to the pilot plants. The effluent water from the wastewater treatment pilot plant and the supernatant deriving from the PHA production pilot plant are subjected to tertiary ultrafiltration treatment to produce water suitable for reuse. Part of this treated water is fed to the N and P recovery system, while most of it is collected in a storage tank located outside the laboratory and then sent to the storage tanks of the Department of Agricultural, Food and Forestry Sciences. Part of the sludge produced by the wastewater treatment pilot plant will be sent to the fermentation processes for PHA production while the remaining part will be used to produce compost.

In summary, as shown in Figure 3, the following streams are produced from the raw wastewater entering the laboratory:

- Water for reuse in green areas and experimental land;
- Sewage sludge to be composted as a soil improver;
- PHA powder for bioplastics production;
- Biochar and zeolite enriched in N and P as soil improver.

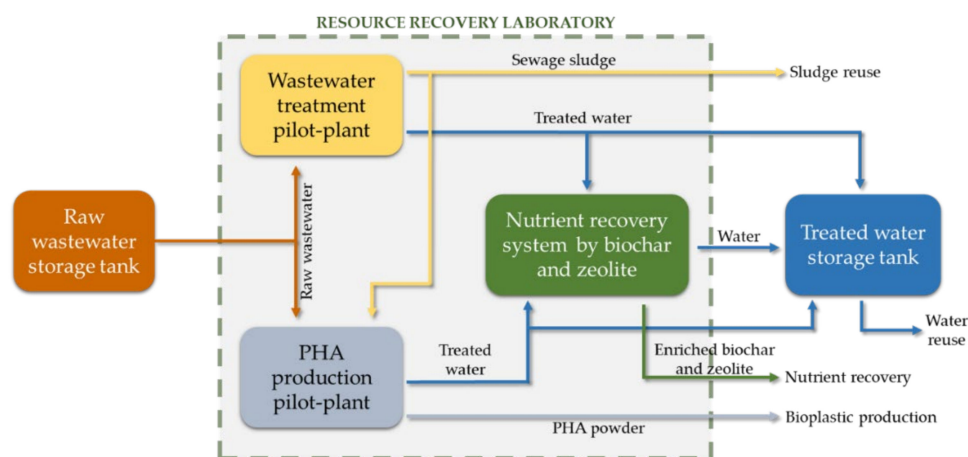


Figure 3. Schematic representation of the resource recovery laboratory.

The plants and systems realized in the laboratory will be described in the sections below.

2.2.1. Sewage Sludge Reduction Technology

For the main wastewater treatment line, it was decided to use a conventional activated sludge (CAS) configuration with the integration of an oxic-settling-anoxic/anaerobic (OSA)

process. The CAS-OSA system allows to reduce the production of sewage sludge compared to the CAS system [21]. The choice to adopt this sludge reduction technology derives from the need to provide a technologically simple and economically sustainable solution to the problem of sludge overproduction that our society is facing. In fact, in recent years, the production of sewage sludge has increased significantly, with production in Europe in 2020 of about 13 million tons of dry matter, about 34% more than the sludge production recorded in 2015 (9.7 million tons of dry matter) [19,20]. The increase is mainly due to the increase in the amount of civil wastewater treated within the WWTPs and the stricter limits on treated wastewater [22,23].

The increase in sludge production has important environmental and operational consequences. Despite the recent socio-economic policies aimed at resource recovery [24], the sewage sludge is mainly disposed as waste in landfills, with a consequent risk of soil and groundwater pollution, or incinerated without energy recovery, with the consequent production of greenhouse gases [21]. From an economic point of view, about 50% of the operating costs of the treatment plants is related to sludge management and disposal [20]. In recent years, various technologies have been proposed to reduce the production of sewage sludge, while maintaining a high efficiency of the treatment processes [20].

Among the developed technologies, the OSA process is one of the easiest to implement in a conventional WWTP [21]. Indeed, the process is a modification of a CAS system in which an anoxic/anaerobic reactor (interchange reactor) is inserted in the activated sludge recycling line [25]. The solids that would normally be recirculated by the conventional system are subjected to an anaerobic/anoxic environment before reaching the activated sludge reactor (aerobic reactor). Usually, 10% of the recirculating sludge is sent to the OSA reactor [21,26]. The anoxic/anaerobic conditions promote the biomass stress. The metabolic split between anabolism and catabolism occurs through the OSA process, which slows bacterial growth and biomass production [19]. Under metabolic uncoupling conditions, the growth of microorganisms is inhibited since part of the energy is consumed for their maintenance, thus leading to a reduction in biomass production [27,28].

The pilot wastewater treatment plant present in the resource recovery laboratory can treat 15–45 L/h of raw wastewater and has the following CAS-OSA configuration (Figure 4):

- (a) an anoxic reactor ($V = 146$ L) for denitrification, fed by the incoming raw wastewater, the recirculation system from the aerobic reactor and the recirculation system from the OSA reactor;
- (b) an aerobic reactor ($V = 257$ L) where the main processes for the removal of organic pollutants take place;
- (c) an oxygen depletion reactor (ODR) ($V = 53$ L) inserted in the recirculation system between the aerobic and anoxic reactors which allows to eliminate dissolved oxygen before entering the anoxic reactor;
- (d) a settler ($V = 62$ L) for solid–liquid separation;
- (e) an OSA interchange reactor ($V = 477$ L) in the sludge rewinding system to create the conditions that induce the metabolic splitting between anabolism and catabolism;
- (f) an ultrafiltration membrane system ($V = 48$ L) to increase the quality of the effluent for reuse, equipped with a clean-in-place (CIP) system.

The plant is equipped with four progressive cavity pumps (Novarotors MN 013-2, Sossano, Italy) for the connections between the reactors, two peristaltic pumps (Watson Marlow Qdos 30, Marlow, UK) for the extraction of water from the membrane and for backwashing, a peristaltic pump (Watson Marlow Qdos 60) for sludge wasting, and two air pumps (Nitto LA-120A, Osaka, Japan), one for the aeration of the aerobic reactor and one for aeration of the membrane compartment.

Several plant configurations will be tested in order to evaluate the best solution for reducing sludge production. For example, a Membrane Bioreactor (MBR)-OSA configuration (Figure 5) will be used. Other configurations will be tested during the experimental campaign based on the results obtained.

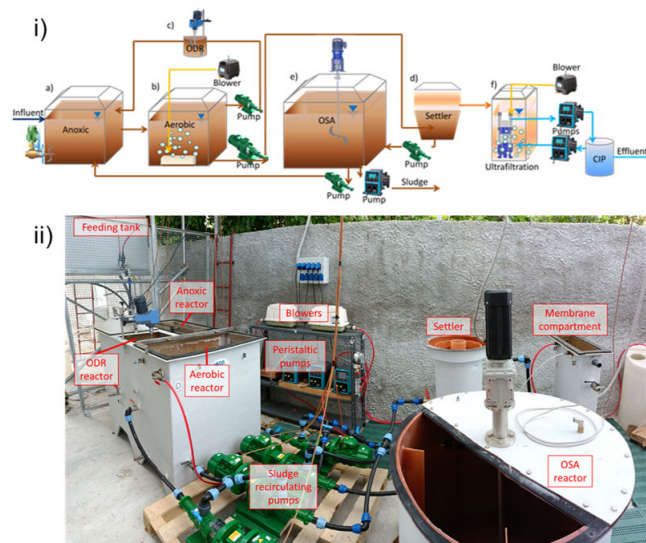


Figure 4. (i) CAS-OSA pilot plant configuration. (a) anoxic reactor, (b) aerobic reactor, (c) oxygen depletion reactor, (d) settling tank, (e) anaerobic OSA reactor, (f) membrane tank. (ii) panoramic view.

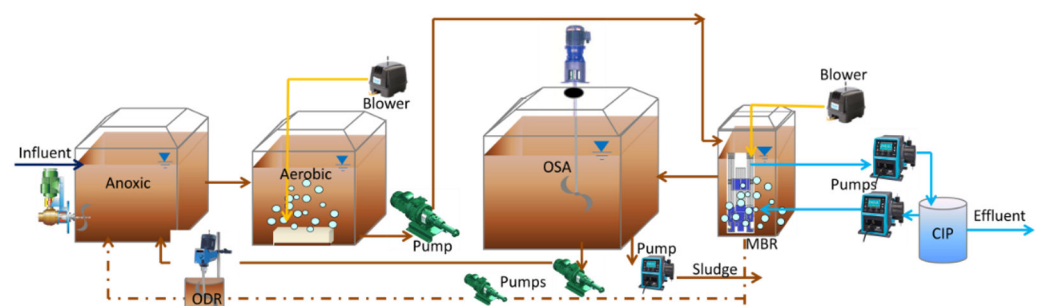


Figure 5. MBR-OSA pilot plant configuration.

2.2.2. Biopolymers Production

PHAs are bio-based and biodegradable polyesters, with thermoplastic properties similar to some petroleum-based polymers, that could replace the conventional plastics on the global market [29,30]. Mixed Microbial Cultures (MMCs) commonly used in biological wastewater treatment have the potential to accumulate intracellular PHA by using volatile fatty acids (VFAs) as precursors [31]. Therefore, within wastewater treatment plants operation, carbon recovery from wastewater is allowed by means of biopolymer synthesis, such as PHAs. The concurrent benefit of biopolymer synthesis is the reduction of the waste sludge amount to be disposed [32,33]. With this aim, a pilot plant for demonstrating PHA production from urban wastewater treatment has been set up in the resource recovery laboratory.

The pilot plant can treat around 40 L/day of the waste activated sludge produced by the pilot plant aimed at sludge reduction (CAS-OSA and MBR-OSA). The system is composed by six main different units as reported below:

- F-SBR for the production of VFAs by sludge acidogenic fermentation;
- Ultrafiltration membrane unit for solid/liquid separation to obtain an optimum fermentation liquid quality;
- N-SBR for ammonium rich stream nitrification, to be used as electron acceptor in S-SBR famine phase;
- S-SBR for the selection of a biomass with high PHA accumulation capacity through aerobic feast and anoxic famine cycles;
- Ultrafiltration membrane unit for solid/liquid separation to enhance biomass selection;

- (f) A-SBR for fed-batch PHA accumulation using biomass from S-SBR and VFAs from F-SBR.

The flowchart of the process is reported in Figure 6. The plant configuration reported above was adapted from previous studies [34,35], in which the VFAs rich streams used as carbon source were produced by the acidogenic fermentation of mixed primary and secondary sludge or cellulosic primary sludge from wastewater sieving. This process scheme combines PHA rich biomass production and nitrogen removal via nitrite from ammonium rich streams such as anaerobic digestion sludge reject water.

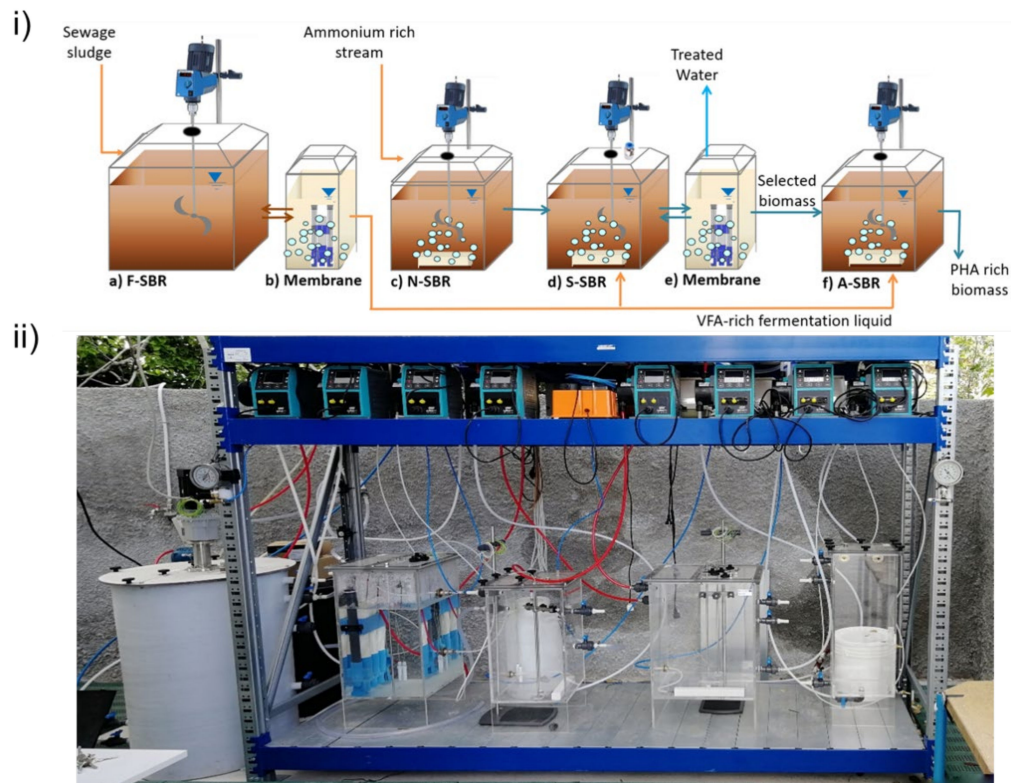


Figure 6. (i) Schematic view of pilot plant configuration. (a) Sequencing batch fermentation reactor (F-SBR). (b,e) Ultrafiltration membrane tank. (c) Sequencing batch nitrification reactor (N-SBR). (d) Selection sequencing batch reactor (S-SBR). (f) Accumulation sequencing batch reactor (A-SBR). (ii) Panoramic view of pilot plant.

The novelty of such a configuration is the use of an ultrafiltration membrane unit in order to improve the PHA accumulation potential of the selected biomass. This and the use of high SRT waste activate sludge (WAS) as substrate for VFAs production are the main contributions of the study carried out in this pilot plant to advancing the state of the art in PHA production from urban wastewater. Indeed, WAS produced from high SRT processes are usually not regarded as a good feedstock for PHA production processes because it is difficult to ferment, and low VFA yields are obtained compared to primary sludge or low SRT waste activated sludge that has a higher biodegradability and thus a better acidogenic potential [36,37].

2.2.3. Nutrient Recovery by Biochar and Zeolite

Nitrogen (N) and phosphorus (P) are key nutrients for soil nutrition and plant growth. It is of paramount importance to recover such nutrients due to the growing demand for food, the depletion of phosphorus (P) mines and the increasing costs for fertilisers [38,39]. Treated wastewater still contains nutrients, including N and P, which can be recovered by different methods [40]. Adsorption by natural adsorbents is an efficient and cost-effective method to recover N and P from wastewaters. Adsorbed nutrients, then, can be desorbed

to be used as fertilisers [41]. Alternatively, nutrient enriched adsorbents can be applied to the soil as amendments to improve soil fertility [42].

Zeolites are a promising option for using as low-cost adsorbent material for wastewater treatment and nitrogen recovery [43]. Zeolites are microporous crystalline tectosilicates with net negative charge due to isomorphous substitutions of Si^{4+} by Al^{3+} [44]. Currently, the most used ion exchange media for NH_4^+ removal is the natural zeolite clinoptilolite (in the activated Na-form) which has been showed to have an NH_4^+ exchange capacity, on average, of $25 \text{ mg N-NH}_4^+ \text{ g}^{-1}$ from mono-component NH_4^+ solution and treated wastewater.

Biochar is one of the most widely used solid materials for phosphorus recovery. It is obtained by pyrolysis of biomass, generally waste, at high temperatures ($300\text{--}800 \text{ }^\circ\text{C}$) and in the absence of oxygen [45]. Biochar consists mainly of aromatic-type carbon and is characterised by large specific surface area ($200\text{--}1000 \text{ m}^2 \text{ g}^{-1}$), low density and high porosity [46].

Within the Wider Uptake project, the research group of the University of Palermo aims to recover N and P from wastewater coming from the student's dormitory and canteen by using zeolite and biochar. To this aim, experimental trials, at both batch and pilot scale, are carried out to evaluate the adsorption and desorption ability of zeolite and biochar as well as their resistance and durability.

At pilot scale, N and P will be recovered from the effluent flow rate by means of adsorption columns (Figure 7). Specifically, several columns have been built and filled with adsorbent materials (zeolites and biochar). The columns are made up of polymethylmethacrylate, with internal diameter of 5 cm and height of 20 cm, thus having a total volume of 0.39 L. The columns operate with downward flow and treated wastewaters is brought into the columns using a peristaltic pump (Watson Marlow—Qdos 30 Universal). The flow rate is set at 0.52 L h^{-1} . To keep the columns in an optimal state of cleanliness, a backwash tank with an upward flow is provided. Zeolite and biochar inside the columns are handled differently. Zeolites will be washed by 1 M NaCl solution to desorb the recovered NH_4^+ and to be regenerated, thus allowing the reuse of the zeolites for multiple cycles. Once exhausted, zeolites will be removed from the column and applied to soil as conditioner. The columns with biochar do not provide for a backwash because P is mainly retained irreversibly and only slightly adsorbed, thus making the desorption not economically recommended [47]. However, when biochar is exhausted, it will be applied to soil as both slow-release fertiliser and CO_2 sink [48].

The columns are equipped with different sampling points to evaluate the kinetic of nutrient adsorption.

2.3. Water Reuse (Treated Water Transport, Storage Tank, Irrigation Systems, Irrigation Area (Grass Area Building 6, Agriculture Area))

The treated water from the plants is collected in a storage tank located outside the resource recovery laboratory. From the tank, the water is transported along a HDPE pipeline ($d = 40 \text{ mm}$) to the water storage tanks ($V = 18,000 \text{ L}$) (Figure 8e)) located in the agricultural department. Subsequently, water is fed to the irrigation systems (Elettropompe Speroni mod. 2CM 25/160A Hp 3) represented by four green areas located along the main avenue of the campus in front of the Engineering Department buildings (green area 1: 1700 m^2 ; green area 2: 610 m^2 ; green area 3: 950 m^2 ; green area 4: 280 m^2) (Figure 8a,d,f). The system consists of 9 zones with turbine irrigators (Hunter mod. PGP) and 4 zones with static irrigators (Hunter 4''). The water can also be fed to the irrigation system of the experimental agricultural field (210 m^2) and to the experimental greenhouse (120 m^2) (Figure 8b,c).

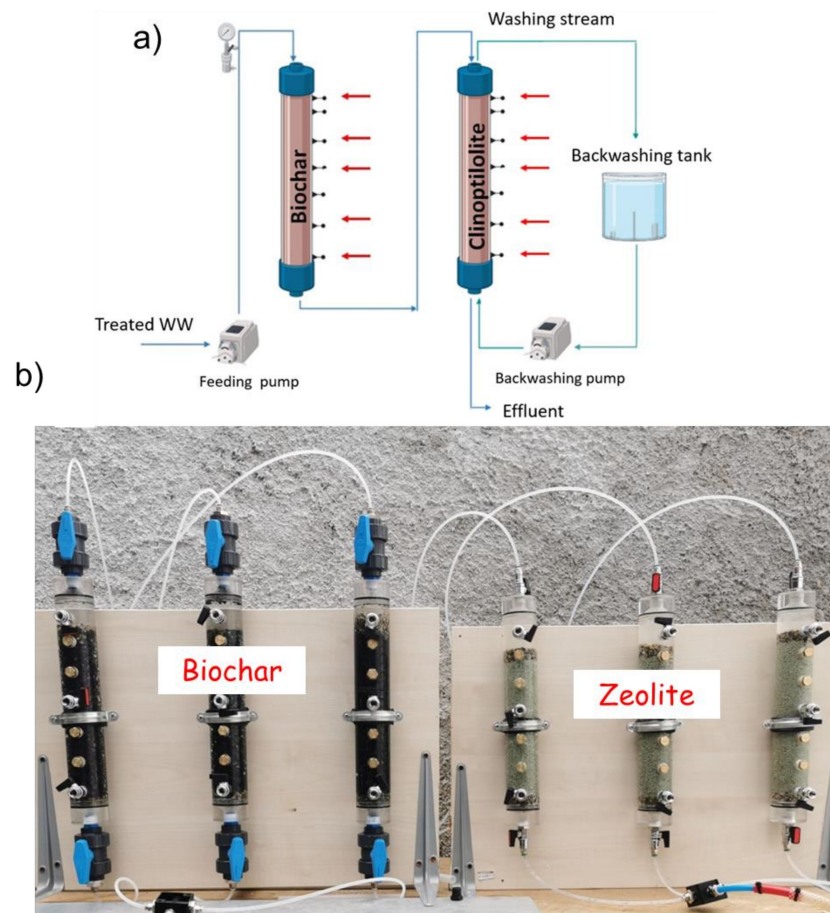


Figure 7. (a) Operating columns scheme; (b) filled columns with biochar and zeolite.



Figure 8. Engineering department green areas and facilities at Palermo University campus: (a,d,f) green areas, (b,c) greenhouse and (e) storage tank for treated water.

2.4. Sludge Composting (Composting Area, Composting Strategies, Compost Usage)

The sludge composting activities will be carried out in an area belonging to the Department of Agricultural, Food and Forestry Sciences. Briefly, several configurations characterized by different composition will be tested in order to optimize the process and the features of the produced material. Each pile will have an overall volume close to 160 L, aeration will be provided by manual turning with a presumed composting time of 90 days. The pile compositions, expressed as percentage in weight, are listed below:

- (1) dried sludge (75%) and bulking agents (25%);
- (2) dried sludge (75%) and bulking agents (25%) amended with worms (*Eisenia Foetida*);
- (3) dried sludge (75%), bulking agents (12.5%) and zeolite (12.5%);
- (4) dried sludge (75%), bulking agents (12.5%) and biochar (12.5%).

The produced compost will be used as amendment on potted plants with the aim to assess the role/effect on plant growth. Figure 9 shows a panoramic view of the area dedicated to composting operations.



Figure 9. Panoramic view of the composting area.

3. Preliminary Results

3.1. Fermentation Batch Tests

For sake of conciseness, only the results of VFA concentration and VFA/solubleCOD (VFA/sCOD) ratio (corresponding to the maximum sCOD value) of the fermented liquid during the batch tests are here presented (Figure 10). In Table 1 the details of the performed batch fermentation tests are reported.

Table 1. Details of the performed batch fermentation tests.

Batch Test	Details
T1	VSS = 4 g/L Uncontrolled pH
T2	VSS = 5.9 g/L Initial pH = 8
T3	VSS = 5.9 g/L Uncontrolled pH
T4	VSS = 5.9 g/L Initial pH = 10
T5	VSS = 2.8 g/L Uncontrolled pH
T6	VSS = 5.9 g/L pH = 10 (continuously adjusted)

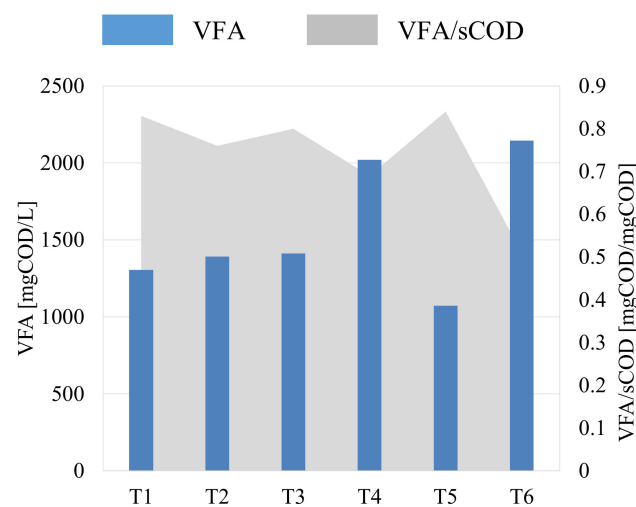


Figure 10. VFA concentration and VFA/sCOD ratio of the fermented liquid during the batch test.

In terms of volatile suspended solid (VSS), the results reported in Figure 11 show that by decreasing the VSS concentration the VFA production decreases consequently. This is mainly due to the reduction of active biomass. According to the data of Figure 10 batch test T4 and T6 have provided the highest VFA value (till to 2145 mg COD/L and 2020 mg COD/L for T4 and T6, respectively) compatible with the PHA production. Indeed, the VFA values obtained during T4 and T6 are both higher than 2 g/L which represents the threshold indicated in literature (among others, [49]) to guarantee the PHA production. This result is mainly due to the fact that alkaline pH (initial for T4 and during the test for T6) have promoted the hydrolysis of organic matter, thus incrementing the sCOD concentration in the fermented liquid. In terms of VFA/sCOD ratio, the values of 0.69 and 0.51 have been obtained for T4 and T6 tests, respectively.

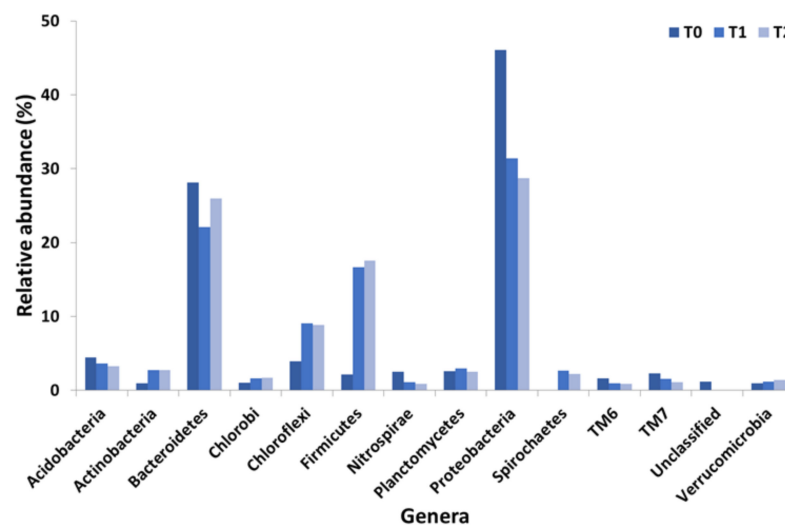


Figure 11. Relative abundance of the most abundant (i.e., having percentage values > 1) bacterial phyla identified by NGS analysis of the V3–V4 region of the 16S rRNA encoding gene in the activated sludge of T0, T1 and T2 conditions, respectively.

Therefore, despite the higher sCOD value obtained for T6 than T4, since the aim here is to produce a VFA rich stream suitable as feedstock for PHA production, T4 operating conditions have been selected as the optimal ones. For more detail on the fermentation batch tests and metagenomic results, the reader is referred to literature [50].

3.2. Metagenomic Results

To characterize the bacterial community structure of the activated sludges operating in T1 and T2, metagenomic analysis—based on Next Generation Sequencing (NGS)—was carried out on total DNA. In particular, metagenomic DNA was extracted, as described by Cinà et al. [51] from samples prepared using the sewage sludge collected from:

- T1 and T2 reactors at the endpoint;
- The sludge recycle line of the real WWTP (the same used to inoculate T1 and T2 reactors) that was considered as the control condition (T0).

The metagenomic DNA was used as a template to amplify the V3-V4 region of the 16S rDNA by using primers previously described by Takahashi et al. [52]. Amplification products were sequenced in one 300-bp paired-end run on an Illumina MiSeq platform (BMR Genomics, Padova, Italy). The raw 16S rDNA data were processed using the QIIME2 environment (<https://qiime2.org/> accessed on 15 September 2021) as paired-end sequences. Overlapping paired-end reads were processed using the plug-in DADA2 for the denoising approach. Unique Amplicon Sequence Variants (ASVs) were assigned and aligned to the Greengenes reference database, with 99% sequence similarity (<https://greengenes.secondgenome.com/> accessed on 15 September 2021). For each sample, the structure of the bacterial community was reported in terms of the percentage of relative abundances of phyla and genera based on (ASVs), as reported in literature [50].

In particular, the most represented (i.e., having percentage values > 1) phyla include Acidobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Planctomycetes, Proteobacteria and TM7 in all the tested conditions (Figure 11). However, the Proteobacteria abundance decreased by about twofold in both T1 and T2 reactors with respect to T0, with a consistent increase of Acidobacteria, Actinobacteria, Chloroflexi, Firmicutes, and Spirochaetes, with the latter present exclusively in T1 and T2 reactors (Figure 11). Concerning the genera, the T0, T1 and T2 reactors share *Acidovorax* and *Rhodobacter* among the most representative genera (Figure 12). Indeed, *Dechloromonas* and *Treponema* genera were present only in T1 and T2 reactors (Figure 12). On the other hand, some of the most represented genera were present only in T0 (e.g., *Cloacibacterium* and *Nannocystis*) or T1 (e.g., *Acquabacterium*) or T2 (e.g., *Macellibacteroides* and *Syntrophomonas*). Thus, the NGS-based study demonstrated changes in the bacterial community of activated sludge in respect of the starting condition. In particular, Acidobacteria, Actinobacteria, Chloroflexi, Firmicutes and Spirochaetes phyla were selectively enriched in T1 and T2 reactors. This finding is in agreement with previous studies highlighting the putative key role of these bacterial phyla, in particular Firmicutes, for VFA production [53–55]. Interestingly, members of the *Treponema* genus (belonging to Spirochetes phylum) are reported to be lignocellulose degrading bacteria and positively correlated with VFA production [56,57]. On the other hand, the *Dechloromonas* genus has been found dominant in the activated sludge of wastewater treatment tanks because of the ability to use VFA and other intermediate compounds as carbon sources [58].

3.3. Adsorption Batch Test

In order to evaluate the best zeolite and biochar for the experimental column phase, preliminary batch tests were carried out at the Agricultural Chemistry laboratories of the Department of Agricultural, Food and Forestry Sciences.

In the case of zeolite, a study was addressed to investigate the effects of acid and alkaline treatment of clinoptilolite on its ammonium adsorption and desorption ability. The treatments applied were based on the following hypotheses: (i) the alkaline treatment (1 M NaOH) aimed to break all covalent bonds between O and H of OH groups in the structure of the tectosilicate and to replace H^+ with Na^+ , thus creating an electronegative bond between O and Na; (ii) the HCl treatment aimed to replace all exchangeable cations of the clinoptilolite surface with H^+ ions. Following the acidic or alkaline pre-treatment, the pre-treated zeolites were treated with three different salts ($NaCl$, $CaCl_2$ or $MgCl_2$) to evaluate the role of the saturating cation on the ability of zeolite in adsorbing NH_4^+ from the NH_4Cl one-component solution, and subsequently, the NH_4^+ recovered after

desorption [59,60]. To our best knowledge, this is the first study to evaluate the effect of both acid and alkaline treatments on the same natural zeolite.

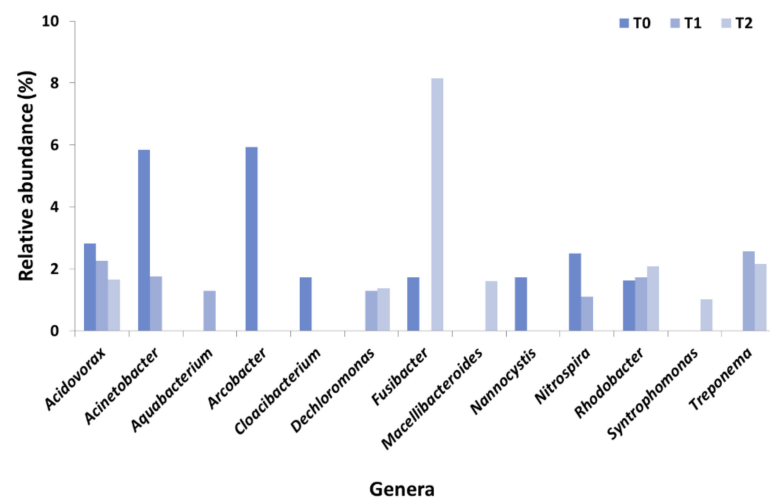


Figure 12. Relative abundance of the most abundant (i.e., having percentage values > 1) bacterial genera identified by NGS analysis of the V3–V4 region of the 16S rRNA encoding gene in the activated sludge of T0, T1 and T2 conditions, respectively.

Batch tests of static adsorption and desorption and of dynamic adsorption were conducted (adsorption kinetics). The results of static adsorption and desorption tests showed that the highest amount of adsorbed NH_4^+ was recorded for untreated and HCl-Na treated clinoptilolite with an average of 11.8 mg of NH_4^+ adsorbed per gram of clinoptilolite (Figure 13). Such a result agreed with that obtained by Lebedynets et al. [61], Bolan et al. [62], Wang et al. [63] and Wen et al. [64] who have observed an adsorption capacity ranging from 8 to 13 mg $\text{NH}_4^+ \text{g}^{-1}$, depending on the particle size of zeolite. The lowest amount of adsorbed NH_4^+ occurred with NaOH-Mg, which was 27% lower than untreated and HCl-Na treated clinoptilolite. The amount of NH_4^+ released after 48 h ranged from 9.4 to 7 mg of NH_4^+ adsorbed per gram of the clinoptilolite previously adsorbed (Figure 14) and depended on the amount of NH_4^+ adsorbed [59].

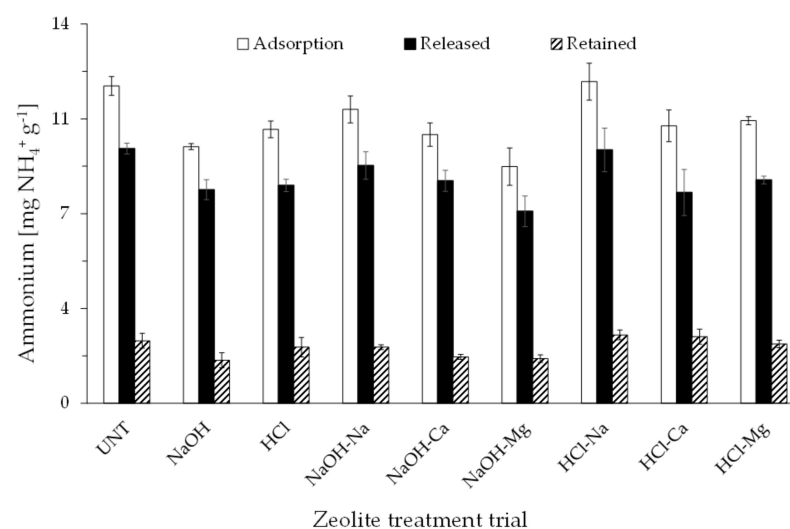


Figure 13. Amount of ammonium adsorbed, released and retained by treated clinoptilolite. Treatments are untreated clinoptilolite (UNT), pre-treated clinoptilolite with 1 M sodium hydroxide (NaOH) or 0.1 M hydrochloric acid (HCl), and treated clinoptilolite with sodium chloride (NaCl), calcium chloride (CaCl_2) magnesium chloride (MgCl_2) after each pre-treatment (NaOH-Na, NaOH-Ca, NaOH-Mg, HCl-Na, HCl-Ca, HCl-Mg). Values are means of three replicates, and bars are standard deviations.

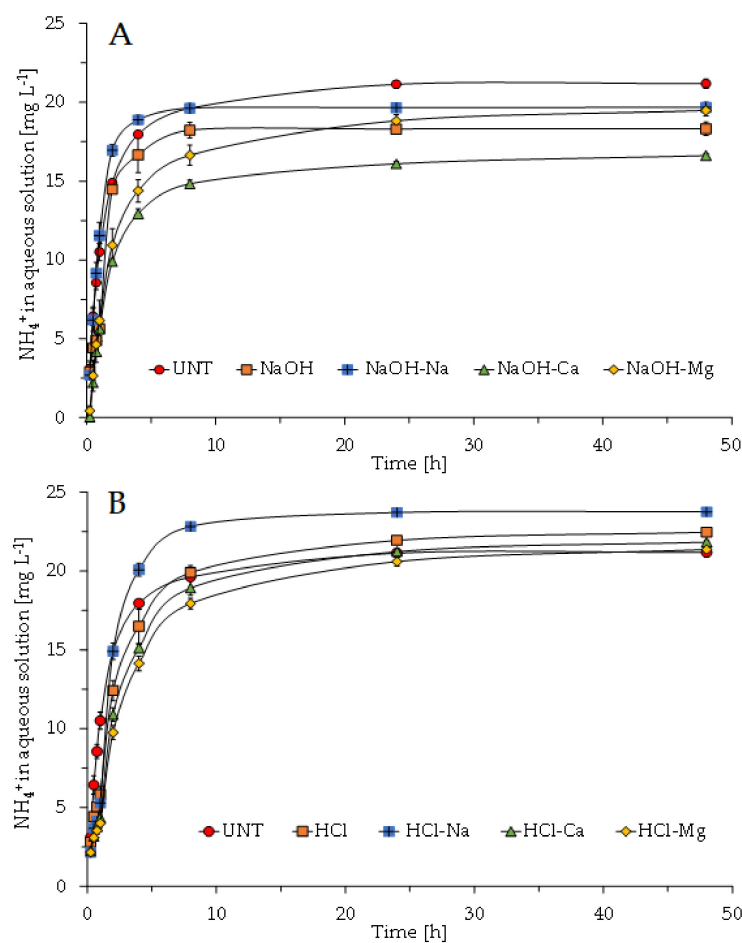


Figure 14. Ammonium adsorption kinetics by treated clinoptilolite during 48 h. Treatments are untreated clinoptilolite (UNT), pre-treated clinoptilolite with 1 M sodium hydroxide (NaOH) or 0.1 M hydrochloric acid (HCl) and treated clinoptilolite with sodium chloride (NaCl), calcium chloride (CaCl_2) magnesium chloride (MgCl_2) after NaOH (A) or HCl (B) pre-treatment. Values are mean of three replicates, and bars are standard deviations.

The results about the adsorption kinetics suggest that the amount of NH_4^+ adsorbed increased with contact time as reported in the literature (Figure 14) [60]. Within the first 15 min, UNT clinoptilolite adsorbed 12% NH_4^+ , HCl-treated clinoptilolite adsorbed 8.6 to 11.2% NH_4^+ , and NaOH-treated clinoptilolite adsorbed 0.2 to 11.6%. Kinetic adsorption of NH_4^+ followed different patterns depending on the pre-treatment. Such results disagreed with those obtained in References [60–68] which reported more than 70% of NH_4^+ adsorption within the first 10 min using natural clinoptilolite. NH_4^+ adsorption by NaOH pre-treated and treated clinoptilolite, except for NaOH-Mg, was almost completed after 8 h, whereas that by HCl pre-treated clinoptilolite was completed within 24 h for all treatments [59].

4. Conclusions

The increasing water demand and the emerging challenges for re-thinking the concept of wastewater treatment plants towards a biorefinery approach, in recent years, have pushed towards the implementation of resource recovery systems. Nevertheless, the widespread application of water-smart solutions is still hampered by several barriers (i.e., technological, regulatory, organizational, social and economic) that should be overcome to allow the requested transition. In this context, the Horizon 2020 project WIDER UPTAKE represents a significant opportunity to demonstrate that the water sector can be pushed toward a more sustainable development. The resource recovery system conceived at

Palermo University in the frame of WIDER UPTAKE is a great chance to demonstrate the feasibility of innovative solutions in the wastewater sector to overcome the existing barriers, thus promoting the implementation of circular economy concept. Indeed, the opportunity to involve students/young generations from the educational programmes is of paramount importance to foster the transition to a circular economy in the water sector. Universities may have a central role in the transition and promote, in the future, sustainable water smart solutions for achieving a higher environmental sustainability level. Despite some case studies related to water resources applying water reuse [69–71] having been presented in the literature, the Palermo University case study is the first case study on WRRF confirming the high role that can be played by universities in promoting sustainability.

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