University of Pavia
Department of Civil Engineering and Architecture
Ph.D. in Design, Modeling, and Simulation in Engineering

RESOURCE RECOVERY FROM WASTEWATER WITH A THERMOPHILIC TECHNOLOGY FOR SEWAGE SLUDGE REDUCTION

Ph.D. Thesis

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PREFACE

I learned about the thermophilic aerobic/anaerobic membrane reactor (TAMR) during my master's thesis. Already in that context, I had the opportunity to understand its operation and management thanks to experimentation with a pilot plant located in a full-scale wastewater treatment plant (WWTP). In addition to a training experience in the academic field, it was a valuable and enriching experience from a personal point of view.

For the first time, I met the working world of WWTPs, almost a bubble independent of the surrounding reality but full of welcoming people ready to help. For the first time, I learned to collaborate with a research group of proactive people, in which everyone, with their defined task, represented a well-oiled cog in the work team. For the first time, I put on a Tyvek® suit (personal protective equipment), used a wrench, cleaned bag filters, disassembled and reassembled ultrafiltration ceramic membranes, I got dirty with thermophilic sludge (also on my face)… in short, as I said earlier, it was also a life experience. Consequently, the choice to continue studying this technology came naturally thanks to the Ph.D. The next chapters of this story will be told in this Ph.D. thesis.

This work was conceived as a "compilation thesis", collecting some research articles and chapters in which I participated as co-author during my Ph.D.

studies in Design, Modeling, and Simulation in Engineering at the Department of Civil Engineering and Architecture of the University of Pavia. Due to copyright issues, some works have been inserted in this document as a preprint, but details on final published versions are provided. The thesis results (Section 4) are preceded by a description of the scientific background (Section 1), the details of the structure (Section 2), and the development timeline (Section 3) of the whole thesis work.

Since of course nothing can be achieved alone, I have many people to thank. First of all, I want to thank the inventor of this technology, Eng. Pier Francesco Ravasio, and my tutor, Prof. Maria Cristina Collivignarelli, trusted me and allowed me to study the interesting world of aqueous waste and water treatment, and discover a new passion. An essential thanks go to all the Professors who have supported me over the years; thanks to their suggestions, ideas, and reviews I grew not only professionally. Last but not least, I would like to thank all the colleagues I collaborated with daily; our important experiences have given rise to valuable working relationships and real friendships.

Francesca Maria Caccamo

ABSTRACT

Today, 1.7 Earths would be needed to satisfy humanity's demand for resources and ecological services in just one year. Minimizing the exploitation of feedstocks and developing a functional recovery of resources in every field, reducing the accumulation of waste, become indispensable. Wastewater treatment plants (WWTPs) are often negatively known to produce biological sewage sludge, a waste whose final fate is increasingly technically and socially debated. This work proposes a technology for the reduction of biological sewage sludge production, a thermophilic aerobic/anaerobic membrane biological reactor (TAMR). The aim can be achieved by (i) prevention in the water line, treating industrial wastewater or aqueous waste with low production of thermophilic sludge; (ii) minimization in the sludge line, directly treating the biological sludge (thickened only or thickened and digested) already produced by WWTPs. Regardless of the approach, TAMR presents two types of residues: an aqueous permeate rich in carbon and nitrogen, and a surplus thermophilic biological sludge in smaller quantities. In this work, after (i) the analysis of high performances on various polluting parameters (COD, surfactants, etc.), and (ii) the process optimization through statistical data processing and study on thermophilic sludge rheology; examples of reuse of TAMR residues have been reported. After the recovery of the nitrogen by stripping, the permeate can be used as an external carbon source in denitrification; while the thermophilic sludge meets the quality requirements for recovery in agriculture. Furthermore, thermal energy production was investigated for future energy recovery at the WWTPs.

ABSTRACT – ITA

In una realtà in cui sarebbero necessari 1.7 pianeti Terra per soddisfare la domanda dell'umanità di risorse e servizi ecologici in un solo anno, diventa indispensabile minimizzare lo sfruttamento delle materie prime e sviluppare un funzionale recupero di risorse in ogni campo, riducendo contestualmente l'accumulo di rifiuti. Gli impianti di trattamento delle acque reflue (WWTPs) sono spesso conosciuti negativamente per la produzione di fanghi biologici di depurazione, un rifiuto il cui destino finale è sempre più tecnicamente e socialmente discusso. Questo lavoro propone una tecnologia per la riduzione della produzione dei fanghi biologici di depurazione, un reattore biologico termofilo aerobico/anaerobico a membrana (TAMR). Lo scopo può essere raggiunto mediamente (i) la prevenzione in linea acque, trattando acque reflue industriali o rifiuti liquidi con una bassa produzione di fango risultante dal processo TAMR; (ii) la minimizzazione in linea fanghi, trattando direttamente il fango biologico (solo ispessito o ispessito e digerito) già prodotto presso i WWTPs. Indipendentemente dalla tipologia di approccio, la tecnologia TAMR presenta due tipi di residui: un permeato liquido ricco di carbonio e azoto, e in minori quantità, un fango biologico di supero estratto dal reattore termofilo. In questo lavoro, dopo aver individuato alte performance su diversi parametri inquinanti (COD, tensioattivi, ecc.), e aver ottimizzato il processo mediante elaborazioni statistiche dei dati e studio della reologia del fango termofilo, sono stati riportati esempi di riutilizzo dei residui di trattamento. Dopo il recupero dell'azoto mediante strippaggio, il permeato può essere impiegato come fonte esterna di carbonio in denitrificazione; mentre il fango termofilo rispetta i requisiti di qualità per il recupero in agricoltura. Inoltre, è stata approfondita la produzione di energia termica da parte della biomassa termofila, in vista di un eventuale recupero energetico presso i WWTPs.

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- **Figure 3.2** Workflow of the PhD work. TAMR: Thermophilic aerobic/anoxic membrane reactor; UF: ultrafiltration system; WW: wastewater; WWTP: Wastewater treatment plant.
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LIST OF PUBLICATIONS

In this "compilation thesis", several documents in which I participated as a co-author are inserted. The details of every single paper/chapter (reference, website, type of document, policy) and the preprint or the editorial PDF are reported in Section 4 (Results). References of publications inserted are also listed below:

1. Collivignarelli, M. C., **Caccamo, F. M.**, & Carnevale Miino, M. (**2022**). An Innovative Technology to Minimize Biological Sludge Production and Improve Its Quality in a Circular Economy Perspective. Emerging Pollutants in Sewage Sludge and Soils. In: Núñez-Delgado, A., Arias-Estévez, M. (eds) Emerging Pollutants in Sewage Sludge and Soils. *The Handbook of Environmental Chemistry*, vol 114. Springer, Cham.
2. Collivignarelli, M. C., Carnevale Miino, M., **Caccamo, F. M.**, Baldi, M., & Abbà, A. (**2021**). Performance of full-scale thermophilic membrane bioreactor and assessment of the effect of the aqueous residue on mesophilic biological activity. *Water*, 13(13), 1754.
3. Collivignarelli, M. C., Pedrazzani, R., Bellazzi, S., Carnevale Miino, M., **Caccamo, F. M.**, Baldi, M., Abbà, A., & Bertanza, G. (**2022**). Numerical Analysis of a Full-Scale Thermophilic Biological System

and Investigation of Nitrate and Ammonia Fates. *Applied Sciences*, 12(14), 6952.

4. Collivignarelli, M. C., Bellazzi, S., Carnevale Miino, M., **Caccamo, F. M.**, Calatroni, S., Durante, A., & Baldi, M. (**2022**). Influence of Heavy Metals on the Rheology of a Thermophilic Biological Sludge for nutrients Recovery: Effect of Iron, Copper, and Aluminium on Fluid Consistency. *Waste and Biomass Valorization*, 1-10.
5. Collivignarelli, M. C., Todeschini, S., Bellazzi, S., Carnevale Miino, M., **Caccamo, F. M.**, Calatroni, S., Baldi., M., & Manenti, S. (**2022**). Understanding the Influence of Diverse Non-Volatile Media on Rheological Properties of Thermophilic Biological Sludge and Evaluation of Its Thixotropic Behaviour. *Applied Sciences*, 12(10), 5198.
6. Collivignarelli, M. C., Abbà, A., **Caccamo, F. M.**, Carnevale Miino, M., Durante, A., Bellazzi, S., Baldi, M., & Bertanza, G. (**2021**). How to produce an alternative carbon source for denitrification by treating and drastically reducing biological sewage sludge. *Membranes*, 11(12), 977.
7. Collivignarelli, M.C., **Caccamo, F.M.**, Bellazzi, S., Abbà, A., Bertanza, G.. (**2023**). Assessment of the Impact of a New Industrial Discharge on an Urban Wastewater Treatment Plant: Proposal for an Experimental Protocol. *Environments*, 10(7),08.

8. Collivignarelli, M. C., Abbà, A., Miino, M. C., **Caccamo, F. M.**, Argiolas, S., Bellazzi, S., Baldi, M., & Bertanza, G. (**2021**). Strong minimization of biological sludge production and enhancement of phosphorus bioavailability with a thermophilic biological fluidized bed reactor. *Process Safety and Environmental Protection*, 155, 262-276.

9. Collivignarelli, M. C., Miino, M. C., Cillari, G., Bellazzi, S., **Caccamo, F. M.**, Abbà, A., & Bertanza, G. (**2022**). Estimation of thermal energy released by thermophilic biota during sludge minimization in a fluidized bed reactor: Influence of anoxic conditions. *Process Safety and Environmental Protection*, 166, 249-256.

1 INTRODUCTION AND SUMMARY OF THE WORK

According to the European directive 86/278/EEC (European Commission, 1986), the "sludge" deriving from the wastewater treatment consists mainly of residues (i) from domestic or municipal wastewater treatment plants (WWTPs) and other WWTPs treating wastewater with a composition like that of domestic and urban wastewater, and (ii) from septic tanks and other similar wastewater treatment devices.

The management of sewage sludge has become an increasingly relevant problem over the years due to (i) the ever-increasing production for population growth and more restrictive limits in the WWTP effluents (European Commission, 1991; Ferrentino et al., 2023), and (iii) the subsequent difficult disposal/recovery (Siddiqui et al., 2023).

Directive 2018/851/EU (European Commission, 2018), Legislative Decree 152/06 (Repubblica Italiana, 2006) and subsequent amendments identified a hierarchy in waste management, including sewage sludge: (i) prevention and minimization of the production, (ii) matter recovery and reuse of the residues, (iii) energy recovery and, finally (iv) safe disposal of the waste.

The sustainable management of sewage sludge faces a global challenge to "reconcile" two sides of the same coin: the desire to prevent pollution and the reuse of a precious resource. Over the last 50 years, four main practices in sewage sludge management have developed: (i) a declining interest in disposal "slowed down" by economic and legislative challenges on landfilling, (ii) a dominant interest in land application (organic fertilizer and soil conditioner in agriculture) as main recycling strategy, (iii) a growing interest in resource recovery from sewage sludge, such as nutrients and heavy metals extracted and separated from contaminants, and (iv) a stable interest in energy recovery mainly by anaerobic digestion (Bagheri et al., 2023; Siddiqui et al., 2023). Although the main form of matter recovery is in agriculture as a soil improver, this interest is declining. The complicated regulatory aspect and the poor acceptance by people have together made agronomic use increasingly difficult to practice. Both the fear of pathogens and micropollutants, as well as the inconvenience caused by bad smells in the fields, are widespread (Chojnacka et al., 2023).

One downside is the focus of scientific research on optimizing individual technologies with the assessment of individual risks or benefits. Since sewage sludge management is multifaceted, a more widespread and integrated approach is desirable in order not to overshadow non-negligible issues (Bagheri et al., 2023). Therefore, the combined use of technologies for the significant minimization of sewage sludge production and approaches for the correct and sustainable management of the produced sludge has become an urgent worldwide priority. Timely intervention by simultaneously applying all the hierarchy required by law is the only effective way to optimize the management of sewage sludge from a circular and sustainable perspective. First of all, the choice of the most suitable sludge reduction technology must be made on a case-by-case basis, considering technical and economic factors, and carefully evaluating the pros and cons resulting from its application.

The prevention and minimization of the production of biological sewage sludge represent the first step of the hierarchy which can be pursued in two distinct ways: (1) by adopting processes capable of treating water with a minimum production of residual/excess sludge; (2) provide in situ (within the WWTPs) treatments to minimize the amount of sludge produced (Collivignarelli et al., 2019; Ferrentino et al., 2023). The thermophilic aerobic/anoxic membrane reactor (TAMR) is a technology capable of responding to both scenarios.

TAMR is an advanced biological process that allows simultaneous exploitation of the thermophily's advantages in auto-thermal conditions and of membrane biological reactor (MBR) systems, guaranteeing a low specific sludge production. The process can be applied (i) to industrial aqueous waste in the water line and (ii) to sewage sludge in the sludge line.

In the water line, industrial aqueous waste/high-strength wastewater with different pollutants, particularly rich in COD, is treated in aqueous waste treatment plants or specially authorized urban WWTPs. To maintain the thermophilic conditions ensured by the exothermic oxidation/degradation processes, the substrate fed to TAMR must be highly concentrated in organic matter; the urban wastewater would certainly not allow it to reach auto-thermal energy.

The plant layout will consist of TAMR first, followed by mesophilic treatment, usually a traditional activated sludge process (CAS). The organic substrates, which have already undergone thermophilic treatment (aqueous waste pre-treatment), are further treated by the downstream CAS. The two types of biotas have different fields of application, which complement each other. The "food", that is "rejected" by TAMR and left as a residual substrate, is well biodegraded by downstream mesophilic treatment. The industrial aqueous waste, after thermophilic pre-treatment, is discharged into the sewer and undergoes a final mesophilic treatment in the urban WWTP, together with the urban wastewater.

In the case of sludge-line, TAMR can be applied to treat pre-thickened or pre-thickened and digested biological sewage sludge from municipal WWTPs. Unlike the previous application, the system does not operate in complete aerobiosis but, to ensure correct and complete hydrolysis of the organic substance, aerobic and anoxic conditions must be alternated. In this application, TAMR can also be used for the co-treatment of sludge and industrial aqueous waste (Collivignarelli et al., 2019).

Regardless of the substrate fed to TAMR, the process outputs are two: (i) the liquid permeate, and (ii) the extracted thermophilic sludge (or excess sludge).

Both the permeate and the extracted sludge can be reused within the WWTP itself or recovered outside the plant.

Permeate is the most significant output in terms of flow rate. It is (i) free of suspended solids (thanks to the presence of ultrafiltration membranes which allow keeping all the thermophilic biomass inside the biological reactor), (ii) rich in ammonia nitrogen (thanks to the strong ammonification activity), and (iii) rich in highly biodegradable COD by the mesophilic biomass. This substrate is suitable (i) for the recovery of ammonia nitrogen by stripping, and (ii) for application in the denitrification process in the water line of WWTPs, replacing external sources of synthetic organic carbon.

The excess sludge has a daily extraction rate of at least two orders of magnitude lower than the permeate and represents the excess biomass of the thermophilic biological system. Excess sludge has excellent qualitative characteristics, thanks to (i) a high content of carbon and phosphorus and (ii) a high degree of humification and sanitization due to the operating temperatures of the process. The excess sludge, resulting from the application of TAMR in the sludge line, can be assimilated to high-quality sludge suitable for spreading in agriculture according to the standards of the Lombardy regional legislation.

In Lombardy (Northern Italy) two full-power TAMR plants have been in operation for years for the aqueous waste treatment (to minimize the sludge produced by intervening on the water line); while the start-up of a third plant for the treatment and direct minimization of biological sewage sludge is underway.

The inclusion of TAMR in an existing WWTP has the potential to convert it into an integrated technological structure with reduced sludge output as waste. TAMR technology aims to evolve the WWTPs of contaminated water into real resource production plants, with a view to a sustainable and circular economy. This approach closely follows the one proposed in recent years by several researchers: a change of perspective in conceiving conventional WWTPs as "water resource recovery facilities", in which nutrients, organic matter, and other valuable substances can be completely recovered and reused (Coats and Wilson, 2017; Cornejo et al., 2019; Lizarralde et al., 2018; Regmi et al., 2019).

Finally, this work intended to propose alternatives to recover the residues resulting from TAMR, a technology for the prevention/minimization of the production of biological sewage sludge, after monitoring and optimizing the process performance. This thesis project is divided into 4 work steps (WSs) focused on TAMR technology: (i) monitoring and performance study, (ii) process optimization, (iii) residues and energy recovery, and (iv) dissemination of results. In WS1, a description of TAMR technology and its strengths, results of TAMR monitoring, evaluation of treatment performance, and removal yields of polluting parameters were reported with a focus on low specific sludge production. WS2 focused both on the rheology of thermophilic sludge as a key factor for optimal hydrodynamic behavior in the biological reactor and on the statistical analysis of monitoring and performance data to support and facilitate plant management. In WS3, examples of possible reuses of TAMR residues, both in the same WWTP and outside the plant, and an estimation of thermal energy released by thermophilic biota during sludge minimization, were studied. WS4 aimed to promote the results of WS1, WS2, and WS3 to other researchers and technical stakeholders. A detailed description of the work is reported in Section 2.

2 WORK STRUCTURE AND EXPECTED RESULTS

This thesis project was divided into four main work steps (WSs) on TAMR technology:

- WS1: Monitoring and performance study,
- WS2: Process optimization,
- WS3: Residues and energy recovery, and
- WS4: Dissemination of results.

This work responded to diverse Sustainable Development Goals (SDGs) (United Nations, 2021) listed below:

- **SDG6** – "Ensure availability and sustainable management of water and sanitation for all"; in particular with the targets 6.3 (By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally), and 6.6 (By 2030, protect and restore water-related ecosystems, including mountains, forests, wetlands, rivers, aquifers, and lakes);
- **SDG9** – "Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation"; in particular, with

the targets 9.4 (By 2030, upgrade infrastructure and retrofit industries to make them sustainable, with increased resource-use efficiency and greater adoption of clean and environmentally sound technologies and industrial processes, with all countries taking action in accordance with their respective capabilities) and 9.5 (By 2030, upgrade infrastructure and retrofit industries to make them sustainable, with increased resource-use efficiency and greater adoption of clean and environmentally sound technologies and industrial processes, with all countries taking action in accordance with their respective capabilities).

- **SDG12** – "Ensure sustainable consumption and production patterns"; in particular, with the targets 12.2 (achieve the sustainable management and efficient use of natural resources), 12.4 (achieve the environmentally sound management of chemicals and all wastes throughout their life cycle, in accordance with agreed international frameworks, and significantly reduce their release to air, water, and soil in order to minimize their adverse impacts on human health and the environment), and 12.5 (substantially reduce waste generation through prevention, reduction, recycling and reuse).

The description of the activities done in every single WS is reported below.

WS1 - MONITORING AND PERFORMANCE STUDY

WS1 provided a general overview of the problem of sewage sludge produced by civil and industrial WWTPs. The "waste management hierarchy", proposed by European and national legislation and which provides a first step in the reduction of the sludge produced at WWTPs, was recalled. WS1 showed how the prevention/minimization of sludge production can be pursued thanks to two different approaches: (i) adopting processes for industrial water treatments with a minimum production of residual sludge; (ii) providing treatments, within the WWTP, on the biological sludge already produced to minimize its quantities. TAMR technology, adaptable to both needs, was presented, underlining strengths and fields of application. A detailed description of the activities done in WS1 and expected outcomes are reported below.

WS1.1 - MONITORING AND PERFORMANCE OF FULL-SCALE TAMRS FOR AQUEOUS WASTE TREATMENT

In WS1.1, monitoring activities were focused on two full-scale TAMRs treating high-strength industrial wastewater. The full-scale plants were respectively located in an urban WWTP also authorized for the pre-treatment of aqueous waste; and in a platform for the treatment mainly of aqueous waste. In both cases, a CAS mesophilic treatment was present downstream of

TAMR; in the first case within the WWTP, and in the second case outside the platform. In WS1.1, based on the monitoring of TAMRs, the high performance on chemical pollutants (COD, N-NO_x, non-ionic, and anionic surfactants) and the low sludge production were evaluated. Furthermore, the transformations of nitrogen in the biological reactor have been extensively studied. The fluorescence in situ hybridization (FISH) tests have not identified nitrifying bacteria in the thermophilic sludge, so the uncommon ammonia removal was found to be caused by stripping due to the pure oxygen injection rather than microbiological transformation.

WS1.2 - MONITORING AND PERFORMANCE OF A PILOT-SCALE TAMR FOR SEWAGE SLUDGE MINIMIZATION

WS1.2 showed the application of TAMR in a WWTP sludge line to minimize the sewage sludge already produced. In WS1.2, a pilot-scale TAMR was fed continuously with biological sludge extracted from a conventional activated sludge system. More than 9 months of experimentation were carried out to evaluate the application of TAMR in WWTPs as possible effective treatment for minimization and quality improvement of sewage sludge. Monitoring and performance study revealed: (i) a high removal of COD and volatile solids, (ii) a low specific production of thermophilic sludge, and (iii) a high accumulation of inorganic phosphorus in the thermophilic biological sludge. A cost analysis showed that the application of TAMR after pre-thickening, without further treatments, could save considerably compared to the current situation (with anaerobic digestion and dewatering in the sludge line).

WS2 - PROCESS OPTIMIZATION

WS2 focused on the optimization of the TAMR process, both through statistical analysis of monitoring and performance data, and through the study of the rheological behaviour of the thermophilic biological sludge. WS2 aims to improve the efficiency and management of the process through concrete tools and targeted actions (e.g., statistical data processing and rheological tests) directly applicable to water utilities. Optimizing the operational activities of existing WWTPs through practical approaches by reducing the consumption of energy and reagents ensures more sustainable and circular WWTPs. In general, if a process works well, there are positive effects in all directions, including on the plant economy (lower sudden and unforeseen management costs) and worker safety (less dangerous situations triggered by malfunctions). Below is a detailed description of the work carried out in WS2 and the expected results.

WS2.1 - STATISTICAL ANALYSIS OF MONITORING DATA

WS2.1 examined the TAMR process for the treatment of aqueous waste. A multivariate statistical analysis was applied, highlighting a correlation between the monitored input pollutants and the calculated performance of the thermophilic process. Statistical models can help the utility operator predict (i) the pollutant load fed and (ii) the performance of the thermophilic biological system in the presence of a limited amount of monitored data. WS2.1 demonstrated a useful tool for the WWTP (or aqueous waste treatment plant) manager to decide the correct mix of aqueous waste to feed to the biological reactor.

WS2.2 - RHEOLOGICAL BEHAVIOR OF THERMOPHILIC SLUDGE

WS2.2 investigated the rheological properties of thermophilic sludge, studying both its thixotropic behavior and the influence of various non-volatile solids (calcium carbonate, sand, and sodium bentonite) and some heavy metals (iron, copper, and aluminum). The rheological behavior represents a key factor for the application of computational fluid dynamics models (CFD), indispensable tools for understanding the hydrodynamics of reactors. WS2.2 underlined how the study of rheology can optimize TAMR management under several aspects: (i) hydrodynamics of bioreactors, (ii) energy consumption (in sludge pumping), (iii) membrane performance (sludge-water separation by filtration), and (iv) mass transfer efficiency of aeration systems. In general, the performance of the biological process is strongly influenced by the rheological behavior of the thermophilic sludge inside the reactor.

WS3 - RESIDUES AND ENERGY RECOVERY

In WS3, examples of possible reuses of TAMR residues, both in the same WWTP and outside the plant, were explored. The outputs of the thermophilic process are (i) an aqueous substrate rich in carbon and nitrogen, the permeate of the filtration system; and (ii) the sludge extracted from the reactor, a small fraction of the thermophilic biomass reproduced. The monitoring of the chemical-physical and microbiological parameters and the experimental tests, carried out on the outputs and described in the WS3, were useful tools for identifying the recovery alternatives for both "waste" products. In WS3, the production of thermal energy during sludge minimization was also investigated. In this way, an integrated structure is created that fully falls within the concept of circular economy. A detailed description of the studies done in WS3 and the expected outcomes are reported below.

WS3.1 - REUSE OF RESIDUES

In WS3.1, the reuse of permeate, outside or inside the WWTP that produced it, as an alternative carbon source for denitrification processes in conventional activated sludge systems was investigated. Based on the respirometric tests (OUR – oxygen uptake rate; AUR – ammonia uptake rate; NUR – nitrate uptake rate), (i) an excellent biological treatability of the permeate by the mesophilic biomass, excluding any inhibiting effect, and (ii) denitrification kinetics comparable to those of methanol, were observed. After stripping in an acid tower for ammonia recovery, the permeate could be recirculated in a WWTP water line, replacing purchased external carbon sources and guaranteeing economic savings. WS3.1 proceeded with a qualitative characterization of residual thermophilic sludge from sludge minimization treatment. The excess sludge could be destined for recovery in agriculture as high-quality sludge according to the Lombardy legislation. Contextually, the transformation mechanisms and bioavailability of phosphorus for land application were investigated: the phosphorus precipitated almost completely in inorganic form and accumulated in the thermophilic sludge; in this form could be directly assimilated by crops.

WS3.2 - THERMAL ENERGY PRODUCTION

TAMR works at temperatures even higher than 50 °C, not due to external heating, but thanks to exothermic degradation processes of the biodegradable organic substance. The thermophilic process is thermally self-sustaining, and temperatures are often too high (>55°C) for probes immersed in thermophilic sludge, used for the continuous measurement of process parameters (dissolved oxygen, temperature, redox potential, etc.). The full-scale plants are equipped with cooling systems to keep the sludge temperature below 50°C. To date, this thermal energy has not yet been exploited; however, in WS3.2, an estimation of thermal energy released by thermophilic biota during sludge minimization was explored and a strict dependence of the thermal energy produced by the anoxic hours was detected. The WS3.2 can represent a reference for future studies on optimizing the conditions to maximize the release of thermal energy produced by TAMR, in view of the subsequent energy recovery.

WS4 - DISSEMINATION OF RESULTS

WS4 aimed to promote the dissemination of results obtained in the project through a poster presentation at conferences, scientific publications, and Ph.D. thesis writing. The main stakeholders that were interested in WS4 were researchers from other academic institutions and technicians of water utilities. A detailed description of the activities done in WS4 and the expected outcomes are reported below.

WS4.1 - SCIENTIFIC PUBLICATIONS

WS4.1 enclosed the activity of research paper writing during the entire project. In total, 9 articles/chapters were published in international peer-review Journals and Books, indexed in Scopus and/or Web of Science. A poster has been also presented at the "9th IUPAC International Conference on Green Chemistry – ICGC 2022" (Athens, 05 Sep – 09 Sep 2022).

WS4.2 - PHD THESIS

In close collaboration with the Supervisor, WS4.2 concerned the activity of Ph.D. thesis writing, promoting the sharing of information in the research group TEC2.0, Laboratory of Water and Waste Treatment Processes and Technologies "Elisa Gazzola", Department of Civil Engineering and Architecture, University of Pavia.

3 WORK DEVELOPMENT

This thesis project was composed of 4 WSs on TAMR technology. **Figure 3.1** shows, through a flowchart, the two applications of TAMR: (1) Water-line application for prevention of biological sewage sludge production, and (2) Sludge-line application for minimization of biological sewage sludge produced.

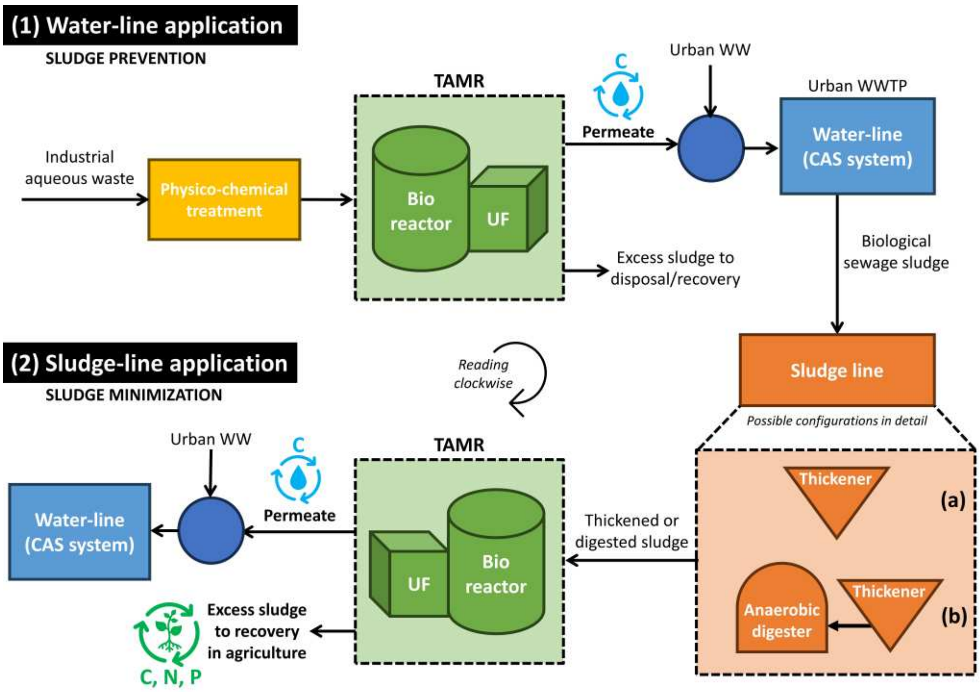

Figure 3.1 Flow chart of TAMR applications. TAMR: Thermophilic aerobic/anoxic membrane reactor; UF: ultrafiltration system; WW: wastewater; WWTP: Wastewater treatment plant; C: carbon recovery; N: nitrogen recovery; P: phosphorous recovery.

Figure 3.2 represents the workflow of WSs: WS1 (Monitoring and performance study), WS2 (Process optimization), and WS3 (Residues and energy recovery) were carried out in parallel. WS4 (Dissemination of results) covered the results of all WSs.

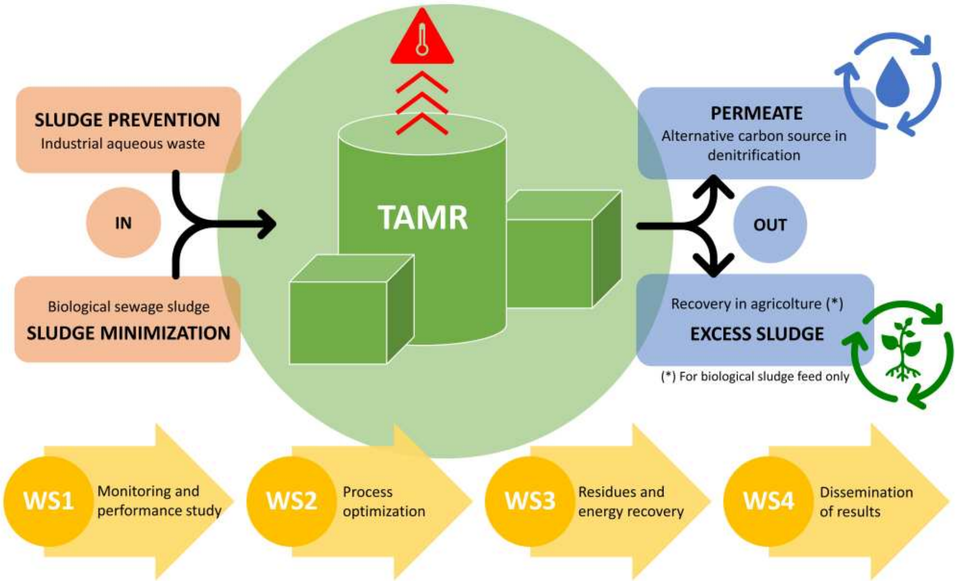

Figure 3.2 Workflow of the PhD work. TAMR: Thermophilic aerobic/anoxic membrane reactor; UF: ultrafiltration system; WW: wastewater; WWTP: Wastewater treatment plant.

Figure 3.3 reports the Gantt timeline of the thesis project. The list of WSs and their duration is reported below:

- **WS1 - MONITORING AND PERFORMANCE STUDY: 19 MONTHS**
  - WS1.1 - MONITORING AND PERFORMANCE OF FULL-SCALE TAMRS FOR AQUEOUS WASTE TREATMENT: **6 MONTHS**

  - WS1.2 - MONITORING AND PERFORMANCE OF A PILOT-SCALE TAMR FOR SEWAGE SLUDGE MINIMIZATION: **13 MONTHS**
- **WS2 - PROCESS OPTIMIZATION: 9 MONTHS**
  - WS2.1 - STATISTICAL ANALYSIS OF MONITORING DATA: **3 MONTHS**
  - WS2.2 - RHEOLOGICAL BEHAVIOR OF THERMOPHILIC SLUDGE: **6 MONTHS**
- **WS3 - RESIDUES AND ENERGY RECOVERY: 32 MONTHS**
  - WS3.1 - REUSE OF RESIDUES: **29 MONTHS**
  - WS3.2 - THERMAL ENERGY PRODUCTION: **3 MONTHS**
- **WS4 - DISSEMINATION OF RESULTS: 27 MONTHS**
  - WS4.1 - SCIENTIFIC PUBLICATIONS: **23 MONTHS**
  - WS4.2 - PHD THESIS: **4 MONTHS**

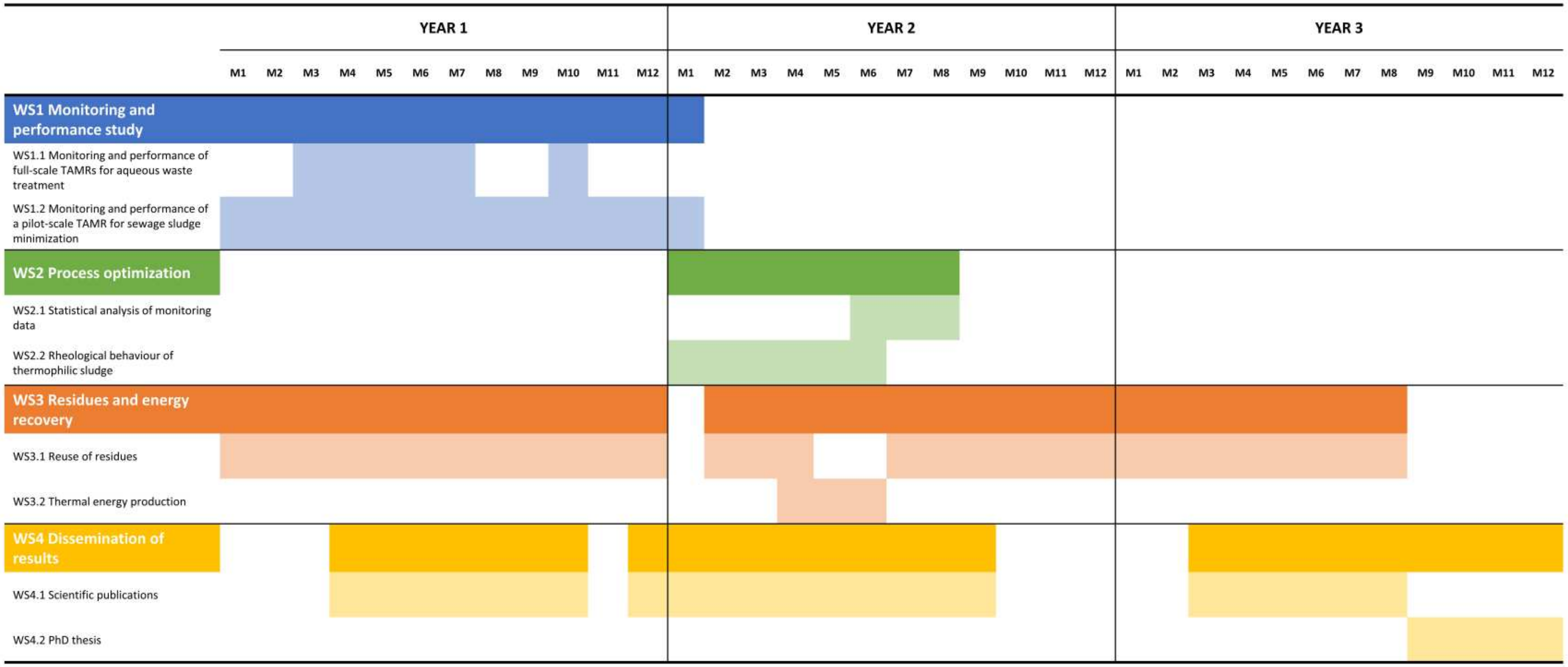

Figure 3.3 Gantt diagram of timeline of thesis' work. M: months; TAMR: Thermophilic aerobic/anoxic membrane reactor.

4 Results

Publication 1

Topic: WS1 - Monitoring and performance study

- WS1.1 - Monitoring and performance of full-scale TAMRs for aqueous waste treatment
- WS1.2 - Monitoring and performance of a pilot-scale TAMR for sewage sludge minimization

WS3 - Residues and energy recovery

- WS3.1 - Reuse of residues

Reference: Collivignarelli, M. C., **Caccamo, F. M.**, & Carnevale Miino, M. (2022). An Innovative Technology to Minimize Biological Sludge Production and Improve Its Quality in a Circular Economy Perspective. Emerging Pollutants in Sewage Sludge and Soils. In: Núñez-Delgado, A., Arias-Estévez, M. (eds) Emerging Pollutants in Sewage Sludge and Soils. *The Handbook of Environmental Chemistry*, vol 114. Springer, Cham.

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An innovative technology for minimize biological sludge production and improve its quality in a circular economy perspective

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Abstract

In the coming years, the production of biological sewage sludge is set to increase. According to European legislation, the management of sludge, as well as other waste, must follow a hierarchical approach according to which the first place in order of priority is represented by the prevention/minimization of the production. Over the last few years, thermophilic aerobic processes proved to be effective in minimizing the production of sludge within the wastewater treatment plants (WWTPs). Thermophilic aerobic/anoxic membrane reactor (TAMR) technology combines the advantages of thermophilic aerobic treatments with those of biological membrane processes. This work reviews the literature concerning the application of TAMR focusing on the prevention of the production of biological sludge and on the improvement of its quality for the purpose of a possible recovery in agriculture in a circular economy perspective. The results show that the process is mature and effective for full-scale application in conventional WWTPs.

Keywords Thermophilic Membrane Reactor; Sludge minimization; Aqueous waste; Agricultural reuse; Circular Economy

1. Introduction

A growing production of biological sewage sludge (BSS) and a simultaneous worsening of the qualitative characteristics are the consequences of the imposition of more restrictive limits, as European Directive 91/271/EEC and subsequent amendments (European Commission 1991, 1998)), on the effluents of wastewater treatment plants (WWTPs) (Mininni et al. 2015). In 2015, European urban WWTPs produced 9.7 million tons of dry matter of BSS (Collivignarelli et al. 2019b). Therefore, a sustainable management of sludge are nowadays desirable and above all mandatory objectives.

In fact, Directive 2018/851/EC (European Commission 2018) identified a hierarchy in waste management, therefore also applicable to BSS: (i) prevention and minimization of the production, (ii) matter recovery and reuse, (iii) energy recovery and finally (iv) safe disposal of waste. The prevention/minimization of the production of BSS at the source is an aspect of primary importance not only because the legislation requires it, but also because it can guarantee many non-negligible benefits including the reduction of costs incurred by WWTPs. As reported in literature by (Bertanza et al. 2014, 2015; Collivignarelli et al. 2015c; Zhao et al. 2019), the management of the sludge represents about 50% of the total operating costs at the WWTPs that produce it. In addition to the economic aspect, the environmental impact linked to the treatments, transport and final disposal of the sludge must also be considered.

According to the Italian Higher Institute for Environmental Protection and Research, in 2017, Italian urban WWTPs produced about 3.2 million tons of sludge (about 0.8 million tons of dry matter) (Ispra 2019; Mininni et al. 2019). 47.7% was sent for recovery and 50.6% for disposal, recording a 1.4% decrease in landfill disposal in favour of recovery compared to the previous year (Ispra 2019). The European Directive 86/278/EEC (European Commission 1986) aimed at encouraging the use of good quality sludge in agriculture by banning the use of untreated sludge on agricultural land to avoid any harmful effects caused by the presence of pathogens and organic contaminants (Zhang et al. 2014; Liu et al. 2018). The practice of reuse can be fully integrated into a circular economy vision (Kacprzak et al. 2017; Ashekuzzaman et al. 2019). Concerning this aspect, in 2020 the European Commission adopted a new Action Plan for the Circular Economy to promote the sustainable use and reuse of resources (European Commission 2020). In the urban water management system, one of the main actions needed to implement a circular economy approach is the transformation of WWTPs into water resource recovery plants (WRRFs) (Cornejo et al. 2019; Collivignarelli et al. 2019d). To do this, the prevention and minimization of the production of BSS represents the first step that can be pursued in two distinct ways: (i) adopting processes capable of treating the water with a minimum production of residual sludge; (ii) provide in situ treatments to minimize the quantities of sludge produced (Collivignarelli et al. 2019b). This work aims to provide an overview of the results obtained testing the thermophilic aerobic/anoxic membrane reactor (TAMR) technology which can guarantee both the approaches described above.

2. The technology

TAMR is an advanced biological process that simultaneously combines a pure oxygen membrane bioreactor (MBR) system and a thermophilic treatment in autothermal conditions). Therefore, according to literature (LaPara and Alleman 1999; Kurian et al. 2005; Ziemba and Peccia 2011; Lloret et al. 2013; Collivignarelli et al. 2017a), the application of the this combined process, in addition to having a low environmental impact as a biological technology, has the following advantages: (i) drastic reduction of the sludge produced, (ii) high removal rates of slowly biodegradable compounds in mesophilic conditions, (iii) excellent flexibility in case of organic overload, (iv) high compactness of the system, (v) inhibition of pathogens, and (vi) possibility of energy recovery. The process can be applied both in the water line and in the sludge line of a WWTPs. In case aqueous waste are fed, TAMR operated only in aerobic conditions while BSS also required an anoxic phase to effectively minimize the BSS production. Thermophilic conditions (47-53 °C) are maintained thanks to the exothermic degradation processes of the thermophilic microorganisms. To ensure the self-heating of the process, the feed must be rich in organic matter and therefore, the water line application should be in WWTPs authorized for the treatment of aqueous waste, as a simple urban sewage would not guarantee the self-heating of the thermophilic process (Figure 1). In the case of sludge line application, the TAMR can be used both to co-treat sewage sludge and aqueous waste and to treat only BSS from conventional active sludge (CAS) systems.

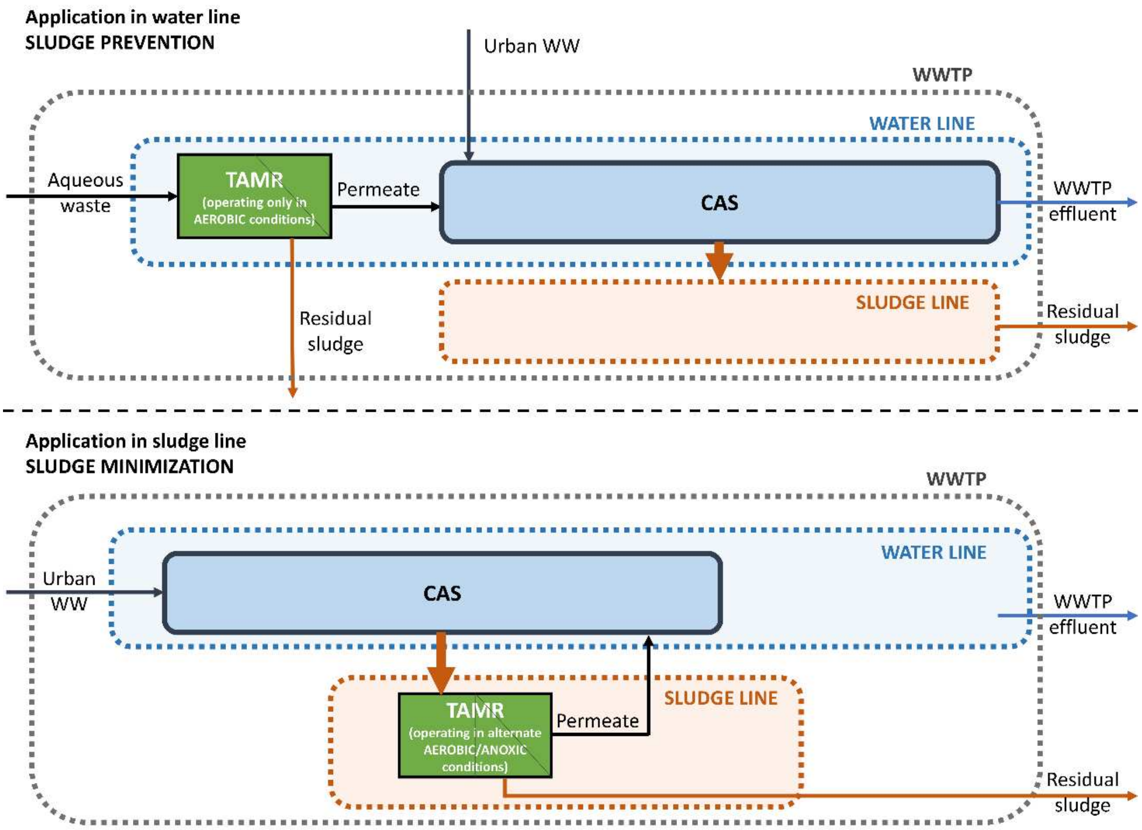

Figure 1: *Application of TAMR in water and sludge line. CAS: conventional active sludge. WW: wastewater. (Original from authors, not previously published)*

The TAMR produces (i) residual sludge (Section 3.1 and 3.2), and (ii) aqueous permeate (Section 3.3). In Lombardy (Italy), there are currently two full-scale TAMR plants for the treatment of aqueous waste (sludge prevention through water line intervention).

3. Sludge prevention/minimization

3.1 Residual sludge production

The residual thermophilic sludge represents the excess sludge of the thermophilic biological system and can have a percentage of dry matter up to 19% (Collivignarelli et al. 2015b, 2019c, 2021b). Its production is clearly lower in terms of mass and volume than that of the permeate. Table 1 shows the results of the specific production of thermophilic sludge obtained mainly during experiments at the semi-industrial scale of the TAMR technology both on diverse aqueous waste and on BSS. In the case of aqueous waste treatment, specific sludge production data monitored in full-scale plants are also available. These results are lower than those achievable with a mesophilic MBR (0.10-0.19 $kg_{VSS\ produced}\ kg_{COD\ removed}^{-1}$) (Lee et al. 2003; Kurian et al. 2005) and close to those reported in the literature for aerobic thermophilic processes (0.08 $kg_{VSS\ produced}\ kg_{COD\ removed}^{-1}$) (Kurian et al. 2005). Even the granular anaerobic processes have higher values than the TAMR technology: for example, the specific production of sludge in a UASB reactor that treats sewage sludge is equal to 0.1 $kg_{VSS\ produced}\ kg_{COD\ removed}^{-1}$ (Chang and Lin 2004).

Table 1. Specific production of sludge in TAMR technology. WW: wastewaters; R: real scale; S: semi-industrial scale; TP: total phosphorus; TN: total nitrogen.

Substrate	Scale	Specific sludge production ($kg_{VSS\ produced}\ kg_{COD\ removed}^{-1}$)	References
Aqueous waste			
pharmaceuticals and detergents production WW and landfill leachate	R	0.092-0.101	(Collivignarelli et al. 2015b)
saline WW, neutral/acid/basic WW, landfill leachate, solvent WW, and slurries	R	0.08-0.09	(Collivignarelli et al. 2019c)
WWs with highly recalcitrant pollutants (e.g., surfactants, solvents, pharmaceutical products)	R	0.052 (*)	(Collivignarelli et al. 2021b)
high strength WWs	S	0.09	(Collivignarelli et al. 2018)
high strength WW mainly containing dyes, surfactants, and solvents	S	0.04	(Collivignarelli et al. 2017a)
WW with high concentrations of COD, TP, TN, chloride, acetic acid, methylene chloride, ethanol	S	0.016	(Collivignarelli et al. 2014)
Aqueous waste and sewage sludge (*)	S	0.04	(Collivignarelli et al. 2019a)

(*) expressed in $kg_{VS\ produced}\ kg_{COD\ removed}^{-1}$

(**) mixture composed of 30% sewage sludge and of 70% of aqueous waste.

3.2 Sludge quality improvement

In general, the Italian legislation on the recovery of sludge in agriculture imposes some stricter limit values (such as on total chromium, lead, arsenic, agronomic parameters and some organic contaminants) compared to other legislations, including the French and German ones. In particular, in the current legislation in Lombardy (Italy) (Lombardy Region. 2019), a distinction is required between "suitable sludge" and "high quality sludge". Sludge suitable for spreading in agricultural fields must comply with the limit values set by current Italian legislation, while “high-quality” sludge requires more stringent limit values than national ones.

Regarding the thermophilic sludge residue, the only criticality could be represented by the insufficiency of organic carbon, which can be solved by mixing other BSS with the thermophilic sludge normally with high concentrations of COD (Collivignarelli et al. 2019a).

However, in an experiment involving the treatment of industrial wastewater with high concentrations of chlorides and perfluoroalkyl, although most of the COD introduced was oxidized in the TAMR, only a minor but still significant part (6-12%) remained in the thermophilic sludge (Collivignarelli et al. 2021a).

A high concentration of phosphorus in the crystalline phase has been identified in the thermophilic sludge. In the thermophilic reactor, the chemical precipitation of total phosphorus takes place in the form of salts, such as vivianite and hydroxyapatite (Collivignarelli et al. 2014, 2018). In agreement with the scientific literature (Liu et al. 2011), this results could be related to the increase in pH induced by the aeration of the reactor which allowed the crystallization of phosphorus (Collivignarelli et al. 2018).

A nitrogen accumulation mechanism in the reactor was also observed due to (i) the absorption of nitrogen by the biomass, (ii) adhesion to mud and (iii) precipitation of ammoniacal nitrogen in the form of struvite (Collivignarelli et al. 2014, 2021a).

As regards the presence of pathogenic microorganisms, thermophilic processes generally guarantee greater safety than mesophilic ones, thanks to higher process temperatures (Ziemba and Peccia 2011; Lloret et al. 2013). Therefore, thermophilic extracted sludge could be suitable for spreading in agriculture thanks to the high content of carbon, nitrogen, phosphorus and potassium, the excellent degree of humification and sanitation that guarantees a healthy and safe recovery of the sludge. Table 2 shows the main qualitative characteristics of the thermophilic sludge extracted from TAMR.

Table 2. Qualitative characteristics of mixed liquor. TN: total nitrogen; TP: total phosphorus.

Results on nutrients	**References**
Organic carbon accumulation (6-12% of COD fed)	(Collivignarelli et al. 2015c, 2019a, 2021a)
Nitrogen accumulation (8-10% of VSS; 8-24% of TN fed)	(Collivignarelli et al. 2014, 2015b, 2019a, 2021a)
Accumulation by chemical precipitation of phosphorus as inorganic salts (70-80% of TP fed)	(Collivignarelli et al. 2014, 2015a, 2018, 2021a)
Other results	
High concentration of total solids (up to 190 kg m^{-3} in the full-scale applications)	(Collivignarelli et al. 2015b, 2019c, 2021b)
Absence of foaming phenomena during the treatment of liquid waste (real laundry wastewater rich in TAS e MBAS)	(Collivignarelli et al. 2019e)
Sanitation thanks to high temperatures (> 45°C)	(Collivignarelli et al. 2014, 2015b)

4. Possibility of permeate reuse

Among the residues, the permeate is the most significant from a quantitative point of view. The ultrafiltration membranes allow to keep all the biomass inside the biological reactor, obtaining a permeate totally solids-free substrate (Collivignarelli et al. 2015c). In addition, it is rich in ammoniacal nitrogen thanks to excellent ammonification activity by the thermophilic bacteria in TAMR (Collivignarelli et al. 2017b, 2019a). Despite the excellent performance of TAMR process (COD removals up to 90% (Collivignarelli et al. 2014, 2015b, 2019a)), permeate contains significant concentrations of well biodegradable COD by a mesophilic biomass, confirming the complementarity between mesophilic and thermophilic processes for the biodegradation of organic substances (Collivignarelli et al. 2015b, a, 2017a, 2018).

Therefore, this substrate can first be subjected to a stripping treatment for the recovery of ammonia nitrogen in the form of ammonium sulphate and possibly, considering the good biodegradability of the organic substance by mesophilic biomass, recirculated in the denitrification reactor of a CAS to improve the kinetics of nitrate removal, in place of external sources of carbon of synthetic origin (Collivignarelli et al. 2014, 2017b, 2021a, b).

5. Tips for future research and applications

Considering the depletion of world natural reserves of phosphorus, it would be interesting to investigate the bioavailability of this nutrient in the sludge extracted from TAMR to evaluate the direct assimilation by crops in case of agricultural reuse.

Another aspect that would be interesting to investigate is the application of the technology on BSS resulting from the treatment of industrial wastewater and aqueous waste. In this case, the Authors suggest to evaluate the performance of TAMR to minimize BSS production considering feed with diverse characteristics and comparing the results with those obtained treating urban BSS. At the same time examine a possible toxic and chronic effect of these substrates on the thermophilic sludge represent an interesting point that should be further investigated.

The authors also suggest to study the up-grade of the process. For instance, introduce mobile support material in the reactor to developed attached biomass it could be an aspect to be explored. The traditional suspended biomass already present and the new adherent biomass developed on supports with a high specific surface would thus work simultaneously, guaranteeing a hybrid biomass process. The support materials introduced into the thermophilic reactor could also be recovered through recycling operations according to a circular economy perspective applied to integrated urban water cycle.

6. Conclusions

The TAMR technology ensures the prevention/minimization of the production of BSS and guarantees the recovery of the residues produced. The excess sludge extracted from the thermophilic biological reactor could be destined for recovery in agriculture thank to its content of nutrients (organic carbon, nitrogen, and phosphorus) and greater protection against the pathogenic load. At the same time, the permeate can be reused as an external carbon source in a post-denitrification process, after stripping to produce ammonium sulphate, thanks to the high content of ammonia nitrogen and well biodegradable organic carbon by mesophilic biomass. In this way, both residues obtained from the TAMR acquire an economic value as products, guaranteeing the important possibility of closing the cycle linked to the management of wastewater and BSS in a circular economy perspective.

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PUBLICATION 2

TOPIC: WS1 - Monitoring and performance study

- WS1.1 - Monitoring and performance of full-scale TAMRs for aqueous waste treatment

WS3: Residues and energy recovery

- WS3.1 - Reuse of residues

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Performance of Full-Scale Thermophilic Membrane Bioreactor and Assessment of the Effect of the Aqueous Residue on Mesophilic Biological Activity

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Abstract: To date, the management of high-strength wastewater represents a serious problem. This work aims to evaluate the performance on chemical pollutants and on sludge production of one of the two full-scale thermophilic membrane bioreactors (ThMBRs) currently operational in Italy, based on monitoring data of the last two and a half years. Removal yields on COD, N-NO_x, non-ionic and anionic surfactants (TAS and MBAS), increased with the input load up to 81.9%, 97.6%, 94.7%, and 98.4%, respectively. In the period of stability, a very low value of sludge production (0.052 kg_{VS} $kgCOD^{-1}$) was observed. Oxygen uptake rate (OUR) tests allowed us to exclude the possibility that mesophilic biomass generally exhibited any acute inhibition following contact with the aqueous residues (ARs), except for substrates that presented high concentrations of perfluoro alkyl substances (PFAS), cyanides and chlorides. In one case, nitrifying activity was partially inhibited by high chlorides and PFAS concentration, while in another the substrate determined a positive effect, stimulating the phenomenon of nitrification. Nitrogen uptake rate (NUR) tests highlighted the feasibility of reusing the organic carbon contained in the substrate as a source in denitrification, obtaining a value comparable with that obtained using the reference solution with methanol. Therefore, respirometric tests proved to be a valid tool to assess the acute effect of AR of ThMBR on the activity of mesophilic biomass in the case of recirculation.

Keywords: thermophilic; wastewater; surfactants; denitrification; sludge production; biological activity; nitrogen uptake rate

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1. Introduction

To date, the management of high-strength wastewater (WW) represents a very serious problem [1]. Conventional wastewater treatment plants (WWTPs) do not present suitable treatments for treating WW with high organic content and possible recalcitrant pollutants such as color [2,3], surfactants [4,5], solvents [6,7], perfluoro alkyl substances (PFAS) [8–10] and heavy metals [11,12]. These WWs must be treated in special treatment lines built on site or located in authorized WWTPs.

Chemical–physical treatments (e.g., coagulation-flocculation) are the most commonly used because of the recalcitrance of these pollutants which tend to inhibit the activity of the biomass with which they come into contact [13–15]. However, these treatments have significant disadvantages. For instance, coagulation–flocculation required the use of chemical reagents and produced large quantities of chemical sludge in which some of these pollutants (e.g., heavy metals) are concentrated [16,17]. This aspect causes an increase in costs and is against the principle of the circular economy of minimizing the use of resources and optimizing the recovery of materials [18–20].

For some years, it has been known that it is possible to biologically treat high-strength WW by exploiting the advantages provided by operating at thermophilic temperatures [21–23]. Recalcitrant pollutants can pass unaltered by the conventional active sludge (CAS) systems because they are not biologically degradable at mesophilic temperatures, e.g., perfluorooctanesulfonic acid (PFOS) [24] but also cause the inhibition of the biomass present [13–15]. However, the high resistance of thermophilic systems combined with the excellent degradative capacity of the thermophilic biomass make these treatments an excellent alternative to traditional physicochemical processes [25,26]. For instance, Pugazhendhi et al. [27] proved that thermophilic biological treatment can reduce the level of tetracycline in manure to about 96% in 72 h. In our previous study on a pilot plant, thermophilic aerobic treatments removed 10–56% and 85–96% of non-ionic and anionic surfactants (TAS and MBAS), respectively, starting from a total surfactant concentration up to 222 mg L^{-1} and 753 mg L^{-1} [28]. Furthermore, in thermophilic biological systems, the excess sludge is generally less than an order of magnitude compared to that produced in CAS systems [29]. This is a strong advantage considering the importance of minimizing the production of sewage sludge at the source [30,31] and the increasingly stringent legislation on the disposal of sludge, especially in agriculture [32–34].

The main disadvantage of thermophilic biological systems is the poor sedimentability of the sludge compared to CAS [22]. One of the possible solutions is to couple ultrafiltration (UF) membranes to the biological treatment [21]. A full-scale thermophilic membrane bioreactor (ThMBR), operating in aerobic conditions, for the treatment of high-strength WW has been operational in Italy for several years, adopting this solution [35,36].

In Italy, a second ThMBR become operational 2018. Data from July 2018 to December 2020 were analyzed and removal yields on COD, N-NO_x, TAS, and MBAS in relation to the loading rate of pollutants were investigated. Sludge production has been calculated and compared with literature data of other mesophilic and thermophilic processes. Moreover, respirometric tests were used as tool to assess the effect on activity of mesophilic biomass of AR of ThMBR. Diverse WWs were separately treated in pilot ThMBR to selectively evaluate the influence of pollutants in ARs (such as cyanides, PFAS and chlorides) on oxygen uptake rate (OUR), ammonia uptake rate (AUR) and nitrate uptake rate (NUR) in the case of permeate recirculation in a mesophilic CAS system.

2. Materials and Methods

2.1. Thermophilic Membrane Bioreactor (ThMBR): Characteristics and Monitoring

The full-scale ThMBR is located in a WWTP authorized to treat both high-strength and urban WW in Lombardy (Italy). High-strength WW was pre-treated with physicochemical process to effectively remove heavy metals and increase the biological treatability, and then by ThMBR (Figure 1). The ThMBR is composed by: (i) a biological reactor where pure oxygen is injected to maintain the O_2 concentration equal to 1.5–3 mg L^{-1} (our previous results indicated a consumption equal to 1.1–1.2 kgO_2 $kgCOD_{removed}{}^{-1}$ [25]), and (ii) a recirculation line where a UF unit (six vessels with 99 ceramic membranes; cut-off: 300 kDa) operating with 3–5 bar of pressure is located. The total volume of the system is 1700 m^3. Temperature (48–50 °C) is maintained thanks to exothermic reactions produced by biomass during the degradation of the organic substance, and it is controlled by a heating/cooling system. To date, only one other ThMBR with similar characteristics is operational in Italy, as described in our previous study [35].

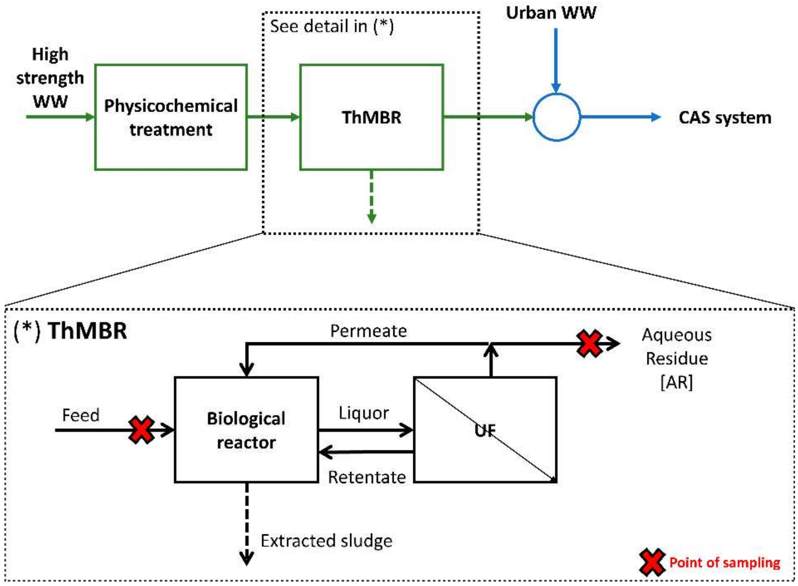


Figure 1. Localization and details of ThMBR in the pretreatment line for high-strength WW.

The AR of the process (permeate) is mixed with urban WW and treated by a CAS system. Therefore, to avoid an excessive overload of the CAS located downstream of the pre-treatment station, it is important that the ThMBR system operates efficiently and effectively in the removal of chemical pollutants. For this reason, monitoring is essential to understand and verify what the real-scale performance of the process actually is.

In Table 1, the main characteristics of high-strength WW treated in ThMBR are reported. The ThMBR was activated in January 2018 to treat industrial WW with highly recalcitrant organic substances (e.g., surfactants, solvents, pharmaceutical products). To ensure significant results, only data from July 2018 (after the system reached complete stability) to December 2020 have been analyzed. For chemical and physicochemical analysis, samples were taken weekly. In 2018–2020, about 62 ± 3 m^3 d^{-1} of high strength WW was fed to the ThMBR, with an average hydraulic retention time (HRT) of the system equal to 30 ± 1.5 d. COD reached 47,913 ± 2568 mg L^{-1} in 2018–2020 with the highest average value (53,220 ± 4911 mg L^{-1}) in 2019. High strength WW fed to the reactor also showed a high concentration of TAS, MBAS, N-NO_x and chlorides. The high conductivity (22,692 ± 1537 µS cm^{-1}) is effectively endured by the ThMBR due to strong resistance of thermophilic biological systems [37].

Table 1. Summary of flowrate, HRT, chemical and physicochemical parameters of high-strength WW fed to the ThMBR during the monitoring period. HRT: hydraulic retention time; TAS: non-ionic surfactants; MBAS: anionic surfactants; TP: total phosphorus. n: number of data points.

	2018 [n: 27]	**2019 [n: 52]**	**2020 [n: 52]**	**2018–2020 [n: 131]**
Q [m^3 d^{-1}]	64.2 ± 7.0	52.4 ± 3.3	71.1 ± 6.2	62 ± 3
HRT [d]	28.5 ± 3.0	34.1 ± 2.0	26.6 ± 2.4	30 ± 1.5
COD [mg L^{-1}]	46,137 ± 3201	53,220 ± 4911	43,529 ± 3370	47,913 ± 2568
TAS [mg L^{-1}]	498 ± 125	326 ± 108	839 ± 90	565 ± 73
MBAS [mg L^{-1}]	198 ± 99	707 ± 228	649 ± 116	579 ± 109
N-NH_4^+ [mg L^{-1}]	420 ± 69	406 ± 89	210 ± 61	332 ± 48
N-NO_x [mg L^{-1}]	337 ± 129	624 ± 165	575 ± 224	545 ± 115
TP [mg L^{-1}]	198 ± 130	316 ± 84	161 ± 53	231 ± 49
Chlorides [mg L^{-1}]	3416 ± 636	4338 ± 668	3225 ± 653	3718 ± 403
Conductivity [µS cm^{-1}]	18,757 ± 2432	22,180 ± 2049	25,322 ± 2877	22,692 ± 1537

2.2. Respirometric Tests

2.2.1. Aqueous Residues

These tests aim to evaluate the effect on mesophilic biomass of the AR of the ThMBR, after the treatment of diverse matrices, not only in this specific situation but also in the case of a future application of the process in other WWTPs.

In this case, usually, high-strength WW products fed to the real ThMBR were previously mixed and homogenized making it impossible to separate the effect of the different polluting matrices. Therefore, a continuous flow pilot plant (1 m^3) with the same characteristics of the full-scale scale ThMBR was used to treat four diverse high-strength WW products. The management conditions were kept similar to those used in the real scale (HRT = 25–30 d; O_2= 1–3 mg L^{-1}; T= 48–50 °C) and the AR extracted from the recirculation line was studied by respirometric tests. Chemical and physicochemical characteristics of the ARs are reported in Table 2. In the case of WW1 and WW2, diverse ARs, due to diverse phases of experimentation and diverse fed concentrations, were considered (ARX-a,b,c). AR3-a represents the residue after WW3 was preatreated in UASB and then in ThMBR. AR3-b refers to WW3 directly treated in ThMBR.

2.2.2. Practical Procedure

As reported by several authors [38–41], respirometric tests (OUR, AUR, and NUR) can be used to evaluate the effect of a substrate on the heterotrophic and autotrophic mesophilic biomass. In our study, respirometric analyses were used to assess the effect of AR of ThMBR on mesophilic biological activity of the CAS system. All tests were conducted at 20 $\pm$ 1 °C.

Biomass used in OUR and AUR analysis was withdrawn directly from the oxidation/nitrification reactor of the same WWTP. In order to later express oxygen uptake as a function of COD, diverse organic substrate solutions were prepared by diluting AR with distilled water in various ratios [40]. In exogenous OUR tests, 500 mL of biomass was aerated for 30 min (up to O_2 concentration of 6.5–7.5 mg L^{-1}) and then mixed with 500 mL of an organic substrate solution. Then, the aeration was stopped, and the system with the 1L mixture was hermetically closed to avoid the dispersion of the oxygen into the atmosphere. Continuous stirring was maintained (300–400 RPM) and nitrification phenomena were inhibited by addition of allylthiourea. pH was maintained at 7.0–7.5 adding H_2SO_4 drop by drop as necessary. The specific oxygen uptake rate (SOUR) was evaluated considering the VSS concentration in the batch reactor and the slope of the curve of oxygen consumption [38]. To evaluate the respiration of the biomass, no substrate was added in endogenous OUR. The SOUR was calculated according to the following equation:

$$\text{SOUR } (\text{mgO}_2\ \text{g}_{\text{VSS}}{}^{-1}\ \text{h}^{-1}) = \text{SOUR}_{\text{exogenous}} - \text{SOUR}_{\text{endogenous}} \tag{1}$$

To better highlight the behavior trend of the SOUR as a function of the COD and evaluate the acute effect of the substrate on the mesophilic biomass, the results were linear fitted.

In AUR, 500 mL of biomass was aerated for 30 min, mixed with 500 mL of AR and enriched of ammonia using $(NH_4)_2SO_4$ to obtain an initial N-NH_4^+ concentration in the 1L blend of 50–60 mg L^{-1}. Continuous stirring (300–400 RPM) and aeration (O_2 concentration of 6.5–7.5 mg L^{-1}) were maintained in the mixture. pH was maintained at 7.0–7.5 adding H_2SO_4 adding H_2SO_4 drop by drop as necessary. Tests were conducted for 6 h and 20 mL was sampled every hour. AUR was evaluated considering the VSS concentration in the batch reactor and the slope of the curve of N-NO_2^- and N-NO_3^- production, according to Holm et al. [38]. The WW that usually enters the oxidation/nitrification reactor of the same WWTP, properly enriched by ammonia as previously described, was used as reference in AUR tests.

Table 2. Summary of chemical and physicochemical characteristics of AR extracted from the pilot-scale ThMBR.

	Source 1			Source 2			Source 3		Source 4
Problematic Aspects of the Untreated High-Strength WW									
	Cyanides and Chlorides			PFAS and Chlorides			COD		COD and High Content of TS
Characteristics of aqueous residue after treatment in ThMBR									
	AR1			AR2			AR3		AR4
	AR1-a	AR1-b	AR1-c	AR2-a	AR2-b	AR2-c	AR3-a *	AR3-b **	
COD [mg L^{-1}]	12,028 ± 2221 [n: 15]	16,408 ± 1502 [n: 4]	13,120 ± 6355 [n: 4]	4841 ± 568 [n: 18]	5992 ± 171 [n: 11]	5778 ± 421 [n: 24]	7151 ± 1148 [n: 3]	8105 ± 1394 [n: 16]	2350 ± 505 [n: 16]
N-NH_4^+ [mg L^{-1}]	488.5 ± 96.0 [n: 14]	196.1 ± 47.2 [n: 4]	207.6 ± 45.6 [n: 4]	773.6 ± 113.6 [n: 18]	928.8 ± 83 [n: 11]	1063.1 ± 82.3 [n: 24]	862.0 ± 190.2 [n: 4]	429.2 ± 125.2 [n: 16]	636.5 ± 65.0 [n: 17]
N-NO_x [mg L^{-1}]	9.5 ± 2.2 [n: 14]	10.1 ± 0.7 [n: 4]	6.3 ± 2.3 [n: 4]	8.2 ± 0.5 [n: 18]	13.3. ± 10.2 [n: 11]	7.3 ± 0.5 [n: 24]	7.2 ± 1.9 [n: 4]	9.5 ± 1.4 [n: 16]	4.1 ± 0.9 [n: 15]
TP [mg L^{-1}]	109.0 ± 34.1 [n: 15]	35.5 ± 8.4 [n: 4]	15.0 ± 1.9 [n: 4]	86.2 ± 11.0 [n: 18]	72.1 ± 11.7 [n: 11]	82.0 ± 10.3 [n: 24]	271.5 ± 38.8 [n: 4]	98.1 ± 8.9 [n: 16]	9.87 ± 2.71 [n: 15]
Chlorides [mg L^{-1}]	3163 ± 458 [n: 12]	2475 ± 2807 [n: 2]	5827 ± 2093 [n: 3]	2631.4 ± 695.0 [n: 11]	4677 ± 1970 [n: 4]	7320 ± 623.2 [n: 24]	n.a.	n.a.	79.7 ± 22.4 [n: 14]
Cyanides [mg L^{-1}]	341.3 ± 61.4 [n: 14]	536.0 ± 55.7 [n: 4]	398.8 ± 233.2 [n: 3]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFAS [mg L^{-1}]	n.d.	n.d.	n.d.	46.1 ± 25.0 [n: 3]	265.6 ± 105.8 [n: 3]	3893 ± 1686 [n: 4]	n.d.	n.d.	n.d.
pH [–]	8.2 ± 0.1 [n: 14]	8.1 ± 0.1 [n: 4]	8.1 ± 0.1 [n: 4]	8.1 ± 0.1 [n:18]	8.2 ± 0.1 [n: 11]	8.2 ± 0.1 [n: 10]	8.2 ± 0.2 [n: 4]	8.5 ± 0.2 [n: 3]	7.2 ± 0.2 [n: 16]
Conductivity [μS cm^{-1}]	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	7736 ± 642 [n: 17]

n.a.: not available; n.d.: not detected; TS: total solids. a-b-c in WW1 and WW2 denote diverse ARs due to diverse phases of experimentation and diverse fed concentrations. *: WW3 was pre-treated in UASB and then in ThMBR. **: WW3 was treated directly in ThMBR.

NUR tests aim to evaluate the feasibility of using the permeate as an external source of organic carbon for denitrifying bacteria. Biomass was withdrawn directly from a full-scale denitrification reactor of the same WWTP. A volume of 500 mL of biomass was aerated for 30 min and mixed with 500 mL of an organic substrate solution. To be able to later express NUR as a function of COD, diverse 500 mL organic substrate solutions were prepared by diluting AR with distilled water in various ratios. The 1L blend was enriched with nitrates using KNO_3 to obtain an initial N-NO_x concentration of 40–50 mg L^{-1}. Continuous stirring (300–400 RPM) was maintained and the system was hermetically closed to avoid the solubilization of the atmospheric oxygen. pH was maintained at 7.0–7.5 adding H_2SO_4 drop by drop as necessary. Tests were conducted for 6 h and 20 mL was sampled every hour and filtered. NUR was evaluated considering the VSS concentration in the batch reactor and the slope of the curve of N-NO_2^- and N-NO_3^- consumption. Methanol, properly diluted with distilled water, was used as reference organic substrate solution in NUR tests.

2.3. Analytical Procedures

COD was measured according to ISPRA 5135 method [42]. Total solids (TS), volatile solids (VS) and volatile suspended solids (VSS) were determined following APAT-IRSA-CNR 2090 method [43]. N-NH_4^+ and N-NO_2^- were studied following APAT-IRSA-CNR 4030 [44] and APAT-IRSA-CNR 4050 [45] methods, respectively. N-NO_3^- concentrations were studied according to EPA 300.1 1997 [46]. N-NO_X was calculated as the sum of N-NO_3^- and N-NO_2^-. Cyanides were measured following APAT IRSA CNR 4070 method [47]. Total phosphorus (TP) was analyzed according to EPA 3051A 2007 [48] and EPA 6010D 2018 [49]. PFAS were determined according to EPA 3550C 2007 [50] and EPA 537 2009 [51] methods. MBAS were analyzed using ISO 7875-1 1996 [52] and ISO 7875-2 1996 [53]. TAS were measured following UNI 10511-1 1996 method [54].

3. Results and Discussion

3.1. Performance on Chemical Pollutants and Sludge Production

The performance of the ThMBR on the removal of COD, TAS and MBAS was evaluated. The main aspect that can be highlighted is that the removal of chemical pollutants strongly depended on the pollutant load rate. In the monitored period, the food:microorganism (F/M) ratio was 0.030 ± 0.003 g_{COD} g_{VS}^{-1} d^{-1}. This low ratio is related to the high recalcitrance of the organic substance (e.g., surfactants, solvents, pharmaceutical products) present in the high-strength WW fed to ThMBR, which, on the other hand, would not be treatable with a conventional mesophilic process.

With an organic loading rate (OLR) between 1.5 and 2 kg m^{-3} d^{-1}, the COD removal efficiency was on average 78.2%. However, this efficiency increased up to 81.9% in the presence of an OLR greater than 3 kg m^{-3} d^{-1} (Figure 2a). These results confirm our previous findings: almost 77% of COD fed was effectively removed by the other real-scale ThMBR plant operating in Italy [35]. Moreover, Simstich et al. [21] reported 83% of COD removal when treating industrial wastewater by a thermophilic MBR operating in aerobic conditions.

N-NO_x was significantly removed according to the increase in loading rate. In the case of a load rate higher than 45 g m^{-3} d^{-1}, an average of 97.6% of N-NO_3 and N-NO_2 were denitrified (Figure 2b). At thermophilic temperatures (>45 °C), denitrification takes place by a microbial biomass comparable or even superior to the mesophilic one in terms of diversity and resistance [55]. Although pure oxygen is dosed in the ThMBR, the denitrification phenomenon can be attributed to the concomitant presence of (i) a high quantity of organic substance given the very high COD of the fed matrices and (ii) some areas of the reactor that remain without adequate oxygenation. These results highlight that a study of the hydrodynamic behavior of the reactor by a residence time distribution (RTD) analysis and/or a computational fluid dynamic (CFD) analysis, as already done in conventional WWTP [56,57], is greatly required. The presence of the same denitrification phenomena was also observed in the other full-scale ThMBR in our previous study [35].

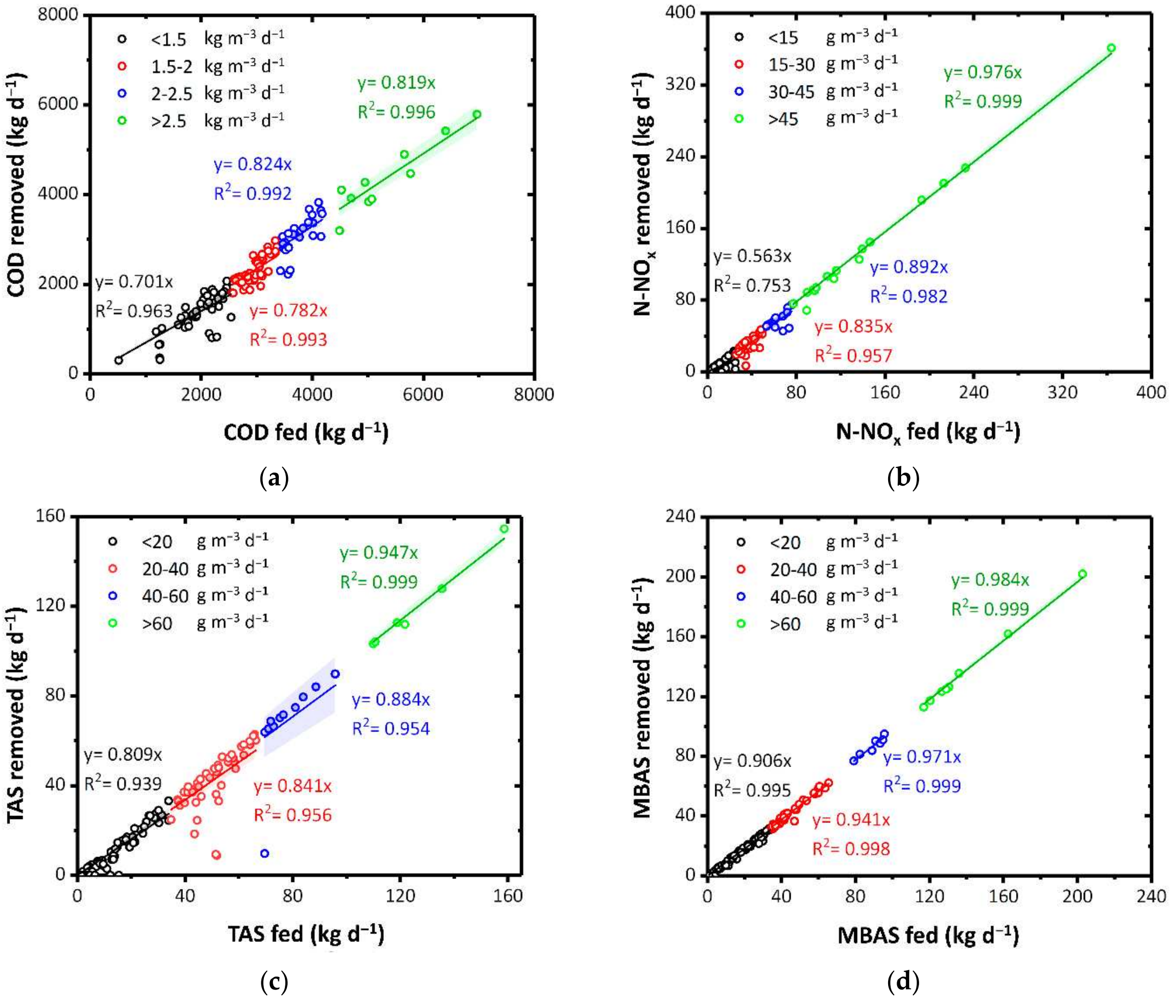
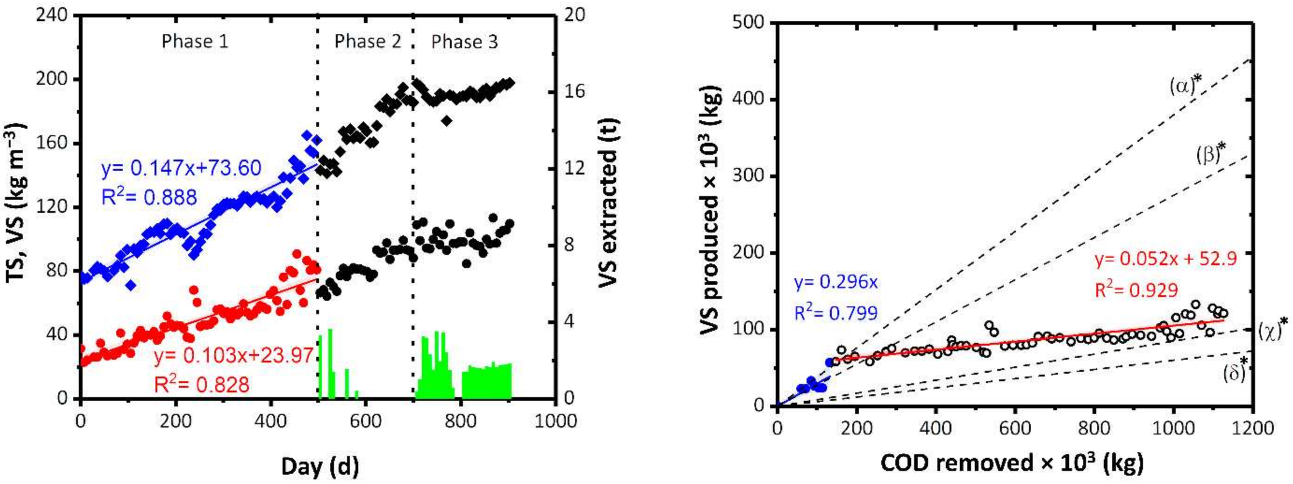


Figure 2. (**a**) COD, (**b**) N-NO_x (**c**) TAS, and (**d**) MBAS removal as a function of pollutants fed. Colors highlight diverse load rates. The colored bands represent the 95% confidence interval of the linear fitting.

A similar result to COD and N-NO_x was also observed for TAS (Figure 2c) and MBAS (Figure 2d), with maximum removal (94.7% and 98.4%, respectively) corresponding with a fed load rate greater than 60 g m^{-3} d^{-1}. In thermophilic membrane biological processes, the direct proportionality between the COD removal yields and the OLR is in accordance with what was reported in our previous studies on pilot plants [28]. However, in this work the same relationship applied to full scale plants has been shown and not only for COD but also for N-NO_x, and surfactants (TAS and MBAS). Precisely, the TAS that could be usually difficult to degrade by biological systems in mesophilic conditions [25,58,59] were instead effectively removed by ThMBR (>84.1%) with fed load rate higher than 20 g m^{-3} d^{-1}. This aspect should not be attributed to the presence of a UF membrane. In fact, due to their molecular weights (from 200 to 400 Da), surfactants can pass through the pores [60]. Instead, these results can be related to the greater ability of thermophilic microorganisms to degrade recalcitrant chemical pollutants such as TAS [25]. MBAS were almost totally removed (94.1–98.4%) by ThMBR. Treating real laundry WW in the other full-scale ThMBR, lower removal yields for TAS and MBAS were obtained (47.8% and 49.5%, respectively) probably due to lower initial surfactant concentrations [25].

Furthermore, even in the presence of significant incoming TAS load rate (up to 100 g m^{-3} d^{-1}), no inhibition of the biomass degradation activity was highlighted, unlike what has generally been observed in mesophilic systems in the presence of surfactants even in low concentrations (30 mg L^{-1}) [13]. This result can be related to the greater resilience of thermophilic biomass towards potentially inhibiting substances for mesophilic biomass present in CAS systems [28].

The concentration of TS and VS in the biological reactor was monitored. In Figure 3a, the concentrations of solids in the tank and the sludge extractions are reported. Three different periods can be identified. In period 1 (duration: 500 days), no extractions were carried out and the concentration of TS and VS in the reactor reached 160 kg m^{-3} and 80 kg m^{-3}, respectively. In period 2, occasional extractions were carried out and the increase in the concentration of TS and VS up to 185 kg m^{-3} and 90 kg m^{-3}, respectively, was observed. For management reasons, in period 3, the concentration of solids in the tank was kept almost constant thanks to periodic extraction of sludge (about 20 t per week).



(**a**) (**b**)

Figure 3. (**a**) Concentration of TS and VS in the biological reactor during the monitoring period and amount of VS extracted. ⧫ indicates TS, • represents VS and green bars indicate VS extracted. (**b**) VS produced by the process as a function of COD removed and comparison with theoretical value of (α) CAS [61,62], (β) mesophilic MBR [63–65], (γ) thermophilic MBR [29], and (δ) value of the other full-scale ThMBR [35]. The specific productions of VS used as comparison were the mean values of those reported in the literature. Blue • represents VS in the start-up phase while ○ represents VS in the subsequent phase. The colored bands represent the 95% confidence interval of the linear fitting. *: values are expressed in $kg_{VSS}\ kg_{COD}{}^{-1}$.

To determine the specific production of VS in relation to the COD removed, period 1 was chosen as the reference as the concentration of sludge accumulated in the tank was not influenced by the extractions (as in periods 2 and 3). The daily production of TS and VS was 0.147 $kg_{TS}\ d^{-1}$ and 0.103 $kg_{VS}\ d^{-1}$, respectively (Figure 3a). Comparing COD removed with the production of VS, the specific sludge production can be approximated with two linear functions. A specific production of 0.296 $kg_{VS}\ kgCOD^{-1}$ has been observed that corresponds with very low COD removals (substantially in the first period after the start-up phase) while subsequently, the production of VS dropped to 0.052 $kg_{VS}\ kgCOD^{-1}$ (Figure 3b).

In the literature, specific sludge production is generally expressed considering the VSS produced. Therefore, the comparison VSS-VS is then made by overestimating the specific production obtained in the present study. The value obtained in this study was lower by one order of magnitude than 0.25–0.51 $kg_{VSS}\ kgCOD^{-1}$ produced by CAS systems [61,62], and 0.19–0.36 $kg_{VSS}\ kgCOD^{-1}$ [63–65] produced by mesophilic membrane biological reactors (MBRs). The present result was comparable with the value of sludge production by thermophilic MBRs reported in the literature (0.02–0.1 $kg_{VSS}\ kgCOD^{-1}$) [29] and with of the other real ThMBR reported in our previous study (0.08–0.09 $kg_{VSS}\ kgCOD^{-1}$) [35].

3.2. *Influence of Aqueous Residues on Mesophilic Biological Activity*

3.2.1. Effect on Decomposing and Nitrifying Biomass

The effect of aqueous residues of ThMBR on mesophilic biomass was first evaluated by means of respirometric tests. The results show a different behavior depending on the initial matrix treated by the ThMBR and therefore on the characteristics of the AR tested. The AR1-a and AR1-b substrates, although rich in cyanides and chlorides, showed a substantial good degradability by the mesophilic biomass, not showing evident acute inhibitory effects of biological activity (Figure 4a). Instead, the AR1-c resulted in minimal toxic-inhibiting activity on biomass. This can be evidenced by the average increase in SOUR with decreasing COD, following dilution of the substrate. This result can be attributed to the fact that this matrix had a similar concentration of cyanides but about double the concentration of chlorides compared to the other AR1s tested. In our study, the initial ratios of SOUR with non-diluted AR were 0.45–1.2 $gCl^- \, g_{VSS}{}^{-1}$ for AR1-a, 0.05–1.75 $gCl^- \, g_{VSS}{}^{-1}$ for AR1-b and 0.6–2.5 $gCl^- \, g_{VSS}{}^{-1}$ for AR1-c. The presence of salinity in WW is a factor that strongly influences the respiration of mesophilic biomass, especially when the biomass is not acclimatized. For example, Pernetti and Palma [14] found that in batch mode, salt/VSS ratios between 0.37 $g_{salt} \, g_{VSS}{}^{-1}$ and 30.7 $g_{salt} \, g_{VSS}{}^{-1}$ produced respiration inhibition between 4% and 84%, respectively. On the contrary, Bassin et al. found that Cl^- had a positive effect on the settling properties as antagonist filamentous bacteria were inhibited by high salt concentrations, but only when the increase in Cl^- concentration was gradual [66].

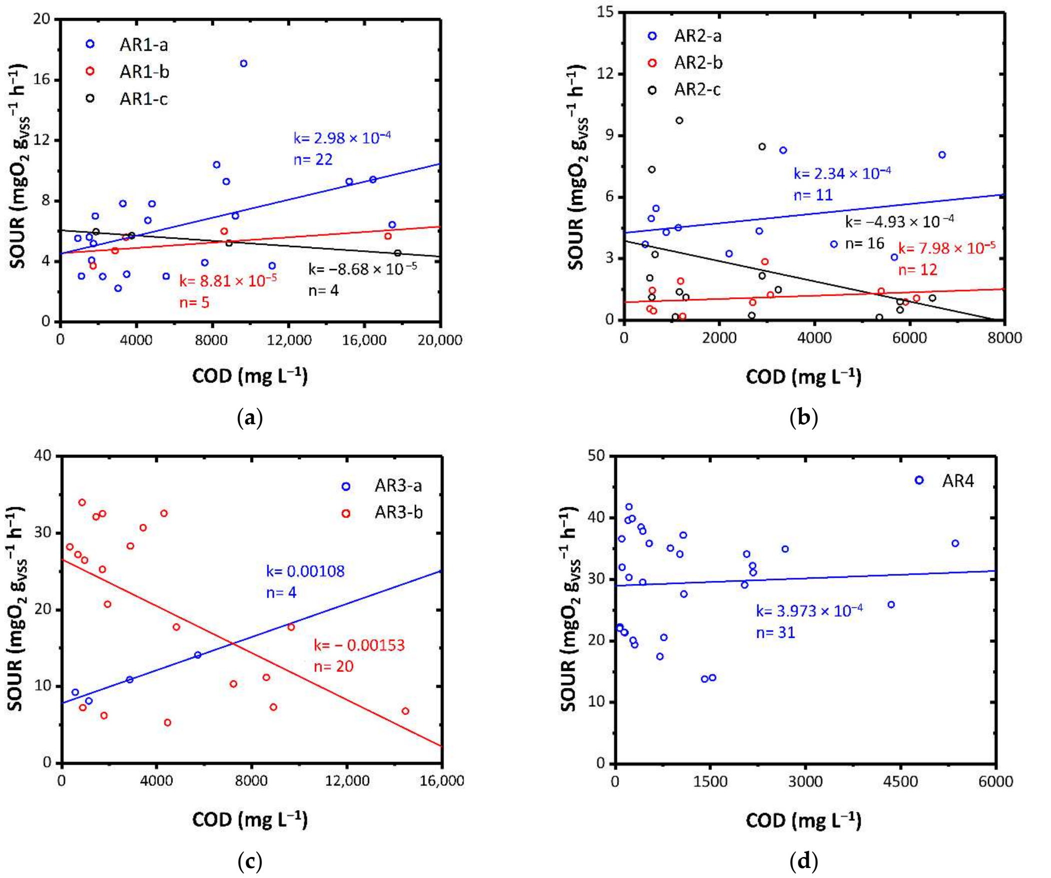


Figure 4. SOUR as a function of COD for: (**a**) AR1-a, AR1-b and AR1-c; (**b**) AR2-a, AR2-b and AR2-c; (**c**) AR3-a, and AR3-b; (**d**) AR4. Colors highlight diverse load rates. k: angular coefficient of the linear fitting expressed as $mgO_2 \, L \, g_{VSS}{}^{-1} \, h^{-1} \, mgCOD^{-1}$; n: number of data points.

AR2 had a significant concentration of chlorides and PFAS. AR2-a and AR2-b, however, did not cause acute inhibition phenomena (Figure 4b). Instead, AR2-c produced a toxic-inhibiting effect on biomass, even more evident than that obtained with the AR1-c substrate.

This result could be attributed to two aspects: (i) the high concentration of chlorides (1.1–2.6 gCl^{-} g_{VSS}^{-1} in SOUR with non-diluted AR2-c); (ii) the very high concentration of PFAS (0.4–1.9 gPFAS g_{VSS}^{-1} in SOUR with non-diluted AR2-c). While some types of PFAS (e.g., PFOS) would seem not to have a toxic effect on the mesophilic biomass of CAS [67], exposure to high concentrations of other PFAS (e.g., Perfluorooctanoic acid-PFOA) has shown negative impacts on microbial growth and organic substance removal performance [15].

AR3-a and AR3-b exhibited a diverse effect (Figure 4c). While AR3-a showed excellent biodegradability in the mesophilic conditions, AR3-b showed an acute inhibitory toxic effect. This result could be attributed to the fact that in the case of the AR3-a, the WW3 had been pre-treated through an anaerobic biological process and then fed to the ThMBR while in the case of the AR3-b, it was directly fed to the ThMBR. The first case may have favored the removal of some interfering substances which instead remained in the AR3-c. This aspect will be the subject of future studies.

AR4 showed a good biodegradability in the mesophilic field highlighted by the average decrease in SOUR with decreasing COD, following dilution of the substrate (Figure 4d). The good biodegradability was also favored by the low concentration of chlorides within the initial matrix (0.01–0.03 gCl^{-} g_{VSS}^{-1} in SOUR with non-diluted AR4).

The impact of AR2-c and AR4 on nitrification activity was also investigated. The WW entering the oxidation/nitrification reactor, properly enriched with $N\text{-}NH_4^{+}$, was used as the reference substrate. AR2-c showed a slight acute inhibition (1.35 ± 0.84 mgN g_{VSS}^{-1} h^{-1}) compared to the reference solution (1.81 ± 1.15 mgN g_{VSS}^{-1} h^{-1}) (Figure 5). This could be traced back to the high concentration of chlorides and PFAS which also had a minimal inhibitory effect in the decomposing microorganisms. Recent studies have shown that PFOS and PFOA can reduce nitrifying activity [68]. An absence of inhibitory phenomena and an increase in nitrification kinetics up to 4.2 ± 0.1 mgN g_{VSS}^{-1} h^{-1} was observed with AR4. Therefore, in this case the substrate had a positive effect of stimulating the mesophilic biomass and the phenomenon of nitrification.

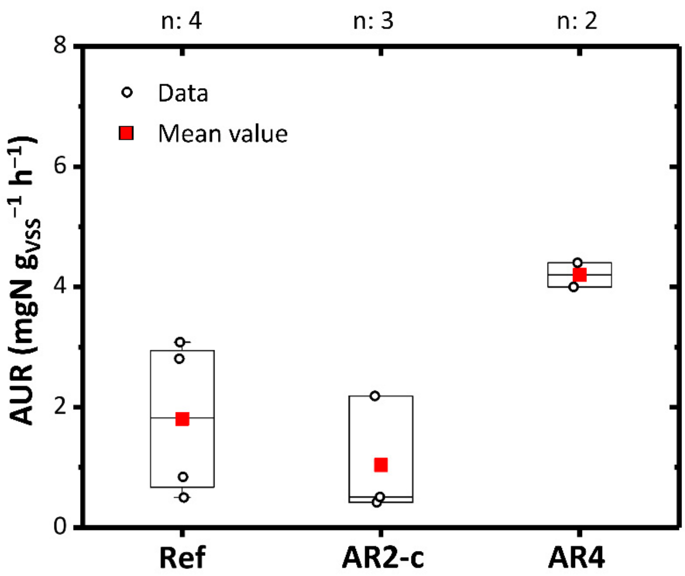


Figure 5. AUR with AR2-c and AR4. Ref: Reference value. Boxplots represent the distance between the first and third quartiles while whiskers are set as the most extreme (lower and upper) data point not exceeding 1.5 times the quartile range from the median. n: number of data points.

3.2.2. Impact on Denitrifying Biomass

The effect of the AR on denitrifying microorganisms was tested only for the AR4. The goal was to identify any acute toxic effect of the substrate against the denitrifying heterotrophic biomass and determine the feasibility of reuse the organic substance, contained within the aqueous residue, as an external source of carbon in denitrification.

As the dilution increased, the NUR increased until it reached the value of 3.5 mgN g_{VSS}^{-1} h^{-1} that corresponded to the COD/N-NO_x ratio equal to 15–17.5 (Figure 6). This can be attributed to a slight acute inhibitory effect on the denitrifying biomass of the undiluted aqueous residue. However, the further increase in dilution reduced the NUR (2–2.5 mgN g_{VSS}^{-1} h^{-1} with 200 mg L^{-1} of COD), due to the significant lowering of the COD/N-NO_x ratio. The maximum NUR is comparable to that obtained with the reference solution 4.5 ± 1 mgN g_{VSS}^{-1} h^{-1} (source of carbon: methanol) and to that reported in the literature for other industrial WW. For instance, Liwarska-Bizukojć et al. [69] reported around 2.0–6.0 mgN g_{VSS}^{-1} h^{-1} as the denitrification rate using industrial WW and around 3.2 mgN g_{VSS}^{-1} h^{-1} using methanol as an external carbon source.

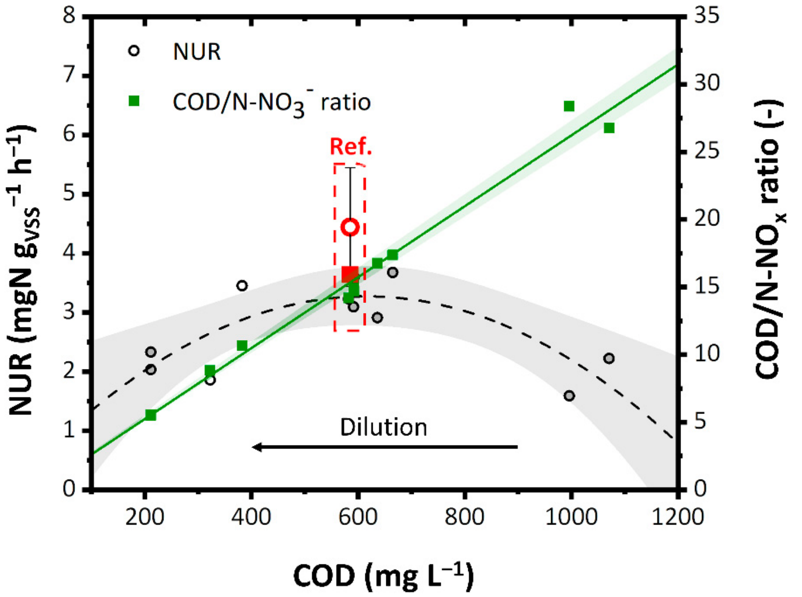


Figure 6. NUR as a function of COD and COD/N-NO_x ratio. Ref: Reference value. The colored bands represent the 95% confidence interval of the linear fitting.

4. Conclusions

Full-scale ThMBR proved to be very effective in pollutants removal, demonstrating an increase in performance as the input load increases. COD, N-NO_x, TAS, and MBAS removal yields were up to 81.9%, 97.6%, 94.7%, and 98.4%, respectively. The denitrification phenomenon can be attributed to some areas of the reactor that remain without adequate oxygenation and the presence of a high quantity of organic substance given the very high COD of the fed matrices. Very low daily sludge production has also been evaluated (TS and VS were 0.147 kg_{TS} d^{-1} and 0.103 kg_{VS} d^{-1}, respectively). Comparing COD removed and VS production, a specific production of 0.296 kg_{VS} $kgCOD^{-1}$ has been observed, especially corresponding to the first period after the start-up phase, while subsequently, the production of VS drops down to 0.052 kg_{VS} $kgCOD^{-1}$.

OUR tests allowed us to exclude the possibility that mesophilic biomass generally exhibited any acute inhibition following contact with the tested substrates except for AR1-c, AR2-c and AR3-b. In these cases, due to the presence of high concentrations of cyanides and chlorides (AR1-c), PFAS and chlorides (AR2-c) and probably other interfering substances (AR3-b), an acute toxic-inhibiting effect was evidenced. The AR2-c matrix itself showed a slight decrease in nitrifying activity (1.35 ± 0.84 mgN g_{VSS}^{-1} h^{-1}) compared to the reference solution (1.81 ± 1.15 mgN g_{VSS}^{-1} h^{-1}) probably due to high PFAS and chlorides concentration, while the substrate AR5 determined a positive effect, stimulating the mesophilic biomass and the phenomenon of nitrification. NUR tests on AR4 highlighted the feasibility of reusing the organic carbon contained in the substrate as a source in denitrification and obtaining a value comparable with that obtained when using the reference solution with methanol.

Although this work focuses only on the evaluation of the acute effect of the aqueous residue on the mesophilic biological activity, respirometric tests proved to be a valid tool to assess the impact of AR on the activity of mesophilic biomass in the case of recirculation. As a future perspective, aspects such as the OUR as a function of time with continuous feeding could be evaluated, to also consider the physiological acclimatization of the biomass to the organic substrate, and thus determine the medium-term effects of RA on mesophilic biomass.

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Nomenclature

AR: Aqueous residue; AUR: Ammonia uptake rate; CAS: Conventional active sludge; CFD: Computational fluid dynamic; HRT: Hydraulic retention time; MBAS: anionic surfactants; MBR: Membrane biological reactor; NUR: Nitrate uptake rate; OLR: Organic loading rate; OUR: Oxygen uptake rate; PFAS: perfluoro alkyl substances; PFOA: Perfluorooctanoic acid; PFOS: perfluorooctanesulfonic acid; RTD: Residence time distribution; SOUR: Specific oxygen uptake rate; TAS: Non-ionic surfactants; ThMBR: Thermophilic membrane bioreactor; TP: Total phosphorus; TS: Total solids; UF: Ultrafiltration; VS: Volatile solids; VSS: Volatile suspended solids; WW: Wastewater; WWTP: Wastewater treatment plant.

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PUBLICATION 3

TOPIC: WS1 - Monitoring and performance study

- WS1.1 - Monitoring and performance of full-scale TAMRs for aqueous waste treatment

WS2: Process optimization

- WS2.1 - Statistical analysis of monitoring data

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Article

Numerical Analysis of a Full-Scale Thermophilic Biological System and Investigation of Nitrate and Ammonia Fates

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Abstract: Thermophilic biological processes proved to be effective in aqueous waste (AW) and high-strength wastewater treatment. In this work, the monitoring of a full-scale aerobic thermophilic biological plant treating various high-strength AW in continuous mode is reported. This paper aims to: (i) provide models to help the AW utility manager in predicting the load of fed pollutants and performances, and (ii) fully investigate nitrogen transformations in biological reactor. Based on the results, the thermophilic sludge in the studied plant was able to degrade Chemical Oxygen Demand (COD) and remove nitrate nitrogen with very high efficiency (79.3% and 97.1, respectively). The monitoring was conducted following a statistical approach and searched for the possible correlations between the input parameters and the efficiency of removal of the plant. Moreover, a multivariate linear regression was carried out highlighting that the yield value of the removal of COD and nitrogen forms, apart from ammonia, was well explained (R^2 = 0.9) by the linear regression against the other monitored parameters. As far as nitrification is concerned, there was, on the one hand, an increase in ammonium ions due to the hydrolysis of the organic substance that occurs in the reactor, and on the other hand, a stripping of the same ammoniacal nitrogen in the form of NH_3. While nitrates were effectively removed, according to fluorescent in situ hybridization tests, sludge proved to be formed by minute flocs, where bacteria responsible for the oxidation of ammonium and nitrite seem to be unable to grow.

Keywords: thermophilic biological reactor; aqueous waste; high-strength wastewater; thermophilic biota; respirometric tests; biological sludge; management tool

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1. Introduction

The disposal of aqueous waste (AW) represents a critical problem due to the high variability of their physico-chemical characteristics [1]. Biological processes in conventional wastewater treatment plants (WWTPs) are not able to remove certain types of pollutants and in some cases, the plant's biota could also be inhibited [2,3]. Therefore, proper pre-treatments are needed.

To date, several additional treatments are available for increasing the acceptance of conventional biological processes, reducing acute or chronic toxicity effects due to the presence of high refractory organic compounds [4,5].

Despite their large use, physico-chemical treatments present several drawbacks, such as high operating costs related to chemical agents and the need for highly specialized personnel in their management [6]. On the contrary, biological processes have greater management simplicity, lower costs, and a lower probability of the formation of dangerous treatment by-products [7].

Among the biological treatments, the scientific community agrees that thermophilic treatments can perform high yields of removal additionally in the case of recalcitrant pollutants [8,9]. While mesophilic bacteria are widely used in the treatment of urban wastewater and work in a temperature range of 15–30 °C, thermophilic biota are widely used for the treatment of AW and find optimal conditions at temperatures between 50–60 °C [10]. For instance, in previous works, a thermophilic biological process was combined with an ultrafiltration membrane system to overcome sludge settleability issues and maximize performance, guaranteeing purification yields of the organic load of 95% [11]. This technology also granted the ability to treat different types of pollutants in high-strength AW in terms of COD content and surfactants [12] being applied in two full-scale plants [13,14].

This process was recently tested for biological sludge minimization, providing excellent results in terms of Total Solids (TS) and Volatile Solids (VS) reduction, and granting minimization of biological sludge production in WWTPs up to 90% [15]. This reduction was assured by the degradative and reproductive kinetics of a thermophilic biomass. In fact, thermophilic bacteria have a lower tendency to reproduce than traditional mesophilic bacteria [16].

So far, the statistical-based characterization of AWTP has received relatively poor attention in the literature. Harrou et al. [17] applied a statistical approach to derive alerts conditions in the management of the plant. The same strategy has been presented in Aguado and Rosen [18], but the analysis has been carried on simulated data. Muszyński et al. [19] reported a statistical study to correlate the amount of VS and the environmental data (COD and BOD_5). O'Brien and Teather [20] developed a predictive model successfully applied to estimate the pollutant concentrations in a biological system (activated sludge). Therefore, our approach represents a clear novelty, since it increases the number of environmental variables if compared to previous studies of a plant of novel conception, based on a thermophilic biological reactor.

In this work, the monitoring of a full-scale aerobic thermophilic biological plant treating various high-strength aqueous waste (AW) in continuous mode is presented. The main novelty points of this work are:

- The application of multivariate statistical analysis to evaluate possible correlation between several pollutants fed and performances of the process to give a useful tool to aqueous waste treatment plant (AWTP) utility manager;
- The adoption of respirometric tests to confirm results obtained by monitoring data analysis and to fully understand ammonia fate in the reactor;
- A microbiological characterization of thermophilic biota presents in a full-scale thermophilic membrane biological reactor, focusing on nitrogen oxidation.

2. Materials and Methods

2.1. Characteristics of the Aqueous Waste Treatment Plant (AWTP)

Monitored AWTP was generally fed with a wide range of high-strength AW: saline, neutral, acid, or wastewater basic high strength wastewater, landfill leachate, solvents wastewater, and wastewater rich of pharmaceutical compounds. The water line is composed by both chemical-physical and biological treatments (Figure 1). This work focuses only on thermophilic system composed by a thermophilic biological reactor (TBR) followed by ultrafiltration (UF) membranes.

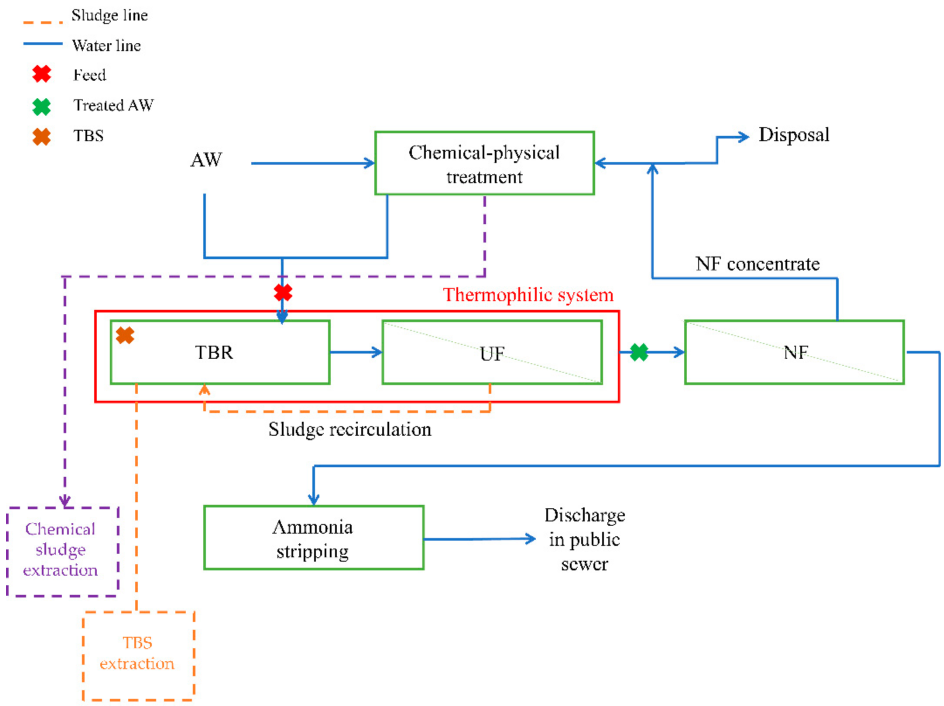


Figure 1. Scheme of the AWTP and sampling points (X). AW: Aqueous waste; NF: Nanofiltration; TBR: Thermophilic biological reactor; TBS: Thermophilic biological sludge; UF: Ultrafiltration.

As can be seen from the process scheme, aqueous waste has two treatment options based on its characteristics. The "light" waste is fed directly to the TBR, while the more resistant waste is pre-treated in two chemical-physical compartments in order to prevent a possible inhibition effect on thermophilic biomass (T = 48 °C). The TBR has a volume of 1000 m^3 and is characterized by an average inlet flow rate Qin = 170 $m^3 d^{-1}$, with a hydraulic retention time HRT of almost 6.0d. To obtain a more compact sludge structure, a dissolved oxygen concentration of 1.5–2 mg L^{-1} in the reactor is guaranteed by the injection of pure oxygen. The biological tank have a diverse oxygen concentration. This management strategy allows both the aerobic removal of the organic substance in zones with oxygen concentration (2 $mgO_2\ L^{-1}$), and the denitrification in anoxic zones (<1 $mgO_2\ L^{-1}$) [13,14]. As previously mentioned, the TBR is coupled with an ultrafiltration system due to the poor sedimentation of the thermophilic sludge. Therefore, the platform is equipped with two parallel UF units and a nanofiltration unit (NF). The UF unit consists of three vessels with 99 ceramic membranes (300 kDa cut-off) with an operating pressure between 3 and 5 bar. As shown in Figure 1, the retentate UF is recirculated in the biological tank. NF and ammonia stripping compensate the further treatments.

2.2. Monitoring Plan

Monitoring activity was focused on the thermophilic system (TBR + UF) from 2017 to 2021, sampling feed and permeate of ultrafiltration (Figure 1) three times a week for five years. Analyses of COD, nitrogen species (Ntot, N-NO_x, N-NH_4^+), total phosphorus (Ptot), total solids (TS), and volatile solids (VS) were performed according to standard methods [21]. The flow rates into and out of the plant, as well as the consumption of pure oxygen required for the organic substance degradation (C_{O2}), were all monitored daily. Finally, the extraction of sludge from the TBR was monitored, as this operation was necessary to avoid overloading of the thermophilic process. Based on the monitored value, the sludge load (Cf) and the sludge age (θ) were calculated according to Masotti [22].

Respirometric tests (ammonia uptake rate–AUR; nitrogen uptake rate-NUR) were carried out twice a week for a total period of 4 months. Over a period of two months, samples of activated sludge were collected weekly to be submitted for microbiological analysis by means of the fluorescent in situ hybridization technique (FISH), in order to investigate the bacterial populations colonizing the plant. Special attention was paid to the oxidation of ammonia nitrogen. The FISH technique was chosen due to its peculiar ability to ensure visualisation of the floc without altering its morphology. This ensured it was possible to highlight viable bacterial populations and, in turn, target micro-organisms (in this case, AOB, NOB and ANAMMOX).

2.3. Data Processing

To determine the removal efficiency of COD, nitrogen, and total phosphorus, mass balances were performed weekly based on fed and extracted loads.

All parameters were studied by a multiple linear regression analysis. Firstly, to obtain correct modeling, the data were normalized for the maximum value of the series to obtain a series of homogeneous values between 0 and 1. Correlations between measured parameters and performances have been computed with both Pearson's and Spearman's rank correlation [23].

The overall behavior of the thermophilic system has been described with a set of multivariate linear regressions, where each physicochemical parameter of the plant has been modeled as a linear combination of the others. A stepwise regression method has been applied by Matlab function *stepwisefit* [24] to select the variables that are more strongly jointly correlated with each of the plant parameters. Given the i-th parameter x_i, it is described with the model:

$$x_i = \alpha_i + \sum_{j \neq i} \beta_{ij} x_j \quad (1)$$

If m parameters are measured, the stepwise procedure allows selecting the $m_i \leq m - 1$ parameters that are more statistically significant to predict each x_i.

2.4. Respirometric Tests

To evaluate the activity of nitrifying and denitrifying biota AUR and NUR tests were performed. Tests were carried out according to Collivignarelli et al. [13].

In the case of NUR tests, the AW feed to the thermophilic system was used as an organic substrate. The thermophilic biomass sampled in the TBR was used to carry out the NUR and AUR tests. Two diverse biomass/substrate (B/S) ratios were tested: 1/1 and 1/7 to investigate the kinetics of NUR both under the standard test conditions (B/S ratio = 1/1) and by reproducing the real plant conditions (B/S ratio = 1/7).

2.5. Fluorescent In Situ Hybridization

After collection, samples were added with absolute ethanol (1:1) and stored at −20 °C until measurement. DNA probes (vermicon AG, Munchen, Germany) for the detection of viable bacteria, ammonia oxidizers (Nitri-VIT®), nitrite oxidizers (Nitri-VIT®), and ANAMMOX (VIT® Anammox) were applied. After hybridization, slides were observed under fluorescence microscopy (Axioscope, Zeiss, Milano, Italy) by using the FS09-Blau Anreg. FITC LP 1046-281 filter set (excitation BP 450-490 nm; beamsplitter FT 510; emission: LP 515 nm) and the F11-002 Grün Anreg. TRITC LP 1046-281 filterset (excitation BP 546/12 nm; beamsplitter FT 580; emission: LP 590 nm respectively).

3. Results and Discussion

3.1. Monitoring of the Biological Thermophilic System

As can be seen in Table 1, the loads of fed pollutants and performances of biological thermophilic process were reported. Biomass was able to treat high COD loads, with a concentration of two orders of magnitude higher, up to 50,000 mg L^{-1}, than conventional activated sludge (CAS) systems where the concentration of COD in input is almost 200–400 mg L^{-1} [17–20], with almost constant performance throughout 5 years of monitoring around 80%. As regards to nitrite and nitrate, removal of yields higher than 95% were granted for the entire monitoring period. Focusing on ammonia removal, a negative value was recorded. This result is confirmed in our previous study [11] where the enhancement of ammonia concentration during the thermophilic biological treatment was related to a strong ammonification phenomenon. If compared with the performance values of a CAS plant, the ability of the TAMR plant to obtain very high removal yields of nitrates is highly relevant [25]. The removal and production yields relating to nitrates and ammonia, respectively, are then reflected in the abatement yields of total nitrogen. In the five years of monitoring, the performances on total nitrogen were the most variable, going from a minimum value of 62% for 2019 to a maximum value of 76.7% recorded in 2021. Comparing the feed and the permeate, significant removal of phosphorus is pointed out at 82%. This result agrees with our previous study [13], in which the accumulation of phosphorus in the sludge in inorganic form was pointed out. The capacity of the TAMR plant, therefore, allows it to accumulate phosphorus inside the sludge making it rich in nutrients and, therefore, susceptible for possible recovery in agriculture.

Table 1. Loads of pollutants fed and performance of the thermophilic system. n: number of data.

Parameters	2017 (n = 51)	2018 (n = 52)	2019 (n = 52)	2020 (n = 38)	2021 (n = 51)	2017–2021 (n = 244)
COD IN (kg w^{-1}) μ COD (%)	30,560 ± 1543 77.6 ± 2.2	33,683 ± 1580 80.9 ± 1.5	34,366 ± 1925 77.7 ± 1.9	30,423 ± 1883 80.3 ± 2.0	29,297 ± 1225 80.1 ± 2.6	31,665 ± 1631 79.3
N-NO_X IN (kg w^{-1}) μ N-NO_X (%)	1503 ± 151.8 95.4 ± 1.8	1808.7 ± 157.7 97.5 ± 0.7	1937 ± 164.5 97.7 ± 0.6	1722 ± 207.8 99.2 ± 0.2	1982 ± 185 95.7 ± 3.6	1791 ± 173 97.1
N-NH_4^+ IN (kg w^{-1}) μ N-NH_4^+	280.7 ± 26.63 −64.9 ± 29.5	382.5 ± 46.3 −63.8 ± 48.8	468.6 ± 42.66 −103.7 ± 38.9	462.1 ± 57.0 −40.1 ± 17.1	237.2 ± 39.6 −21.3 ± 20.9	366 ± 42.4 −58.6
Ntot IN (kg w^{-1}) μ Ntot (%)	3243.5 ± 241.7 70.2 ± 2.3	4020.4 ± 463.3 69.6 ± 3.6	3569 ± 417.8 62.0 ± 3.3	3039 ± 241.7 67.1 ± 4.5	2983 ± 420 76.7 ± 3.6	3371 ± 357 69.1
Ptot IN (kg w^{-1}) μ Ptot (%)	336.1 ± 58.5 81.0 ± 6.46	245.8 ± 48.2 80.1 ± 5.1	326.3 ± 43.0 84.6 ± 1.9	279.3 ± 47.4 85.3 ± 6.2	299.3 ± 37.6 80.1 ± 2.6	297 ± 47 82.2
C_{O2} (kgO_2 CODrem^{-1})	1.47 ± 0.1	1.26 ± 0.1	1.28 ± 0.1	1.66 ± 0.3	1.44 ± 0.1	1.42 ± 0.1
TS (kg m^{-3})	205.2 ± 4.6	220.6 ± 5.5	208.2 ± 6.2	180.1 ± 6.4	186.1 ± 7.2	200 ± 6.0
VS (kg m^{-3})	52 ± 2.7	50.2 ± 2.6	47.7 ± 5.4	47.1 ± 3.6	48.2 ± 3.2	49 ± 3.5
Cf (kg_{BOD} (kg_{TS} d)$^{-1}$)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
θ (d)	252 ± 19	206.9 ± 19.7	184.6 ± 15.9	232.0 ± 2.6	233.1 ± 2.3	221.7 ± 11.9

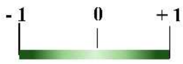
The specific oxygen consumption, C_{O2} was influenced by the biodegradability of the organic matter fed. It remains constant for all years monitored and is equal to 1.42 kgO_2 CODrem^{-1}, this value represents a significant index of the operating conditions of the plant, in fact it allows to quantify the oxidizing agent necessary to degrade 1 kg of organic substance. TS in the TBR was 180.1–205.0 g L^{-1} and the value of VS remained constant over the years of monitoring at around 47.1–52.0 g L^{-1}. These very high solids concentrations allow us to consider this operating reactor as a fluidized bed process [26]. Sludge load (Cf) and sludge age (θ) provided an indication of the operating conditions of the thermophilic biological process. The sludge load remained almost constant for all monitoring years (0.02 kgBOD (kgSST d)$^{-1}$). θ indicates the number of days in which biomass remained in the biological system. The average value (221.7 ± 11.9 d) was one order of magnitude higher than a traditional CAS plant (5–20d) [22]. This aspect was due to a lower production of VS typical of thermophilic system [9], which helped to produce a lower amount of excess sludge and more stabilized [27]. This aspect was extremely important for AW utility managers from an economic point of view since sludge production from biological systems represented one of the major cost items in plant management [28]. Over monitored years, θ strongly decreased in 2018 and 2019 to 206.9 ± 19.7 d and 184.6 ± 15.9 d, respectively, increasing again in 2020 and 2021 up to 233.1 d. This result was due to a higher sludge extraction in 2018 and 2019 (8.4 ± 1.6 t_{TS} w^{-1} and 9.1 ± 1.5 t_{TS} w^{-1}, respectively) with respect to other monitored years (2017: 6.4 ± 0.8 t_{TS} w^{-1}; 2020: 5.8 ± 0.2 t_{TS} w^{-1}; 2021: 5.4 ± 0.2 t_{TS} w^{-1}).

3.2. Data Analysis

3.2.1. Pearson and Spearman Correlation Coefficients

Pearson moment correlation coefficient and Spearman rank correlation coefficient were calculated to evaluate a possible correlation between the monitored parameters and calculated performances of the thermophilic biological process. Spearman's correlation index S is a non-parametric statistical measure of correlation, it measures the degree of relationship between two variables. The Pearson correlation index P between two statistical variables is an index that expresses a possible linearity relationship between them.

From the analysis of Pearson's correlation index (Figure 2), a positive correlation between the COD fed at the system and nitrates were pointed out (0.44), This consideration explains the value of 0.36 between the yield of removal nitrates and the yield of removal COD. The COD entering the plant is positively correlated to the amount of oxygen consumed (0.42) and the total nitrogen treated (0.36). CODin and COD are related to oxygen consumption; as the organic substance increases, the amount of oxygen needed to degrade it also increases. It is also possible to note some negative correlations, such as the ammonia entering the plant seeming to negatively influence the total nitrogen and COD abatement yields, suggesting a treatment upstream of the biological process. High concentrations of ammonia entering the TBR can lead to a reduction in total nitrogen removal yields and COD [29].



	COD IN	µ COD	N-NO_x IN	µ N-NO_x	N-NH_4^+ IN	Δ N-NH4+	Ntot IN	µ Ntot	Ptot IN	µ Ptot	C_{O2}	TS	VS
COD IN	1.00												
µ COD	0.24	1.00											
N-NO_x IN	0.44	− 0.04	1.00										
µ N-NO_x	0.13	0.36	0.19	1.00									
N-NH_4^+ IN	0.29	− 0.16	0.21	0.12	1.00								
Δ N-NH4+	− 0.01	0.09	− 0.02	− 0.06	0.20	1.00							
Ntot IN	0.36	-0.09	0.45	0.04	0.52	0.16	1.00						
µ Ntot	− 0.10	0.38	0.12	0.22	− 0.20	0.14	0.22	1.00					
Ptot IN	0.18	0.01	0.03	− 0.02	− 0.04	-0.06	− 0.05	− 0.05	1.00				
µ Ptot	0.06	0.02	− 0.05	-0.04	0.05	0.01	− 0.07	− 0.11	0.57	1.00			
C_{O2}	0.42	0.24	0.17	0.19	0.12	0.03	0.07	− 0.03	0.15	0.09	1.00		
TS	0.23	0.13	0.02	0.08	0.12	0.00	0.23	− 0.02	− 0.09	− 0.06	− 0.06	1.00	
VS	0.02	0.03	− 0.10	− 0.11	− 0.03	0.06	-0.06	− 0.03	0.11	0.05	0.00	0.29	1.00

Figure 2. Pearson's correlation matrix.

Spearman's correlation matrix shows very similar results (Figure 3). Further, in this case, there is a negative correlation between ammonia entering the plant and the reduction yields of removal COD and total nitrogen. We can see that the correlation coefficient passes from the value of −0.16 in the matrix P to a value −0.29 in the matrix S. These results confirm the negative correlation between these two parameters, ammonia, and fed COD. It is possible to see a solid positive correlation between the nitrates fed to the system and the total nitrogen fed. This correlation can be explained considering that nitrates are the most considerable portion of the nitrogen forms treated by the thermophilic biological system. Also, a negative correlation index of total phosphorus removal vs. TS (−0.03) was pointed out.

	COD IN	µ COD	$N\text{-}NO_x$ IN	µ $N\text{-}NO_x$	$N\text{-}NH_4^+$ IN	Δ N-NH4+	Ntot IN	µ Ntot	Ptot IN	µ Ptot	C_{O2}	TS	VS
COD IN	1.00												
µ COD	0.17	1.00											
$N\text{-}NO_x$ IN	0.42	− 0.10	1.00										
µ $N\text{-}NO_x$	0.02	0.23	0.31	1.00									
$N\text{-}NH_4^+$ IN	0.34	-0.29	0.20	0.02	1.00								
Δ N-NH4+	− 0.03	0.17	− 0.02	0.09	0.13	1.00							
Ntot IN	0.44	-0.20	0.55	0.00	0.50	0.02	1.00						
µ Ntot	− 0.11	0.38	0.12	0.26	− 0.29	0.31	0.18	1.00					
Ptot IN	0.16	0.00	0.03	− 0.03	-0.03	− 0.12	− 0.05	− 0.07	1.00				
µ Ptot	0.03	0.09	− 0.08	− 0.03	− 0.08	0.06	− 0.14	− 0.02	0.69	1.00			
C_{O2}	0.48	0.25	0.16	0.16	0.11	− 0.07	0.07	-0.04	0.19	0.15	1.00		
TS	0.24	0.10	0.04	-0.24	0.12	0.02	0.23	− 0.03	− 0.16	− 0.20	− 0.07	1.00	
VS	0.02	0.12	− 0.13	− 0.09	− 0.13	0.16	− 0.10	0.06	0.03	0.04	− 0.01	0.36	1.00

Figure 3. Spearman's correlation matrix.

From the analysis of the two correlation matrices, a positive correlation emerges between all the polluting parameters monitored at the entrance to the TBR. This management choice is positively reflected in the performance analysis, shown in Table 1, which shows very high-performance values for all the polluting parameters analyzed except for ammonia nitrogen.

If Pearson (P) < Spearman (S), this means a monotone correlation, but not a linear one [30]. Results could help in the future in the correct modeling of the observed parameters, giving information on the type of correlation between the data, whether linear or monotone. The negative correlation index between the total phosphorus abatement yields and the total solids concentration increase in the Spearman's matrix means the correlation between the two data is monotone but not linear.

The results obtained can be compared with the results presented by Muszyński et al. [19], in which the bacterial species are correlated with the COD and BOD_5 removal yields in our work instead the Spearman and Pearson matrices are made between the diverse environmental parameters of the plant.

3.2.2. Multivariate Linear Regression

Tables 2 and 3 show the matrices of coefficients of a set of multiple linear regressions, where each parameter is seen as a function of the other parameters monitored by the plant. It was decided to differentiate the parameters between two diverse groups: (i) fed loads of each polluting chemical parameter, and (ii) the yields of removal together with process parameters.

Table 2. Coefficients of multivariate linear regressions of input loads. Rows are indicative of the modeled parameters, while the columns represent coefficients of the model. α_i, intercept value with ordinate axis.

	COD IN	N-NO$_X$ IN	N-NH4+IN	Ntot IN	Ptot IN	α_i
COD IN	-	0.2927	0.1762	-	0.1119	0.4
N-NO$_X$ IN	0.4199	-	-	0.5212	-	0
N-NH4+IN	0.1532	-	-	0.7021	-	0
Ntot IN	-	0.2262	0.3015	-	-	0.02
Ptot IN	0.3595	-	-	−0.2545	-	0.4

Table 3. Coefficients of multivariate linear regressions of yields and physical process parameters. Rows are indicative of the modeled parameters, while the columns represent coefficients of the model. α_i, intercept value with ordinate axis.

	μ COD	μ N-NO$_X$	Δ N-NH$_4^+$	μ Ntot	μ Ptot	C_{O2}	TS	VS	α_i
μ COD	-	0.2722	-	0.1858	-	0.1563	0.1047	-	0.31
μ N-NO$_X$	0.3223	-	-	-	-	-	-	−0.0994	0.73
Δ N-NH$_4^+$	-	-	-	1.3883	-				0
μ Ntot	0.735	-	-	-	-	−0.1661	-	-	0.16
μ Ptot	-	-	-	-	-	-	-	-	0.55
C_{O2}	0.3959	-	-	−0.1039	-	-	-	-	0.09
TS	0.147	-	-	-	-	-	-	0.3268	0.43
VS	-	0.0493	-	-	-	-	0.2711	-	0.13

From Table 2 it is possible to highlight that Ptot and N-NH$_4^+$ removal yields were never used as a parameter useful for modeling, and Ptot was not the result of the linear regression of any other parameter.

Figure 4 shows the comparisons between the values measured in the plant and the values estimated by the multiple linear regressions. Thanks to these comparisons, it is possible to appreciate the excellent goodness-of-fit of the regression models. In particular, the yield value of removal COD and nitrogen forms, apart from ammonia, is well explained by the linear regression against the other monitored parameters. In regard to the COD entering the plant and the COD efficiency, the relationships between measured and estimated values have an angular coefficient of 1.059 and 1.003 with an R^2 of 0.98 and 0.99, respectively.

Similar results were obtained by observing the graphs of the input and the reduction of nitrates. In this case the slope of the interpolating line was 0.923 and 0.982 associated with R^2 values of 0.928 and 0.996, respectively. Also, in this case, it is possible to state that the multiple linear regression model allows estimating the data with an excellent degree of precision. In the case of N-NO$_X$, however, the model tends to slightly underestimate the predicted value compared to the observed one.

This type of analysis allows the AW utility manager to predict the number of certain pollutants or performances of the thermophilic biological system in presence of a limited amount of data.

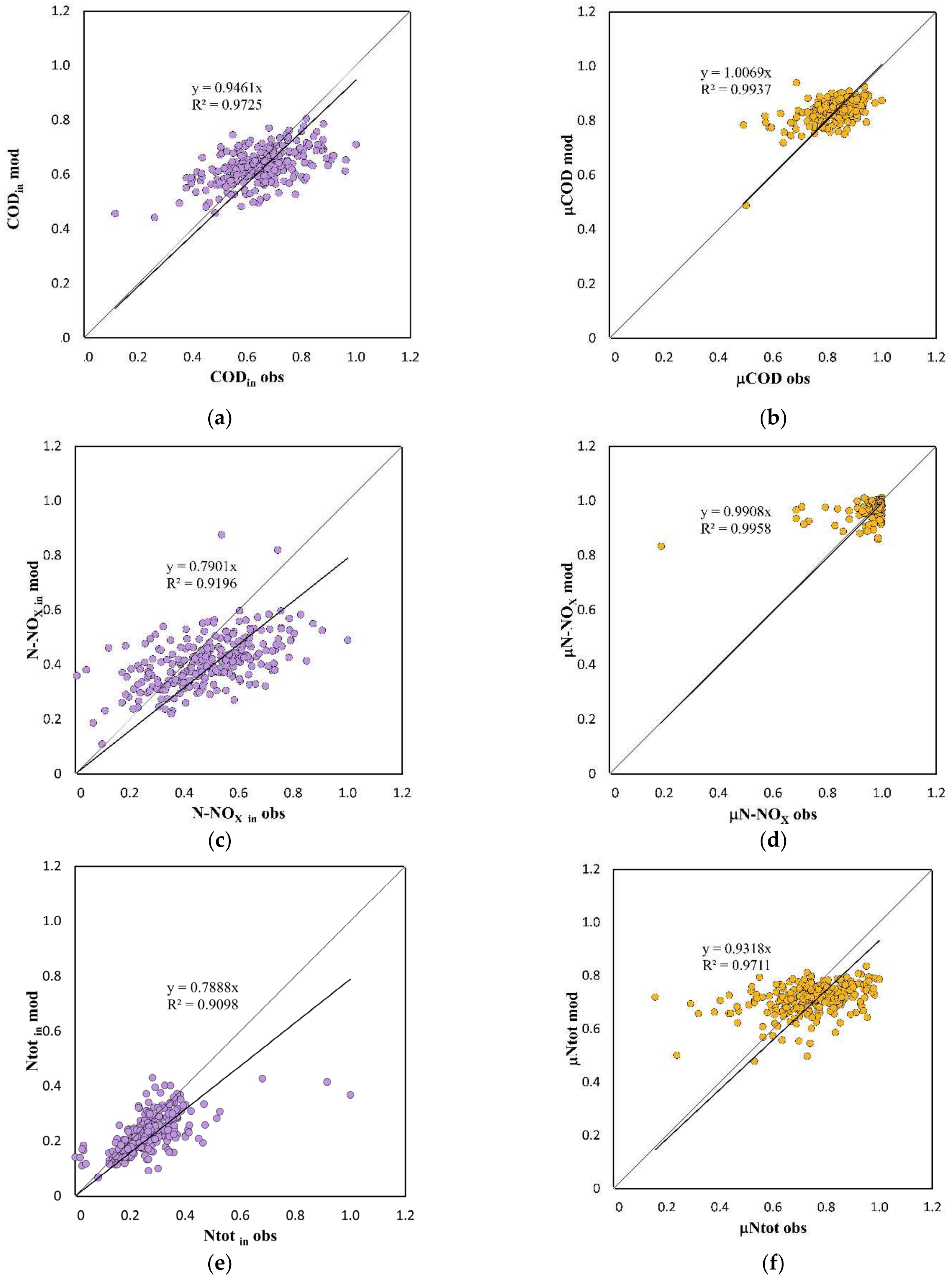


Figure 4. *Cont.*

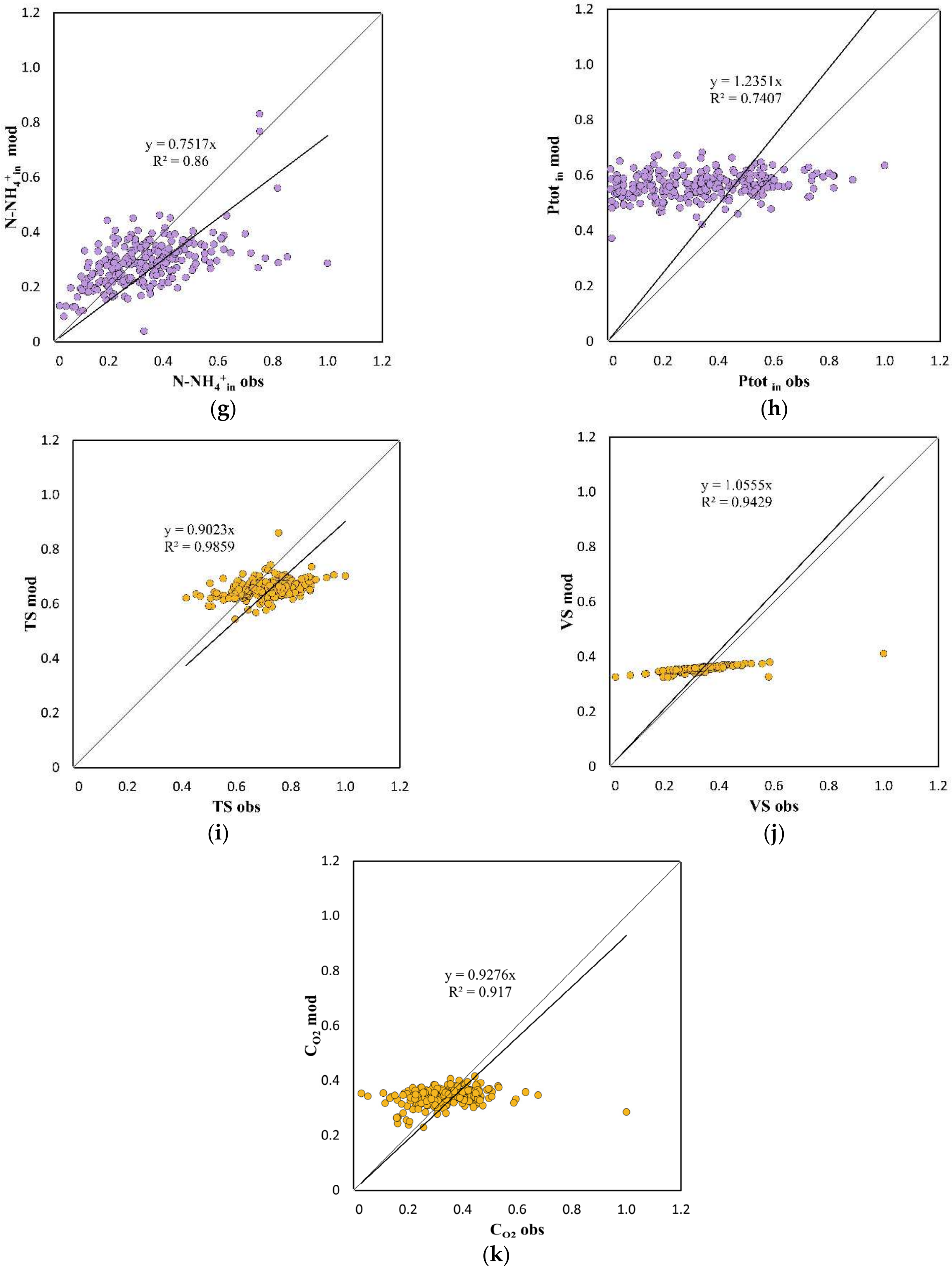


Figure 4. Multivariate linear regressions: comparison between normalized observed value and modelized values Figures from (**a**–**k**) show the trend of the measured values and of the modeled values considering both the concentrations of pollutants in input and their removal efficiency.

3.3. Respirometric Tests

Ammonia Uptake rate tests (AUR) tests were carried out to evaluate the nitrifying activity of the thermophilic biomass. In respirometry tests, no nitrification activity was detected. This study showed that uncommon ammonia removal was caused by stripping due to pure oxygen injection rather than microbiological transformation. The microbiological analyses highlighted the absence of nitrifying biomass (see Section 3.3). Indeed, these results completely agree with previous literature findings. At thermophilic temperatures, above 45 °C, nitrification processes do not take place and the bacteria due to this process are totally inhibited [31].

The nitrogen uptake rate (NUR) findings confirm the ability of the thermophilic biomass in denitrifying nitrogen in thermophilic conditions (Figure 5). Data from the monitoring of the system suggested nitrates removal higher than 95%.

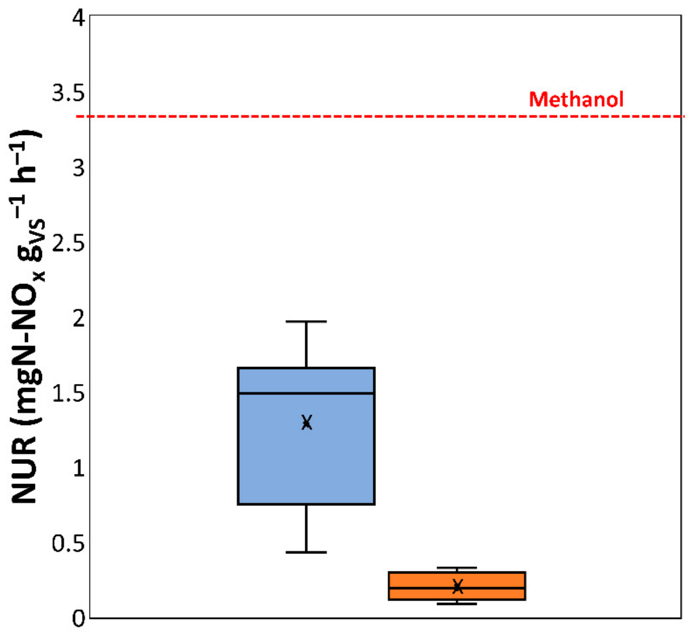


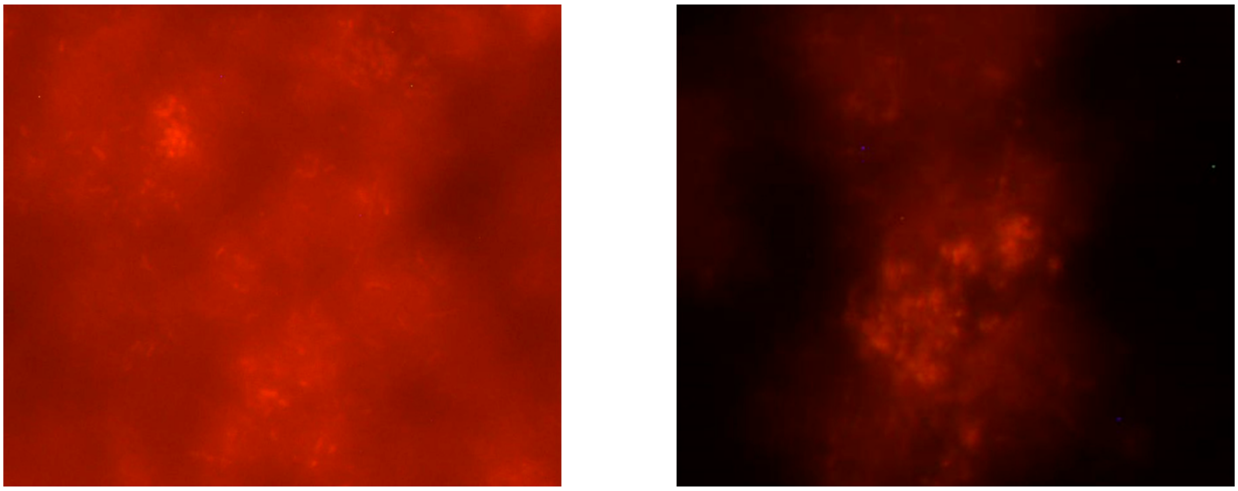
Figure 5. Results of NUR tests and comparison with the literature value [13,32] obtained with methanol dosage as a carbon source.

The potential of thermophilic bacteria to decrease nitrates over time was confirmed by the findings. Tests carried out under standard conditions have very high NUR kinetics (1–1.7 mgN-NO_X gVS^{-1} h^{-1}). The tests carried out to keep the test reports equal to the real plant ratio showed definitely lower values, equal to 0.21 mgN-NO_X gVS^{-1} h^{-1}. The two values are lower than the value found in the literature, relating to mesophilic biomass with carbonaceous source methanol, an excellent substrate according to the literature [28].

To obtain feedback on the very high nitrate abatement performance, it is necessary to pay attention to the concentration of solids in the tank. In fact, as can be seen in Figure 5, the denitrification kinetics of the thermophilic biomass taken under the real plant conditions is equal to 0.21 mgN-NO_X gSV^{-1} h^{-1}. The very high removal yields are, therefore, guaranteed by the high concentration of VS in the TBR (50 kg m^{-3}). Considering the obtained kinetics and the content of VS, the biological system was able to remove almost 1680 kg of nitrates in the HRT of the system, which was 94% of the fed N-NOx at the same time 1791 kg. This aspect confirms the results obtained by monitoring of the full-scale system.

3.4. Biota Characteristics

In any case, no AOB (Ammonia Oxidizing Bacteria), NOB (Nitrite Oxidizing Bacteria), and anammox bacteria were identified. If we compare the results with the results of the analysis carried out on samples from the same AWTP plant, which was the subject of a publication by Abbà et al. [33], despite the large time interval between the two measurements, we note the continued absence of nitrifying bacteria. Figure 6 displays a picture of the flocs, which have a diameter always lower than 150 µm and are quite dense, without any bone of filaments, as previously highlighted. Red spots represent the viable bacteria.



(**a**) (**b**)

Figure 6. Micrographs of the sludge after fluorescent in situ hybridization, under red fluorescence. Red spots represent viable bacteria clusters (magnification: 1000×). Photos (**a**,**b**) representing the same sample at two different times.

The biomass of this plant proves to be quite peculiar, given the particular process conditions that lead to the development of minute flocs and a bacterial community whose populations are present in different percentages compared to conventional activated sludge. The bacteria responsible for the oxidation of ammonium and nitrite seem to be unable to grow.

4. Conclusions

In this work, a full-scale aerobic thermophilic biological plant treating various high-strength AW was monitored for five years. The process scheme and operating conditions, in terms of input load, temperature, dissolved oxygen concentration, and salt content make the biomass of the plant studied capable of degrading organic matter with a very high efficiency (up to 80%) and to remove nitrate nitrogen with equally high efficiency (up to 99%). Based on collected data, a multivariate statistical analysis was applied highlighting a correlation between several pollutants fed and performances of the process. This can be a useful tool for the manager of AWTPs for deciding the correct mixture of AW to feed to the biological reactor. The respirometric tests (AUR and NUR) confirmed results obtained by monitoring data analysis and helped to fully understand ammonia fate in the reactor. As far as nitrification is concerned, there is an increase in ammonium ions due to the hydrolysis of the organic substance that occurs in the reactor and, while, on the other, a stripping of the same ammonia nitrogen in the form of NH_3 as confirmed by the respirometric tests. Based on fluorescent in situ hybridization tests, no AOB, NOB, and anammox bacteria were identified in a 50 °C pure oxygen thermophilic biomass.

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TOPIC: WS2: Process optimization

- WS2.2 - Rheological behavior of thermophilic sludge

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ORIGINAL PAPER

Influence of Heavy Metals on the Rheology of a Thermophilic Biological Sludge for nutrients Recovery: Effect of Iron, Copper, and Aluminium on Fluid Consistency

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Abstract
Currently, thermophilic membrane biological reactors (TMBRs) are used to treat industrial wastewaters and biological sewage sludge with the aim of nutrients recovery. The performance of the biological process is strongly influenced by rheological behaviour of the thermophilic biological sludge (TBS) inside the reactor. Considering the high concentration of heavy metals in matrices fed to the reactors, this work aims to evaluate the influence of heavy metal types and concentration on rheological properties of TBS. Sludge has been extracted from a full-scale TMBR and conditioned with Fe^{3+}, Cu^{2+}, and Al^{3+}. Rheological measures have been conducted and constants *k* and *n* of Herschel–Bulkley model were used to define the rheological properties of TBS. Rheological properties of high consistency TBS ($0.06 \le k_0 < 0.2$ and $0.6 \le n_0 < 0.8$) were not significantly affected by the conditioning with Fe^{3+}, Cu^{2+}, and Al^{3+}. In case of TBS with initial low consistency ($0.02 \le k_0 < 0.06$) and behaviour more similar to Newtonian fluids ($0.8 \le n_0 < 1$), Fe^{3+} and Al^{3+} determined a significant increase in consistency. On the contrary, the addition of Cu^{2+} reduced *k* of conditioned TBS with a lower impact on the distance for Newtonian behaviour (*n*). This work demonstrates the strong influence of Fe^{3+}, Cu^{2+}, and Al^{3+} on the rheological properties of TBS depending on the initial consistency of the sludge, and the types and dosage of heavy metals.

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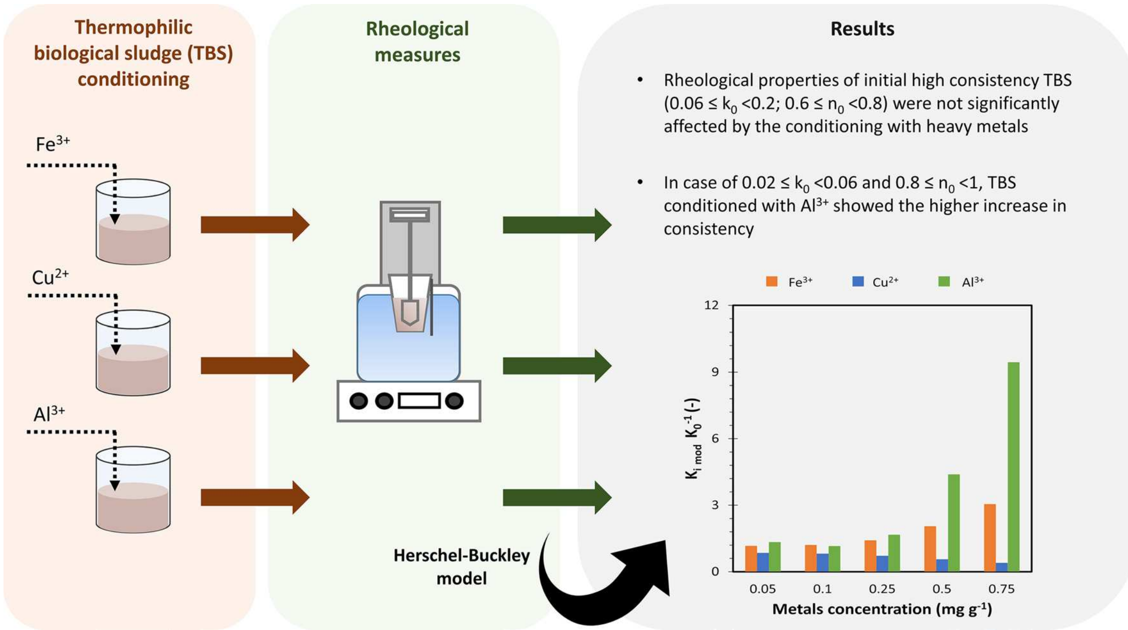
Graphical Abstract



Keywords Sludge conditioning · Aqueous waste · Biological process · Thermophilic treatments · Sludge rheology · Biological sewage sludge

Statement of Novelty

The rheological behaviour of sludge represents a key factor for optimal hydrodynamic behaviour in biological process. Several studies deal with rheological properties of biological sludge, but they are mainly focused on the influence of parameters such as temperature, pH and total solids on mesophilic sewage sludge. The novelty of this study is that the influence of heavy metals concentration on the rheological properties of a thermophilic biological sludge has been studied. This work can serve as a reference for (i) further studies on parameters influencing the rheological behaviour of thermophilic sludge, and (ii) for an optimal management of full-scale thermophilic membrane biological reactors predicting the effects of feeding waste with a high heavy metal content.

Introduction

Biological reactors nowadays represent a valid solution for the treatment of wastewater (WW) and aqueous waste (AW), as amply demonstrated by the literature and evidenced by their diffusion in industrial plants [1]. Thanks to their tolerance to high concentration of pollutants, thermophilic membrane bioreactors (TMBRs) have been recently promoted and used to treat high-strength industrial WWs and biological sewage sludge (BSS) to recover nutrients, both in organic and inorganic form [2–4]. However, as for conventional biological processes, the characterization of the system with suitable quantitative methods remains a crucial point to optimize the process, improving performances and minimizing costs.

For instance, performance of biological processes is strongly influenced by the hydrodynamic behaviour of sludge in reactor. To date, computational fluid dynamics (CFD) models are solid and well-founded approaches that represent a fundamental tool for the design of biological reactors [5]. They can also be effectively used in monitoring the hydraulic behaviour of already built systems to detect dead volumes and flow bypasses [6–8]. However, fluid dynamic models are based on many parameters such as, for instance, shear stress, shear rate and apparent viscosity. Therefore, the knowledge of rheological properties represents a necessary requirement to compute representative CFD models [8, 9].

Moreover, the rheological properties of activated sludge have been investigated since they severely impact many aspects of biological systems management [10]. Typical examples are sludge pumping, bioreactor hydrodynamics, mass transfer efficiency of aeration systems, sludge-water separation by decantation and filtration [11–14]. Therefore, knowing the relationships between the rheological parameters of the biological reactor and the input of the plant allows also to program the feeding process to optimize performance and reduce costs [15].

TMBRs are generally fed with industrial AW and BSS very variable in terms of composition [2–4]. In urban BSS fed to TMBRs, heavy metals generally do not represent an issue with concentration in the order of 100–1000 mg kg^{-1} of dry matter [3]. On the contrary, in AW, the content of heavy metals can be very high depending mainly by the industrial process in which they originate. For instance, aqueous residue from the treatment of metal surface can contain heavy metals over 50,000 mg L^{-1} [16] and must be therefore subjected to a coagulation/flocculation pre-treatment to reduce content. In general, the biota in thermophilic conditions showed good activity even in the presence of high concentrations of heavy metals but the maximum acceptable concentration is highly dependent on the type of cation. For instance, Cu^{2+} presents toxicity on thermophilic biota at concentration of one order of magnitude lower than Al^{3+} (almost 0.3 vs. 1 mg g^{-1}) [17, 18].

Several previous studies were focused on the influence of physico-chemical parameters on rheological behaviour of TBS highlighting a strong dependence from total solids content, pH, and temperature while a not clear impact of aeration conditions was pointed out [19–21]. Other studies also showed a significant impact of pre-treatment. For instance, Riley and Forster [22] demonstrated that thermophilic aerobically digested sludge showed yield stresses one order of magnitude lower than not-digested sludge with similar TS concentration. Also Dai et al. [19] found that higher sludge retention time in anaerobic digester stimulates lower levels viscosity and consistency index with higher value of flow behaviour index.

However, literature lacks information about the influence of specific pollutants as heavy metals on rheological behaviour of biological sludge, representing a huge gap especially for a proper management of TMBRs.

In this work, the influence of heavy metals on the rheological properties of the TBS are presented and discussed. The shear stress and the apparent viscosity of TBS, extracted from a full-scale TMBR, have been evaluated as a function of the increasing concentration of iron (Fe^{3+}), copper (Cu^{2+}), and aluminium (Al^{3+}) and of the initial consistency of the sludge. This work can serve as a reference for (i) other researchers, answering questions as how do heavy metals affect the rheology of a TBS? Does the effect depend on the type of metal tested? and for (ii) water utilities, providing helpful information to predict the effects of feeding AW with high heavy metals content on the rheological properties of TBS in a full-scale plant.

Materials and Methods

Rheological Model

In literature, several models that interpolate shear stress—shear rate evaluated by experimental measurements in order to obtain the relative flow curve are available [23]. Most of these models adopt a power-low curve including an offset to account for stress yield *y*. When considering TBS from a membrane system, Herschel–Bulkley model (Eq. 1) can provide an adequate adaptation of experimental data of these sludge samples because characterized by a high concentration of solids [20, 24].

$$\tau = \tau_0^{HB} + k\dot{\gamma}^n \quad (1)$$

τ (Pa) is the shear stress and γ ˙represents the share rate (s^{-1}). τ_0 (Pa) represents the yield shear stress and quantifies the amount of stress that the fluid may experience before it yields and begins to flow. *k* and *n* are characteristic constants of the fluid. *k* indicates the consistency of the fluid and the higher the more viscous the fluid is. The exponent *n*, less than 1, expresses the non-Newtonian behaviour; the more *n* differs from the unit, the more the characteristics of the fluid differ from the Newtonian ones.

Sludge Characteristics

TBS was collected from a full-scale TMBR located in Mortara (Lombardy, Italy). The plant treats AW containing also high concentration of heavy metals. The system (1700 m^3) is composed by a biological reactor and an ultrafiltration line (membrane cut-off: 300 kDa) operating with 3–5 bar of pressure. In the biological reactor, aerobic conditions (1.5–3 mg ${L^{-1}}_{O2}$) are maintained injecting pure oxygen. Thanks to spontaneous exothermic reactions due to organic matter degradation by thermophilic biota [25], the temperature is maintained constant at almost 49 °C. Full details of the characteristics of this biological plant are available in our previous study [26].

To evaluate the effect of initial consistency of the fluid and level of non-Newtonian behaviour, full-scale plant was monitored until the initial rheological conditions of thermophilic sludge changed. Two extractions were made: (i) one in low consistency sludge conditions and (ii) the other one with high-consistent sludge. Extracted TBS were diverse in term of rheological properties but not in terms of physico-chemical characteristics and heavy metals concentration (which was almost zero) (Table 1). In fact, to easy the conditioning procedure, samples were taken after a period of almost no feeding of heavy metals-rich AW in the reactor.

Preparation of Heavy Metals Solutions

$CuSO_4$, $FeCl_3$ and $AlCl_3$, purchased from Sigma-Aldrich (Merck Group, MO, USA), were used to prepare doping solutions with copper, iron, and aluminium, respectively, in ultrapure water (Purite Select; SUEZ Water Purification

Table 1 Characteristics of high and low consistency TBS extracted from the full-scale plant

	Low consistency sludge	High consistency sludge
Rheological properties		
k_0 (–)	0.02–0.06	0.06–0.2
n_0 (–)	0.8–1	0.6–0.8
Heavy metals concentration		
Fe^{3+} (mg g^{-1})	0.023–0.025	0.025–0.026
Cu^{2+} (mg g^{-1})	0.001–0.002	0.001–0.003
Al^{3+} (mg g^{-1})	0.033–0.036	0.034–0.035
Other physico-chemical characteristics		
TS (g kg^{-1})	170–180	170–180
VS/TS (–)	0.48–0.50	0.47–0.49
COD (mg kg^{-1})	37,500–38,500	41,000–42,000
N_{tot} (mg kg^{-1})	4000–4500	3900–4200
P_{tot} (mg kg^{-1})	250–300	270–320
pH (–)	6.5–7	6.5–7

TS: total solids; VS: volatile solids; COD: chemical oxygen demand; N_{tot}: total nitrogen; P_{tot}: total phosphorus

Systems Ltd, France). To avoid change in total solids content of the sludge samples after conditioning, highly concentrated heavy metals solutions (80–300 mg g^{-1}) were prepared.

Practical Procedure

The TBS was withdrawn from the full-scale plant and delivered to the laboratory in a plastic tank within 1 h after sampling. To evaluate the effect of the increase in the concentration of heavy metals on the rheological features, the TBS was conditioned with the alternative addition of Fe^{3+}, Cu^{2+}, and Al^{3+} by testing up to a concentration of 1.35 mg g^{-1}, 0.65 mg g^{-1}, and 0.52 mg g^{-1}, respectively. This choice was made: (i) considering typical concentration of Fe^{3+}, Cu^{2+}, and Al^{3+} in TBS when heavy metals-rich AW are fed (data not shown), and (ii) to study the phenomenon in a wider spectrum testing heavy metals also in extreme conditions of concentration. After heavy metals dosage, the conditioned TBS was mixed for 4 h before carrying out the rheological measurement to completely homogenize the sample.

The rheological tests were carried out using rheometer RC20 (RheoTec) with a configuration CC25DIN of coaxial cylinders. Spindle radius measured 12.5 mm while the internal radius of the measuring cylinder was 13.56 mm. The working principle of the instrument is based on the sliding of the TBS in the cavity between the coaxial cylinders, thanks to the spindle rotation with a fixed share rate, while the external cylinder is held, needs a torque that is measured by the instrument [20]. Each rheological test was performed with fixed shear rates maintained for 150 s and increased step by step. Shear stress and apparent viscosity were recorded every 15 s (ten data sets for each share rate). The following shear rates were tested: 25 s^{-1}–50 s^{-1}–100 s^{-1}–200 s^{-1}–400 s^{-1}–600 s^{-1}–800 s^{-1}–1000 s^{-1}. The mean and confidence interval of the shear stress measurements for each applied shear rate were calculated.

To better simulate real conditions at full-scale, the temperature during test was maintained at 48–50 °C using a thermostatic bath. The scheme of the apparatus is reported in Fig. 1.

Data Analysis

In the present study, for the calculation of the initial/breaking shear stress, yield was considered as a deterministic parameter in the interval 0–3.99 Pa with steps of 0.05 Pa.

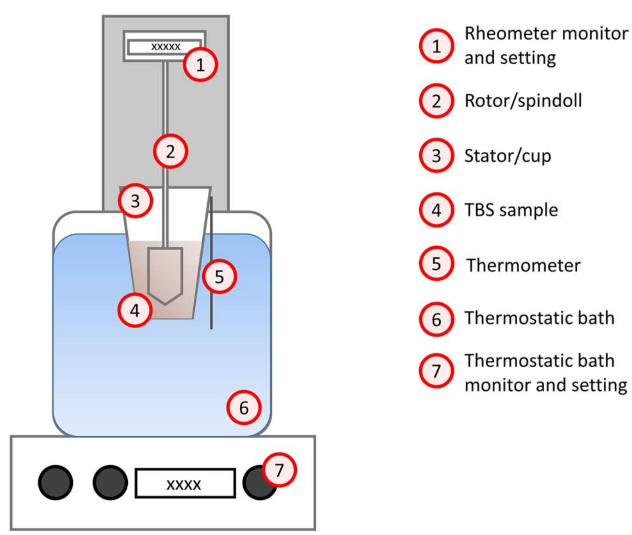


Fig. 1 Scheme of the apparatus (rheometer and thermostatic bath) used in the tests

For each of these values, the regression coefficient R^2 was estimated and used for the choice of τ_0 that produce a best fitting. The accuracy of the interpolation was estimated through an additional statistical descriptor which is the standard estimation error.

The Herschel-Buckley model was used to estimate k_i and n_i of conditioned samples and results were referred to k and n of not-conditioned sludge (k_0 and n_0) in which heavy metals were almost absent (Table 1). $k_i\ k_0^{-1}$ were fitted with 2-nd degree polynomial function (in case of Fe^{3+} and Al^{3+}) and linear function (in case of Cu^{2+}) as a function of heavy metals dosage. To evaluate the influence of initial rheological characteristics of sludge, data has been also analysed grouping results in two diverse cases depending on initial k_0 and n_0. Moreover, to better highlight the influence of the metal in the change of rheological properties, $k_{i\ mod}$ and $n_{i\ mod}$ were calculated for diverse predetermined iron, copper, and aluminium concentrations based on fitting functions previously found.

Results and Discussion

Influence of Initial Rheological Properties

Iron (Fe^{3+})

In Fig. 2, values of $k_i\ k_0^{-1}$ and $n_i\ n_0^{-1}$ as a function of Fe^{3+} concentration are showed considering also diverse initial rheological condition of TBS.

The effect of the Fe^{3+} dosage on the rheological properties of the TBS was diverse depending on the initial characteristics of the sludge. In case of a TBS with a higher initial consistency value of the fluid ($0.06 \le k_0 < 0.2$) and a behaviour more distant from the Newtonian one ($0.6 \le n_0 < 0.8$), it was not possible to highlight significant variations in the rheological parameters when varying of the tested metal concentration (Fig. 2).

On the contrary, the Fe^{3+} concentration seemed to have a greater influence on the rheology of the TBS with initial characteristics typical of a Newtonian fluid ($0.8 \le n_0 < 1$) and characterized by a low consistency index ($0.02 \le k_0 < 0.06$). As the metal concentration increased beyond 0.4 mg g^{-1}, the consistency index increased considerably ($k_i\ k_0^{-1}$ up to 5.29 with 1.13 mg g^{-1} of Fe^{3+}), and the behaviour of the fluid deviated more than that typical of Newtonian fluids ($n_i\ n_0^{-1}$ dropped down to 0.75).

This increase can be attributed to the strong coagulating effect caused by the reaction of the iron in contact with the TBS. The dosage of Fe^{3+} determined the formation of bridge bonds that reduce the negative zeta-potential typical of biological sludge [27], leading to the formation of aggregates more resistant to shear stress. The consequent limitation of Brownian movements of particles increased the consistency index and reduced the value of n [28–30]. However, this result was apparently in contrast with Shrestha et al. [31] who highlighted a reduction in the viscosity of the biological sludge in case of $FeCl_3$ dosage. However, difference of results can be mainly attributed to three aspects: (i) the diverse dosage method (continuous in the previous one, impulse in our study), (ii) the diverse type of sludge tested

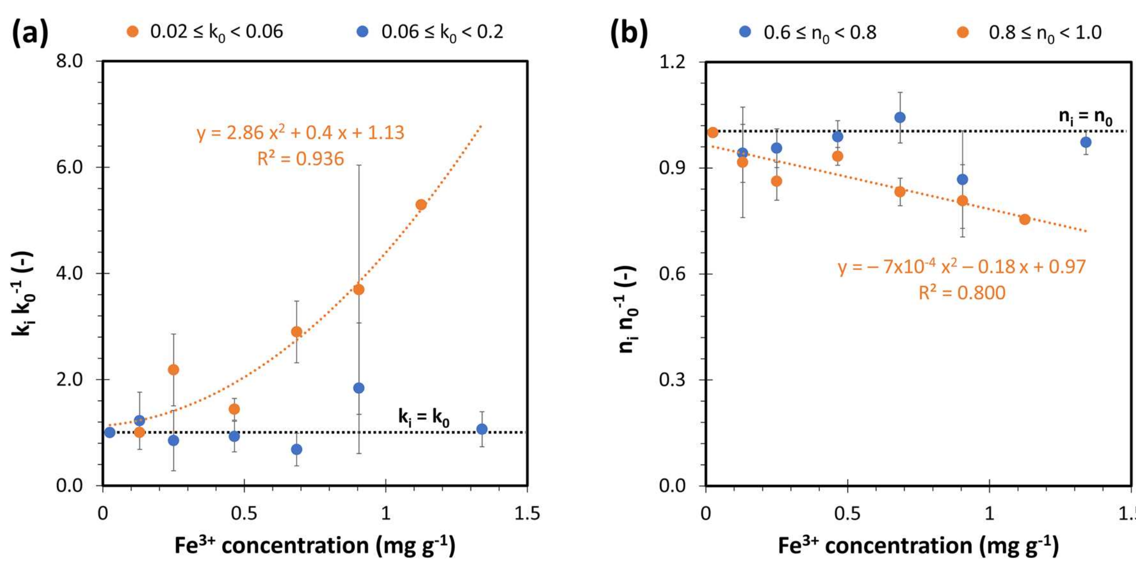


Fig. 2 **a** $k_i\ k_0^{-1}$ and **b** $n_i\ n_0^{-1}$as a function of $Fe.^{3+}$ concentration. Orange lines represent the 2-nd degree fitting while black lines represent the unchanged situation ($k_i = k_0$ or $n_i = n_0$). The bars represent the 95% confidence interval ($n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article)

(mesophilic in the previous study), and (iii) the Fe^{3+} dosage of at least one order of magnitude lower in the previous study compared to this one.

The different behaviour of TBS with a higher initial consistency and more distant from the Newtonian fluids ($0.06 \leq k_0 < 0.2$; $0.6 \leq n_0 < 0.8$) and that with a lower initial consistency and behaviour closer to the Newtonian ones ($0.02 \leq k_0 < 0.06$; $0.8 \leq n_0 < 1$) can be attributed to the diverse initial zeta-potential. In the first case, the zeta-potential can be considered very low, given the high initial consistency due to a limited phenomenon of Brownian motion of the particles [28, 29]. Therefore, the addition of cations did not cause an appreciable variation of the rheological properties. In the second case, the low initial consistency, determined by the strongly negative zeta-potential, produced a more appreciable coagulating phenomenon upon the addition of Fe^{3+}.

Copper (Cu^{2+})

In Fig. 3, values of $k_i\ k_0^{-1}$ and $n_i\ n_0^{-1}$ as a function of Cu^{2+} concentration are presented considering also diverse initial rheological condition of TBS.

Unlike Fe^{3+}, Cu^{2+} showed a different effect on the rheological properties of the TBS. The dosage of copper caused a behaviour that was qualitatively and quantitatively dependent on the initial consistency and distance from the Newtonian behaviour.

In the case of TBS with higher initial consistency value and a fluid behaviour more distant from the Newtonian ($0.06 \leq k_0 < 0.2$; $0.6 \leq n_0 < 0.8$), it was not possible to highlight significant variations in the rheological parameters as the metal concentration (Fig. 3).

On the contrary, the concentration of Cu^{2+} seemed to have a greater influence on the rheology of the TBS with initial characteristics typical of a Newtonian fluid ($0.8 \leq n_0 < 1$) characterized by a low consistency index ($0.02 \leq k_0 < 0.06$). As the metal concentration increased, the consistency index decreased considerably ($k_i\ k_0^{-1}$ up to 0.542 with about 0.65 mg g^{-1} of Cu^{2+}, maximum tested concentration), and the behaviour of the fluid tended to slightly deviate from that typical of Newtonian fluids ($n_i\ n_0^{-1}$ grown up to 1.09).

This decrease in viscosity can be attributed to the lack of coagulating capacity of copper and its toxic-inhibiting effect also in lower concentrations [32]. The stress condition imposed by the presence of Cu^{2+} could have determined the release of soluble microbial products (SMP) by the biomass [33, 34]. These, together with the extracellular polymeric substances (EPS) could cause the reduction of the consistency of the fluid (k). These results are confirmed by Chen et al. [35] which found in their study that the release of EPS determined a reduction in sludge viscosity increasing sludge settleability.

The absence of perceived decrease in consistency as the Cu^{2+} dosage increased in the presence of a TBS with a higher initial consistency value and a fluid behaviour more distant from the Newtonian one ($0.06 \leq k_0 < 0.2$; $0.6 \leq n_0 < 0.8$) is to be attributed to the more marked initial presence of bridge bonds which are only minimally reduced by the formation of substances with surfactant power.

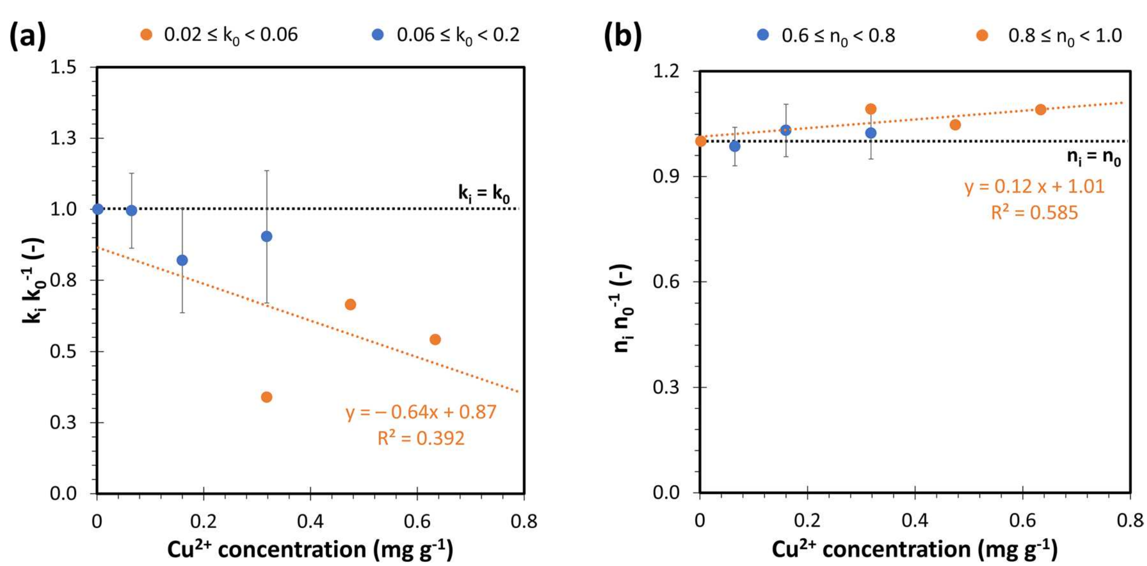


Fig. 3 **a** $k_i\ k_0^{-1}$ and **b** $n_i\ n_0^{-1}$ as a function of Cu.$^{2+}$ concentration. Orange lines represent the 1-st degree fitting while black lines represent the unchanged situation ($k_i = k_0$ or $n_i = n_0$). The bars represent the 95% confidence interval ($n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article)

Aluminium (Al^{3+})

In Fig. 4, values of $k_i\ k_0^{-1}$ and $n_i\ n_0^{-1}$ as a function of Al^{3+} concentration are showed considering also diverse initial rheological condition of TBS.

In terms of behaviour, the effect of the Al^{3+} dosage was similar to that obtained with the addition of Fe^{3+}. Also in this case, in the case of TBS with higher initial values of the fluid consistency ($0.06 \le k_0 < 0.2$) and greater distance from the Newtonian behaviour ($0.6 \le n_0 < 0.8$), no significant rheological changes have been highlighted (Fig. 4).

On the contrary, as for Fe^{3+}, the concentration of Al^{3+} has a greater influence on the rheology of the TBS with initial characteristics typical of a Newtonian fluid ($0.8 \le n_0 < 1$) and with a low consistency index ($0.02 \le k_0 < 0.06$). As the metal concentration increases beyond 0.2 mg g^{-1}, the consistency index increases considerably ($k_i\ k_0^{-1}$ up to 4.82 with 0.52 mg g^{-1} of Al^{3+}, maximum tested concentration), and the behaviour of the fluid deviates more than that typical of Newtonian fluids ($n_i\ n_0^{-1}$ decreased to 0.74).

This result can be compared to what has already been stated for Fe^{3+} (Sect. 3.1.1.): the addition of the metal leads to the formation of aggregates which increase the consistency index by binding to the negatively charged sites on the TBS surface and stepped away the behaviour of the sludge from that typical of Newtonian fluids [36].

Similarly to what was observed for Fe^{3+} (Sect. 3.1.1.), the diverse behaviour between the TBS with higher initial consistency and non-Newtonian properties ($0.06 \le k_0 < 0.2$; $0.6 \le n_0 < 0.8$) and that with a more limited initial consistency and more close to the Newtonian one ($0.02 \le k_0 < 0.06$; $0.8 \le n_0 < 1$) can be attributed to the different initial zeta-potential (very close to zero in the first case, markedly negative in the second) [28, 29]. The addition of Al^{3+} in the first case did not cause an appreciable variation of the rheological properties while in the second case produced a more appreciable formation of bridge bonds producing aggregated particles more resistant to shear stress [28–30].

Comparison of the Influence of Metals Dosed

Considering the interpolating models (2-nd degree for Fe^{3+} and Al^{3+}, 1-st degree for Cu^{2+}) identified for each single metal, the $k_{i\ mod}\ k_0^{-1}$ and $n_{i\ mod}\ n_0^{-1}$ at diverse predetermined metal concentrations were calculated, by way of comparison (Fig. 5).

Only $0.02 \le k_0 < 0.06$ and $0.8 \le n_0 < 1$ cases were evaluated considering that rheological properties of initial high consistency TBS were not significantly affected by the conditioning with heavy metals. The diverse behaviour of Fe^{3+} and Al^{3+} compared to Cu^{2+} was highlighted.

In the first two cases, the coagulating effect of the metal substantially increased the consistency of the fluid away from the typically Newtonian behaviour. TBS conditioned with Al^{3+} showed a more pronounced effect than Fe^{3+} (C: 0.05 mg g^{-1}), with $k_{i\ mod}\ k_0^{-1}$ reaching 1.33 and 1.16, respectively. In case of higher concentration (C: 0.75 mg g^{-1}), the effect of Al^{3+} was almost third times the effect of Fe^{3+} ($k_{i\ mod}\ k_0^{-1}$ equals to 9.44 and 3.04, respectively).

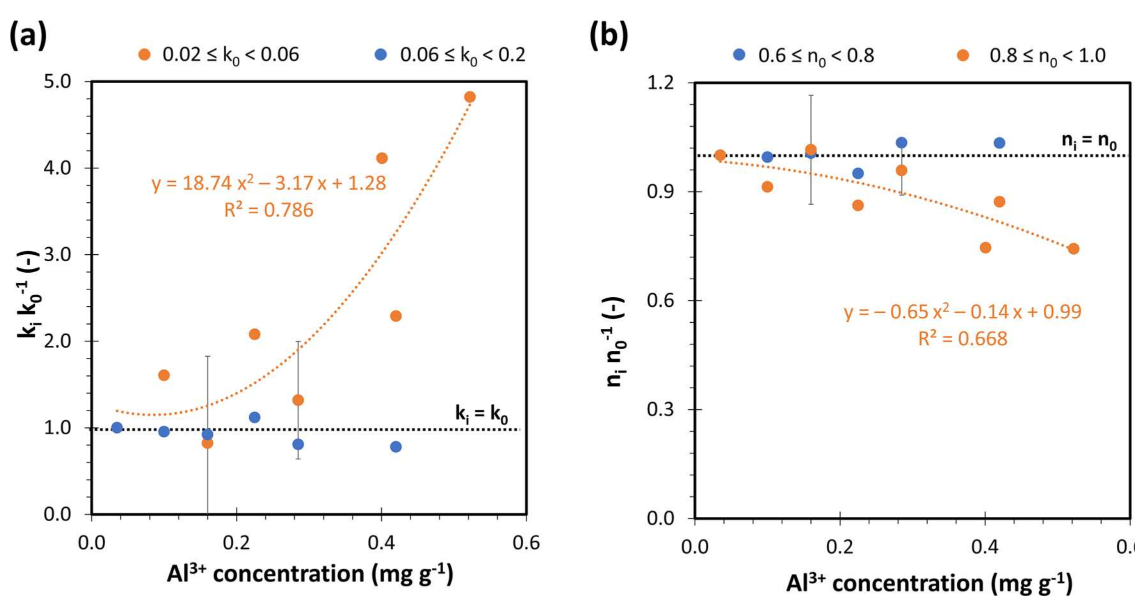


Fig. 4 **a** $k_i\ k_0^{-1}$ and **b** $n_i\ n_0^{-1}$ as a function of Al^{3+} concentration. Orange lines represent the 2-nd degree fitting while black lines represent the unchanged situation ($k_i = k_0$ or $n_i = n_0$). The bars represent the 95% confidence interval ($n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article)

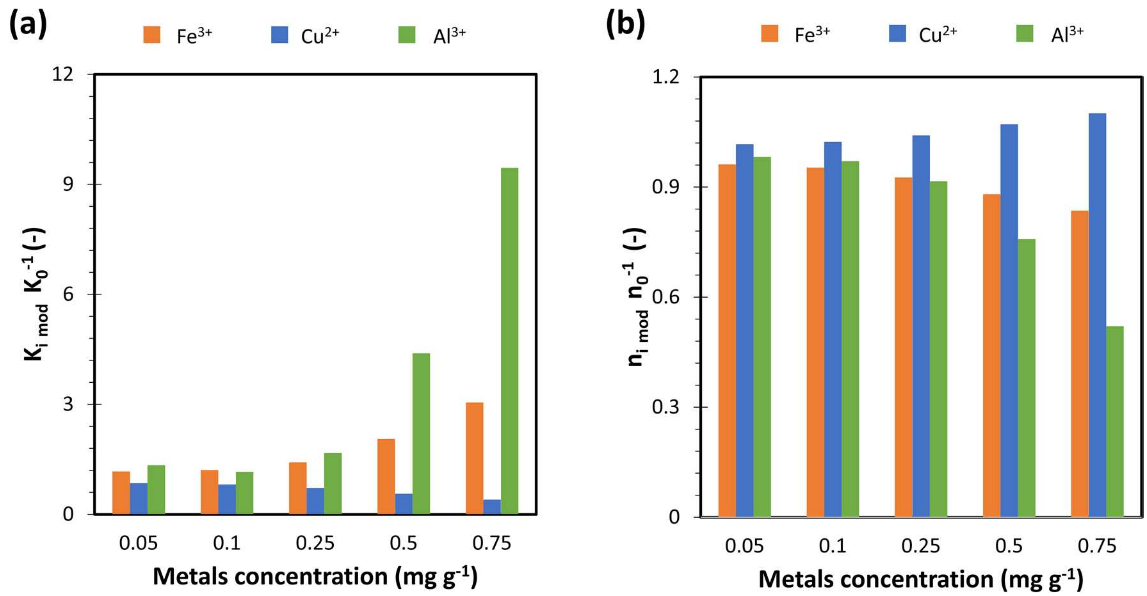


Fig. 5 **a** $k_{i\ mod}\ k_0^{-1}$ and **b** $n_{i\ mod}\ n_0^{-1}$as a function of Fe^{3+}, Cu^{2+}, and Al.$^{3+}$ concentration in case of $0.02 \le k_0 < 0.06$ and $0.8 \le n_0 < 1$ (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article)

Instead, TBS conditioned with Cu^{2+} showed an opposite effect due to the absence of the coagulating phenomenon associated with an inhibitory phenomenon of biomass [32]. In presence of 0.05 mg g^{-1} of copper, the model estimated that $k_{i\ mod}\ k_0^{-1}$ dropped to 0.84 with an almost constant value of n ($n_{i\ mod}\ n_0^{-1}$ equals to 1.02). Increasing Cu^{2+} concentration up to 0.75 mg g^{-1}, $k_{i\ mod}\ k_0^{-1}$ decreased to 0.39 and not significant differences in *n* have been evaluated ($n_{i\ mod}\ n_0^{-1}$: 1.1).

Conclusions

This study demonstrates the strong influence of Fe^{3+}, Cu^{2+}, and Al^{3+} on the rheological properties of TBS depending on the initial consistency of the sludge and the types and dosage of heavy metals. In case of TBS with initial low consistency ($0.02 \le k_0 < 0.06$) and behaviour similar to Newtonian fluids ($0.8 \le n_0 < 1$), Fe^{3+} and Al^{3+} determined a significant increase in consistency, especially in high concentration. In case of TBS conditioned with 0.05 mg g^{-1} of Fe^{3+} or Al^{3+}, with $k_{i\ mod}\ k_0^{-1}$ reached 1.16 and 1.33, respectively. On the contrary, the addition of Cu^{2+} on initial low consistency TBS, markedly reduced k with a lower impact on the distance for Newtonian behaviour (n). In presence of 0.05 mg g^{-1} of copper, the model estimates that $k_{i\ mod}\ k_0^{-1}$ dropped to 0.84 with an almost constant value of n ($n_{i\ mod}\ n_0^{-1}$ equals to 1.02). Rheological properties of initial high consistency TBS ($0.06 \le k_0 < 0.2$ and $0.6 \le n_0 < 0.8$) were not significantly affected by the conditioning with Fe^{3+}, Cu^{2+}, and Al^{3+}. Therefore, this study provides helpful information for the proper management of thermophilic biological systems giving details about expected changes in rheological characteristics of sludge based on types and amount of heavy metals fed. This knowledge represents a crucial point considering that sludge consistency affects hydrodynamic behaviour in reactors and therefore performances of the process.

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Author Contributions MCC: conceptualization, methodology, funding acquisition, resources, supervision. SB: formal analysis and investigation, writing-original draft preparation, visualization. MCM: writing-original draft preparation, writing-review and editing, visualization. FMC: writing-original draft preparation. SC: writing-original draft preparation. AD: formal analysis and investigation. MB: conceptualization, methodology, supervision.

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Data Availability All data generated or analysed during this study are included in this published article.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Understanding the Influence of Diverse Non-Volatile Media on Rheological Properties of Thermophilic Biological Sludge and Evaluation of Its Thixotropic Behaviour

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Abstract: In this study, the rheological properties of thermophilic biological sludge (TBS) have been investigated evaluating the influence of non-volatile solids (NVS). Calcium carbonate, sand, and sodium bentonite were separately added to the sludge to evaluate the effect of concentration and type of NVS. Results show that TBS consistency coefficient significantly enhanced increasing sodium bentonite concentration. On the contrary, calcium carbonate and sand showed relatively small influence on the rheological properties of TBS. Thixotropic behaviour of TBS has also been investigated and is more pronounced at higher shear rate (1000 s^{-1}). Double exponential fitting model was the best choice to represent thixotropic behaviour in case of low (100 s^{-1}) and high shear rate (1000 s^{-1}), while a single-exponential model represents the best option in case of medium shear rate (400 s^{-1}).

Keywords: thermophilic sludge; non-volatile solids; sewage sludge; rheology; sludge conditioning; thixotropic behaviour

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1. Introduction

Knowledge of the rheological properties of biological sludge (BS) is essential for optimizing the management of a high-strength wastewater treatment plant for several aspects: (i) membrane performance [1], energy consumption (e.g., in sludge pumping), hydrodynamics of bioreactors [2], oxygen transfer by aeration systems [3], and sludge sedimentation [4].

The hydrodynamic behaviour of the BS is closely related to its characteristics, which include the relative concentration of solids, the nature of the wastewaters and the treatment process to which it is subjected. At low concentrations of total solids (TS), the sludge behaviour can be reasonably approximated in most cases as a Newtonian fluid with a linear relationship between shear stress and strain rate and negligible yield stress [5]. Therefore, measured viscosity is rather independent of the shear rate at given temperature and pressure values, and the flow curve is represented by a straight line. As the concentration of TS increases, the sludge deviates from the Newtonian behaviour and can assume shear-thinning or shear-thickening behaviour. For such a non-Newtonian sludge the measured viscosity becomes dependent on the shear rate and the ratio of shear stress to shear rate, so-called apparent viscosity, is introduced [6]. The rheological characterization of non-Newtonian sludge leads to practical difficulties in interpreting the behaviour of mixed liquor. The Ostwald–de Waele model, the Bingham model, the Herschel and Buckley model, and the Casson model are most commonly used to express non-Newtonian relationships between shear stress and shear rate for activated sludge. They can provide a suitable description of the sludge properties for practical applications [7].

Several studies have shown that temperature significantly influences rheological characteristics [6,8]. For instance, Baroutian et al. [6] studied the dependence of the rheological properties of a BS on temperature in a range from 25 °C to 55 °C, finding a reduction of the viscosity value with increasing temperature. As already described in the literature, the rheological parameters of a sludge depend upon other process parameters such as pH [9,10], TS concentration, [11,12], and volatile solids (VS) concentration [13]. Treatments to which the sludge is subjected also influence the rheological properties. Several differences have been reported in the literature between non-digested mesophilic sludge [9,14], digested mesophilic sludge [14,15], and thermal hydrolysed sludge [14,16]. For instance, digestion influences the rheological properties reducing viscosity of BS due to VS degradation [9,14,15]. Also thermal hydrolysis processes can lead to a reduction of the consistency coefficient of BS thanks to the breaking of the long chains of fatty acids inside the sludge [14,16]. These differences highlight how those processes to which the BS is subjected can strongly influence its rheological behaviour. In spite of how the knowledge that the rheological behaviour of BS is increasing, most of the studies were conducted in mesophilic conditions—though thermophilic sludge can also be used to biologically treat high strength wastewaters. With high temperature conditions (T: 45–48 °C), a biomass was selected that had several advantages such as a higher degradation rate of the organic substrate, lower excess sludge production and rapid inactivation of pathogens [17]. However, as a drawback, thermophilic biological sludges (TBS) have poor settling properties which make it necessary to couple membranes in case of operation with high biomass concentration [18].

Data on the rheological properties of TBS are limited to the influence of temperature and TS, without focusing, for example, on non-volatile solids (NVS). Furthermore, the modelling of the thixotropic behaviour of a TBS represents a new topic in the international scientific panorama. In the technical literature relatively few studies have been found linking shear stress and time in a deterministic way [19–22].

This work aims to study how diverse types and concentrations of NVS affect the rheological properties of a complex biological substrate such as TBS. Understanding the influence of diverse types of NVS on the rheology of TBS represents a key point to reliably characterize the hydrodynamic behaviour of the matrix. Furthermore, the time–response of TBS was investigated for applied shear stress to evaluate and characterize the thixotropic properties. The investigation approach and obtained data aim at providing practical information for facility managers to deal with practical problems occurring during the treatment process while assuring suitable levels of the plant efficiency.

2. Materials and Methods

2.1. Thermophilic Biological Sludge (TBS)

TBS was sampled in a full-scale membrane reactor fed with high-strength wastewaters. Thermophilic biological reactor operated at a temperature of almost 48–50 °C and was followed by ultrafiltration membranes for sludge separation. Details about the full-scale thermophilic membrane reactor configurations are reported in our previous study [23]. After sampling, TBS was delivered to the laboratory in a plastic tank within 1 h to preserve the biological activity and qualitative properties of the sludge. The main characteristics of the unconditioned sludge are reported in Table 1.

Table 1. Characteristics of unconditioned TBS. u.m.: unit of measure; (*): calculated as a difference between TS and VS.

Parameter (u.m.)	Value	Analytical Method
Rheological Properties		
k (–)	0.012–0.22	-
n (–)	0.51–1.1	-
Physico-Chemical Properties		
pH (–)	7.5–8	[24]
TS (kg m^{-3})	175–185	[25]
VS (kg m^{-3})	85–95	[26]
NVS (kg m^{-3})	85–95	*
COD (mg L^{-1})	180,000–190,000	[27]
Ntot (mg L^{-1})	4000–5000	[28]
Ptot (mg L^{-1})	45–50	[29]
N-NH_4^+ (mg L^{-1})	150–160	[30]
N-NO_3^- (mg L^{-1})	250–300	[31]
N-NO_2^- (mg L^{-1})	40–55	[32]

2.2. Choice of Non-Volatile Media

The main aim of this work is to investigate the rheological properties of TBS during the operating conditions of existing treatment plants at full scale and how these properties can be affected by most frequent causes of malfunctioning in order to provide a useful dataset that can be of help for managers to adopt remedial measures and assure suitable plant efficiency levels. For this reason, it was decided to test three types of NVS species usually already present within a WWTP because the article aims to provide useful information to the operator of a thermophilic biological plant for the treatment of high-strength wastewaters. The non-volatile media tested were:

- Calcium carbonate, as it represents a residual product of the reaction of lime in water and is very frequently used in full-scale high-strength wastewater treatment plants;
- Sand, to understand if malfunctions of the sand removal processes, which result in a high number of solid particles entering the thermophilic biological reactor, can affect the rheology of the BS;
- Sodium bentonite, as it can limit the filtering properties of membranes. For this reason, understanding the influence of this substance in biological membrane processes is a fundamental aspect for the optimization of treatment.

2.3. Rheological Properties

2.3.1. Evaluation of Sludge Consistency

In literature, several mathematical models are available in order to describe the rheological properties of BS [33]. Most of these models adopt a power-low curve that includes an offset to account for the yield stress (τ_0). When considering thermophilic biological sludge from a membrane system [34], the Herschel–Buckley model (Equation (1)) [35] can provide an adequate representation of the experimental data because BS is characterized by a high concentration of solids [36]. Denoting with τ the shear stress and with $\dot{\gamma}$ the shear rate, the model reads:

$$\tau = \tau_0^{HB} + k\dot{\gamma}^{\,n} \tag{1}$$

where k and n represent the consistency coefficient and the flow behaviour index, respectively. When n equals to 1 a Newtonian behaviour is obtained, n lower than 1 indicates pseudoplastic properties, and n higher than 1 indicates a dilatant behaviour. The more n differs from 1, the more fluid deviates from Newtonian properties. τ_0^{HB} indicates the yield stress.

For every different dosage of diverse type of NVS, the estimated values k_i and n_i of Herschel–Buckley model were related to the corresponding parameters k_0 and n_0 of unconditioned sludge.

2.3.2. Evaluation of Thixotropic Behaviour

Three different models, referred to as single, double, and triple exponentials, were used to interpolate the measured shear stress values over time (Equations (2)–(4)) and were then compared to evaluate which one provides the best fitting to the experimental curve.

$$\tau = Ae^{-\alpha t} \tag{2}$$

$$\tau = Ae^{-\alpha t} + Be^{-\beta t} \tag{3}$$

$$\tau = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\chi t} \tag{4}$$

2.4. Experimental Procedure

To evaluate the effect of increasing TS concentration on rheological behaviour, TBS was conditioned with calcium carbonate, sand, and sodium bentonite that were separately added. Calcium carbonate (particle size 1–3 μm) and sodium bentonite (particle size 35–105 μm) in powdered form were purchased (Heiltropfen, United Kingdom) while sand (particle size 0.16–0.24 mm) was sampled as a grit removal residue in a full-scale wastewater treatment plant (WWTP). Sand was washed with distilled water to avoid the presence of organic matter; it was subsequently dried at 105 °C in an oven (Memmert, Germany) to avoid the presence of water which could alter the test.

TBS was conditioned by adding NVS from 90 kg m^{-3} up to 190 kg m^{-3} of concentration. After that, the conditioned TBS was mixed for 4 h at 50–80 rpm to completely homogenize the sample before performing the rheological measurement. The value of VS, on the other hand, remained unchanged compared with the unconditioned sample.

Rheological tests were performed using RC20 coaxial cylinders rheometer (RheoTec, Germany) with a CC25DIN configuration (i.e., spindle radius 12.5 mm; stator internal radius 13.56 mm). The following shear rate values were tested: 25 s^{-1}, 50 s^{-1}, 100 s^{-1}, 200 s^{-1}, 400 s^{-1}, 600 s^{-1}, 800 s^{-1}, and 1000 s^{-1}. The experimental procedure to acquire data for evaluating the flow curve of the testing sludge sample was carried out by maintaining each shear rate value for 150 s before increasing it at the next measurement step. Shear stress and apparent viscosity were acquired every 15 s (i.e., ten datasets for each share rate step). The shear stress values measured at each time and plotted in the following figures should not be intended as instantaneous values as they represent the average of the measures taken within the corresponding sampling time interval of 15 s. The mean and confidence interval of the shear stress measurements were calculated for each applied shear rate. Moreover, before carrying out the shear stress measurement at different shear rate steps, each sludge sample was preliminary subjected to a fixed shear rate (i.e., 400 s^{-1}) for a constant time (i.e., 300 s) to cause structure breakdown and reduce the possible thixotropic influence on the rheological measurements.

In a subsequent step, to evaluate a possible thixotropic property of TBS, the shear stress and the apparent viscosity were recorded at three diverse shear rates (100 s^{-1}, 400 s^{-1}, and 1000 s^{-1}), each of which has been maintained for a suitably long-time interval (i.e., 1200 s). Shear rate values have been selected to provide an overview of the sludge behaviour within the investigated range. These values have been considered sufficient to obtain suitable information at this early stage of investigation, while the number of investigated shear rate values will be enhanced in subsequent studies.

It must be pointed out that other experimental techniques can be applied for the thixotropic analysis of a non-Newtonian fluid, such as the 3-intervals thixotropy test (3ITT) from the multiple interval thixotropic test protocol (miTT) [37]. The 3ITT test can be conveniently used for applying an instant shear stress/shear rate deformation in order to simulate effects of pumping and stirring during food processing [38]. In any case, common full-scale applications of TBS in wastewater treatment plants involve overall steady state operating conditions with negligible shear stress/shear rate mean local variations.

To reproduce full-scale operating conditions, the temperature during all tests was kept at 48 ± 2 °C using a thermostatic bath (VELP Scientifica, Italy).

2.5. Data Processing

All parameters of Equation (1) were estimated by fitting the experimental data. The yield stress was considered as a deterministic parameter ranging in the interval (0–3.9 Pa) with steps of 0.05 Pa [36]. For each of these values, the coefficient of determination R^2 was estimated for the measured shear stress–shear rate values. The yield stress that maximizes R^2 has been assigned to the parameter τ_0 in Equation (1). The accuracy of the fitting of model was assessed through the standard estimation error. For each concentration (say i) of NVS, k and n parameters of the Herschel–Buckley model were related to the corresponding parameters of the unconditioned sludge.

For the description of the thixotropic properties, the parameters of exponential models (Equations (2)–(4)) have been estimated with a non-linear least square constrained algorithm, as implemented by the *lsqcurvefit* function of Matlab®.

The parameter Δ (Equation (5)) was used to compare fittings of different exponential models in diverse conditions.

$$\Delta = m_{line} - 1 \tag{5}$$

where m_{line} represents the coefficient of linear interpolation of model data as a function of experimental data. If Δ is positive the model overestimates experimental data, and vice versa models were also compared using the Akaike's Information Criteria (AIC) index [39] (Equation (6)), which represents a method for evaluating and comparing statistical models. It is defined as:

$$\text{AIC} = 2p - 2\ln(L) \tag{6}$$

where p represents the number of parameters in the statistical model and L is the maximized value of the likelihood function of the estimated model. In this study, given the Gaussian distribution of the error, L has been considered equal to the sum of the normalized residues squared. The AIC index provides a measure of the quality of the estimate considering both the goodness of fit and the mathematical complexity of the model. Generally, models with the lowest AIC are preferred.

3. Results

3.1. Dependence of Rheological Parameters from Non-Volatile Media

Preliminary tests (see Supplementary Materials) highlighted how non-volatile media can potentially affect the rheological behaviour of TBS sampled in diverse reactors. Two TBS samples were extracted from two diverse full-scale plants (with similar conditions of feeding and operational temperature) and both the consistency coefficient, and the flow behaviour index were calculated to characterize the sludge from a rheological point of view. Results show that TBS with lower TS and NVS content exhibited a higher consistency coefficient, thus suggesting a possible relation between NVS and rheological properties.

Therefore, the influences of non-volatile media concentration and type were considered and the relation between these factors and TBS rheology was deeply studied. Figure

1 shows the results of the tests carried out by varying the concentration of diverse non-volatile media in TBS.

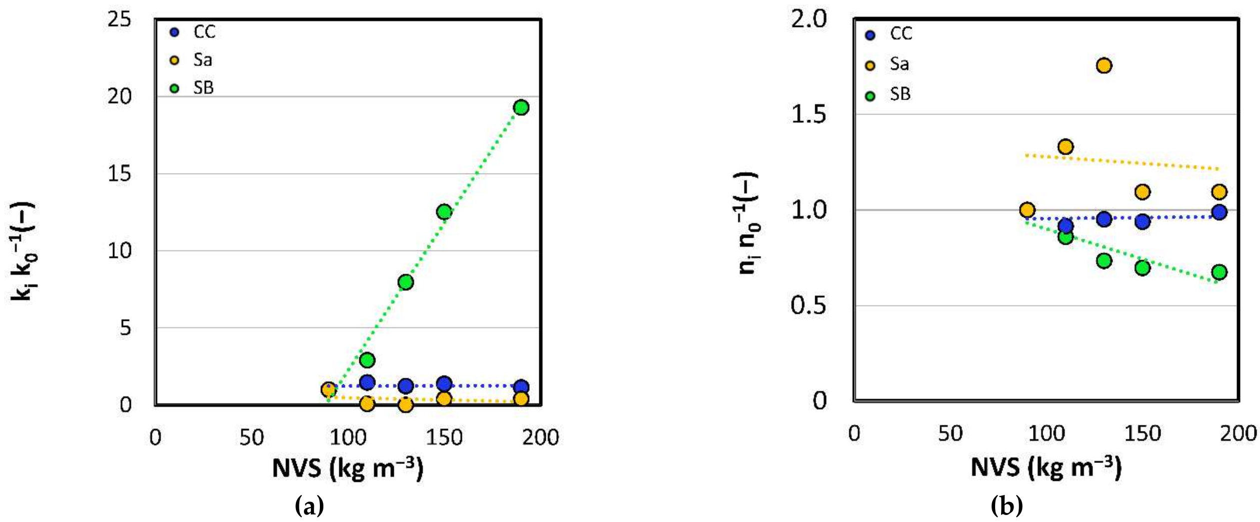


Figure 1. (**a**) Variation of consistency coefficient of conditioned TBS with respect to unconditioned sludge $k_i\ k_0^{-1}$ as a function of NVS concentration. (**b**) Variation of flow behaviour index of conditioned TBS with respect to unconditioned sludge $n_i\ n_0^{-1}$ as a function of NVS concentration. Dot lines represents the linear tendency. CC: calcium carbonate; Sa: sand; SB: sodium bentonite.

The consistency coefficient (k_i) of the fluid conditioned with calcium carbonate was nearly constant despite a strong increase in the concentration of solids inside the tested sludge sample (Figure 1a). The variation of parameter k with respect to that of the unconditioned sample (k_0) was expressed by the non-dimensional ratio $k_i\ k_0^{-1}$ that reached 1.1. However, this increase was limited when compared with an increase in NVS concentration greater than 110% (from 90 kg m^{-3} to 190 kg m^{-3}). Moreover, $n_i\ n_0^{-1}$ also remained almost the same. It reduced from a value of 1 (unconditioned situation) to 0.98 at 190 kg m^{-3} concentration of NVS (Figure 1b), showing an almost negligible influence of this type of non-volatile media on the rheology of TBS within the investigated concentration range.

In the case where the sample was conditioned with sand, the sludge consistency coefficient reduced to 0.5 the value of the unconditioned sludge (Figure 1a). Apart from two values of the flow behaviour index n, obtained with NVS concentration in the range 120–140 kg m^{-3}, deserving further investigation, the distance from the Newtonian behaviour was relatively small (i.e., $n_i\ n_0^{-1} \approx 1.1$) if compared with an increase in NVS concentration greater than 110% (Figure 1b).

Sodium bentonite produced the greatest influence on TBS rheology among the three types of NVS (see Supplementary Materials). Increasing NVS from the initial 90 kg m^{-3} up to 190 kg m^{-3}, the ratio $k_i\ k_0^{-1}$ reached the value of 19.29 (Figure 1a). Concerning the flow behaviour index (n), adding sodium bentonite made the conditioned sludge behave like a pseudoplastic fluid as the ratio $n_i\ n_0^{-1}$ was always lower than 1 for all NVS concentrations, reaching the minimum value of about 0.67 at the highest NVS concentration (Figure 1b).

3.2. Evaluation of Sludge Response after Prolonged Imposed Shear Rate

In this case the experiment was aimed at investigating the possible thixotropic behaviour of a TBS unconditioned with a high solids content [40,41]. Tests were carried out on unconditioned sludge sample subjected to a constant shear rate for a duration of 1200 s. The thixotropic properties were evaluated for three shear rate values that are assumed to be representative of the sludge behaviour over a wide range of operating conditions: 100 s^{-1}, 400 s^{-1}, 1000 s^{-1}.

The experimental values of the shear stress τ as a function of the time t are presented in Figure 2. As previously pointed out, the shear stress value plotted at each time represents the average measure within the corresponding time interval of 15 s which was assumed to be long enough to smooth out transient effects due to a change in the spindle rotation.

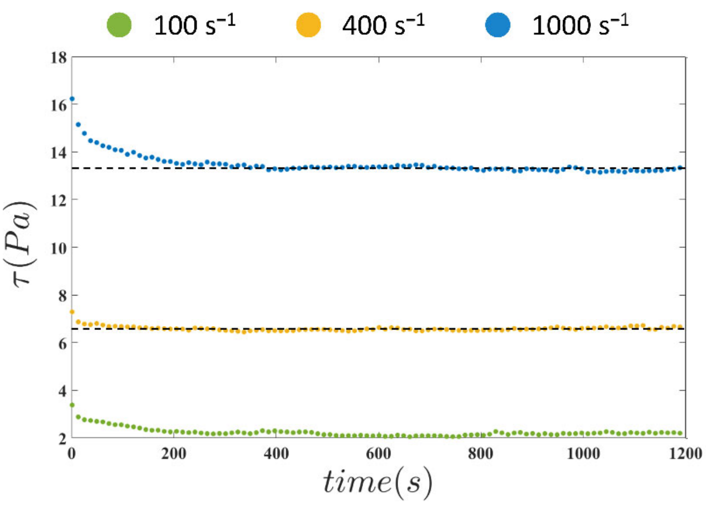
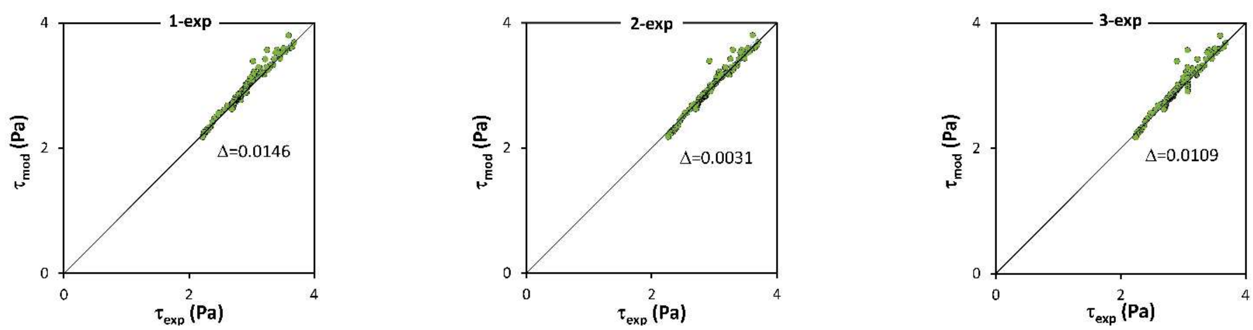


Figure 2. Shear stress as a function of time for different values of applied shear rate.

We observed an initial phase with decreasing shear stress values which was quite pronounced in the test carried out at maximum shear rate (i.e., 1000 s^{-1}); the shear stress dropped from 16 Pa to 13.5 Pa, showing a reduction of about 15.3% in the first 300 s from the beginning of the test. After that, the measured shear stress became almost stable. Such behaviour can be explained by the fact that at higher shear rates a great part of the microscopic structures were suddenly broken, therefore fewer changes in the measured behaviour occurred at subsequent instants. In any case, an analogous behaviour can be observed also at the lower shear rate (i.e., 100 s^{-1}) where the shear stress decreased from about 3.5 Pa to 2 Pa leading to a reduction of 43% within the same time interval. Such a behaviour was probably related to relatively complex microscopic aspects that deserve further investigation.

The three exponential models previously described (Equations (2)–(4)) have been used to describe the shear stress as a function of time for different values of applied shear rate and results were compared (Figure 3). All the exponential models provided a suitable fitting and showed the inclination to slightly overestimate the higher shear stress. In general, the calculated values of the parameter Δ confirmed the suitability of the three models to fit experimental data, but double exponential model (Equation (3)) provided values of Δ below 0.01 in all three testing conditions.



(**a**) (**b**) (**c**)

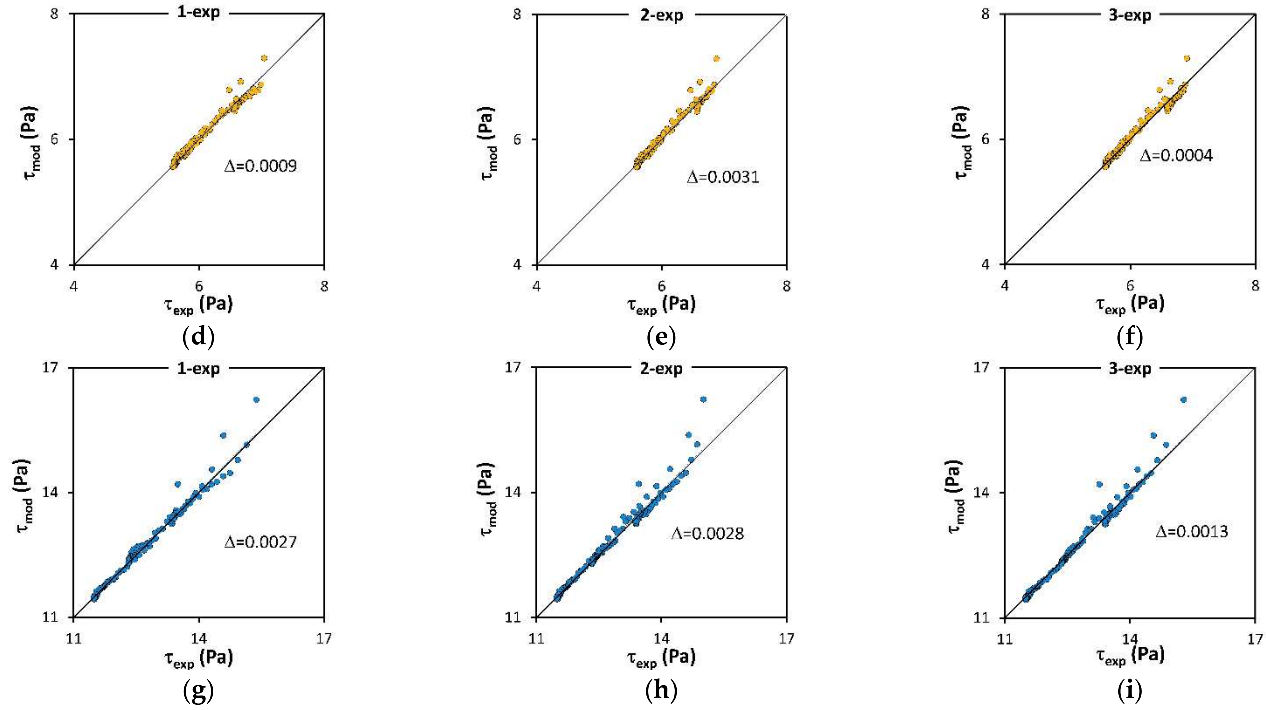


Figure 3. Comparison between experimental data and single, double, and triple-exponential models to describe the shear stress as a function of time. (**a**–**c**) are referred to 100 s^{-1}. (**d**–**f**) are referred to 400 s^{-1}. (**g**–**i**) are referred to 1000 s^{-1}.

4. Discussion

4.1. Dependence of Rheological Parameters from Non-Volatile Media

Preliminary rheological comparison of two TBS coming from two diverse high-strength wastewater treatment plants highlighted how the results are apparently not in line with previous literature findings [14]. As shown in the Supplementary Materials, TBS with higher TS concentrations but lower VS was characterized by a lower consistency index with respect to that which characterized lower concentrated sludge. Therefore, NVS influence on the rheological properties of a biological matrix has been investigated.

In this work, three types of non-volatile media were tested. Firstly, calcium carbonate ($CaCO_3$) was tested as it represents a residual product of the lime reaction in water and its presence is very common in plants with chemical-physical processes (as in this specific case). Results highlight how calcium carbonate induced a relatively small effect on the rheological parameters of TBS, also increasing the concentration of NVS from 90 kg m^{-3} up to 190 kg m^{-3}. This result can be attributed to the absence of chemical interaction of $CaCO_3$ with the water present inside the sludge. In fact, as reported by Behzadfar et al. [42], the influence of calcium carbonate on the rheological properties of fluids varies depending on particle size. They observed that higher increases in viscosity were appreciable with higher particle sizes of calcium carbonate [42]. In our study particle size was too small (1–3 μm) to lead to a significant change on sludge rheological properties. Based on these results, the potential dosage before biological processes of lime composed of small-particle $CaCO_3$ will produce circumscribed effects on the rheological parameters of TBS.

Sand was tested as NVS to understand if the rheology of BS can be affected by malfunctions of grit removal processes, which determine high quantity of granular materials entering the biological reactor. Results agree with Adeyinka et al. [43] who have explained that sand does not influence the consistency coefficient of a fluid given absence of chemical interaction between silica and water. On the contrary, Mangesana et al. [44] have highlighted how fluid viscosity can be influenced by sand dimensions, increasing with particle size. In our study, results were obtained focusing only on sand with size of 0.16–0.24 mm, therefore different outcomes with sands of diverse sizes cannot be excluded. Moreover, an increase in consistency due to the contribution of interaction between biomass and sands over the long-term cannot be excluded, and other studies should be further developed. Probably, based on these results, the potential malfunction of grit removal processes will produce circumscribed effects on the rheological parameters of TBS, even if some experimental points in Figure 1b show a deviation from the above-described behaviour and deserve further investigation.

Sodium bentonite is a non-volatile media reducing the filtering properties of membranes [45]. For this reason, understanding the influence of this substance in membrane biological processes is a key aspect for process optimization. In this work, results highlight how sodium bentonite strongly influences rheological parameters of the investigated biological mixture, enhancing the sludge consistency coefficient about 20 times when increasing NVS concentration from 90 kg m^{-3} to 190 kg m^{-3}. These results agree with previous findings of Hamida et al. [46], which highlighted how even small concentrations of sodium bentonite in water produce a viscous thixotropic fluid with low filtration capacity. However, to date no results on a thermophilic biological substrate are available. This result represents a key tool for water utility operators to optimize the operating costs of the plant (e.g., pumping costs, washing of the membranes) and performance of the thermophilic biological process.

4.2. Evaluation of Sludge Response after Prolonged Imposed Shear Rate

Plots of the shear stress as a function of time at a fixed shear rate were evaluated, highlighting the thixotropic behaviour of the investigated sludge sample. In particular, the obtained R^2 values are 0.9738, 0.9899, 0.9911, respectively for the speeds of 100 s^{-1}, 400 s^{-1} and 1000 s^{-1}. These results can be related to the different speed of bond rupture in the biological matrix. In exponential models, parameters α, β and χ can represent the diverse phases of rupture of the surface tension of the sludge. In two-exponential model, the parameters A and α can be associated with a faster phase describing the macro-breaking of the ionic bonds and hydrogen bonds present in the sludge, while the other two B and β can be related with a slower phase indicating the breaking of weak bonds inside the biological structure.

The three-exponential model tried to explain the breaking of the bonds inside the sludge flake at 3 temporal moments. As can be seen from Figure 3, at the higher shear rates (i.e., 400 s^{-1} and 1000 s^{-1}) the three-exponential model has the lowest value of the parameter Δ and for this reason it seems able to better mimic the 3 phases of sludge breaking.

Each exponential model was characterized by an intrinsic complexity given by the number of parameters to be fitted. The AIC index was calculated to combine the information on the accuracy that can be achieved by a model and the number of its tuning parameters (Table 2).

Table 2. Values of AIC index for different models in diverse shear rate conditions. (*): best conditions.

	AIC Index		
Shear Rate (s^{-1})	**Single Exponential**	**Double Exponential**	**Triple Exponential**
100	−19.859	−39.862 *	14.883
400	−55.641 *	−41.035	−26.070
1000	102.200	89.300 *	132.827

Based on the results, for shear rate equal to 100 s^{-1} and 1000 s^{-1}, the double-exponential model was the best choice for describing the thixotropic behaviour (−39.862 and 89.300) while in case of 400 s^{-1}, lower AIC was reached using single-exponential model despite the difference with double-exponential model is very limited (−55.641 vs. −41.035). Therefore, balancing accuracy and mathematical complexity of the model, double-exponential model can be suitably adopted to describe thixotropic behaviour of TBS in this range of shear stress. The tested matrix shows a first phase in which breaking of particles bonds results in a rapid decrease of its viscosity and a second phase where the decrease of the viscosity value is slower.

5. Conclusions

This work aimed to quantify the influence of non-volatile media on rheological properties of TBS and to better explore the response of thermophilic sludge in case of prolonged shear rate. The results show a significant dependence of TBS from sodium bentonite concentration, while calcium carbonate and sand produced relatively lower influence. TBS response to diverse shear rates for longer period highlighted a thixotropic behaviour which is much pronounced at higher shear rate (1000 s^{-1}). Double exponential fitting model was the best choice in terms of interpolation accuracy of the results and mathematical complexity for describing thixotropic behaviour in case of low (100 s^{-1}; AIC: −39.826) and high shear rate (1000 s^{-1}; AIC: 89.300), while single-exponential model represented the best option in case of medium shear rate (400 s^{-1}) but with limited difference with double-exponential model (−55.641 vs. −41.035).

This study represented a crucial step in gaining insight into the rheological properties of TBS, with special reference to thixotropic behaviour. Future research is needed to clarify a few aspects that have been pointed out in this work. The acquired experimental findings will be useful for managers of high-strength wastewater treatment plants, providing important information for improving the process efficiency.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/article/10.3390/app12105198/s1, Section: Dependence of rheological parameters from the thermophilic sewage sludge composition; Figure S1: Shear stress of TBS sampled from (a) plant 1 and (b) plant 2 as a function of imposed shear rate. Section: Dependence of rheological parameters from the type of NVS; Figure S2: Viscosity as a function of shear rate during TBS conditioning with (a) calcium carbonate, (b) sand, and (c) sodium bentonite. Unconditioned situation is represented by 180 g L^{-1} of TS concentration.

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Nomenclature

AIC: Akaike information criterion; BS: biological sludge; COD: chemical oxygen demand; N-NH_4^+: ammonia nitrogen; N-NO_2^-: nitrite; N-NO_3^-: nitrate; Ntot: total nitrogen; NVS: non-volatile solids; Ptot: total phosphorous; TBS: thermophilic biological sludge; TS: total solids; VS: volatile solids; 3ITT: three interval thixotropic test; WWTP: wastewater treatment plant

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- WS3.1 - Reuse of residues

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How to Produce an Alternative Carbon Source for Denitrification by Treating and Drastically Reducing Biological Sewage Sludge

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Abstract: Minimizing the biological sewage sludge (BSS) produced by wastewater treatment plants (WWTPs) represents an increasingly difficult challenge. With this goal, tests on a semi-full scale Thermophilic Alternate Membrane Biological Reactor (ThAlMBR) were carried out for 12 months. ThAlMBR was applied both on thickened (TBSS) and digested biological sewage sludge (DBSS) with alternating aeration conditions, and emerged: (i) high COD removal yields (up to 90%), (ii) a low specific sludge production (0.02–0.05 $kg_{VS\ produced}/kg_{COD removed}$), (iii) the possibility of recovery the aqueous carbon residue (permeate) in denitrification processes, replacing purchased external carbon sources. Based on the respirometric tests, an excellent biological treatability of the permeate by the mesophilic biomass was observed and the denitrification kinetics reached with the diluted permeate ((4.0 mgN-NO_3^-/(g_{VSS} h)) were found comparable to those of methanol (4.4 mgN-NO_3^-/(g_{VSS} h)). Moreover, thanks to the similar results obtained on TBSS and DBSS, ThAlMBR proved to be compatible with diverse sludge line points, ensuring in both cases an important sludge minimization.

Keywords: wastewater treatment plant; sludge minimization; carbon recovery; thermophilic membrane reactor; nitrate uptake rate tests; respirometric tests; circular economy

1. Introduction

In the European Community, the gradual implementation of the urban wastewater (WW) directive determined an increasing quantity of biological sewage sludge (BSS) production due to a significant increase in discharges into public sewers and the request for greater efficiency of the purification process [1,2]. For the EU-27, in 2005 about 11 million tons of dry sludges were produced [3] and this amount is expected to have now exceeded 13 million tons of dry matter [4,5]. From the data published by ISPRA (Italian Superior Institute for Environmental Protection and Research) in 2018, the urban WW treatment activities produced approximately 3.1 million tons of sludge in Italy alone. Approximately 800 thousand tons generated by the treatment of industrial WW were also produced [6].

For Europe's decisions oriented to circular economy processes, identification of long-term technical-economic strategies that are able to provide answers to this problem is very important [7]. First of all, it is necessary to follow the waste hierarchy detailed in Directive 2018/851/EC, which establishes the rules and priorities in the treatment and management of waste in order to ensure the lowest environmental impact: (i) prevention; (ii) preparation for use; (iii) recycling; (iv) energy recovery; and (v) final disposal [8]. As the legislation points out, the use of landfill disposal should be discouraged, as it is not an effective and efficient approach [9]. The reuse of BSS in soils and the incineration appear to be the main routes adopted [4].

In Europe, in 2015, despite the strong regulatory uncertainties, the main destination was represented by spreading in agriculture (45%), followed by incineration (27%), composting and other forms of reuse (13%), disposal in landfills (8%), and other forms of disposal (3%) [3]. In 2016, in Italy, according to the data published by ARERA (Regulatory Authority for Energy, Networks and the Environment), the BSS recovery has exceeded the disposal (almost, 80% vs. 20%). The 70% of recovered BSS were reused in agriculture, by direct spreading or by composting amending products, while a residual percentage was destined for co-incineration in waste-to-energy plants or cement factories [10].

The prevailing operation of reuse BSS in agriculture is not exempt from critical points such as the presence of harmful substances originally contained in WW [11–13] and the low acceptance by the population, mainly related to the odor impact [14,15]. Furthermore, for instance in Italy, several managers of wastewater treatment plants (WWTPs) reported increasing difficulties in the reuse of BSS as soils conditioner due to the absence of adequate outlets in their respective territories. This has led to a resumption of landfilling or an increase in extra-regional and cross-border flows [10].

Considering that BSS disposal represents a deep problem in the environmental sector, technologies that minimize BSS are of fundamental importance [16]. BSS minimization can be achieved through two main approaches: (i) reducing the production in water line of WWTPs, or (ii) applying technologies in sludge line acting on BSS already produced by biological processes [17].

In this study, results of an innovative biological process applied in sludge line are presented. The Thermophilic Alternate Membrane Biological Reactor (ThAlMBR) is an advanced biological membrane system used to lysate and oxidize the excess BSS produced by WWTP through thermophilic bacteria, under controlled conditions of temperature and aeration. Previous experiments on ThAlMBR pilot plant mainly concerned the treatment of industrial aqueous waste [18–20]. With regard to BSS, only two preliminary experiments have been carried out on TBSS [21,22], never on DBSS. A recent experimentation was conducted on TBSS to evaluate the minimization of BSS produced by a municipal WWTP owing to the application of ThAlMBR. The reduction of BSS was quantified in 89% and 92%, in terms of total and volatile solids fed to the system, respectively [23]. These previous studies have made it possible to approach the optimal operative conditions for the process (hydraulic retention time, organic loading rate (OLR), temperature, etc.), which, however, have never been thoroughly tested for long periods, as was the case in this experimentation which lasted 12 months.

Furthermore, there are no full scale plants equipped with this technology for the treatment of BSS while two aqueous waste treatment plants have been built. The first plant was able to remove almost 90% of COD with a specific sludge production of 0.08–0.09 $kg_{VSSproduced}/kg_{CODremoved}$ (VSS: volatile suspended solids) [24]. The second full scale removed 78.2% of COD (operating with an organic loading rate (OLR) between 1.5 and 2 $kg/(m^3d)$, and almost 82% of COD in case of an OLR greater than 3 $kg/(m^3d)$. In this case, a specific sludge production of 0.052 $kg_{VSproduced}/kg_{CODremoved}$ was highlighted [25].

Regardless of the substrate being fed, ThAlMBR produces a carbonaceous aqueous residue (permeate). In recent work, the feasibility of reuse permeate, from ThAlMBR fed with aqueous waste, in denitrification processes as alternative carbon source was proved [25]. If the amount of organic substance in the untreated WW is too low in relation to the required COD:N ratio, the addition of an external carbon source becomes necessary to increase the yields of the denitrification process [26,27]. This criticality can occur with post-denitrification but also with pre-denitrification schemes where the incoming effluent is particularly lacking in organic matter. Generally, the purchase of an external carbon source (e.g., methanol, ethanol, acetic acid, glucose), necessary to increase denitrification kinetics [27,28] represents the most significant costs in WWTPs management together with the disposal of the BSS produced [29,30].

As alternative sources of carbon, in order to guarantee economic savings and a lower environmental impact, WW from food, confectionery, dairy, and beverage production processes can be used, owing to the high content of organic carbon that is easily biodegradable [31,32]. An important disadvantage could be the inconstancy of both qualitative and quantitative characteristics of this industrial WW, related to the variety of production cycles [28].

In this work, biological treatability, with oxygen uptake rate (OUR) tests, and denitrification kinetics, with nitrate uptake rate (NUR) tests, were evaluated. The feasibility of reusing the permeate within the WWTP itself as an alternative caron source in denitrification, to move forward a water resource recovery facility (WRRF), from the limited vision of a WWTP intended for WW treatment only [33,34] was explored.

Moreover, the minimization of BSS production by means of ThAlMBR was investigated first, focusing also on its possible placement in the WWTP sludge line. Subsequently, attention was paid to the possible total or partial replacement of external carbon sources in the WWTP water line. Tests on a semi-full scale plant were carried out for 12 months. ThAlMBR was applied both on thickened (TBSS) and digested biological sewage sludge (DBSS).

2. Materials and Methods

2.1. ThAlMBR Pilot Plant

ThAlMBR pilot plant consisted of a thermally insulated biological reactor (volume: 1 m^3). An ultrafiltration (UF) system allowed the separation of the oxidized aqueous residue (permeate) from the active thermophilic biomass. The UF retentate could be either recirculated upstream of the UF membranes or introduced into the thermophilic biological reactor. The membrane line included seven tubular ceramic membranes with a molecular cut-off of 300 kDa. More detailed information was reported by Collivignarelli et al. [19]. The presence of materials with a particle size of the order of a few mm could obstruct the membranes, therefore, before entering the UF unit and in correspondence with the recirculation line, bag filters are positioned to ensure a coarse filtration.

The experimentation was conducted alternating aerobic/anoxic conditions to promote cell lysis processes and biological oxidation of the fed BSS. Due to the formation of biological foams, aerobic/anoxic cycles were balanced preliminary testing diverse solutions. 2 h of anoxic conditions followed by 6 h of aerobic conditions represent the optimal and stable solution in terms of reduction of biological foams and was chosen to perform the tests. During the aerobic phases, ThAlMBR worked with pure oxygen injected in the recirculation line. The dissolved oxygen and the temperature of the biological reactor were monitored by a submerged probe (Oxymax W COS31, Endress + Hauser, Reinach, Switzerland), and maintained between 2–6 mg/L and 47–53 °C for oxygen and temperature, respectively.

The system worked in autothermal mode owing to the development of heat produced by the oxidation reactions of the BOD (exothermic reactions). The plant was also equipped with a heat exchanger, necessary for cooling the reactor in case the exothermic oxidation reactions raise the temperature over 50 °C.

This work considers results obtained only during stable operating conditions, neglecting the fluctuations due to the start-up phase.

2.2. ThAlMBR Placement in WWTP Sludge Line

ThAlMBR pilot plant was tested in two urban WWTPs located in Northern Italy (130,000 and 70,000 population equivalent, respectively). Two WWTPs with conventional active sludge (CAS) system, with a complete sludge line, and without industrial drains in WW entering the WWTPs, were chosen. As shown in Figure 1, two scenarios were identified as the ThAlMBR was introduced at diverse points of sludge lines to evaluate the feasibility of the process in minimizing the BSS. In scenario A (Figure 1a), the sludge line featured a static thickener, a dynamic thickener with polyelectrolyte dosage, an anaerobic digester, and a dewatering treatment by centrifuge. In this scenario, the pilot plant was placed downstream of the static thickener. In Scenario B (Figure 1b), ThAlMBR was placed downstream of the anaerobic digester.

In both cases, the permeate was investigated as alternative carbon source for denitrification processes.

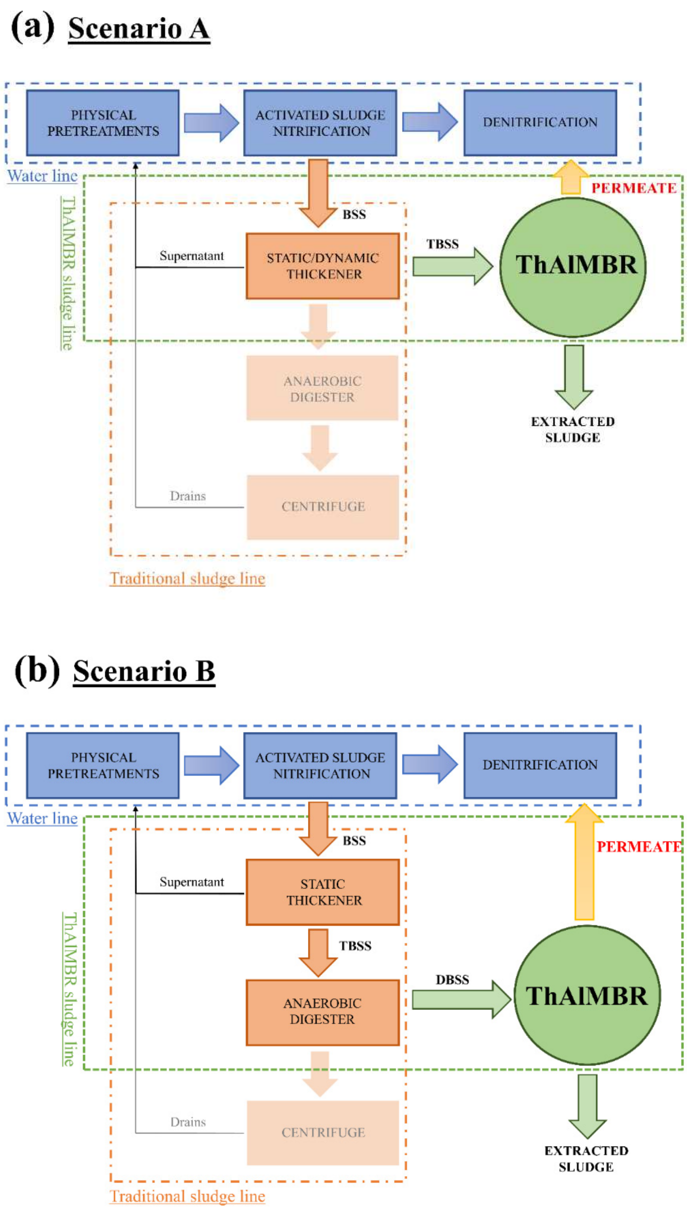

Figure 1. Applications of ThAlMBR in sludge line. (**a**) Scenario A: downstream of the thickener, (**b**) Scenario B: downstream of the anaerobic digester. BSS: biological sewage sludge, TBSS: thickened biological sewage sludge, DBSS: digested biological sewage sludge, VS: volatile solids.

2.3. TBSS and DBSS Treated by ThAlMBR

TBSS was sampled after the static thickener of plant A, before the addition of polyelectrolyte as a flocculant at the dynamic thickener (Scenario A, Figure 1a). DBSS was sampled after the anaerobic digestion of BSS produced by plant B (Scenario B, Figure 1b).

Table 1 shows the chemical and chemical-physical parameters of the two BSS fed to the process. The average values of the measurements taken during the experimentation, and the confidence interval were reported.

Table 1. Chemical and physico-chemical parameters of TBSS and DBSS.

Parameter	TBSS	DBSS
	Mean Value ± Confidence	**Mean Value ± Confidence**
COD [mg/L]	32,348 ± 1488	35,223 ± 2706
TN [mg/L]	1173 ± 84	914 ± 260
$N\text{-}NH_4^+$ [mg/L]	548 ± 51	251 ± 77
$N\text{-}NO_x$ [mg/L]	3.4 ± 1.2	n.d.
TS [g/L]	20 ± 2	24 ± 4
VS [g/L]	14 ± 1	14 ± 2
VS/TS [%]	71 ± 2	57 ± 4
pH [-]	5.5 ± 0.1	6.5 ± 0.3
Electrical conductivity [µS/cm]	2586 ± 182	3345 ± 439

COD: chemical oxygen demand; TN: total nitrogen; TS: total solids; VS: volatile solids; n.d.: not detected.

These substrates were almost equivalent from a chemical point of view. The only difference concerned the value of the inert solid residue, greater in DBSS due to the accumulation of inert material in the digester where samples were taken, and the ammoniacal nitrogen concentration, more than double in TBSS. The pH of TBSS was slightly more acidic, but this had no effect on the pH of the process which has settled around neutrality (Table 2).

Table 2. Operative conditions in the thermophilic reactor with TBSS and DBSS.

Operative Parameter	TBSS	DBSS
	Mean Value ± Confidence	**Mean Value ± Confidence**
TS [kg/m^3]	62 ± 2	77 ± 6
VS [kg/m^3]	28 ± 1	30 ± 2
VS/TS [%]	45 ± 1	41 ± 3
HRT [day]	10 ± 1	10 ± 1
OLR [kgCOD/(m^3 d)]	3.2 ± 1.7	3.4 ± 0.2
T [°C]	50 ± 2	48 ± 2
pH [-]	6.7 ± 0.1	7.1 ± 0.4

TS: total solids; VS: volatile solids; HRT: hydraulic retention time; OLR: organic loading rate; T: temperature.

2.4. Operative Conditions

Table 2 shows the operative conditions inside ThAlMBR during the experimentation with TBSS and DBSS.

TBSS and DBSS were fed continuously for 10 months and 2 months, respectively. Initially, only the minimization of TBSS and the reuse of the permeate produced were taken into consideration. Following the obtained results, it was decided to deepen the study on the treatment of DBSS to evaluate the possible double applicability of ThAlMBR, representing the second phase a consolidation of an already tested process on TBSS. This explains the diverse duration of the two experimentation phases.

The total solids (TS) in the reactor were kept no more than 80 kg/m^3 and their increase was managed through controlled extractions of thermophilic sludge. In fact, a total solids value greater than 100 kg/m^3 could have compromised the hydrodynamics, the mechanicals, and the hydraulics of the pilot plant, as well as the change in rheological characteristics of the thermophilic sludge with consequent management problems.

In both sludges the OLR was the same (almost 3 $kg_{COD}/(m^3 d)$). The hydraulic retention time (HRT) of the system was maintained around 10 d. The pH was close to neutrality, an optimum condition for thermophilic bacterial species.

2.5. Analytical Methods

The analytical parameters, in the fed TBSS and DBSS, in the thermophilic sludge and in the permeate, were monitored using official methods recognized internationally. COD was determined with the method proposed by ISO 6060: 1989 [35], total nitrogen (TN) was monitored with CNR-IRSA [36]. For ammoniacal nitrogen ($N\text{-}NH_4^+$) the method of APAT IRSA-CNR 4030 A1:2003 was used [37]. Organic nitrogen was calculated starting from the measurements of TN and $N\text{-}NH_4^+$, the nitric and nitrous nitrogen being negligible. Total solids (TS) were determined using UNI EN 14346:2007 [38] and volatile solids using UNI EN 15169:2007 [39].

Electrical conductivity was daily measured using the probe WTW-IDS, model TetraCon® 925 (Xylem Analytics Germany Sales GmbH & Co, Mainz, Germany). pH was daily measured using the probe WTW-IDS, Model SenTix® 940 (Xylem Analytics Germany Sales GmbH & Co, Mainz, Germany).

2.6. Respirometric Tests

2.6.1. OUR Tests

OUR tests were performed as an index of metabolic-enzymatic activity of a biological system, as suggested by Hagman et al. [40] and Kristensen et al. [41]. The respirometric tests of OUR were used to evaluate the biological treatability of the permeate in the mesophilic field. A mesophilic biomass from a traditional CAS process was used and endogenous OUR tests with biomass alone were first conducted to understand its health status. Subsequently, exogenous OUR tests were carried out by contacting the mesophilic biomass and the substrates (BSS fed or ThAlMBR permeate), in equal volume ratio. Total of 500 mL of biomass was aerated for 30 min up to a dissolved oxygen concentration of about 7 mg/L and then the aeration was stopped, mixed with 500 mL of substrate and the beaker was hermetically closed to prevent the entry of oxygen from the external environment. The substrate either undiluted or suitably diluted with distilled water was added with the aim of evaluating a possible toxic-inhibiting effect of the substrate against the mesophilic biomass. Continuous stirring was maintained (about 400 RPM). All tests took place at room temperature. The OUR value was calculated considering the concentration of VSS in the tested sample and the slope of the oxygen consumption curve [41].

To globally assess the performance of ThAlMBR process, an index has been proposed, GPI (global process index), which is a dimensionless number between −1 and 2. In particular, the index aims to investigate the increase/decrease in the permeate biodegradability compared to the BSS fed to ThAlMBR, in relation to the COD removal performance in ThAlMBR process. The Equation (1) has been used to calculate the GPI:

$$\mathrm{GPI}\ [-] = \frac{\mathrm{OUR_{OUT}} - \mathrm{OUR_{IN}}}{\mathrm{OUR_{OUT}} + \mathrm{OUR_{IN}}} + \frac{\mathrm{COD_{IN}} - \mathrm{COD_{OUT}}}{\mathrm{COD_{IN}} + \mathrm{COD_{OUT}}} \tag{1}$$

where:

- $\mathrm{OUR_{IN}}$ [$\mathrm{mg_{O_2}/(g_{VSS}\ h)}$] represents the OUR of the BSS fed to ThAlMBR;
- $\mathrm{OUR_{OUT}}$ [$\mathrm{mg_{O_2}/(g_{VSS}\ h)}$] represents the OUR of ThAlMBR permeate;
- $\mathrm{COD_{IN}}$ [mg/L] represents the COD of the BSS fed to ThAlMBR;
- $\mathrm{COD_{OUT}}$ [mg/L] represents the COD of ThAlMBR permeate.

The more the GPI value was close to 2, the greater was the biodegradability of the permeate compared to that of the starting BSS and the removal of COD carried out by ThAlMBR in the BSS. The more the index value was close to −1 the more the carbon in the permeate showed a poor biological degradation (certainly lower than that of BSS fed to ThAlMBR). GPI remained almost 1 if there is: (i) an excellent degradation of COD with a reduction in the biodegradability of the permeate, or on the contrary, (ii) a reduced reduction of organic carbon and an important improvement of the biodegradation of the permeate. The latter case can be linked to the presence of organic substance which is difficult to biodegrade by the thermophilic biomass in the BSS fed to the process. However, during the stay of the BSS in the reactor, the thermophilic biomass should be able to simplify the structure of the COD making it easily biodegradable in the permeate.

The reference substrate was represented by the BSS entering ThAlMBR. Therefore, with the GPI index it is possible to evaluate how ThAlMBR modified the biodegradability of the treated BSS.

2.6.2. NUR Tests

NUR tests were performed to evaluate the feasibility of using the permeate as an external source of organic carbon for denitrifying bacteria. The biomass used was taken from a denitrification process present in a real WWTP and both methanol (as an external source of organic carbon of reference) and the permeate suitably diluted with distilled water were used as substrates. Ammonia in permeate was stripped before tests to avoid possible inhibition of microorganism. The method described also in our previous study was used [25]. Total of 500 mL of biomass was aerated for 30 min and mixed with 500 mL of substrate, enriched with nitrates using KNO_3 to obtain an initial N-NO_x concentration in the starting sample of approximately 50 mg/L. The system was kept in constant stirring (about 400 RPM) and was hermetically sealed to avoid the solubilization of atmospheric oxygen. The pH was maintained around neutrality with the addition of H_2SO_4 if necessary. The tests lasted a total of 6 h, and every hour 25 mL of sample was taken and filtered for chemical analyses (COD, N-NO_x^-). The NUR was evaluated considering the concentration of VSS in the tested sample and the slope of the nitric and nitrous nitrogen consumption curve was determined.

3. Results and Discussion

3.1. Performance and Sludge Minimization

The organic substrate fed was oxidized by the thermophilic bacteria present inside the reactor. Figure 2 shows the COD removal yields calculated comparing the inlet (TBSS and DBSS) and outlet (permeate) concentrations from the process. Specifically, Figure 2a shows the COD removal in the first part of the experiment with TBSS, in which an average removal efficiency of 92% was achieved. While Figure 2b shows the COD removal yields in DBSS, with an average pilot plant performance of 91%. The results relating to the COD removal yields are comparable between the two sludges. As there were no particularly noticeable differences, it is possible to state the excellent applicability of the ThAlMBR to both TBSS and DBSS.

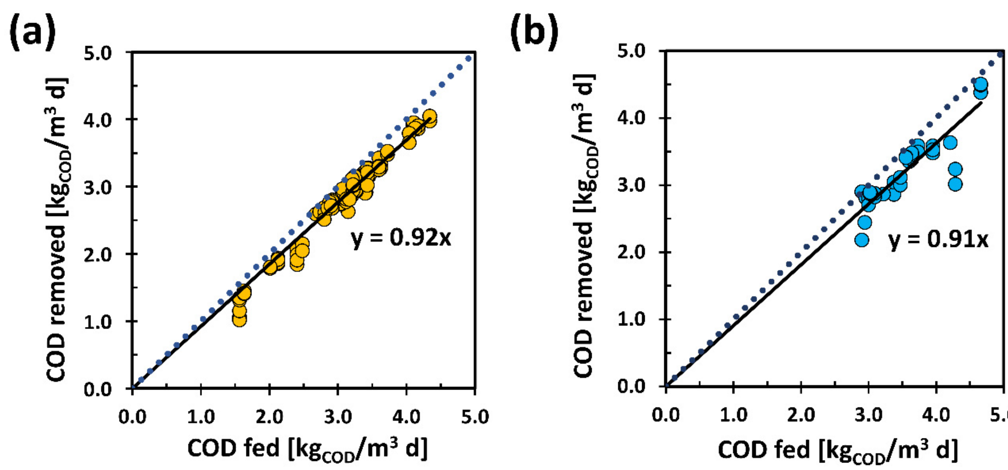

Figure 2. COD removal yields with TBSS (**a**) and DBSS (**b**). The continuous black lines represent the fitting of COD removed as a function of COD fed while the dot blue lines represent the condition of complete COD removal.

It is important to underline that the COD of the permeate did not have significant variations in terms of concentration (2318 ± 155 mg$_{COD}$/L and 3400 ± 855 mg$_{COD}$/L, in case of TBSS and DBSS, respectively). This result was indicative of a stable operation of the process which ensured an output permeate with uniform and unchanged qualitative and quantitative characteristics. It constituted a not negligible aspect in view of a possible reuse of the aqueous residue which continued to maintain an important residual COD considering the treatment with a mesophilic biomass.

The results reported in Figure 2 were obtained under comparable conditions of average OLR: 3.2 ± 1.7 $kg_{COD_{IN}}/(m^3\ d)$ for TBSS and 3.4 ± 0.2 $kg_{COD_{IN}}/(m^3\ d)$ for DBSS. The COD removal yields obtained with ThAlMBR were completely similar or superior to those of the MBR systems reported in the scientific literature. For example, a COD removal efficiency of 62–79% was found treating landfill leachate with an aerobic thermophilic MBR [42]. From other types of aqueous waste, ThAlMBR removed almost 78% of COD with an OLR of 3–6 $kg_{COD_{IN}}/(m^3\ d)$ [43] and proved to be able to remove up to 94% with HRT of 10 d [20]. Considering the treatment of thickened biological sludge by means of a thermophilic process with alternating oxygen cycles, COD removal values of 57% were achieved with an average HRT of 20 days and an OLR of approximately 1.4–1.8 $kg_{COD_{IN}}/(m^3\ d)$ [21]; even higher up to 85% with HRT of 13–14 days and OLR of almost 2 $kg_{COD_{IN}}/(m^3\ d)$ [22].

ThAlMBR is usually a powerful ammonia producer, as has already been observed in previous experiments [18,22], capable of converting organic nitrogen into ammonia through transamination reactions [44]. Similar behavior was also observed in this experiment on TBSS and DBSS. The conversion of organic nitrogen, introduced with BSS, into ammoniacal nitrogen is clearly visible in Figure 3. The subdivision of total nitrogen into its various forms has been represented, as average values on the experimental phases. The contribution of $N\text{-}NO_X$ during the experimentation had a negligible weight given its concentrations involved between 1 and 10 mg/L. Therefore, in the distribution of nitrogen forms, only the forms of organic and ammoniacal nitrogen were considered.

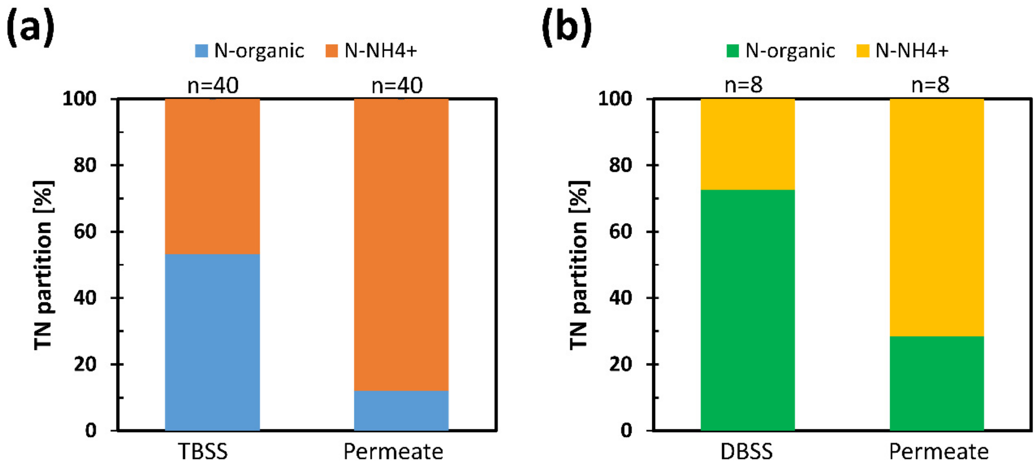

Figure 3. TN partition (**a**) in TBSS and (**b**) in DBSS. TN: total nitrogen, TBSS: thickened biological sewage sludge, DBSS: digested biological sewage sludge, n: number of data.

In TBSS, the total nitrogen entering ThAlMBR is made up equally of organic and ammoniacal nitrogen, while in DBSS the prevalent form of nitrogen is organic (about 70%). In both cases, the ammonification phenomena due to the thermophilic biomass is evident in the permeate output. The ammonification process was investigated in more detail by evaluating the production yields of ammoniacal nitrogen and calculating the amount of organic nitrogen converted. The results are shown in Figure 4.

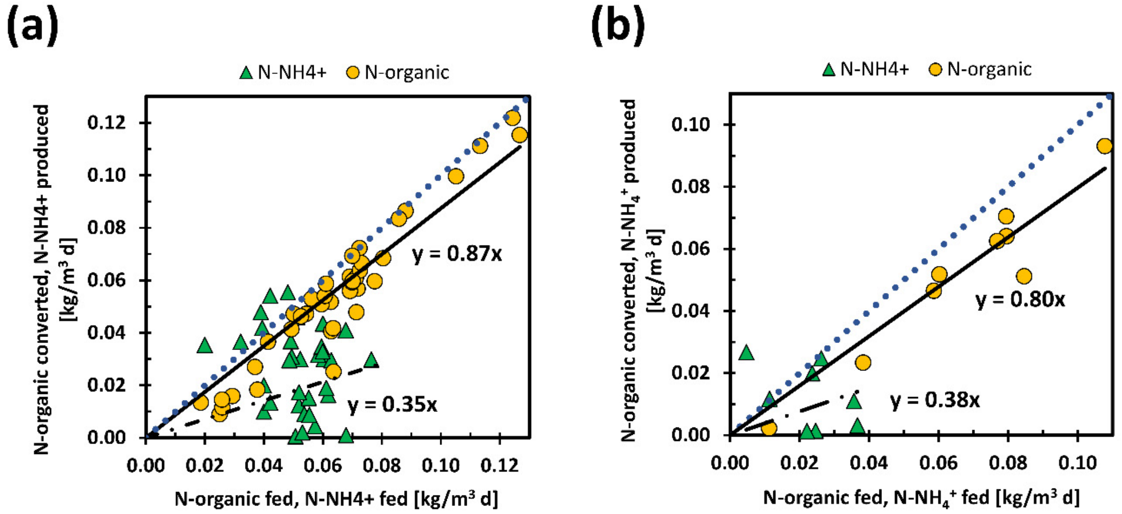

Figure 4. Conversion rates of N-organic in N-NH_4^+ with TBSS (**a**) and with DBSS (**b**). The black lines represent the fittings while the dot blue lines represent the condition of complete pollutant removal.

A reduction of organic nitrogen between inlet and outlet in the 80–90% range was evaluated, 87% and 80% for TBSS and DBSS, respectively. About ammoniacal nitrogen, production yields of 35% for TBSS and 38% for DBSS were obtained. In ThAlMBR, the amount of ammonia produced should not deviate much from the amount of organic nitrogen converted, although percentage differences of approximately 40–50% were observed in this experimentation. A portion of the organic nitrogen was certainly used by thermophilic bacteria for metabolic functions, but, in particular the production of ammoniacal nitrogen seemed to be underestimated, as was also observed in our previous tests [45]. This can be attributed to the stripping phenomena to which ammonia was subjected following the high temperatures and pH of the process, sometimes higher than neutrality.

For the evaluation of the specific sludge production in ThAlMBR, no distinction between the periods of feeding TBSS and DBSS has been done, as the process did not present significant differences. For the calculation of the specific production of sludge (i) the thermophilic biomass extractions carried out to keep the value of TS of the process approximately constant and (ii) the quantity of sludge lost during ordinary and extraordinary maintenance operations of the plant were considered. Ordinary maintenance operations mainly concerned the daily cleaning of the pre-filters present both in the recirculation line and in the UF line. The specific sludge production was therefore calculated considering, in addition to the TS and vs. extracted and lost, also the COD removed. ThAlMBR sludge production was 0.05–0.08 $kg_{TSproduced}/kg_{CODremoved}$ and 0.02–0.05 $kg_{VSproduced}/kg_{CODremoved}$. Results were comparable to those of some previous experiments on industrial aqueous waste: 0.04 $kg_{VSproduced}/kg_{CODremoved}$ [19,45] and 0.09 $kg_{VSproduced}/kg_{CODremoved}$ [18]. However, the results of this experimentation were better than those reported by Simstich et al. [46] for thermophilic aerobic MBRs where the specific sludge production were 0.07–0.29 $kg_{MLSS}/kg_{CODremoved}$ and 0.03–0.09 $kg_{MLVSS}/kg_{CODremoved}$ (MLSS: mixed liquor suspended solids, MLVSS: mixed liquor volatile suspended solids).

For this same type of process, Suvilampi and Rintala [47] indicated values equal to 0.12–0.16 $kg_{TSS}/kg_{CODremoved}$. On the other hand, for a mesophilic MBR the sludge production can be generally higher than the thermophilic conditions, such as 0.10 $kg_{VSSproduced}/kg_{CODremoved}$ [48] up to 0.19 $kg_{VSSproduced}/kg_{CODremoved}$ [49].

3.2. OUR Tests

Aerobic biomass uses oxygen to perform catabolic/anabolic functions and to activate the biological oxidation processes of organic pollutants. For comparing the biodegradability of different substrates, OUR data are an important tool. Highly biodegradable substrates determined a high demand for oxygen in the short term, on the other hand, poorly biodegradable substrates have much lower oxygen consumption rates. One of the objectives of the experiment was to evaluate the possible reuse of the permeate as an external carbon source in a denitrification process in a WWTP water line. Therefore, the biodegradability of the aqueous residue produced has been compared with that of the fed substrate. OUR tests were performed using a mesophilic biomass taken from a CAS system. In the discussion of the results, no distinction is made between TBSS and DBSS as the trend of the results was comparable. Figure 5 shows the results of the OUR and GPI values obtained from the various tests.

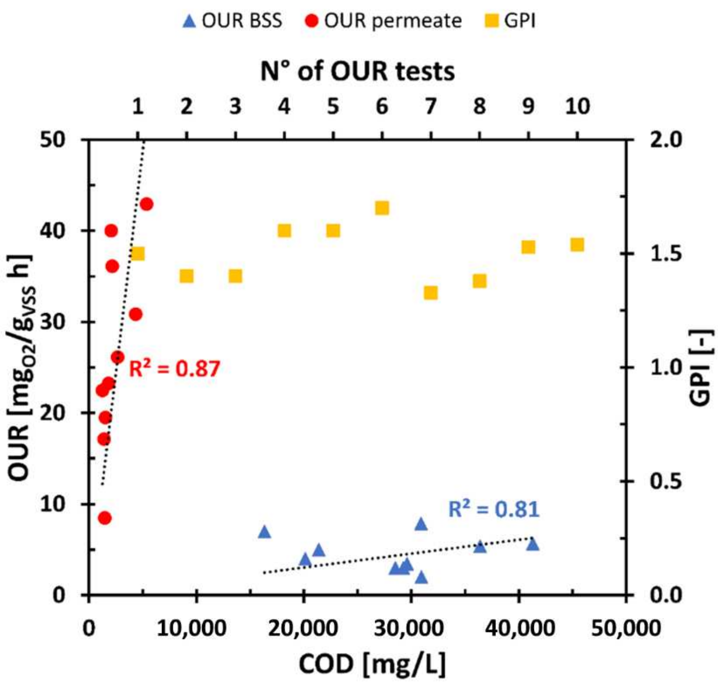

Figure 5. Trend of OUR and GPI. Dot black lines represent the linear fitting of OUR values as a function of COD. Yellow squares indicate the GPI values in ten diverse OUR tests.

The results obtained showed that OUR of BSS fed was always lower than the output permeate. OUR of the permeate reached an average value of 31.1 $\pm$ 6.6 $mg_{O_2}/(g_{VSS}\ h)$ against an average value of 4.0 $\pm$ 1.1 $mg_{O_2}/(g_{VSS}\ h)$ of the fed BSS. Similar results were obtained by Collivignarelli et al. [18], using a permeate deriving from the treatment of industrial aqueous waste. This difference between inlet and outlet to the reactor seemed to be due to an increase in biodegradability achieved by the thermophilic process. Despite the important reduction of COD carried out by ThAlMBR, the permeate contained residual organic substances highly biodegradable from the mesophilic biomass. ThAlMBR oxidize the fraction of COD more difficultly biodegradable from a mesophilic biomass, leaving in the permeate substances more easily biodegradable from a CAS.

The GPI values were also shown in Figure 5. GPI has always assumed positive values, between 1.25 and 1.5. These results showed a constant trend of the excellent performance of the process, both in terms of COD removal and increase in biodegradability. In this way, the excellent availability of usable organic carbon from a mesophilic biomass in the permeate was confirmed, already visible from the OUR values.

The most important result was the demonstration of an increase in the biodegradability of the permeate compared to that of the fed BSS. The COD of the permeate (around 2000–3000 mg/L), despite being of an order of magnitude lower than the COD of the fed BSS, was found to be more degradable by the mesophilic biomass. Therefore, ThAlMBR guaranteed an important reduction of COD but at the same time remaining organic substance was highly biodegradable promoting possible reuse of the permeate.

Any inhibiting substances present within the permeate, resulting from the catabolism processes of COD, can affect the OUR value. In this regard, respirometric tests were conducted on the fed BSS and the permeate diluted at diverse concentrations. The trends are shown in Figure 6.

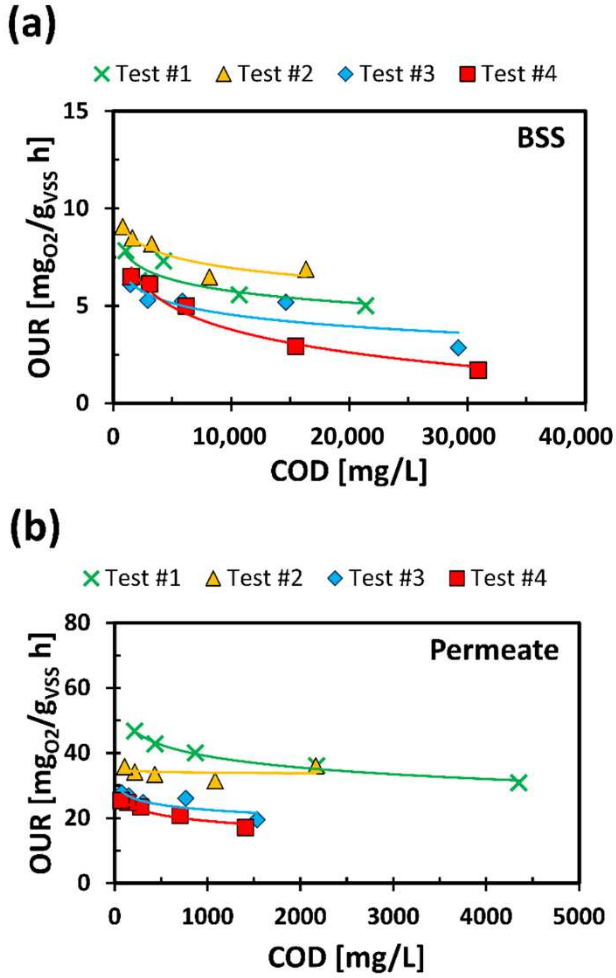

Figure 6. OUR trend with COD, for fed BSS (**a**) and permeate (**b**). Continuous lines represent the fitting of OR as a function of COD.

Each curve represents a test carried out on the same BSS or permeate sample, respectively. For each test, the sample was tested not diluted (associated with the major COD), and at least with three dilutions (COD decreasing with the dilution increasing). As the dilution factor increased, the OUR values increased and therefore the biodegradability of the tested substrates. Trends of this type are characteristic of substrates with a toxic-inhibiting effect on biomass [50]. This effect is linked to the presence of harmful substances in the tested substrate which could disturb and, in the worst cases, inhibit the biomass put in contact with the substrate. The results showed that the toxic-inhibiting effect can be reduced by suitably diluting the analyzed substrate and the increase in OUR with the dilutions was not excessively marked, demonstrating a reduced acute toxicity for mesophilic biomass.

The feedback obtained from OUR tests, in addition to supporting the thesis of a good complementarity between CAS system and a thermophilic process, has shown that ThAlMBR process does not worsen the toxic-inhibiting effect of the permeate against a traditional mesophilic biomass, compared to those of BSS.

3.3. NUR Tests

Through the NUR tests it was possible to verify the effect of the permeate on the denitrifying biomass, which is an important aspect in case of reusing the permeate as an external source of carbon in denitrification process. As for the OUR, NUR tests were performed by diluting the permeate. For the dilutions, the water entering the denitrification section of a full scale WWTP was used to recreate the real operating conditions. The results of the NURs are reported in Figure 7.

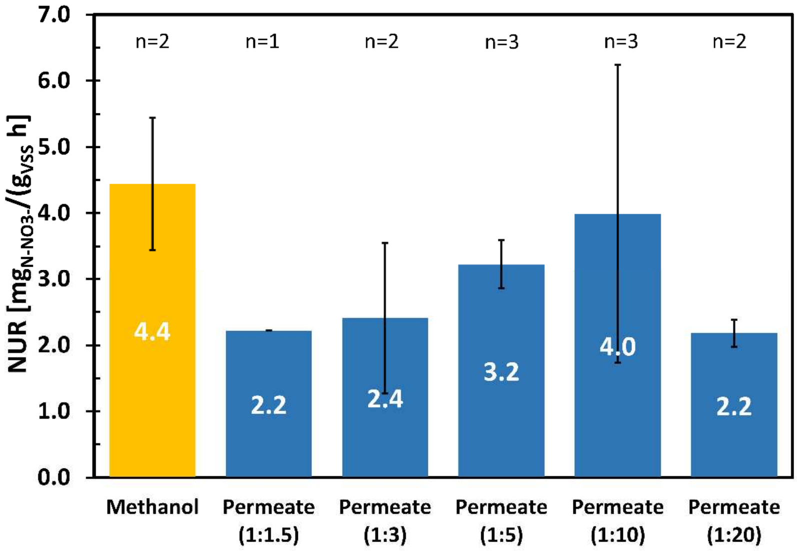

Figure 7. Results of NUR tests. Each test was conducted with the substrate fed to denitrification. The dilution value has been indicated in brackets. n: number of tests.

The substrates used in the NUR tests were permeate in diverse dilutions and methanol. The latter was a highly biodegradable carbonaceous substrate used as a reference, usually purchased in the WWTPs to increase the denitrification kinetics in the post-denitrification processes. The test carried out with methanol could represent a typical situation found in a real WWTP. Different permeate dilutions were tested: 1:1.5, 1:3, 1:5, 1:10, 1:20.

The highest NUR value was obtained with methanol. Of all tests carried out with the permeate, the best result was obtained by diluting the permeate 1:10, as it is comparable with the NUR value of methanol. Tests carried out at lower permeate dilutions (1:1.5; 1:3; 1:5) showed a lower denitrification rate, probably due to a slight toxic-inhibiting effect of the substrate on mesophilic bacteria (resolved by increasing the dilution). The excessive dilution of the permeate (1:20) determined a lower NUR probably due to a low amount of bioavailable COD, necessary for heterotrophic bacteria to carry out denitrification.

The results obtained proved that the permeate can be potentially used as an alternative carbon source in a denitrification process, with results not significantly different from those obtained using methanol. Furthermore, the NUR of the permeate was found to be in line with and in some cases higher than those found in the literature using other types of industrial WW (Table S1).

4. Conclusions

The results of the semi-full scale ThAlMBR allowed to confirm its applicability in the minimization of BSS. In addition, the versatility of the technology was demonstrated for the first time by testing a double location in WWTP sludge lines, both downstream of a thickener and downstream of an anaerobic digester. Specific production of sludge between 0.02 and 0.05 $kg_{VSproduced}/kg_{CODremoved}$ was obtained. The possible recovery of the permeate aqueous residue deriving from ThAlMBR was then investigated by means of OUR and NUR tests. The permeate, owing to (i) its constant qualitative and quantitative characteristics, (ii) its excellent biological treatability in the mesophilic field, and (iii) denitrification kinetics comparable to those obtained using methanol (4.0 mgN-NO_3^-/(g_{VSS} h) with a dilution ratio of 1:10), can be advantageously reused as an alternative carbon source in a post-denitrification process. After stripping in an acid tower for ammonia recovery, the permeate could be recirculated in the WWTP water line hosting ThAlMBR. In this way (i) economic savings, (ii) greater storage safety, and (iii) recovery operation in a circular economy approach applied to water treatment can be guaranteed.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/membranes11120977/s1. Table S1: NUR values obtained from diverse carbon sources.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

BSS	Biological sewage sludge
CAS	Conventional activated sludge
DBSS	Digested biological sewage sludge
HRT	Hydraulic retention time
NUR	Nitrate uptake rate
OLR	Organic loading rate
OUR	Oxygen uptake rate
ThAlMBR	Thermophilic alternate membrane biological reactor
TBSS	Thickened biological sewage sludge
TN	Total nitrogen
TS	Total solids
UF	Ultrafiltration
VS	Volatile solids
WW	Wastewater
WWTP	Wastewater treatment plant

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Assessment of the Impact of a New Industrial Discharge on an Urban Wastewater Treatment Plant: Proposal for an Experimental Protocol

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Abstract: Assessing the compatibility of industrial discharges with the biological process of a municipal wastewater treatment plant (WWTP) may represent a critical task. Indeed, either focusing only on chemical characterization or ecotoxicity tests designed to assess the impact on surface waters may lead to questionable or misleading conclusions. The feasibility of an industrial connection to the sewer should better take into account the features of the downstream WWTP, in particular by studying the potential effects on the biomass of that specific plant. With this aim, a multi-step experimental protocol applicable by water utilities has been proposed: (step 1) calculation of the flow rate/load ratio between industrial discharge (ID) and urban wastewater (WW); (step 2) analysis of the modified operating conditions of the biological stage; (step 3) experimental assessment of the impact of the ID on the WWTP biomass by means of respirometric tests. An application of this protocol is presented in this work as a case study, namely a new ID (average flowrate 200 m^3 d^{-1}) coming from an aqueous waste treatment plant (AWTP) to be connected to the public sewer. The integrated evaluation of results showed that no negative impacts could be expected on the downstream urban activated sludge WWTP (treating a flow rate of around 45,000 m^3 d^{-1}).

Keywords aqueous waste; public sewer; respirometry; respirogram; multi-OUR

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1. Introduction

Both domestic wastewater (WW) from residential settlements and services, and industrial WW, deriving mainly from commercial activities and generated by production processes, are discharged into the public sewers to be treated by a wastewater treatment plant (WWTP) [1–3]. National regulations have imposed emission limits for WW discharge into sewers, as in Italy with the Legislative Decree No. 152 of 2006 (Third part, Annex 5, [4]). Considering the industrial WW, attention is therefore placed on the quantitative and qualitative characteristics of the industrial discharge (ID) which mixes with the other WW conveyed by the sewer. For example, according to the Italian legislation, the emission limits in sewer per product unit referred to specific production cycles are reported (Third part, Annex 5, [4]).

In addition to necessitating various limits to be respected by several parameters, the Italian law requires the same toxicity test to be applied for discharges both into surface water and into the sewer. The acute toxicity test is mandatory and must be performed on *Daphnia magna*, and, in addition, on *Ceriodaphnia dubia*, *Selenastrum capricornutum*, bioluminescent bacteria, or organisms such as *Artemia salina*, for saltwater discharges, or other organisms. For discharges into sewer, the sample is considered toxic when, after 24 h, the number of immobile organisms is equal to or greater than 80% of the total. If more than one toxicity test is performed, the worst result should be considered (Third part, Annex 5, [4]).

The toxicity tests thus imposed appear appropriate for assessing the suitability of a final discharge into a surface water body. Conversely, for a more suitable and reliable evaluation of the compatibility of a discharge into the sewer, the experimental assessment using the biomass present in the downstream WWTP (such as respirometry tests) could be suggested. Furthermore, a non-negligible operational aspect is that the respirometric does not require complex and expensive equipment [5]. In this way, attention is paid to the urban WWTP and the biological treatment that must purify the WW that is discharged into the sewer.

The scientific literature reports that respirometry could be effectively applied, in biological treatments of real WWTP, for (i) daily management and/or operational control to evaluate a possible plant upgrade [6–8], (ii) performance diagnosis [2,9], (iii) detection of toxicity caused by influent WW [1,10–12] and (iv) WW characterisation/COD fractionation [2,3,13]. Arias-Navarro et al. [9] applied respirometric tests to a real WWTP serving an equivalent population of 730,000 and the results revealed that the WWTP was operated at low efficiency and under overload. With a similar aim, in the work of Vitanza et al. [14], an activated sludge model was calibrated using respirometric results obtained from three WWTPs. After the calibration, the simulation of the operation of one of the plants was performed and the goodness of the simulation demonstrated that the model was able to predict WWTP performance. Respirometry techniques were used by Aguilar et al. [12] to evaluate the toxic and inhibitory effect of several heavy metals on the activated sludge collected from a WWTP which also treated industrial WW. The results showed that toxicity caused by heavy metals studied follows the order: Hg ≫ Zn > Cr > Pb > Ni [12].

This work proposes an experimental protocol to be used to assess the compatibility of a new ID to be discharged in a sewer system served by an activated sludge WWTP. The study of the quantitative and qualitative characteristics of ID is proposed to examine and predict any possible impact on the WWTP. The focus is placed on the biological sector, through the control of compliance with the operating conditions and the measurement, through respirometry, of the influent WW biodegradability/toxicity towards autotrophic and heterotrophic biomass.

This study is aimed at responding to some needs of the WW treatment world, such as those expressed by Mainardis et al. [15]:

(i) the proposal and development of a technical-scientific experimental methodology, shared and universally applicable by all water utilities, also to guarantee homogeneous and unambiguous comparisons between different realities;
(ii) the promotion of greater integration and application of respirometry into WWTP management as a diagnostic tool, in particular for ecotoxicity assessments of new industrial sewerage connections.

As a case study, in this work, the application of the proposed procedure is presented for the assessment of the ID of an aqueous waste treatment plant (AWTP) to be discharged into a public sewer served by an activated sludge WWTP.

2. Materials and Methods

2.1. Case Study and ID Characteristics

The studied ID was produced in a new AWTP which encompassed both chemical-physical and thermophilic biological treatments. The aqueous waste treated came mainly from pharmaceutical and galvanic processes. The new ID of the AWTP had to be discharged, with an average flowrate of 200 m^3 d^{-1}, through an authorized pipeline, into the public sewer. In Table 1, the main characteristics of ID are reported. According to the permission, the ID had to be stopped (i) in case of rain, with the aim to not overload the sewage system, (ii) in the case of maintenance to sewage sections of interest and (iii) under specific circumstances upon request by the urban WWTP manager.

The public sewer, as usual, reached the urban WWTP, located downstream of the AWTP, as shown in Figure 1a. The urban WWTP treated an average WW flow rate of about 45,000 m^3 d^{-1} in a water-line typical of a conventional activated sludge (CAS) plant, consisting of primary sedimentation, pre-denitrification, oxidation and nitrification in a single compartment, and finally secondary sedimentation.

Table 1. Qualitative characteristics of AWTP industrial effluent. n: number of data.

	Mean Value	Confidence Interval
pH (−)	10.1 [n: 12]	±0.24
COD (mg L^{-1})	2143 [n: 12]	±85
BOD_5 (mg L^{-1})	317 [n: 12]	±77
TN (mg L^{-1})	154 [n: 5]	±50
N–NO_3^- (mg L^{-1})	15.5 [n: 12]	±3.5
N–NH_4^+ (mg L^{-1})	15.4 [n: 12]	±3.5

The monitoring data of AWTP refer to the year 2019.

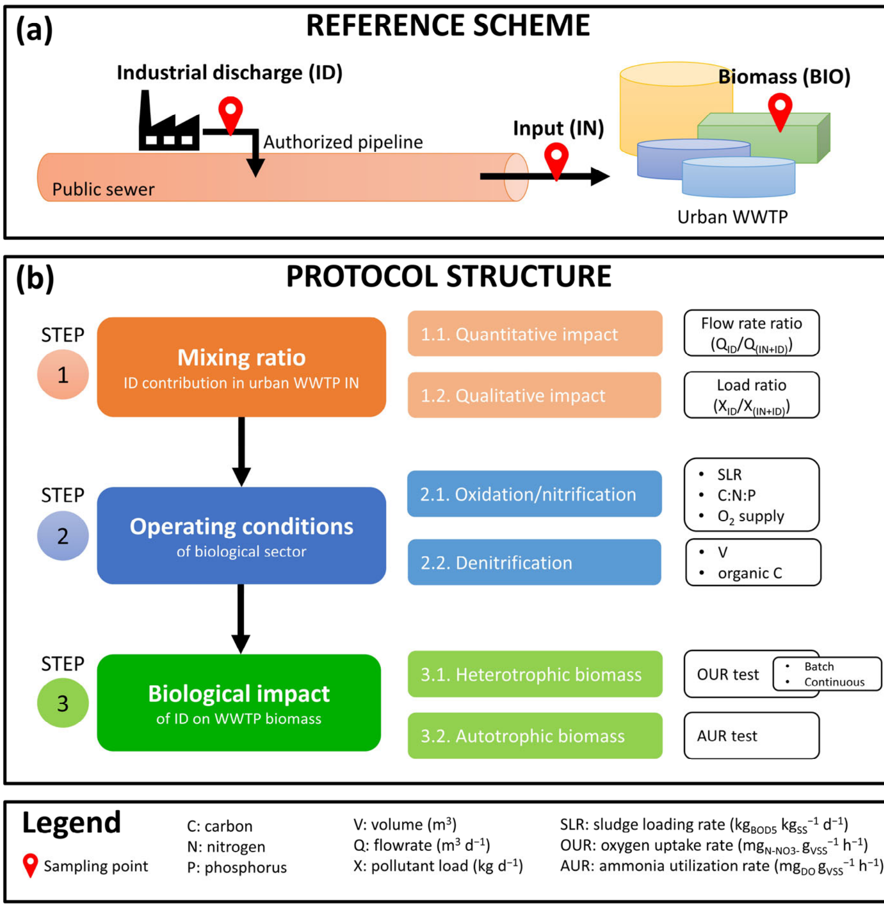

Figure 1. (**a**) Reference scheme of the case study and (**b**) structure of the experimental protocol. ID: industrial discharge; IN: WW entering the urban WWTP; BIO: autotrophic and heterotrophic biomass sampled in WWTP; WWTP: wastewater treatment plant.

2.2. Experimental Protocol Structure

The purpose of the experimental protocol is to evaluate the quantitative and qualitative impact of a new ID on a CAS WWTP. First, the sampling points must be carefully identified:

(i) ID: industrial water which must be discharged into the public sewer.
(ii) IN: WW entering the WWTP (without ID).
(iii) BIO: biomass in the oxidation-nitrification tank.

In the case of a WWTP equipped with primary sedimentation, the IN sample should coincide with the entrance to the biological compartment (primary sedimentation exit), for greater precision in the evaluation of the polluting loads (BOD_5, COD, etc.) that really impact on the biological activity. In this study, the IN sample was taken upstream of the primary sedimentation, considering slightly higher pollutant loads in favour of safety. The purification efficiency of primary sedimentation has therefore been neglected.

If the WWTP had separate oxidation and nitrification compartments, with dedicated biomass separation units, two biomass samples from both reactors would have been required. In this WWTP, a single biomass sample was taken due to the configuration of the process in a single tank.

A further possibility, without considering the sampling point ID, could be to study only the sampling point IN during two different conditions: (i) in the presence of ID and (ii) in the absence of ID. To make the first condition possible, it was necessary (i) to interface with the AWTP staff for the request to the competent authority for a temporary ID permit, (ii) to wait sufficient time to find the ID at the sampling point IN. The minimum time interval needed was calculated starting from the length of the sewer section between the ID and the WWTP.

Figure 1b shows the structure of the experimental protocol.

- Step 1 concerns the estimation of the weight of the ID on the WW to be treated in the WWTP. We assessed (i) the quantitative impact of the ID on the WWTP through the ratio between the ID flowrate Q_{ID} and the mixed WW flowrate $Q_{(IN+ID)}$ (or Q_{INNEW}), and (ii) the qualitative impact of the ID on the WWTP through the ratio between the mass load of selected pollutants in the ID (X_{ID}) and the mixed WW ($X_{(IN+ID)}$ or X_{INNEW}). The pollutants of concern should be identified as the most significant and critical for the WWTP.
- Step 2 includes a check of the operating conditions in which the oxidation/nitrification and denitrification reactors should work with the addition of the new ID into the sewer. First, it is necessary to check the volumes available, in order to verify any possible under-sizing caused by the ID. Subsequently, for the oxidation/nitrification stage, the following checks should be carried out: (i) the new sludge loading rate (SLR) has to guarantee the correct performance of the nitrification process; (ii) the new BOD:N:P ratio should not deviate too much from the optimal one for aerobic systems equal to 100:5:1 (BOD/N = 20, BOD/P = 100) [16–19]; (iii) the capacity of the present air supply system has to cover the increase in the oxygen demand for the oxidation processes. For denitrification, the availability of organic carbon with respect to the new load of $N{-}NO_3^-$ must be envisaged.
- Step 3 involves the study of the ID biological impact on the biomass grown in the urban WWTP and represents a crucial assessment. For a CAS system, the biological activities of heterotrophic and autotrophic biomass can be evaluated through oxygen uptake rate (OUR) and ammonia utilization rate (AUR) tests, respectively. In case potential criticalities arose from Step 1 and Step 2, a more in-depth analysis through the application of continuous respirometry is strictly recommended. In effect, thanks mainly to the longer duration of the test, it is possible to obtain more detailed information on the biological activity, compared to the more immediate and easier to apply batch OUR tests (described below in Section 2.5.2). Indeed, the authors advise to include continuous OUR tests, regardless of the result of point 1.

The step-by-step application of the assessment protocol to the case study is described in the following paragraphs.

2.3. Mixing Ratio Estimate (Step 1)

For the estimation of the future ID contribution on the WW entering the urban WWTP, (i) WWTP monitoring data of the year 2021 and (ii) an estimate of the ID flow rate provided by the AWTP company were considered. The ID quantitative contribution was calculated as follow:

$$\text{ID quantitative impact (\%)}=\frac{Q_{ID}\left(m^3 d^{-1}\right)}{Q_{(IN+ID)}\left(m^3 d^{-1}\right)} \tag{1}$$

where:

- Q_{ID} (m^3 d^{-1}): estimated ID flow rate.
- $Q_{(ID+IN)}$ (or Q_{INNEW}) (m^3 d^{-1}): sum of IN point flow rate, from WWTP monitoring data, and estimated ID flow rate. This represents the overall WW arriving from the sewer and treated by WWTP, if ID is authorised.

As regards the IN-flow rates, the monthly average values were considered.

To study the ID qualitative impact on the urban WWTP, the loads of the most critical polluting parameters for this case study were calculated, namely: chemical oxygen demand (COD), total nitrogen (TN), nitric nitrogen ($N{-}NO_3^-$) and ammoniacal nitrogen ($N{-}NH_4^+$). The authors recommend evaluating the polluting parameters to be taken into consideration on a case-by-case basis, including 5-day biological oxygen demand (BOD_5), total suspended solids, total phosphorus, and other specific pollutants of concern such as heavy metals, surfactants, persistent organic pollutants, etc. The loads were calculated starting from daily concentrations of COD, TN, $N{-}NO_3^-$, $N{-}NH_4^+$ (measured on 24-h composite samples), multiplied by the flow rates. The data of the flow rates and concentrations of the chemical parameters were provided by the management staff of the AWTP and the WWTP. For WWTP data, a public online database is also available from which to download the necessary information. The qualitative contribution of ID was defined by the following equation:

$$\text{ID qualitative impact (\%)}=\frac{X_{ID}\left(kg\ d^{-1}\right)}{X_{(IN+ID)}\left(kg\ d^{-1}\right)} \tag{2}$$

where:

- X_{ID} (kg d^{-1}): load of generic ID polluting parameter.
- $X_{(ID+IN)}$ (or X_{INNEW}) (kg d^{-1}): sum of IN pollutant load, from WWTP monitoring data, and ID pollutant load for the generic parameter. This represents the overall load of WW arriving from the sewer and treated by the WWTP, if ID is authorised.

2.4. Operating Conditions (Step 2)

For the oxidation/nitrification compartment, the following parameters, both for IN and IN_{NEW} (IN + ID) condition, were calculated:

$$\text{1. Sludge loading rate (SLR) } (kg_{BOD_5} kg_{SS}^{-1} d^{-1})= \frac{X_{BOD_5,IN\ BIO}}{SS\ {*}V_{ox}} \tag{3}$$

where:

- $X_{BOD5,IN\ BIO}$ (kg d^{-1}): BOD_5 daily load entering the biological oxidation/nitrification compartment.
- SS (kg m^{-3}): total suspended solids concentration in the oxidation/nitrification tank (hp: equal in both IN and IN_{NEW} phases).
- V_{ox} (m^3): volume of the existing oxidation/nitrification tank.

2. Oxygen supply $\left(kg_{02}\ d^{-1}\right)$= $k*\alpha*X_{BOD_5,IN\ BIO}+\beta*V_{ox}*SS+k*\gamma*X_{nit}$ (4)

where:

- k (−): safety factor to consider for the oscillations of the influent load to the WWTP, assumed equal to 1.5.
- α ($kg_{O2}\ kg_{BOD5removed}$): amount of oxygen required to oxidize 1 kg of organic substance (BOD_5) in the oxidation tank, assumed equal to 0.5 [20].
- $X_{BOD5,IN\ BIO}$ (kg d^{-1}): BOD_5 average daily load entering the biological oxidation/nitrification compartment;
- β ($kg_{O2}\ kg_{SS}^{-1}\ d^{-1}$): amount of oxygen consumed in 1 day by 1 kg of biomass, assumed equal to 0.1 [20].
- V_{ox} (m^3): volume of the existing oxidation/nitrification tank.
- SS (kg m^{-3}): total suspended solids concentration in oxidation/nitrification tank (hp: equal in both IN and IN_{NEW} phases).
- γ ($kg_{O2}\ kg_N$): amount of oxygen required to nitrify 1 kg of ammonia nitrogen, assumed equal to 4.57 [20].
- X_{nit} (kg d^{-1}): nitrogen daily load which must be nitrified, determined by a balance on total Kjeldahl nitrogen (TKN) (Equation (5)):

$$X_{nit}= TKN_{IN}- TKN_{ass}-TKN_{out} \tag{5}$$

where:

- TKN_{IN} (kg d^{-1}): TKN of WW entering the WWTP (if possible, better entering the biological reactor).
- TKN_{ass} (kg d^{-1}): TKN assimilated by the biomass for vital functions, calculated as 5% of BOD_5 removed [21].
- TKN_{out} (kg d^{-1}): TKN limit load in the WWTP effluent and calculated from a concentration limit equal to 2 mg L^{-1}, assumed based on the emission limits of the Italian regulation (Table 2, Annex 5, Part III, Legislative Decree n° 152 of 2006 [4]).

For the pre-denitrification tank the following parameters were determined:

1. Required denitrification tank volume $(V_{DEN})\ (m^3) = \frac{X_{den}}{v_{den,s}*VSS}$ (6)

where:

- X_{den} (kg d^{-1}): nitrogen daily load which must be denitrified, determined by a balance on TN (Equation (7)):

$$X_{den}= TN_{IN}- TN_{ass}-TN_{out} \tag{7}$$

where:

- TN_{IN} (kg d^{-1}): TN of WW entering the WWTP.
- TN_{ass} (kg d^{-1}): TN assimilated by biomass for vital functions, calculated as 5% of BOD_5 removed in the oxidation tank [19].
- TN_{out} (kg d^{-1}): TN limit load in the WWTP effluent and calculated from a concentration limit equal to 8 mg L^{-1}, assumed based on the emission limits of the Italian regulation (Annex 5, Part III, Legislative Decree n° 152 of 2006 [4]).
- $v_{den,s}$ ($kg_{N-NO3^-}\ kg_{VSS}$-1 d^{-1}): denitrification specific rate, determined with the following equation [22,23]:

$$v_{den,s}\left(\frac{kg_{N\text{-}NO_3^-}}{kg_{VSS}\ d}\right)=v_{den,s\ [20\ °C]}*\theta^{(T\text{-}20)} \tag{8}$$

where:

- T (°C): temperature of water in denitrification tank.
- $v_{den,s\ [20\ °C]}$ ($kg_{N-NO3^-}\ kg_{VSS}^{-1}\ d^{-1}$): denitrification rate at 20 °C (standard values: 2.9–3.0 $g_{N-NO3^-}\ kg_{VSS}^{-1}\ h^{-1}$ [24]), assumed equal to 0.07 $kg_{N-NO3^-}\ kg_{VSS}^{-1}\ d^{-1}$.

- θ (−): van't Hoff-Arrhenius coefficient, assumed equal to 1.10 [20].
- VSS (kg m^{-3}): volatile suspended solids concentration in denitrification tank (hp: equal in both IN and IN_{NEW} phases).

$$\text{2. Required organic carbon (OC)}\left(\text{kg d}^{-1}\right)= \frac{BOD_5}{N\text{-}NO_3^-}\ \text{ratio}^*\ X_{DEN} \quad (9)$$

where:

- $BOD_5/N{-}NO_3^-$ ratio (−) necessary to ensure efficient denitrification, assumed equal to 5 [20].
- X_{den} (kg d^{-1}): nitrogen daily load which must be denitrified, determined with Equation (7).

2.5. OUR and AUR Tests (Step 3)

OUR and AUR tests, whose important applicability has been demonstrated in many previous studies [2,25–29], were inevitably included in the protocol structure to study the impact of different substrates on the autotrophic and heterotrophic mesophilic biomass of the urban WWTP. In particular, the aim was to evaluate the effect of the ID on the biological activity of the CAS system.

For all tests, the heterotrophic/autotrophic biomass was sampled directly from the urban WWTP (BIO-sampling point, Figure 1a) and was used shortly after sampling, and after 1 h of re-aeration. The substrates used were sampled only at the IN-sampling point (24-h composite samples), at the urban WWTP, but at two different times: (i) with the presence of ID in WW from the sewer (IN_{NEW}), and (ii) without ID into WW arriving from the sewage system (IN). This approach was possible thanks to collaboration agreements between the AWTP company and the personnel of the WWTP. If this approach was not possible, the authors would have maintained two sampling points IN and ID (as explained in Section 2.2) and recreated the IN_{NEW} substrate in the chemical laboratory by mixing IN and ID. The mixing ratio (quantitative contribution of ID) had been estimated in step 1 of the proposed protocol (Section 3.1). All tests were conducted at room temperature, maintaining continuous stirring of the mixture at about 300–400 rpm, with a magnetic stirrer.

2.5.1. Analytical Methods

COD was measured according to ISPRA 5135 method [30]. For ammoniacal nitrogen ($N{-}NH_4^+$) and TKN, the methods of APAT IRSA-CNR 4030 [31] and APAT IRSA-CNR 5030 [32] were used, respectively. $N{-}NO_3^-$ concentrations were studied according to UNI EN ISO 10304-1:2009 [33]. UNI 11658:2016 [34] was applied for TN. Volatile suspended solids (VSS) were determined following the APAT-IRSA-CNR 2090 method [35]. pH was measured using the probe WTW-IDS, Model SenTix® 940 (Xylem Analytics Germany Sales GmbH & Co, Mainz, Germany). Dissolved oxygen (DO) concentration was measured by WTW Multi-parameter portable meter MultiLine® Multi 3510 IDS thanks to WTW Optical IDS dissolved oxygen sensors FDO® 925 (Xylem Analytics Germany Sales GmbH & Co, Mainz, Germany) (called DO probe in the next sections). The measured DO concentration was transferred to a portable PC via USB connection and the MultiLab® Importer for data acquisition via Excel® software was used.

2.5.2. OUR Test

Respirometry deals with the measurement and interpretation of dissolved oxygen consumption by a biological system to degrade a substrate [36]. The application of respirometry to WWTP can provide information on the characterization of the influential WW biodegradability, therefore, on any inhibitory effect of WW on the WWTP biomass. Both endogenous and exogenous OUR were evaluated in this study. The endogenous OUR represents the oxygen consumed only for biomass respiration, while the exogenous OUR consists in the consumption of oxygen necessary for (i) the oxidation of the organic substance or of the nitrogenous compounds present in the WW and (ii) the cellular respiration of the biomass [15,37].

Batch Test

The great advantage of OUR tests in batch mode is the immediate performance both for the modest instrumentation required (Figure 2) and for the immediacy of execution [2,38]. The experimental set-up was similar to that employed by Borzooei et al. [13] and Capodici et al. [39].

Endogenous OUR tests were first conducted with 500 mL of heterotrophic biomass to study endogenous respiration alone. In exogenous OUR tests, 500 mL of oxidizing biomass was aerated up to a DO concentration of 7.5–8.0 mg L^{-1}, and then mixed with 500 mL of substrate. At this point the aeration was stopped and the laboratory scale batch reactor with a 1 L mixture was hermetically isolated to avoid oxygen exchange with the external environment. During the test, the DO concentration (mg L^{-1}) was measured every 5 s. Each batch OUR test, stopped when the dissolved oxidation concentration was below 2 mg L^{-1}, lasted approximately 10–20 min. Due to the limited duration, the batch OUR was allowed to evaluate "only" (i) the rapidly biodegradable fraction of organic substance and (ii) any acute, therefore immediate, toxic-inhibiting effect of the WW against the biomass.

At the end of the test, the OUR value ($mg_{DO}\ g_{VSS}^{-1}\ h^{-1}$) was calculated according to the following equation:

$$\text{OUR}\ (mg_{DO}\ g_{VSS}{}^{-1} h^{-1}) = \frac{\text{Slope of the DO utilization curve}\ (mg_{DO}\ L^{-1} h^{-1})}{\text{VSS concentration in batch reactor}\ (g_{VSS} L^{-1})} \tag{10}$$

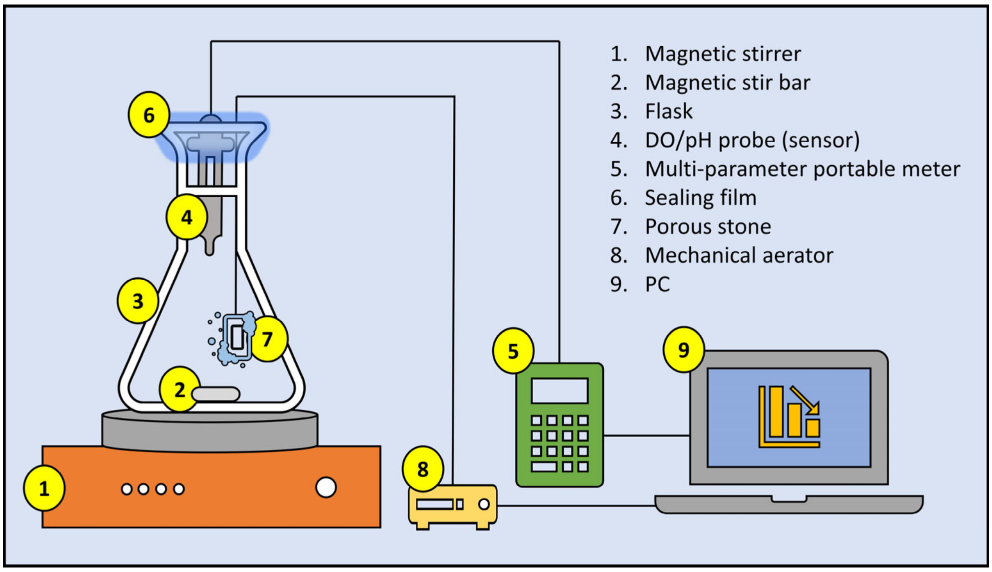

Figure 2. Laboratory respirometric apparatus.

Continuous Test

Continuous OUR tests were carried out with a laboratory scale reactor (2 L), containing 1 L of heterotrophic biomass and 200 mL of substrate. This option was set to maintain a low S/X ratio (biomass/substrate) in the range 0.01–0.2 $g_{COD}\ g_{VSS}^{-1}$, thus considering (i) biomass growth negligible and (ii) endogenous respiration remained approximately constant [40]. Similarly, Mainardis et al. [15] recommended a narrower range equal to 0.01–0.05 $g_{COD}\ g_{VSS}^{-1}$.

The reactor was isolated to avoid oxygen exchanges with the surrounding atmosphere and loss of volume by evaporation (Figure 2). The aeration was always kept in the 2–5 mg L^{-1} range thanks to the connection of the aeration system to an electric mechanism that guaranteed the connection-detachment of the aeration itself. The electrical mechanism was regulated by software installed on a PC and through the DO concentration measured by the DO probe. The latter was in turn connected to the laptop. Of extreme importance was the data acquisition system, which allowed the DO data, measured in the reactor, to be acquired on the PC. In some cases, these tests lasted up to 48 h.

At the end of the test, a succession of decreasing curves (DO consumption curves) was obtained, alternating phases of non-aeration and phases of aeration in the reactor. With the same process described for batch OUR, more final OUR values ($mg_{DO}\ g_{VSS}^{-1}\ h^{-1}$) were calculated. A curve, called respirogram, with the OUR trend over time was drawn [15].

Continuous tests can provide additional information compared to batch tests: (i) possible medium-long term toxic effects caused by the substrate towards the biomass, (ii) evaluation of the different fractions of COD present in the substrate (quickly and slowly biodegradable). Batch tests are quicker to perform, but continuous tests, lasting for several hours, have the great strength of monitoring toxicity over a period as long as the hydraulic retention time of the WWTP [38].

2.5.3. AUR Test

Thanks to the AUR test, it is possible to easily measure the activity of the nitrifying bacteria present in the biomass of CAS plants. The AUR test can be applied to: (i) measure the nitrification kinetics [41], and (ii) evaluate the degree of inhibition (via determination of any inhibitory effect on nitrifying bacteria by sewage containing potentially toxic substances) [42,43].

First, 500 mL of biomass, after aeration, was mixed with 500 mL of substrate to obtain a total volume of 1 L in a batch reactor (Figure 2). Continuous aeration up to DO saturation conditions were maintained in the mixture. The pH was maintained approximately equal to that (7.5–8.5) measured in the mesophilic biomass at the time of sampling in the oxidation tank. Tests were conducted for about 5–6 h and 25 mL of mixture was sampled every hour or half hour. The samples were filtered with 0.45-micron filter paper to separate the biomass. AUR value was determined considering the VSS concentration in the batch reactor and the positive slope of $N{-}NO_3^-$ production curve (negligible concentrations for $N{-}NO_2^-$) as reported in Equation (2):

$$\mathrm{AUR}\ (mg_{N\text{-}NO_3^-}\ g_{VSS}{}^{-1}h^{-1}) = \frac{\text{Slope of N-NO}_3^- \text{ production curve } (mg_{N\text{-}NO_3^-}\ L^{-1}h^{-1})}{\text{VSS concentration in batch reactor } (g_{VSS}L^{-1})} \quad (11)$$

3. Results and Discussion

3.1. Step 1: Mixing Ratio

Table 2 shows flow rates and loads of polluting parameters in terms of (i) average annual values for WWTP input and (ii) available values (either assumed or measured) for ID. The quantitative contribution of ID was defined based on the flow rates. Assuming that the ID was authorized by the competent authority, ID represented 0.45% of all WW arriving at the WWTP ($Q_{INNEW} = Q_{IN} + Q_{ID}$), identifying a low mixing ratio of 1/250. At least from a quantitative point of view, it was possible to state that the ID considered did not represent a problem for the urban WWTP.

The qualitative contribution was obtained from COD and different nitrogenous forms. ID had an almost negligible contribution in terms of TN, $N{-}NH_4^+$, and COD on the WW treated by WWTP, showing no critical issues. Instead, the load of nitrates brought by the industrial WW was significant, on average above 27%. An important load of $N{-}NO_3^-$ could possibly represent a problem for the denitrification compartment, in terms of the insufficient volume available. The subsequent steps envisaged by the protocol had the aim, among others, of further investigating these aspects.

Table 2. Flow rates (m^3 d^{-1}) and loads (kg d^{-1}) of the polluting parameters in ID, IN, IN_{NEW} and mixing ratio of ID in IN_{NEW}. n: number of data.

	ID	IN	IN_{NEW} * (IN + ID)	Mixing Ratio (ID/IN_{NEW})
Q (m^3 d^{-1})	200 ± 50 [set/estimated]	45.575 ± 2053 [n: 12]	45.775 [calculated]	0.45 ± 0.05% [n: 12]
COD (kg d^{-1})	441 ± 43 [n: 12]	12.677 ± 2606 [n: 70]	13.118 [calculated]	3.7 ± 0.7% [n: 12]
TN (kg d^{-1})	28.4 ± 9.3 [n: 5]	1044.6 ± 10 [n: 5]	1073 [calculated]	2.6 ± 0.9% [n: 5]
$N{-}NO_3^-$ (kg d^{-1})	3.0 ± 0.8 [n: 12]	10.1 ± 3.4 [n: 70]	13 [calculated]	27.3 ± 8.5% [n: 12]
$N{-}NH_4^+$ (kg d^{-1})	3.2 ± 0.9 [n: 12]	731.1 ± 94 [n: 70]	734 [calculated]	0.44 ± 0.12% [n: 12]

The monitoring data of AWTP and WWTP refer to the year 2019. * Calculated from the sum of IN and ID mean values.

3.2. Step 2: Operating Conditions of the Biological Stage

Table 3 reports the comparison between geometric and process parameters during the two different conditions studied at the urban WWTP, first in the absence and then with the addition of the new ID in the incoming WW. For the oxidation/nitrification process, no significant changes were observed with the addition of the ID. The main operating conditions have remained unchanged; therefore, the nitrification process should not undergo critical issues, at least due to the aspects just investigated. Only the daily oxygen supply saw a slight increase of 0.8% compared to the starting situation without ID. This increase should not undermine the air supply systems in any way, nor create any problems to be addressed.

More marked variations resulted in denitrification, in particular in the tank volume. Even in the situation without ID, the denitrification volume was undersized for the autumn/winter water temperatures (<15 °C). This criticality has been expanded with the IN_{NEW} condition: at a water temperature of 14 °C, a volume deficit of about 1000 m^3 was estimated. Compared to the previous situation (IN), there was a need for an additional volume of 300 m^3. Despite the under-sizing of the denitrification tank, if the process did not previously present functioning issues, the authors excluded the occurrence of a denitrification crisis with the addition of ID.

As widely known, denitrification by heterotrophic bacteria requires organic carbon (OC) [44–46]. Demand for OC in the IN_{NEW} condition increased by about 4%. Despite this, the BOD_5 entering the biological compartment, therefore the pre-denitrification, has always been more than sufficient to guarantee complete denitrification.

Table 3. Operating conditions at the urban WWTP, without ID (IN) and with ID (IN_{NEW}: IN + ID) into the WW to be treated. SLR: sludge loading rate, V_{OX}: oxidation/nitrification volume, V_{DEN}: denitrification volume, OC: organic carbon.

	IN	IN_{NEW} (IN + ID)
Oxidation/nitrification		
SLR (kg_{BOD5} kg_{SS}^{-1} d^{-1})	0.111	0.109
BOD:N:P	100:26:4	100:26:4
Oxygen supply (kg_{O2} d^{-1})	12′300	12′400
Denitrification		
V_{DEN} (m^3)	6700 (14 °C) 3785 (20 °C)	6990 (14 °C) 3950 (20 °C)

	(Real volume: 6000 m^3)	(Real volume: 6000 m^3)
OC (kg d^{-1})	2500 ($BOD_{5,\ IN\ BIO}$: 4370 kg d^{-1})	2600 ($BOD_{5,\ IN\ BIO}$: 4440 kg d^{-1})

$BOD_{5,IN\ BIO}$ (kg d−1): BOD_5 daily load entering the biological oxidation/nitrification compartment.

3.3. Step 3: Biological Impact on Biomass

3.3.1. Heterotrophic Biomass

The effect of ID of AWTP on heterotrophic biomass of urban WWTP was first evaluated with respirometric tests. Figure 3 shows the results of batch OUR tests. First, to understand the health status of biomass, endogenous OUR, before each exogenous test, was carried out. An average value of 3.8 $mg_{DO}\ g_{VSS}^{-1}\ h^{-1}$ showed a regular respiration of the WWTP mesophilic biomass, according to our previous research [47,48]. VSS in the BIO-sample (Figure 1) were about 1.8–2 g L^{-1}, as adopted in other experimental studies [40].

A domestic WW without industrial aqueous waste was used as the reference substrate (COD: 300–400 mg L^{-1}, in line with domestic sewage COD reported in the literature [49]). The present WW, which includes different industrial discharges, showed lower biodegradability (23 $mg_{DO}\ g_{VSS}^{-1}\ h^{-1}$) than domestic WW (37 $mg_{DO}\ g_{VSS}^{-1}\ h^{-1}$), as expected. Contrarily, the studied ID improved the biodegradability of WW entering the urban WWTP (from 23 $mg_{DO}\ g_{VSS}^{-1}\ h^{-1}$ to 27 $mg_{DO}\ g_{VSS}^{-1}\ h^{-1}$). In general, despite the reduction of OUR values, in respect to the reference, no acute inhibition effects were observed, especially from the ID under study. The reduction in biodegradability between the reference and the IN substrate was due to the integrated and synergistic effect of multiple industrial WWs discharged into the sewer. Nevertheless, in all cases, the mean OUR values were more than acceptable, above 20 $mg_{DO}\ g_{VSS}^{-1}\ h^{-1}$.

Figure 4 reports the results of OUR tests, performed in parallel and continuous modes, with biomass sampled in the oxidation/nitrification tank of the WWTP (BIO-sampling point, Figure 1a). The addition of IN (Figure 4a) and IN_{NEW} (Figure 4b) substrate were tested under the same boundary experimental conditions. Respirograms in Figure 4 show, before the substrate dosage, the endogenous OUR (first point of the discretized respirogram). Time by time, the regular respiration of the biomass must be evaluated, otherwise the test should be invalidated. The values acquired around 2.5–3.5 $mg_{DO}\ g_{VSS}^{-1}\ h^{-1}$ (5–7 mg_{DO} L^{-1} h^{-1} with VSS in BIO-sample of about 1.8–2 g L^{-1}) indicated, as reported in other literature studies [50], a regular endogenous respiration for activated sludge in urban WWTP. After endogenous evaluation, the substrates (COD: 300–350 mg L^{-1}, low S/X ratio guaranteed: 0.03–0.04 $g_{COD}\ g_{VSS}^{-1}$) were dosed and their degradation over time was studied (exogenous OUR—endogenous OUR). The peak values of exogenous OUR, reached after 0.35 h, were similar and equal to 27.2 and 27.0 $mg_{DO}\ g_{VSS}^{-1}\ h^{-1}$, respectively for IN and IN_{NEW}. These were comparable with the results of batch OUR and remained approximately constant from 0.35 h to 0.6–0.65 h for both IN and IN_{NEW}, indicating an immediate and continuous degradation of the easily biodegradable organic substance. After 0.6–0.65 h, exogenous OUR began to decrease, indicating a final phase of degradation of the residual and more slowly biodegradable organic matter. The experimental tests were considered completed once the exogenous OUR reached values close to the endogenous ones.

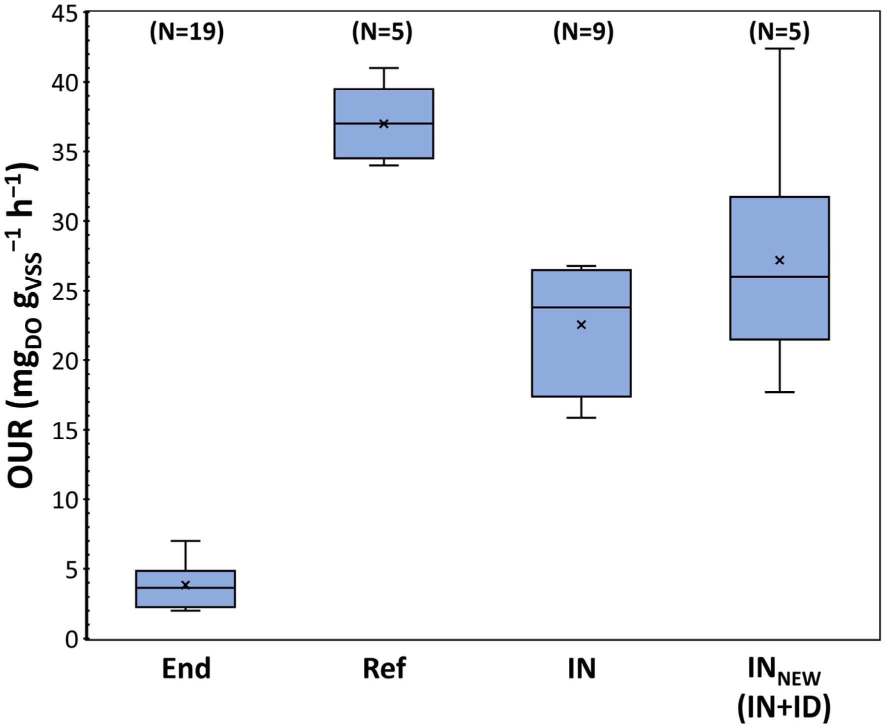

Figure 3. Results of the batch OUR tests carried out with the heterotrophic biomass sampled in the WWTP. End: Endogenous OUR. Ref: Reference value. Boxplots represent the distance between the first and third quartiles while whiskers are set as the most extreme (lower and upper) data point not exceeding 1.5 times the quartile range from the median. The cross represents the mean value. N: number of replicated tests.

In general, exogenous OUR always maintained values above the endogenous, thus indicating the absence of toxic-inhibitory effects towards the biomass. The areas of the exogenous respirograms were about 14.5 mg_{DO} g_{VSS}^{-1} and 16.2 mg_{DO} g_{VSS}^{-1} for IN and IN_{NEW}, respectively. However, the oxygen used by biomass only for the substrate degradation was 11.4 mg_{DO} g_{VSS}^{-1} for IN and 13.6 mg_{DO} g_{VSS}^{-1} for IN_{NEW}. Hence, a slightly higher oxygen consumption was observed for IN_{NEW} substrate degradation. This result was expected, as IN_{NEW} contained an aliquot of ID, which, although reduced, brought with it a series of "more difficult" and slower biodegradable molecules.

For the degradation of substrates, an oxygen consumption in the range of 21–27 mg_{DO} has been observed (area of the degradation "bell" equal to 11–14 mg_{DO} g_{VSS}^{-1}, multiplied by VSS concentration of about 1.8–2 g L^{-1}). Considering (i) COD dosed with substrates equal to 60–70 mg_{COD}, (ii) BOD_5/COD ratio of about 50% for both IN and IN_{NEW}, 30–35 mg_{BOD5} were dosed at the beginning of the tests. A BOD_5 removal of 70–80% was observed in just over 1 h. This result was conceptually correct, as BOD_5 is a measurement corresponding to a contact time of 5 days, and a complete degradation of the substrates in terms of biodegradable organic matter would not have been visible in 1 h. For a simpler and more immediate performance of the continuous test, an approximation was assumed: the tests were stopped at exogenous OUR values close to the endogenous.

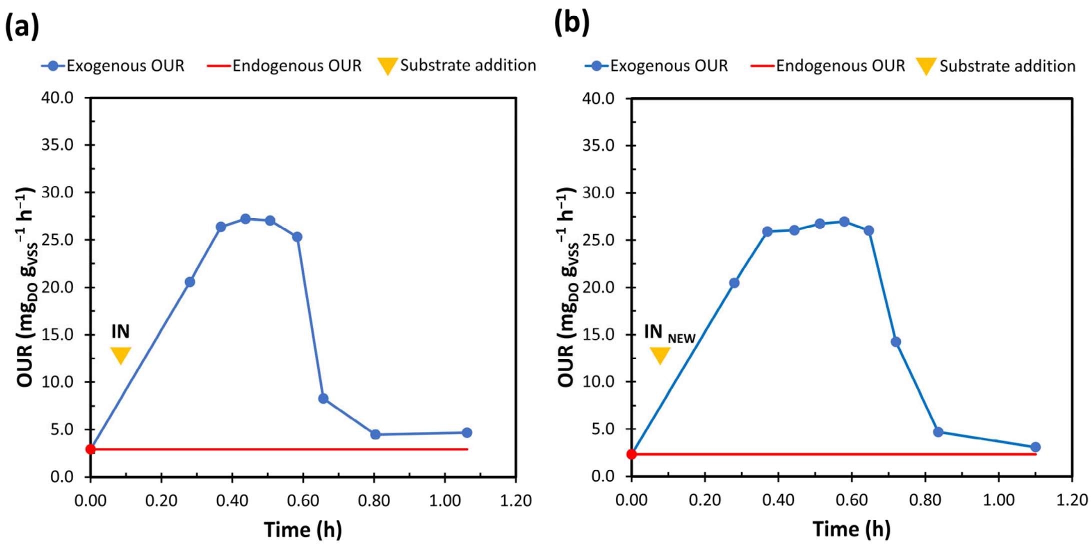

Figure 4. Continuous OUR tests performed in parallel with (**a**) IN and (**b**) IN$_{NEW}$ (IN + ID) substrate.

3.3.2. Autotrophic Biomass

The impact of ID on the nitrification activity of case study biomass was also investigated (Figure 5). A sample of civil sewage, with domestic WW only, was used as the reference substrate. The WW entering the oxidation/nitrification reactor (IN) showed a visible acute inhibition effect (2.1 mg$_{N\text{-}NO3\text{-}}$ g$_{VSS}$$^{-1}$ h^{-1}) compared to the reference substrate (2.9 mg$_{N\text{-}NO3^-}$ g$_{VSS}$$^{-1}$ h^{-1}). As for OUR tests, this could be due to various toxic-inhibiting substances for autotrophic biomass present in many industrial wastes that reach the sewage system. With the addition of the studied ID, a further reduction of the nitrifying kinetics was observed, down to 1.6 mg$_{N\text{-}NO3^-}$ g$_{VSS}$$^{-1}$ h^{-1}. This result could be linked to (i) new inhibitory pollutants for the autotrophic biomass or (ii) excessive increase in loads of substances already present in WW entering the WWTP. In any case, the nitrifying biomass was put in contact with a new substrate (ID contained in IN$_{NEW}$) to which it had not yet acclimatised. After an initial period of instability and transition, thanks to a better acclimatization of the autotrophic biomass, better nitrification kinetics cannot be excluded. However, IN$_{NEW}$-AUR values did not seem to be excessively alarming, since some literature studies have shown a nitrification kinetic with WW (and properly enriched with N–NH_4^+), usually treated by the nitrifying biomass, equal to 1.8 mg$_{N\text{-}NOx}$ g$_{VSS}$$^{-1}$ h^{-1} [26]. In any case, despite these results, the conditions for prohibiting the company from discharging into the public sewer did not seem to exist; however, further insights should not be excluded, for example a more in-depth AUR test campaign.

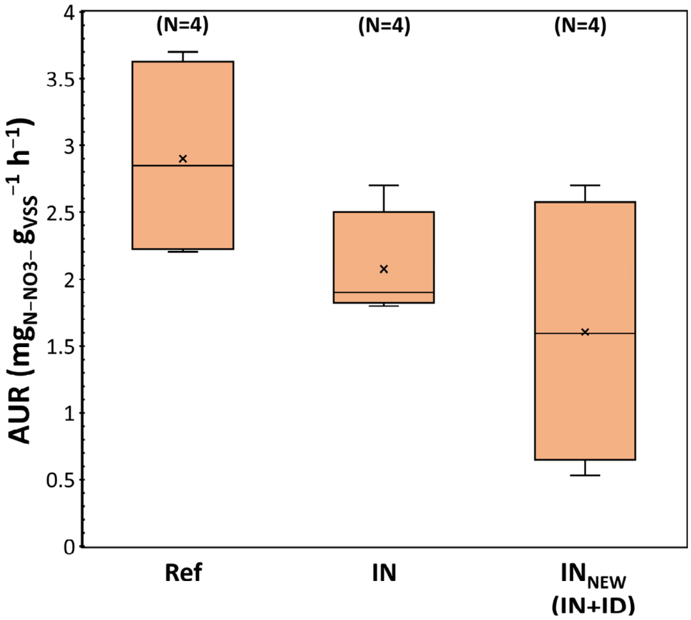

Figure 5. Results of the batch AUR tests with autotrophic biomass sampled in the case study-WWTP. Ref: Reference value. Boxplots represent the distance between the first and third quartiles while whiskers are set as the most extreme (lower and upper) data point not exceeding 1.5 times the quartile range from the median. The cross represents the mean value. N: number of replicated tests.

4. Future Outlooks

This study represented the first approach and first version of an experimental protocol to evaluate the impact of new industrial waste on the urban WWTP. The authors are aware and hope that further steps will be taken in future research, to make this protocol even more complete, reliable, and easily applicable by both the urban WWTP receiving the ID, and by the company responsible for the discharge into the sewer.

The proposed methodology is valid if the industrial WW is discharged with a constant flow rate/pollutant load throughout the day, further investigation is required if significant variations in flow rate and/or load are found. Most urban WWTPs are made up of traditional biological treatments which generally cannot tolerate significant variations in the influent sewage. With the occurrence of a negative impact on the WWTP, the company responsible for the discontinuous effluent could be suggested/requested to insert a homogenization tank before discharge into the sewer.

The adaptation of step 1 to the specific case is of fundamental importance. The choice of pollutants for the assessment of the ID qualitative impact on the WWTP (load ratio) is essential. The most critical pollutants in the ID (such as heavy metals, surfactants, persistent organic pollutants, aromatic and nitrogenous organic solvents, chlorinated solvents, etc.) can only be identified through a case-by-case analysis based on company production processes. The authors recommend not considering in practice only the parameters of this case study, as they refer to the specific situation. A further step could be envisaged, to study the impact of some target pollutants, carried by ID, on the removal efficiencies of the urban WWTP. In this case, the WWTP effluent should also be monitored.

The integrated approach of this experimental protocol aims to make all the realities gravitating around the world of WW treatment more attentive. Greater awareness of companies translates into better management of industrial waste and better care and protection of the environment. Greater safeguarding of urban WWTPs guarantees efficient and optimized treatment, more controlled effluents, and therefore a reduced environmental impact.

5. Conclusions

The possibility to connect to the public sewer a new discharge coming from an AWTP, with an average flowrate of 200 $m^3\ d^{-1}$, was studied in this work by adopting an integrated assessment protocol, based on operating data processing as well as experimental tests. The sewage is treated in an urban WWTP, with a conventional activated sludge process; the average incoming flow rate is 45,000 $m^3\ d^{-1}$. In Italy, for getting the permission to discharge into sewers, an acute toxicity test is mandatory, but the prescribed method is the same used for assessing the toxicity of discharge into natural water bodies. This may lead to wrong evaluations. Contrarily, an effective and reliable evaluation of the compatibility of new discharge with the centralized WWTP should be carried out by considering the site-specific features of the plant and the biological prosses. In this work, the dimensional characteristics of the WWTP (flow rate/load mixing ratio, volumes), the operating conditions (SLR, C:N:P, OC, oxygen supply), and the response and activity of the biomass (both heterotrophic and autotrophic) have been pointed out as fundamental issues to consider. To meet this need, a multi-step experimental protocol for WW utilities has been proposed, where respirometry (OUR and AUR tests) plays a relevant role.

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Abbreviations

AUR	Ammonia utilization rate
AWTP	Aqueous waste treatment plant
BIO	Oxidation/nitrification biomass
CAS	Conventional activated sludge
COD	Chemical oxygen demand
DO	Dissolved oxygen
ID	Industrial discharge
IN	Input to the WWTP
$N-NH_4^+$	Ammoniacal nitrogen
$N-NO_3^-$	Nitric nitrogen
OC	Organic carbon
OUR	Oxygen uptake rate
TN	Total nitrogen
TKN	Total Kjeldahl nitrogen
SLR	Sludge loading rate
SS	Total suspended solids
VSS	Volatile suspended solids
WW	Wastewater
WWTP	Wastewater treatment plant

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PUBLICATION 8

TOPIC: WS1 - Monitoring and performance study

- WS1.2 - Monitoring and performance of a pilot-scale TAMR for sewage sludge minimization

WS3 - Residues and energy recovery

- WS3.1 - Reuse of residues

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Strong minimization of biological sludge production and enhancement of phosphorus bioavailability with a thermophilic biological fluidized bed reactor

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ABSTRACT

Identify sustainable biological sewage sludge (BSS) management represents a current challenge. In this work, pre-thickened BSS taken from a large-scale urban wastewater treatment plant (WWTP) was fed continuously for about 9 months to a semi-industrial scale thermophilic biological fluidized bed reactor (TBFBR) operating in alternate conditions. The BSS treated in TBFBR was strongly reduced (89–92%) and the production was evaluated in 0.0007–0.0023 kg $kg_{CODremoved}{}^{-1}$ d^{-1} and 0.0004–0.0014 kg $kg_{CODremoved}{}^{-1}$ d^{-1} of TS and VS, respectively. The 92% of the P-PO_4^{3-} and P-org precipitated in inorganic form and accumulated in thermophilic biological sludge (TBS). In basic soils with chelating activity, phosphorus contained in TBS presented a higher bioavailability (84.0 ± 25.3%) with respect to untreated sludge. The analysis of four diverse scenarios highlighted that TBFBR located after pre-thickening could save 73–96% of operating costs with respect to current situation.

1. Introduction

Over the years, the problem linked to the disposal of biological sewage sludge (BSS) assumed greater importance due to their growing production. The imposition of more restrictive limits to the discharge of effluents from urban wastewater treatment plants (WWTPs) (European Directive 91/271/EEC (European Commission, 1991) and subsequent amendments (European Commission, 1998)) caused a greater production of BSS with a worsening of its qualitative characteristics. In fact, a better water treatment efficiency involves greater production of BSS with higher level of contamination (Mininni et al., 2015). In Europe, in 2015, 9.7 million tons of dry matter of BSS were produced by urban WWTPs (Collivignarelli et al., 2019b). Therefore, the adoption of processes that allow both significant minimization of sludge production and a correct and sustainable management of them is therefore necessary and urgent.

Concerning legislative aspects, Directive 2018/851/EC (European Commission, 2018), identified a hierarchy in waste management, therefore also applicable to BSS: (i) prevention and minimization of waste production, (ii) matter recovery and reuse of the residual BSS produced, (iii) energy recovery and finally (iv) safe disposal of the residues. The choice of the most appropriate technology for the prevention and minimization of BSS production must be made considering technical and economic factors, carefully evaluating the

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Nomenclature

BSS	Biological sewage sludge
CAS	Conventional activated sludge
COD	Chemical oxygen demand
DM	Dry matter
FA	Phosphorus accumulation
HRT	Hydraulic retention time
MBR	Membrane biological reactor
OC_{AD}	Operating cost of anaerobic digestion
OC_{DEW}	Operating cost of static, dynamic thickening, and dewatering
OC_{SD}	Operating cost of sludge disposal and transport
OC_{TBFBR}	Operating cost of TBFBR
OLR	Organic loading rate
Q	Daily flow rate
SDC	Sludge disposal in agriculture
S_{TS}	Total solids load
S_{VS}	Volatile solids load
TBFBR	Thermophilic biological aerobic/anoxic membrane reactor
TBS	Thermophilic biological sludge
TN	Total nitrogen
TP	Total phosphorus
TK	Total potassium
TS	Total solids
TSS	Total suspended solids
UF	Ultrafiltration
VS	Volatile solids
WRRF	Water resources recovery facility
WW	Wastewater
WWTP	Wastewater treatment plant
X_{TS}	Total solids concentration
$\%_{VS/TS}$	VS/TS ratio

advantages and disadvantages deriving from its application (Kacprzak et al., 2017).

The minimization of BSS production is an aspect of primary importance not only because the legislation requires it, but also because it guarantees many non-negligible benefits. A first positive consequence is the reduction of costs as BSS management represents approximately the 50% of the total operating costs of WWTPs (Bertanza et al., 2015a; Collivignarelli et al., 2015c; Zhao et al., 2019). In addition to the economic aspect, also the environmental impact related to the treatments, transport, and final disposal of BSS should be considered. Therefore, reducing the quantity produced at the source is one of the essential interventions applicable to improve the BSS management from multiple points of view (Bertanza et al., 2014).

According to the Italian Superior Institute for Environmental Protection and Research (Ispra, 2019), in Italy, in 2017, about 3,200,000 tons of wet sludge, (approximately 800,000 tons of dry matter) were produced in urban WWTPs (Mininni et al., 2019). 47.7% of them were sent to recovery options (including energy production, recycling/recovery of organic substances, recovery in agriculture) and 50.6% to disposal operations (mainly including biological or physico-chemical treatment before disposal, landfill, and incineration), recording a 1.4% decrease in landfill disposal in favour of recovery compared to the previous year. In particular, the sludge destined to agriculture reuse represents about 6% of the total BSS recovered (Ispra, 2019).

Council Directive 86/278/EEC (European Commission, 1986) banned the use of untreated BSS on agricultural land and thus encouraged the spreading of good quality BSS to avoid harmful effects on the biosphere. The use of BSS for agriculture should be done with the appropriate controls as, despite the treatments, BSS can contain heavy metals, pathogens, and organic contaminants (Collivignarelli et al., 2015b; Liu et al., 2018; Zhang et al., 2014). For this reason, several countries are implementing stricter legislation to prevent the spread of BSS with harmful compounds and limit the agricultural reuse to high-quality sludge. Besides, the management of BSS for the agronomic reuse is made critical also by a lack of acceptance by the population (Mininni et al., 2015), linked (i) to the fear of organic and inorganic micropollutants possibly present that could threaten the safety and quality of food (Zhang et al., 2019) and (ii) to the bad odours during the spreading of biosolids in agricultural fields due to the presence of volatile organic compounds and ammonia (Collivignarelli et al., 2019c).

Despite these limitations, the use of treated BSS for agronomic purposes as a soil improver is one of the main recycling practices that allows the enhancement of the organic substance and nutrients in cultivated fields increasing the fertility of the soils (Collivignarelli et al., 2020; Sgroi et al., 2018). A rational use of BSS can considerably reduce the dependence on inorganic fertilization (Neczaj and Grosser, 2018) and therefore the carbon footprint generated by the production of inorganic fertilizers (Sharma et al., 2017). Furthermore, the reuse of BSS in agriculture is more sustainable both from an economically and environmental point of view than landfilling (Kacprzak et al., 2017; Zhao et al., 2019). In addition, the practice of reuse can be entirely inserted in the concept of circular economy (Ashekuzzaman et al., 2019; Kacprzak et al., 2017). About this aspect, in 2020 the European Commission adopted a new Circular Economy Action Plan to promote sustainable use/reuse of resources (European Commission, 2020). In urban water management system, one of the main actions required to implement a circular economy approach is the transformation of WWTPs in water resources recovery facilities (WRRFs) (Collivignarelli et al., 2019a; Cornejo et al., 2019). To do this, the prevention and minimization of BSS production is the first step that can be pursued in two distinct ways: (i) by adopting processes capable of treating water with a minimum production of residual BSS; (ii) providing in-situ treatments to minimize the quantities produced (Collivignarelli et al., 2019b).

Following the second approach, a technology could be used directly in the sludge line of a traditional WWTP, which can act on the BSS already produced in the water line. In this way the precarious balance of the processes present in the water line would not be altered. Among the existing technologies, the biological ones are the most interesting from an environmental point of view, because they are more sustainable than chemical processes. Thermophilic biological fluidized bed reactor (TBFBR) is a biological reactor operating in a thermophilic regime and consisting of a membrane filtration. An important limitation of thermophilic biomass is the lack of capacity to settle. The membranes were applied as a solid-liquid separation system to replace sedimentation. This was a successful association of two processes adding the advantages of membrane biological reactor (MBR) to that of thermophilic treatments.

In this work, pre-thickened BSS taken from a large-scale urban WWTP was fed continuously for about 9 months to a semi-industrial scale TBFBR operating in alternate condition. This aeration condition is particularly innovative in a thermophilic process and allowed to exploit of bacterial biomass with different characteristics compared to that present in traditional aerobic thermophilic treatments. Sludge minimization was evaluated and chemical and microbiological characteristics of extracted thermophilic biological sludge (TBS) in a soil spread perspective were compared with untreated BSS. Moreover, phosphorus bioavailability in TBS and transformation pathways into the reactor were studied and discussed. Finally, an

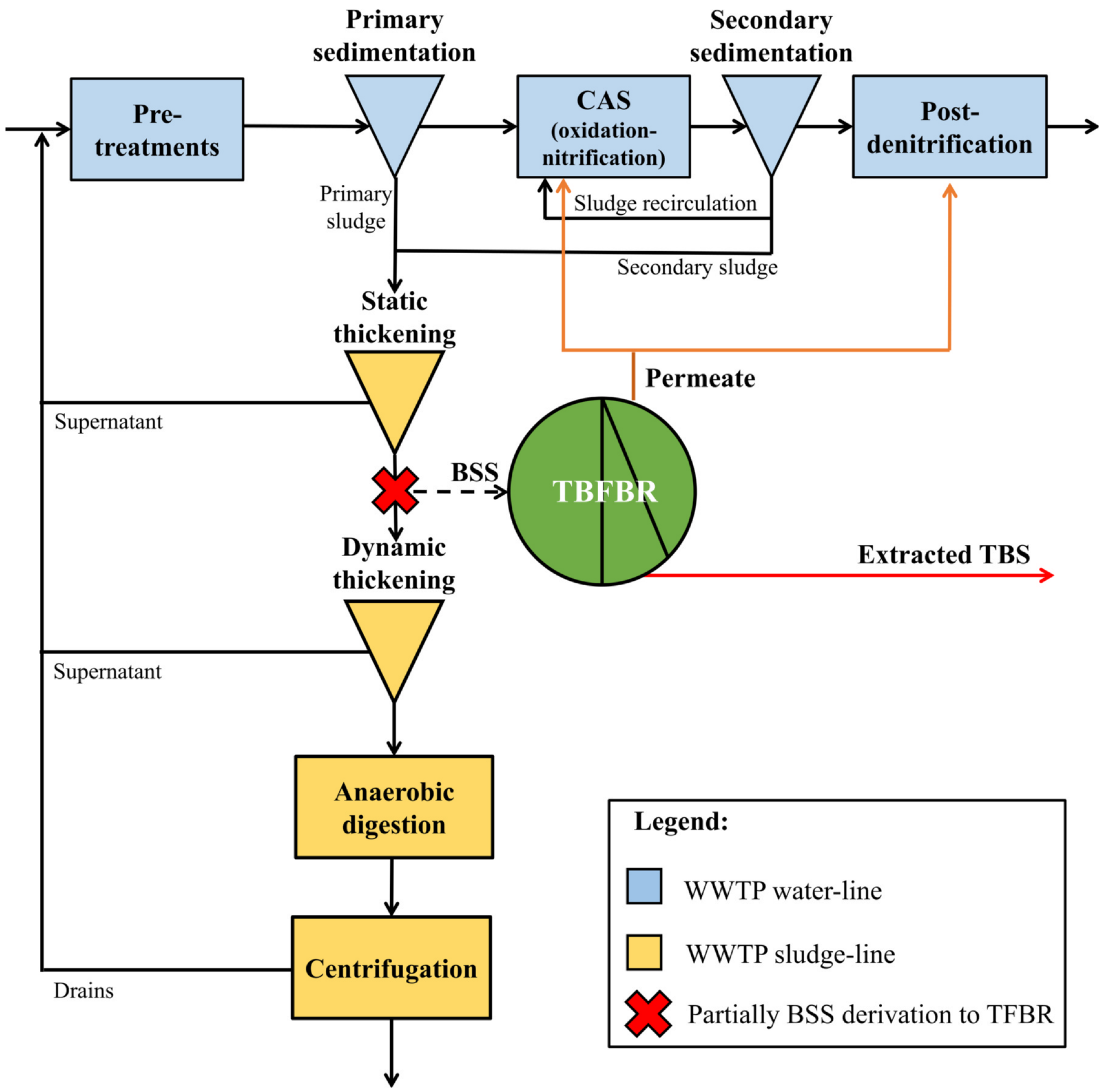

Fig. 1. Block diagram of the real WWTP with the introduction of TBFBR plant in sludge-line.

assessment of management costs in four different scenarios was elaborated to determine the optimal solution.

2. Material and methods

2.1. TBFBR

2.1.1. Localization of the plant

The urban WWTP is located in Lombardy (Italy) and treats the sewage of about 130,000 equivalent inhabitants adopting a conventional activated sludge (CAS) system (Fig. 1). The goal of minimizing the residual BSS of a WWTP was achieved with the application in situ of TBFBR.

2.1.2. TBFBR design and management

TBFBR plant (4.0 × 2.4 × 2.3 m) worked in thermophilic condition and with alternating cycles of aeration with pure oxygen and non-aeration. The main components of the pilot plant were the biological reactor (volume: 1 m^3), the recirculation line and the membrane ultrafiltration (UF) unit, as shown in Fig. 2. The biological reactor was thermally insulated. In the recirculation line a Venturi-type device for direct injection of pure oxygen and a heat exchanger were located. The crossflow UF section consisted of a vessel containing 7 ceramic membranes with 23 channels and cut-offs equal to 300 kDa and a pore size of 10 nm. The permeate could be recirculated in the thermophilic reactor or in the water line of the large-scale WWTP. Before UF unit and heat exchanger for the elimination of coarse particulates, two filters were located.

The start-up phase of the TBFBR lasted about 4 months and had the purpose of achieving the desired operative conditions, and an equilibrium regime to ensure maximum reliability and robustness of the process. Considering that this paper aims to evaluate the reduction of BSS production and the chemical and microbiological characteristic of the residue produced, only stable phase period (5 months) (Fig. S1 (a) is considered, and results of start-up phase are not reported). The chemical characteristics of the TBS present in the reactor at the start of the stable phase period (t_0) are shown in Table S1. In the biological reactor, a condition of intermittent aeration was maintained. In the stable phase, a total of 6 h of anoxia per day were reached, divided into cycles of two hours of anaerobiosis separated by 6 h of aerobiosis (Fig. S1 (b)). Intermittent oxygen cycles were adopted with the aim of (i) promote the cell lysis of sludge during the anoxic phases allowing the breakdown of more complex molecules; (ii) promote the oxidation of lysed compounds during the

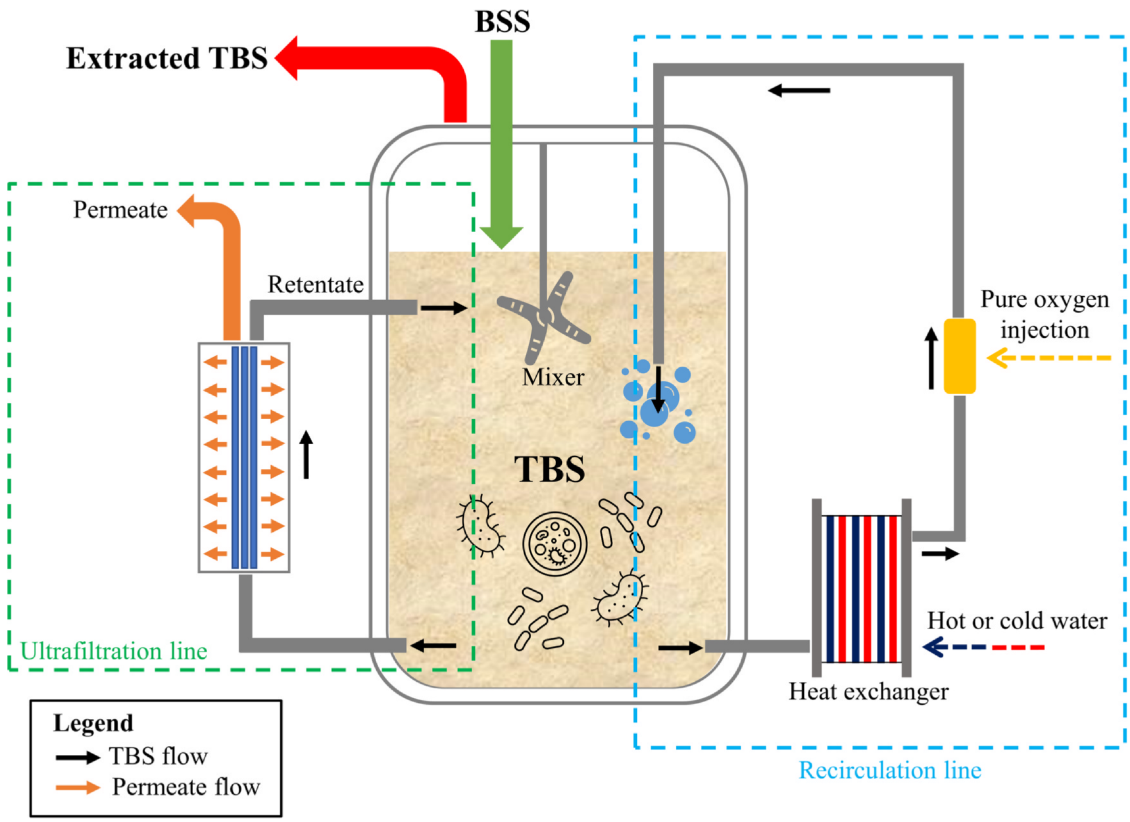

Fig. 2. TBFBR schematic diagram.

Table 1
Operative conditions in the TBFBR. TS: total solids; VS: volatile solids; HRT: hydraulic retention time; OLR: organic loading rate; n: number of data.

Parameter	Value
TS [kg m^{-3}]	66.3 ± 7.5 [n = 52]
VS [kg m^{-3}]	31.7 ± 3.9 [n = 52]
HRT [day]	10 ± 1 [n = 122]
OLR [kg_{COD} m^{-3} d^{-1}]	3.4 ± 0.4 [n = 122]
Temperature [°C]	52 ± 2 [n = 122]
pH [-]	7.0 ± 0.6 [n = 40]

aerobic phases; (iii) avoid the formation of biological foams during the resumption of oxygen dosage (Collivignarelli et al., 2017). The dissolved oxygen and the temperature into the reactor were measured by a submerged probe (Endress+Hauser Oxymax W COS31). The process parameters of TBFBR process are shown in Table 1.

Thermophilic conditions were ensured thanks to exothermic reactions produced by biomass maintaining an organic loading rate (OLR) between about 3 and 4 kg_{COD} m^{-3} d^{-1} thanks to BSS addiction. A heating/cooling system controlled the temperature of the system.

Some extractions of TBS from the system to keep the concentration of solids in the reactor constant were necessary. In fact, values of total solids (TS) higher than 75 g L^{-1} could cause an increase in the viscosity of the TBS, making management and maintenance operations complicated, especially for electromechanical equipment. The specific sludge production was calculated considering the quantity of total solids (TS) and volatile solids (VS) accumulated daily in the reactor and the COD (chemical oxygen demand) removed from the process.

An additional loss of TBS was found in the daily cleaning of the two pre-filters of the pilot-plant. The pre-filters, once disassembled, were weighed before cleaning to quantify the TBS inside them and also consider this contribution.

2.1.3. Fed BSS characteristics

Statically thickened BSS not previously conditioned with polyelectrolyte was tested as substrate in the TBFBR. The physico-chemical characteristics of the substrate fed to the pilot-plant are shown in Table 2.

2.2. Analytical methods

To carry out the analysis on feeding BSS and extracted TBS samples, the legislation imposed by the Lombardy Region (Italy) was used as reference (Lombardy region, 2019). This legislation has stricter limits than Italian legislation (Government of Italy, 1992), which is among the most stringent for land reuse of BSS in the European context (Collivignarelli et al., 2019a). pH, TS, VS were measured according to APAT-IRSA-CNR 2060 (2003), UNI EN 14346 (2007), UNI EN 15169 (2007), respectively. Heavy metals (cadmium, total chromium, mercury, nickel, lead, copper, zinc, arsenic, selenium, beryl), TP and TK were evaluated according to UNI EN 13657 (2004) and UNI EN ISO 11885 (2009). Chromium IV was evaluated using CNR-IRSA 16 (1986) guidelines. Organic carbon and TN were studied according to CNR-IRSA 5 (1988) and CNR-IRSA 6 (1985), respectively. Polycycle aromatic hydrocarbon (PAH), bis(2-ethylhexyl) phthalate (DEHP) and nonylphenols (NP) were measured according to EPA 3550C (2007) and EPA 8270 E (2018). Total polychlorinated biphenyls (PCB) were evaluated using EPA 3550C (2007) and EPA 8082 A (2007) instructions. Polychlorinated dibenzo-p-dioxins and dibenzofurans, dioxin like polychlorinated biphenyls (PCDD/F + DL

Table 2
Physico-chemical parameters of fed BSS. TN: total nitrogen; TP: total phosphorus; TS: total solids; VS: volatile solids; n.d.: not detected; n: number of data.

Parameter	Fed BSS
COD [mg L^{-1}]	33,136 ± 4545 [n = 20]
TN [mg L^{-1}]	1216 ± 138 [n = 20]
N-NH_4^+ [mg L^{-1}]	545 ± 61 [n = 20]
N-NO_x [mg L^{-1}]	2.9 ± 1.1 [n = 20]
TP [mg L^{-1}]	570 ± 162 [n = 20]
TS [g L^{-1}]	19.4 ± 2.6 [n = 20]
VS [g L^{-1}]	13.6 ± 2.6 [n = 20]
VS TS^{-1} [-]	0.70 ± 0.08 [n = 20]
pH [-]	5.5 ± 0.4 [n = 30]
Conductivity [μS cm^{-1}]	2588 ± 423 [n = 20]

PCB) were defined according to EPA 1668C (2010). Toluene was studied with EPA 5030C (2003) and EPA 8260 D (2018) guidelines. Adsorbable organic halogens (AOX) were determined in according to EPA 5035 A (2002) and EPA 8260 D (2018). Hydrocarbons (C10-C40) were measured using UNI EN ISO 14039 (2005).

Faecal coliforms and *Salmonella* spp. were evaluated according to CNR-IRSA 3 (1983) instructions. Phytotoxicity tests were performed in according to UNI EN 10780 (1998). Also, biochemical and biological parameters not regulated by the present legislation were studied. Enterococci and spore count of *Clostridium perfringens* were defined with ISTISAN ISS F 003 A rev. 00 (2007) reports and ISTISAN ISS F 004 A rev. 00 (2007) reports, respectively. Mildews count and yeast count were determined in according to CNR-IRSA 5 (1983) guidelines. Finally, research viable helminth eggs were measured with APAT 1.2.2 Manual 20 (2003).

COD was monitored according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Phosphorus elution tests were carried out following the methods of analysis on fertilizers as required by the Italian Ministry of Agricultural, Food and Forestry Policies (Government of Italy, 2006) to simulate the different types of soil (neutral, acid, basic).

2.3. Phosphorus accumulation and pathways

Starting from the phosphorus analyses on feeding BSS and extracted TBS samples, the mechanisms of transformation and accumulation of phosphorus in the thermophilic bioreactor were evaluated.

The following pathways were considered:

- Pathway (**a**): portion of soluble phosphorus in the form of phosphates P-PO_4^{3-} present in the feeding BSS. It was measured directly in BSS samples with chemical analyses.
- Pathway (**b**): P-PO_4^{3-} not transformed and accumulated in TBS based on monitoring data.
- Pathway (**c**): soluble phosphorus extracted as P-PO_4^{3-} from TBFBR. It was measured directly in permeate samples with chemical analyses.
- Pathway (**d**): P-PO_4^{3-} transformed in organic phosphorus and accumulated in the bioreactor in form of thermophilic biomass.

$$P_{path(d)} = FA^{*}VS_{produced} \qquad (1)$$

Where FA is the factor of phosphorus accumulation by biomass based on measured data in fed BSS (P-organic/VS) and $VS_{produced}$ was evaluated based on sludge production of the system.

- Pathway (**e**): soluble P-PO_4^{3-} converted into inorganic phosphorus. Calculated with the following equation:

$$P_{path(e)} = P_{path(a)} + P_{path(h)} - P_{path(c)} - P_{path(d)} \qquad (2)$$

- Pathway (**f**): inorganic phosphorus fed into the reactor. From the elution tests on phosphorus, the inorganic phosphorus in feeding BSS was equal to 72%.
- Pathway (**g**): not soluble organic phosphorus fed to TBFBR. Value determined from the measured data.
- Pathway (**h**): organic phosphorus transformed in P-PO_4^{3-}. This fraction is calculated with the following equation:

$$P_{path(h)} = P_{path(g)} - P_{path(i)} \qquad (3)$$

- Pathway (**i**): insoluble organic phosphorus leaving the TBFBR considering the quality of the permeate.
- Pathway (**j**): inorganic phosphorus accumulated in TBS. Calculated as follows:

$$P_{path(j)} = P_{path(e)} + P_{path(f)} \qquad (4)$$

2.4. Mass balances and economic evaluation

In mass balance and economic evaluation, four diverse scenarios have been considered:

- S0: current scenario of the existing WWTP with the sludge line consisting in static thickening, dynamic thickening, anaerobic digestion, dewatering.
- S1: scenario with TBFBR treating low strength BSS (TS = 24.5 kg m^{-3}). The sludge line was composed by static thickening, TBFBR, and dewatering.
- S2: scenario with TBFBR treating high strength BSS (TS = 65 kg m^{-3}). The sludge line was composed by static and dynamic thickening, TBFBR, and dewatering.
- S3: scenario with TBFBR treating high strength BSS (TS = 65 kg m^{-3}) and anaerobic digestion. The sludge line was composed by static and dynamic thickening, TBFBR, anaerobic digestion, and dewatering. In this scenario, a 50% flowrate bypass of the TBFBR was assumed to guarantee enough biogas production and evaluate the case of contemporary recovery of matter, by TBFBR, and energy, by anaerobic digestion.

Mass balances were evaluated and compared assuming in all scenarios a volume of 1 m^3 of BSS entering the sludge line. In economic and mass balance evaluation the cost of the dosage of polyelectrolyte was neglected. Considering that in S1, S2, S3 the lower amount of BSS produced would have determined a lower dosage of polyelectrolyte, this assumption minimize the costs in S0 and maximize the costs in other scenarios.

In economic analysis, for every single scenario, two cases have been considered to evaluate a possible range of unitary costs [€ $m^{-3}_{sludge\ in}$]: (i) best case, with the maximization of income and minimization of costs and (ii) worst case, with the maximization of costs and minimization of income. Only operating costs were evaluated, and no depreciation costs were considered.

2.4.1. Static, dynamic thickening and dewatering

The total operating cost of static, dynamic thickening, and dewatering (OC_{DEW}) have been calculated according to Eqn 5.

$$OC_{DEW}\ [€] = (SEC_{ST}{}^{*}V_{IN\ ST} + SEC_{DT}{}^{*}V_{IN\ DT} + SEC_{DEW}{}^{*}V_{IN\ DEW})^{*}EC \qquad (5)$$

Table 3
Values of parameter used in economic analysis and scenario evaluation. (a) assumed equal to energy necessary for dewatering.

		Values		References
		MIN	MAX	
Energy cost [€ kWh^{-1}]	EC	0.06	0.17	(Bertanza et al., 2018)
Income from electric energy sale [€ kWh^{-1}]	ES	0.06	0.22	(Bertanza et al., 2018)
Thickening and dewatering				
Energy required static thickening [kWh m^{-3}]	SEC_{ST}	0.2	0.7	(Campanelli et al., 2013)
Energy required dynamic thickening [kWh m^{-3}][(a)]	SEC_{DT}	1	3	(Campanelli et al., 2013)
Energy required dewatering [kWh m^{-3}]	SEC_{DEW}	1	3	(Campanelli et al., 2013)
TBFBR				
O_2 cost [€ $kgO_2{}^{-1}$]	RC	0.04	0.10	(Bertanza et al., 2018)
O_2 consumed [kgO_2 $kg_{COD\ removed}{}^{-1}$]	R	1.1	1.2	This experimentation
COD removal yield [-]	$\mu_{COD\ TBFBR}$	0.85	0.95	This experimentation
Energy required [kWh m^{-3}]	E	60		(Collivignarelli et al., 2021)
Permeate stripping [€ m^{-3}]	SC	0.5	0.6	Assumption based on real cases
Anaerobic digestion				
Specific mixing power [W m^{-3}]	SEC_{AD}	4.9	7.9	(Campanelli et al., 2013)
Low heating value of CH_4 [kWh Nm^{-3}]	LHV	10		(Tchobanoglous et al., 2003)
Biogas production [m^3 $kg_{VS\ inlet}{}^{-1}$]	BP	0.35	0.50	(Campanelli et al., 2013)
CH_4 in biogas [-]	BC	0.50	0.60	Real cases
Electrical efficiency of cogeneration [-]	μ_{COG}	0.35		(Bertanza et al., 2015b)
Electrical efficiency of mixing [-]	μ_{MIX}	0.80		Real cases
Time of operation of mixer [h d^{-1}]	F	24		Real cases
Sludge transport and disposal				
Transportation cost/per trucks of 30 t [€ km^{-1}]	TC	0.2	0.25	(Bertanza et al., 2016)
Distance covered for agricultural application [km]	D	40	200	(Bertanza et al., 2018)
Sludge disposal [€ $t_{dewatered\ sludge}{}^{-1}$]	SDC	30*	140**	*(Bertanza et al., 2018); ** (Canziani, 2016)

where $V_{IN\ ST}$, $V_{IN\ DT}$ and $V_{IN\ DEW}$ [m^3] represent the inlet volume in static, dynamic thickening, and dewatering, respectively. For the meanings and the values of other parameters, please refer to Table 3. In balances of mass of static thickening in S1, S2 and S3, the yield of water removal was assumed equal to that in S0. In S1, S2 and S3 balances of mass: (i) the yield of water removal of static thickening was assumed equal to that in S0; (ii) dynamic thickening and dewatering allowed to obtain a TS concentration of 65 kg m^{-3} and 240 kg m^{-3}, respectively, as in the current scenario (S0).

2.4.2. TBFBR

The operating cost of TBFBR (OC_{TBFBR}) have been calculated according to Eqn 6:

$$OC_{TBFBR}\quad [€] = EC^{*}E^{*}V_{IN\quad TBFBR} + RC^{*}R^{*}M_{COD\quad IN}{}^{*}\mu_{COD} + V_{PERM\quad TBFBR}{}^{*}SC \tag{6}$$

where $V_{IN\ TBFBR}$ and $V_{PERM\ TBFBR}$ [m^3] represent the inlet volume in TBFBR and the permeate, respectively. $M_{COD\ IN}$ represents the COD [kg] fed to the reactor. For the meanings and the values of other parameters, please refer to Table 3.

2.4.3. Anaerobic digestion

The operating cost of anaerobic digestion (OC_{AD}) have been calculated as follows:

$$OC_{AD}\quad [€] = \frac{SEC_{AD}{}^{*}V_{AD}{}^{*}F^{*}EC}{\mu_{MIX}} - M_{VS\quad IN}{}^{*}BP^{*}BC^{*}LHV^{*}ES^{*}\quad \mu_{COG} \tag{7}$$

where V_{AD} represents the volume of the anaerobic digester [m^3] assuming a hydraulic retention time (HRT) equal to 25d (assumption based on literature real cases and existing WWTP (Bolzonella et al., 2005)). $M_{VS\ IN}$ represents the VS [kg] fed to the anaerobic digester. For the meanings and the values of other parameters, please refer to Table 3. The balance of mass was calculated considering a removal yield of VS equal to 45%.

2.4.4. Sludge disposal and transport

The operating cost of sludge disposal and transport (OC_{SD}) has been determined according to Eqn 8:

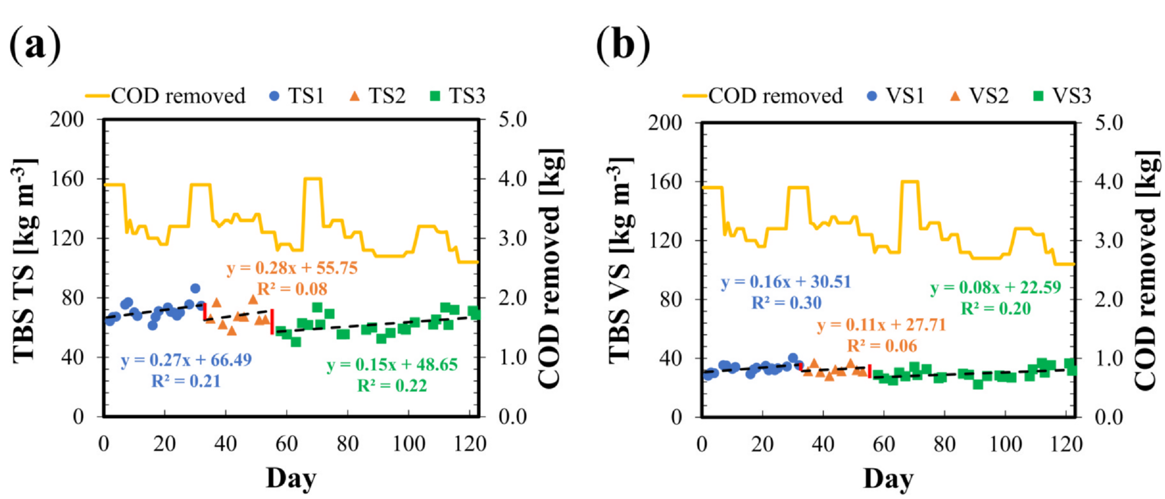

Fig. 3. Concentration of solids in TBFBR and COD removed, expressed in TS (a) and VS (b). Red lines indicate TBS extraction.

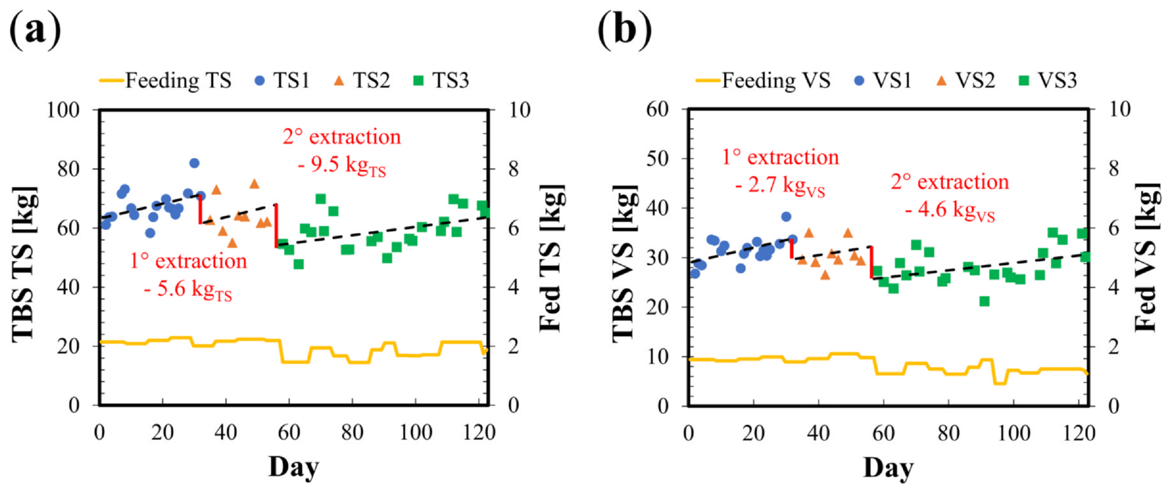

Fig. 4. (a) TS and (b) VS fed and extracted from TBFBR. Red lines indicate TBS extraction.

$$OC_{SD}\ [€] = SP^{*}\left(SDC + TC^{*}D^{*}\frac{1}{TT}\right) \tag{8}$$

where SP represents the production of dewatered sludge in diverse scenarios [t]. TT represents the capacity of transport for each truck and was assumed equal to 30 t of sludge, based on real cases.

3. Results and discussion

3.1. Specific sludge production and TBS extractions

Fig. 3 shows the concentration of TS and VS measured in TBS and the COD removed by TBFBR process. The sludge production was calculated based on TS and VS accumulation in the reactor and considering the two extractions of TBS from the system. For this reason, the trend of the TBS solids has been divided into three periods (Fig. 4(a)). The increase of TS and VS concentration in the different periods was equal to 0.27, 0.28, 0.15 $kg_{TS}\ m^{-3}\ d^{-1}$ (TS1, TS2, TS3, respectively), and 0.16, 0.11, 0.08 $kg_{VS}\ m^{-3}\ d^{-1}$ (VS1, VS2, VS3, respectively) (Fig. 3). The amount of COD removed was 108, 80, 199 kg_{COD}, respectively. The daily specific production of biomass was calculated as 0.0007–0.0023 $kg_{TSproduced}\ kg_{CODremoved}{}^{-1}\ d^{-1}$ and 0.0004–0.0014 $kg_{VSproduced}\ kg_{CODremoved}{}^{-1}\ d^{-1}$. The total specific TBS production was equal to 0.047–0.080 $kg_{TSproduced}\ kg_{CODremoved}{}^{-1}$ and 0.025–0.045 $kg_{VSproduced}\ kg_{CODremoved}{}^{-1}$.

These results are similar to 0.016 and 0.03 $kg_{VSSproduced}\ kg_{CODremoved}{}^{-1}$ proposed for TBFBR in aerobic condition by Duncan et al. (2017) and much lower than that achievable with a mesophilic MBR (in the range 0.10–0.19 $kg_{VSS\ produced}\ kg_{CODremoved}{}^{-1}$), according to Lee et al. (2003) and Kurian et al. (2005). Also, anaerobic granular processes present higher values than TBFBR technology: as example, the specific sludge production in a UASB reactor that treats sewage sludge is equal to 0.1 $kg_{VSSproduced}\ kg_{CODremoved}{}^{-1}$ (Chang and Lin, 2004).

During the first and second extraction, 5.6 kg and 9.5 kg of TS (Fig. 4(a)), and 2.7 kg and 4.6 kg of VS were extracted (Fig. 4(b)), respectively. Compared to 240 kg_{TS} and 170 kg_{VS} (100%) fed to the reactor, the extracted TBS represented 6.3% and 4.3% in term of TS and VS, respectively. A further loss of sludge was found in the daily cleaning operations of the two pre-filters, located in the recirculation line and in the ultrafiltration unit. At each cleaning of both filters, an average of 0.09 kg_{VS} and 0.19 kg_{TS} were removed. Therefore, the reduction of sewage sludge was quantified in 89% (TS) and 92% (VS) of solids fed to the system.

3.2. Qualitative characterization of extracted TBS

3.2.1. Chemical and microbiological parameters

Chemical and microbiological characterization of extracted TBS were carried out. The aim was to evaluate a possible recovery in agriculture in compliance with the limits imposed by current legislation in Lombardy region (Lombardy region, 2019). There is a distinction between "suitable sludge" and "high-quality sludge". The sludge suitable for spreading in agricultural fields must comply with the limit values imposed by the Italian legislation in force and the high-quality sludge requires more stringent limit values than the national ones (Collivignarelli et al., 2019a). These limit values have been reported in Table 4 together with legislative limits of other European countries (Germany and France) for comparison. In general, the Italian national legislation imposes stricter limit values on total chromium, lead, arsenic, agronomic parameters, and some organic contaminants, compared to other French and German legislations.

The analyses were performed on BSS fed to the process and on extracted TBS. The results are shown in Table 4.

The BSS does not reach the VSS TSS^{-1} limit neither for high-quality sludge nor for suitable sludge, as it exceeds in the presence of volatile organic substance. It is unsuitable for recovery in agriculture according to the legislation of the Lombardy. Instead, the extracted TBS resulted, in all parameters, as high-quality sludge. In particular, the agronomic parameters, including TN, TP, TK, and degree of humification are higher in the extracted TBS treated with TBFBR.

Microbiological tests regarding to pathogenic bacteria and other microorganisms (faecal coliforms, *Salmonella* spp., enterococci, *Clostridium perfringens* spores, mildews, yeasts, and viable helminth eggs) was carried out on BSS substrate feeding the TBFBR pilot plant and in the extracted TBS from the bioreactor to evaluate the effect and influence of the process on the microbiological parameters. The results of the microbiological tests are shown in Table 4. The BSS did not respect the limits imposed by Lombardy regional legislation on *Salmonella* spp. and faecal coliforms, not falling within the options for recovery in agriculture. Promising results were obtained in the extracted TBS. Excellent reduction of mildews and bacteria were achieved probably thanks to the high temperatures kept constantly in the reactor (50–54 °C), but less effective on spores was highlighted due to their higher resistance to thermophilic temperature. About the presence of pathogenic microorganisms, thermophilic processes generally grant greater safety than mesophilic ones mainly thanks to higher temperatures and HRT (Skillman et al., 2009; Ziemba and Peccia, 2011). Our results are confirmed by the study of Lloret et al. (2013) on a thermophilic anaerobic digestion which

Table 4
Results of the qualitative characterization tests of BSS and extracted TBS. VSS: volatile suspended solids; TSS: total suspended solids; PAH: polycyclic aromatic hydrocarbons; PCB: polychlorinated biphenyls; PCDD/F: polychlorinated dibenzo-p-dioxins and dibenzofurans; AOX: absorbable organic halogens; DEHP: di(2-ethylhexyl) phthalates; NP: nonylphenols; DM: dry matter; TEQ: toxic equivalent; MPN: most probable number; CFU: colony forming units; n.d.: not detected; n: number of data. In red the measured values that did not respect the limits imposed by the Lombardy regional legislation.

Parameter	Unit of measure	Legislation					Measured values	
		Italy (Government of Italy, 2018, 1992)	France (Government of France, 2020, 1997)	Germany (Government of Germany, 2017, 1992)	Lombardy (Italy) (Lombardy region, 2019)		Fed BSS [This experimentation]	Extracted TBS [This experimentation]
					High quality	Suitable		
pH	–	–	–	–	$5.5 < pH \le 11$		5.1 ± 0.4 [n = 31]	7.3 ± 1.5 [n = 3]
Dry matter (dry residue at 105 °C)	%	–	–	–	–	–	1.9 ± 0.3 [n = 20]	5.2 ± 0.4 [n = 3]
Dry residue at 600 °C	%	–	–	–	–	–	0.6 ± 0.2 [n = 20]	2.7 ± 0.2 [n = 3]
VSS TSS^{-1}	%	–	–	–	<60	<65	70 ± 8 [n = 20]	48.7 ± 1.5 [n = 3]
Heavy metals								
Cadmium	mg kg_{DM}^{-1}	20	20	10	≤5	≤20	<0.5	<0.5 [n = 3]
Total chromium	mg kg_{DM}^{-1}	200	1000	900	≤150	<200	11	56.4 ± 65.9 [n = 3]
Chromium VI	mg kg_{DM}^{-1}	–	–	–	<2		<0.5	0.4 ± 0.1 [n = 3]
Mercury	mg kg_{DM}^{-1}	10	10	8	≤5	≤10	<0.1	0.4 ± 0.3 [n = 3]
Nickel	mg kg_{DM}^{-1}	300	200	200	≤50	≤300	5.5	8.5 ± 14.3 [n = 3]
Lead	mg kg_{DM}^{-1}	750	800	900	≤250	≤750	22	26.5 ± 45.5 [n = 3]
Copper	mg kg_{DM}^{-1}	1000	1000	800	≤400	≤1000	63	176.7 ± 15.3 [n = 3]
Zinc	mg kg_{DM}^{-1}	2500	3000	4000	≤600	≤2500	210	266.7 ± 191.4 [n = 3]
Arsenic	mg kg_{DM}^{-1}	20	–	–	≤10	<20	<0.5	<0.5 [n = 3]
Selenium	mg kg_{DM}^{-1}	–	–	–	≤10		<0.5	< 0.5 [n = 3]
Beryl	mg kg_{DM}^{-1}	–	–	–	≤2		<0.5	<0.5 [n = 3]
Agronomic parameters								
Organic carbon	% DM	>20	–	–	>20		33.8	24.7 ± 0.6 [n = 3]
Total nitrogen (TN)	% DM	>1.5	–	–	>1.5		1.6	3.1 ± 0.1 [n = 3]
Total phosphorus (TP)	% DM	>0.4	–	–	>0.4		2.9	2.4 ± 0.5 [n = 3]
Total potassium (TK)	% DM	–	–	–	–		0.14	1.9 ± 0.4 [n = 3]
Degree of humification	% DM	–	–	–	–		25.1	34.3 ± 8.1 [n = 3]
Organic pollutants								
PAH	mg kg_{DM}^{-1}	6	2–5[a]	–	<6[d]		<2	<2 [n = 3]
PCB	mg kg_{DM}^{-1}	0.8	0.8[b]	0.1[c]	<0.8		<0.5	<0.1 [n = 3]
PCDD/F	ng_{TEQ} kg_{DM}^{-1}	25	–	100	≤25[h]		3.3	6.1 ± 4.9 [n = 3]
Toluene	mg kg_{DM}^{-1}	–	–	–	≤100		<0.1	<0.1 [n = 3]
AOX	mg kg_{DM}^{-1}	–	–	400	<500		<1.92	<1.92 [n = 3]
DEHP	mg kg_{DM}^{-1}	–	–	–	<100		<0.66	<0.66 [n = 3]
NP	mg kg_{DM}^{-1}	–	–	–	<50[e]		<1.98	<1.98 [n = 3]
Hydrocarbons (C10 – C40)	mg kg_{DM}^{-1}	–	–	–	<10,000		n.d.	116.7 ± 115.5 [n = 3]
	mg kg^{-1}	–	–	–	≤1000[i]		<100	<100 [n = 3]
Microbiological parameters								

(continued on next page)

Table 4 (*continued*)

Parameter	Unit of measure	Legislation					Measured values	
		Italy (Government of Italy, 2018, 1992)	France (Government of France, 2020, 1997)	Germany (Government of Germany, 2017, 1992)	Lombardy (Italy) (Lombardy region, 2019)		Fed BSS [This experimentation]	Extracted TBS [This experimentation]
					High quality	Suitable		
Salmonella spp.	MPN g_{DM}^{-1}	1000	8 [f]	–	< 100		260	<32
Faecal coliforms	MPN g_{DM}^{-1}	–	–	–	< 10,000		730,000	1500
Enterococci count	CFU g_{DM}^{-1}	–	–	–	–		15,000,000	110,000
Clostridium perfringens spores	CFU g_{DM}^{-1}	–	–	–	–		9,100,000	4,600,000
Mildews	CFU g^{-1}	–	–	–	–		27,000	80
Yeast	CFU g^{-1}	–	–	–	–		34,000	240
Helminth eggs	P A^{-1} 50^{-1} g^{-1}	–	3 [g]	–	–		Absent	Absent
Biological parameters								
Phytotoxicity test	–	–	–	–	Germination index (30% dilution) must be >60%		87	88 ± 1 [n = 3]

[a]Different values for different compounds (fluoranthene-5, benzo(b)fluoranthene-2.5, benzo(a)pyrene-2);
[b]Sum of seven congeners,
[c]For each congener;
[d]Sum of acenaphthene, fenanthrene, fluorene, fluoranthene, pyrene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, indeno(1,2,3-c,d)pyrene, dibenzo(a,h)anthracene, benz(a)anthracene, chrysene, benzo(e)pyrene;
[e]Sum of nonylphenol, nonylphenol monoethoxylate, nonylphenol diethoxylate;
[f]Value expressed in MPN 10^{-1} g_{DM}^{-1};
[g]Value expressed in eggs 10^{-1} g_{DM}^{-1};
[h]Sum of PCDD/F and dioxine like polychlorinated biphenyls;
[i]In any case, the limit is considered to be respected if the search for carcinogenicity markers provides values lower than those defined in pursuant to note L, contained in Annex VI of Regulation (EC) no. 1272/2008 of the European Parliament and of the Council, of 16 December 2008, referred to in Commission Decision 955/2014 / EU of 16 December 2008, as specified in opinion of the Higher Institute of Health Protocol no. 36565 of 5 July 2006, and subsequent amendments and additions.

successfully reduced *Salmonella* spp., and *Escherichia coli* below detection limits but not *Clostridium perfringens* spores (4.63 $\log_{10}$ spores mL^{-1}). Similar results were also obtained by Aitken et al. (2005).

In the hypothesis of applying TBFBR technology for the treatment of BSS, the excess TBS extracted from the thermophilic biological reactor could certainly be destined for recovery in agriculture as high-quality sludge, acquiring an economic value as a product without neglecting the important possibility of closing the cycle linked to the management of BSS in a circular economy perspective.

3.2.2. Phosphorus accumulation and bioavailability

Phosphorus in BSS occurred in the soluble form of phosphates or in the insoluble form as organic and inorganic phosphorus. Fig. 5 shows the pathways of the different forms of phosphorus introduced in the TBFBR process.

The soluble phosphorus P-PO_4^{3-} in the feeding BSS was mainly transformed into inorganic phosphorus and accumulated in the TBS. The remaining part of phosphates was partially used by microorganisms to produce new biomass (0.14 kg) (in this case an accumulation factor of 0.01 was used) and exit the system in the permeate (0.08 kg). Based on monitoring data, there was no accumulation of phosphates in the reactor.

The inorganic phosphorus accumulated in the TBS, was the preponderant fraction equal to about 6.96 kg. 61.2% represented the fraction originally present in the BSS while 38.8% was precipitated as inorganic phosphorus. The accumulation of phosphorus in the form of inorganic salts in aerobic and anaerobic bioreactors that treated both wastewater (WW) and BSS in thermophilic conditions was frequently reported in the literature (Bolzonella et al., 2012; Collivignarelli et al., 2015a; Liu et al., 2011; Zhang et al., 2021).

In this experimentation the precipitation of P-PO_4^{3-} and P-org in the inorganic form was equal to 92%. Sánchez-Ramírez et al. (2019) observed in a thermophilic anaerobic digestion of BSS a percentage of 77% of phosphorus precipitated with respect to the total available. Similar results were obtained by Watts et al. (2006). In their study after anaerobic digestion the 78–91% of the incoming phosphorus remained bound in the solids, either through precipitation and/or as organic phosphorus.

Almost all the organic phosphorus introduced was converted into P-PO_4^{3-} thanks to operational conditions of the process. The amount of organic phosphorus in output was negligible: only a minimal amount crossed the ultrafiltration membranes. Hence, the predominant mechanism in the system was the accumulation of inorganic phosphorus in TBS, as shown in Fig. 6.

Since the results of the analysis of pathways, the concentration of phosphorus in TBS was evaluated and compared with thickened BSS and sludge taken directly from the oxidation reactor of the CAS system (Fig. 7). The characteristics of CAS and BSS were very similar since the only process that differentiate them is a pre-thickening phase. The results also highlighted a higher concentration of P in TBS (36,811.4 ± 4441.0 mg kg_{DM}^{-1}) with respect to CAS (26,631.7 mg

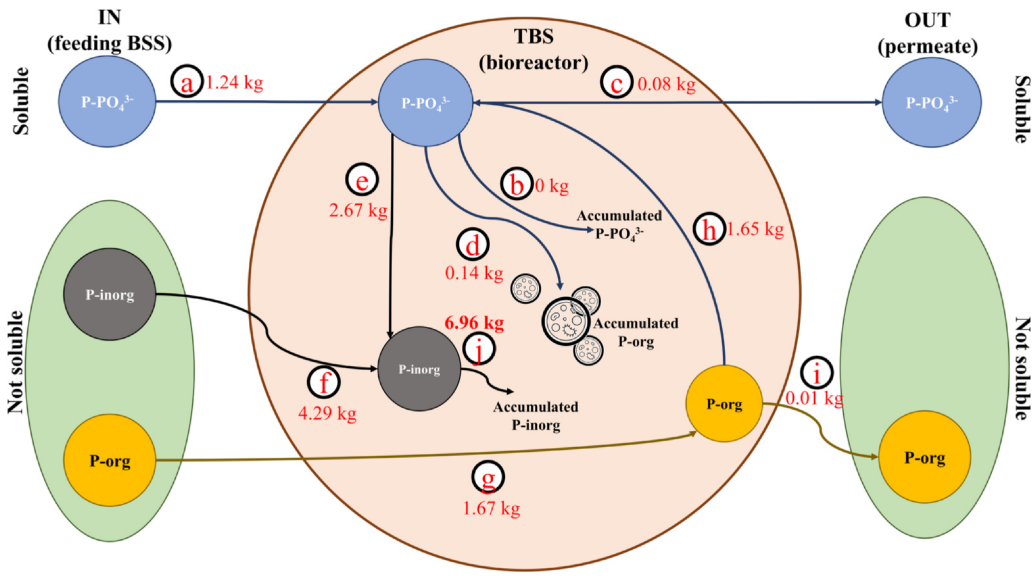

Fig. 5. Pathways of phosphorus. P-org: organic phosphorus, P-inorg: inorganic phosphorus.

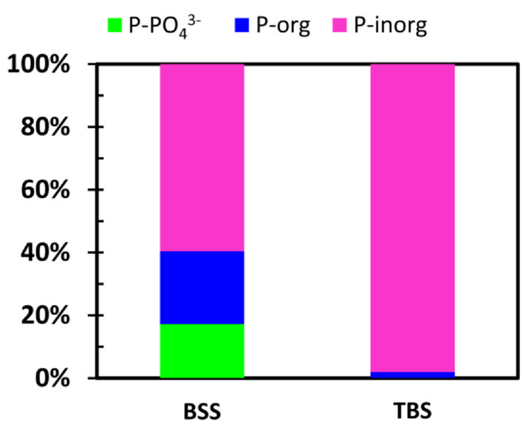

Fig. 6. Quantities of phosphorus in BSS and accumulated in TBS. P-org: organic phosphorus, P-inorg: inorganic phosphorus.

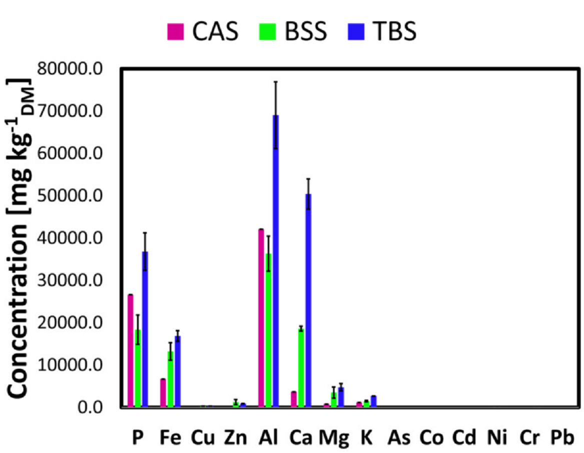

Fig. 7. Element concentrations in CAS, fed BSS and extracted TBS. DM: dry matter. [number of data: 2].

kg_{DM}^{-1}) and BSS (18,381.5 ± 3458.5 mg kg_{DM}^{-1}) thanks to precipitation. Also, a higher concentration in TBS of aluminium, calcium and iron was found: 69,027.4 ± 7865.9 mg_{Al} kg_{DM}^{-1}, 50,421.8 ± 3566.2 mg_{Ca} kg_{DM}^{-1}, 16,877.1 ± 1275.1 mg_{Fe} kg_{DM}^{-1}. Some metals (such as iron and aluminium) can have beneficial effects on certain types of plants (Kaur et al., 2016; Rizwan et al., 2017). These elements can increase resistance to biotic and abiotic stresses, improving crop growth and production (Kaur et al., 2016; Rizwan et al., 2017).

The purpose was to evaluate the bioavailability of phosphorus contained in TBS. In fact, in case of sludge spread on cultivated agricultural fields, evaluate whether the phosphorus present in the extracted TBS could be directly assimilated and used by crops for their biotic functions is extremely important.

Elution tests were performed on three different substrates: CAS, fed BSS and TBS. As shown in Fig. 8, diverse types of extractions (neutral, acid, basic) were tested, as well as the chelating activity on the calcium ion of $Ca_3(PO_4)_2$ induced by the presence of humic and fulvic acids and/or other organic compounds released by the root system of the tree essences. In all samples, the greatest bioavailability of phosphorus was found in basic extraction simulating chelating activity (Petermann test), as shown in Fig. 8(d). The results showed that CAS and fed BSS had good bioavailability values of the phosphorus present, up to values of 73%. The best result was obtained on TBS, with a bioavailability of 84.0 ± 25.3%. This was related with the higher concentration of phosphorus in inorganic form thanks to precipitation phenomena operated by TBFBR.

TBFBR acted on the characteristics of the phosphorus forms present in the TBS, increasing their solubility and therefore their bioavailability. As Zhang et al. (2021) showed, since phosphorus is a dying resource, recovering this nutrient through sludge is an alternative source as a fertilizer in agriculture or as a raw material by the phosphorus refinery industry. Liu et al. (2011) also proposed in their study that sludge from a thermophilic aerobic digestion process can be valuable for land application. In addition to phosphorus, other elements showed excellent bioavailability, up to 70% for cadmium and calcium. However, it should be noted that many of these elements (arsenic, cadmium, and lead) are present only in traces, as shown in Fig. 7.

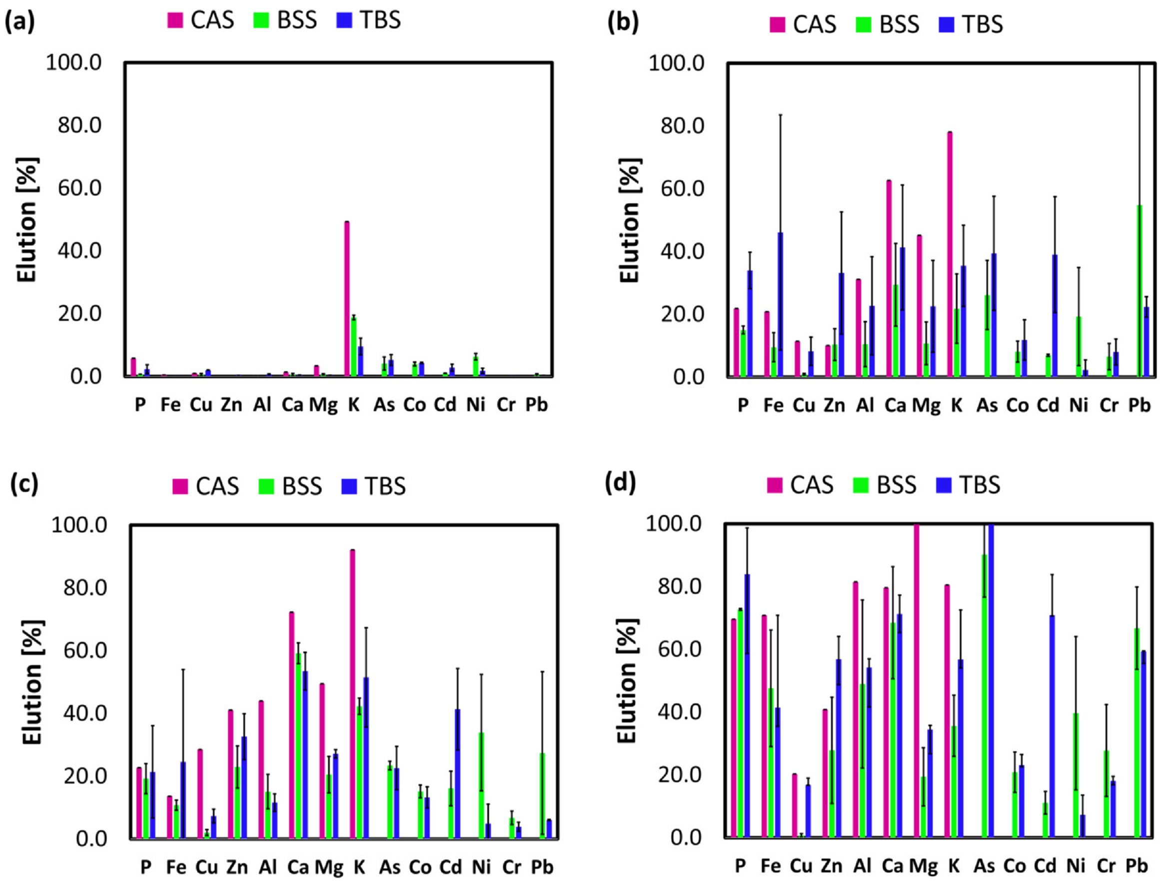

Fig. 8. Elution tests on CAS, BSS and TBS. Soil simulated: **(a)** neutral; **(b)** acidic with chelating activity; **(c)** acidic without chelating activity; **(d)** basic with chelating activity [number of data: 2].

3.3. Mass balances and economic evaluations

Daily flow rate (Q), TS concentration and load, VS load and the ratio VS/TS were calculated in the current scenario (S0) and in three diverse situations: (i) TBFBR treating low strength BSS (S1), (ii) TBFBR treating high strength BSS (S2), and TBFBR treating high strength BSS + anaerobic digestion (S3) (Fig. 9). Current yields of the diverse processes in the sludge line of the WWTP were considered in the balances.

Considering that the TBFBR total costs [€ $kg_{TStreated}^{-1}$] (Fig. S2, Eq. S1) rapidly decreased with the increase of the treated TS from 20 to about 65 kg m^{-3}, a higher concentration of BSS (65 kg m^{-3}) reached by dynamic thickening before treatment in TBFBR was assumed in S2 and S3. Given the function, it was not advantageous to increase the performance of dynamic thickening above 65 kg m^{-3} of TS in the BSS as there was no further economic savings. Starting from 1 m^3 of BSS from the water line, with the same characteristics for each scenario, different results in terms of sludge production could be obtained.

Based on the balances of mass, the ratio between the total costs of each scenario (S1, S2, S3) and the total cost of S0 were evaluated (Fig. 10). S1/S0 varied from 6.0% to 27.2% of S0. The ratio S2/S0 was slightly lower than S1: from 5.8% to 26.7% of S0. In terms of costs, the two scenarios S1 and S2 could guarantee the same savings compared to the reference scenario. The difference is negligible because in the costs evaluation, the sludge disposal in agriculture (SDC) was the most impactful item, rather than the cost of the treatment itself. In the last scenario S3, there was a range of variation in total costs between 19.5% and 91.8% of the current situation. In the worst case, the costs savings was minimum, less than 10%. Co-recovery of energy and matter in S3 was found to be the least convenient solution, while S1 and S2 could grant a cost saving of 73–96% of S0. In S3, sludge spreading was the cost item that had the greatest impact on total costs. A greater quantity of BSS was observed leaving the sludge line. As shown in Fig. 9, in S3 the total solids load (S_{TS}) at the outlet of WWTP was lower than in S0, but more than 3 times higher than in the first two scenarios. Accordingly, from an economic point of view, S3, despite the possibility of guaranteeing savings deriving from energy recovery, was not interesting when compared both to the current situation and to the other scenarios proposed. On the other hand, S1 and S2, excluding the anaerobic digester, ensured greater minimization of BSS production, with consequent lower costs for the WWTP management.

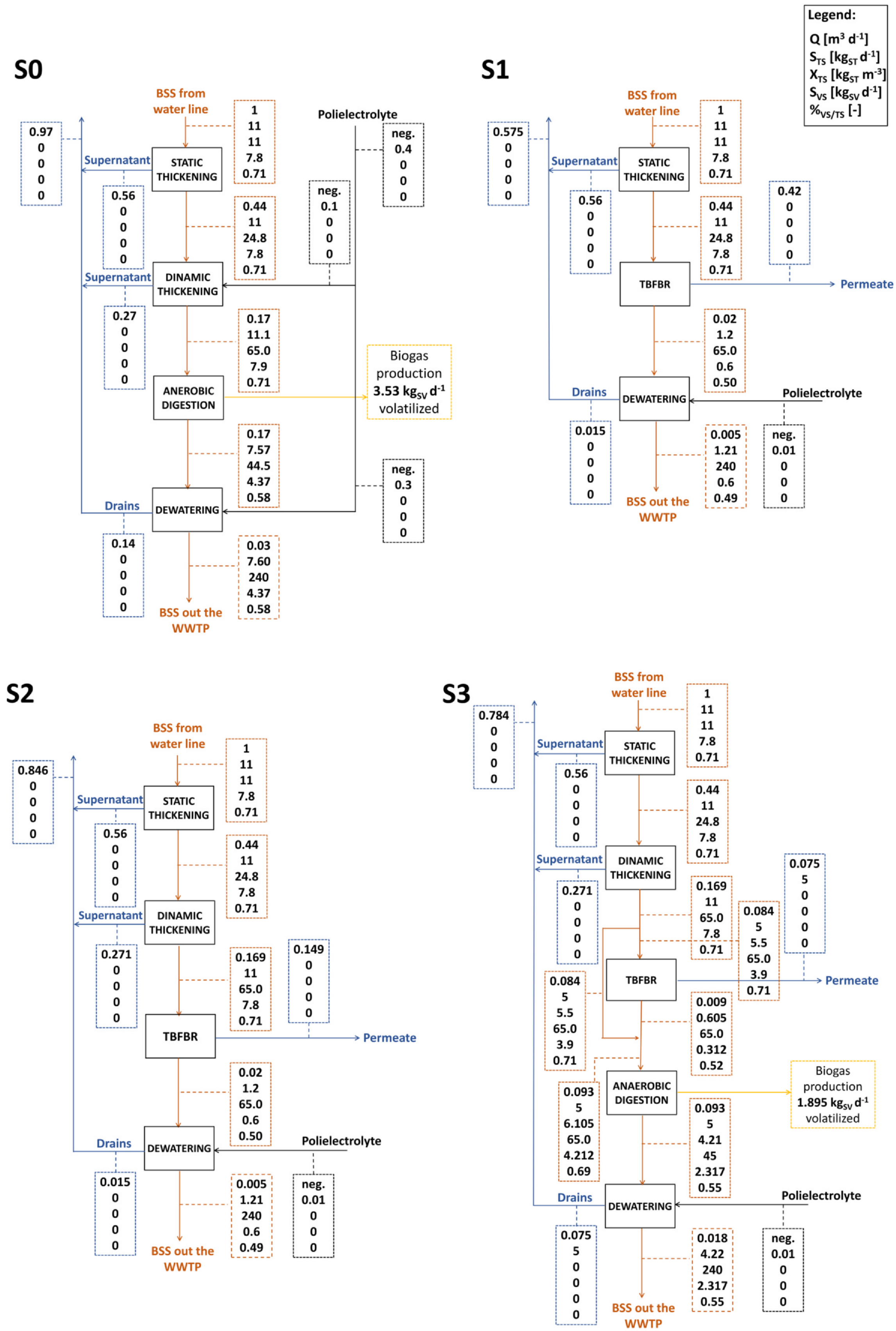

Fig. 9. Mass balances in S0, S1, S2, S3 scenarios. Q: daily flow rate, S_{TS}: TS load, X_{TS}: TS concentration, S_{VS}: VS load, $\%_{VS/TS}$: ratio VS/TS, neg: negligible.

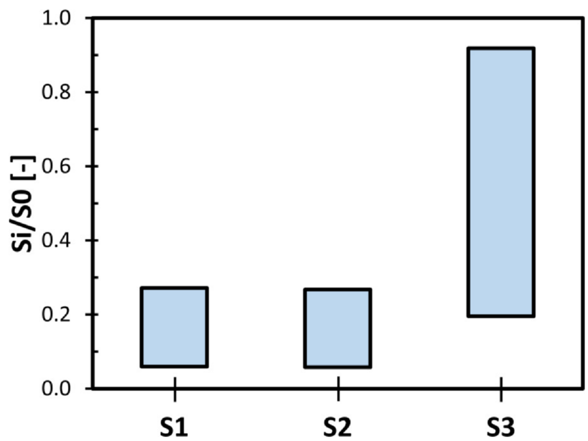

Fig. 10. Ratio between total costs in each scenario and reference situation (S0).

4. Conclusions

The BSS produced by a conventional urban WWTP, treated in TBFBR were strongly reduced (89–94%). The sludge production was evaluated in 0.0007–0.0023 kg $kg_{CODremoved}^{-1}$ d^{-1} and 0.0004–0.0014 kg $kg_{CODremoved}^{-1}$ d^{-1} of TS and VS produced, respectively. The results showed the higher chemical, chemical-physical and microbiological quality of TBS with respect to BSS and demonstrated the classification as "high-quality sludge" for reuse in agriculture, as required by one of the current most restrictive legislation. The 92% of the P-PO_4^{3-} and P-org precipitated in inorganic form and accumulated in TBS. In basic soils with chelating activity, phosphorus contained in TBS presented a higher bioavailability (84.0 ± 25.3%) with respect to BSS (72.7 ± 0.3%) and untreated CAS (69.6%). Other metals also showed excellent bioavailability and some of these (such as iron and aluminium) can have beneficial effects on the vegetative growth of plants, increasing crop production. Also, operating costs evaluation highlighted that the optimal solutions were S1 and S2 granting (73–96%) of operating costs savings with respect to S0. Conversely, it was inadvisable to co-recover energy and matter with anaerobic digestion after TBFBR (S3) due to lower operating costs savings (8–80%). Based on these results, TBFBR could ensure the closure of the WW treatment cycle allowing the transformation of WWTPs into WRRF and fitting the aims of circular economy.

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CRediT authorship contribution statements

Maria Cristina Collivignarelli: Conceptualization, Methodology, Validation, Supervision. **Alessandro Abbà:** Conceptualization, Validation, Writing – review & editing, Supervision. **Marco Carnevale Miino:** Conceptualization, Formal analysis, Investigation, Writing – review & editing, Visualization. **Francesca Maria Caccamo:** Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Stefano Argiolas:** Formal analysis, Investigation, Visualization. **Stefano Bellazzi:** Formal analysis, Investigation, Visualization. **Marco Baldi:** Methodology, Validation, Writing – review & editing. **Giorgio Bertanza:** Conceptualization, Methodology, Validation, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psep.2021.09.026.

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Estimation of thermal energy released by thermophilic biota during sludge minimization in a fluidized bed reactor: Influence of anoxic conditions

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ABSTRACT

Thermophilic biological fluidized bed reactors operating in aerobic/anoxic alternate conditions proved to be a feasible solution for sewage sludge minimization. However, to date, no data about energy released by thermophilic biota (ThBio) are available in literature. This work aims to estimate specific thermal energy released by ThBio highlighting the influence of daily anoxic conditions. A pilot-scale reactor (1 m^3) was fed continuously with mesophilic sewage sludge and monitored for more than four months and a thermophysical model was applied to estimate thermal energy released by ThBio ($k_{T,biota}$ and $k_{T,COD}$). Results suggested that the increase of daily anoxic time stimulated COD removal (92.7 ± 1.3 % vs. 81.3 ± 4.9 %, with 6 h and 0 h of daily anoxic time, respectively). The thermal energy released by ThBio was strictly dependent on anoxic conditions. In fact, increasing anoxic conditions from 0 h d^{-1} to 6 h d^{-1}, $k_{T,biota}$ and $k_{T,COD}$ reduced from 1.8 ± 1.3 Mcal kg_{VS}^{-1} kg_{COD}^{-1} and 26.6 ± 13.7 Mcal kg_{COD}^{-1} to 0.5 ± 0.1 Mcal kg_{VS}^{-1} kg_{COD}^{-1} and 15.6 ± 4.2 Mcal kg_{COD}^{-1}, respectively. Although, biological mechanism responsible of this behaviour is not completely clear, this work can serve as reference for future studies about the optimization of conditions to maximize thermal energy release from ThBio during organic substance degradation alternate aerobic/anoxic, in view of subsequent energy recovery.

1. Introduction

Thermophilic biological processes generally operate at temperature of 45 °C or higher (LaPara and Alleman, 1999). They are well known since the 1930s (in anaerobic conditions) (Rudolfs and Heukelekian, 1930) and the 1950s (in aerobic conditions) (LaPara and Alleman, 1999) but in recent years the interest of scientific community regrowth (Martín et al., 2018; Vital-Jacome and Buitrón, 2021; Yin et al., 2018).

Many applications have been studied and applied for degrading organic substances contained in diverse type of matrices as industrial wastewaters (Lopez-Vazquez et al., 2014), animal manure and sewage (Bi et al., 2019; Juteau, 2006), solid organic waste (Elango et al., 2009), and agro-residues (Cavinato et al., 2010).

Considering that sewage sludge production in wastewater treatment plants (WWTPs) increased significantly in last years and expected to enhance more in future (Collivignarelli et al., 2019; Kominko et al.,

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2017), thermophilic biological processes have been explored and applied as potential opportunity to minimize this production. For instance, anaerobic digesters operating in thermophilic conditions are known and applied in sludge treatments since many years (Buhr and Andrews, 1977). However, recent studies demonstrated the feasibility of minimize sewage sludge in WWTPs also using thermophilic fluidized bed reactor operating in alternate aerobic/anoxic conditions (Collivignarelli et al., 2022). In this case, previous studies focused on pollutants removal and possibility of reuses of residues neglecting to evaluate the thermal energy released by thermophilic biota (ThBio).

The release of thermal energy by thermophilic microorganisms during organic substance degradation is a well-known phenomenon, being also exploited for maintaining reactors in autothermal conditions both in case of aerobic (LaPara and Alleman, 1999) and anaerobic thermophilic digestion (Lettinga, 1995; van Lier, 1996). However, the release of thermal energy by ThBio's metabolic activities in alternate aerobic/anoxic conditions has not been fully investigated especially the influence of anoxic conditions in a biological fluidized bed reactor treating mesophilic sludge.

Thermal energy release during biodegradation of residues represents a key aspect in the new concept of more sustainable and circular WWTPs (Liu et al., 2020). The European parliament (2018) introduced a waste hierarchy, also applicable on WWTPs' residues, which defines energy recovery as priority after minimization and reuse options. Therefore, quantify the specific production of thermal energy released during organic substance removal by thermophilic biological systems represents a crucial point to better exploited this phenomenon in view of thermal energy reuse.

This paper aims to estimate the amount of thermal energy released during the degradation of sewage sludge by ThBio monitoring a pilot-scale reactor for more than four months. The main novelty points of this work with respect to existent literature are (i) the estimation of specific coefficients of thermal energy produced by ThBio ($k_{T,biota}$ and $k_{T,COD}$) during organic substance mineralization; (ii) the study of the influence of anoxic conditions on specific thermal energy released; (iii) the pilot-scale thermophilic biological fluidized bed reactor was coupled with ultrafiltration membranes to operates with high biomass concentration. ThBio was fed with mesophilic sewage sludge operating in a continuous mode to obtain more reliable data.

2. Materials and methods

2.1. Pilot-scale reactor

To evaluate the specific thermal energy released by ThBio, a pilot-scale reactor of 1 m^3 was used. The apparatus consisted of three main components: (i) the reactor, where the biological reaction took place; (ii) the boiler, used to provide heat to maintain the minimum temperature required by the system (50 °C); and (iii) the pipes used to move ThBio in the system (Fig. 1). To avoid high temperature (>60 °C) that could inhibit the activity of ThBio (LaPara and Alleman, 1999), a cooling system with tap water (15 °C) was provided, but not used as the temperature of the reactor did not spontaneously exceed 55 °C during tests.

The reactor was a steel cylinder with an external diameter of 0.95 m^2, isolated with a rock wool panel 5 cm thick. The opening, at the top of the cylinder was 30% of the surface. The boiler tank was a steel cylinder too, with an external diameter of 0.5 m^2, 1.08 m height, and a capacity of 200 L. It was kept at 70 °C by two electrical resistances, 2 kW each, and the envelope was insulated in the same way as the reactor. The steel pipes, not insulated, were of two main nominal diameters (DN), DN32 and DN65, 15 m and 5.3 m long, respectively. To avoid thermophilic sludge settleability issues, ultrafiltration membranes were used to separate ThBio from aqueous phase (permeate) which was extracted from the system. Details on ultrafiltration membranes are reported in our previous works (Collivignarelli et al., 2021a, 2021b).

2.2. Experimental design and management mode

The system was fed with mesophilic mixed sludge (primary + secondary), sampled in a full-scale WWTP (130,000 population equivalents) after a phase of static thickening and before anaerobic digestion. In Table 1, the main characteristics of fed biological sewage sludge are reported.

To obtain robust data, the pilot-scale system operated in continuous mode for one month without anoxic phases to acclimatize the ThBio to the feed (this phase was not considered in data analysis). When stable conditions were reached, the pilot-scale system was monitored for more than four months. The hydraulic retention time (HRT) was imposed equal to 9.5 d, based on satisfactory results in terms of organic substance removal obtained in our previous tests (Collivignarelli et al., 2021b). To evaluate the influence of anoxic phases on thermal energy released by ThBio, diverse aerobic/anoxic conditions were tested (Table 2) avoiding

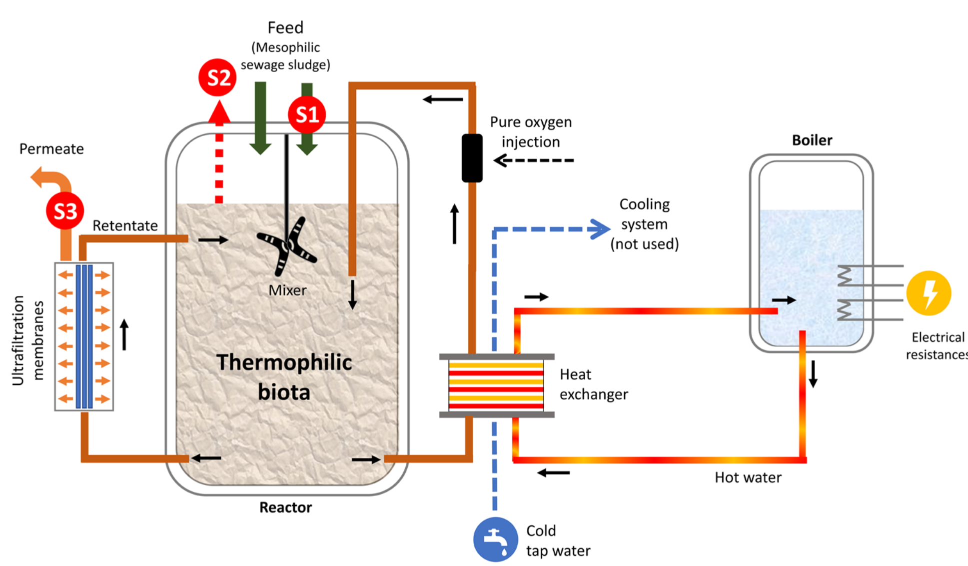

Fig. 1. Scheme of the thermophilic and heating/cooling systems. Sampling point of feed, thermophilic biota (ThBio) and outflow of the system are indicated with S1, S2, and S3, respectively.

Table 1
Characteristics of fed biological sewage sludge. COD: chemical oxygen demand; TP: total phosphorus; TN: total nitrogen; TK: total potassium; TS: total solids; VS: volatile solids; DM: dry matter.

Parameter	u.m.	Value
Chemical and chemical-physical parameters		
COD	mg L^{-1}	30,000–35,000
TP	mg L^{-1}	450–600
TN	mg L^{-1}	1100–1300
TK	mg L^{-1}	20–30
N-NH_4^+	mg L^{-1}	500–600
N-NO_x	mg L^{-1}	1–3
TS	kg m^{-3}	18–22
VS	kg m^{-3}	10–15
Other parameters		
Degree of humification	% DM	23–26
pH	–	5–6
Conductivity	μS cm^{-1}	2000–3000
Phytotoxicity test (expressed as germination index)	%	85–87

the injection of pure oxygen. Based on the experience of the authors, to avoid foams formation and consequent management issues, when more than 2 h of anoxic conditions were imposed daily, they were grouped in 2 h-block separated by 6–10 h of aerobic conditions.

During aerobic conditions pure oxygen injection granted 2 – 4 mg L_{O2}^{-1} in the thermophilic reactor. To maintain the pH in the reactor almost constant (7.0–7.4), 0.1–0.3 L d^{-1} of NaOH was dosed. No extractions of excess sludge have been made during test.

Feed, permeate and thermophilic sludge were sampled three times a week. For all samples, total COD has been analysed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Thermophilic sludge was also investigated volatile solids (VS) content according to (UNI EN 9:, 1516, 2007, 2007).

2.3. Thermophysical model

To understand the magnitude of the thermal energy released by ThBio (Q_{biota}), a model for a heat balance has been applied including in its boundary the apparatus, the outdoor environment, and the boiler. The monitoring of the boiler activity and the sludge temperature allowed to skip the heat exchanger efficiency for the heat balance between the hot water and the sludge. As this value depends on a wide variety of factors (as the configuration, fluid thermophysical properties and the state of wear), a proper approximation could not be achieved. Thereby, a balance between the hot water temperature difference and the sludge temperature has been considered neglecting heat exchanger losses. The energy balance included the difference between total heat losses (Q_{loss}) and the energy provided by the boiler to keep reactor temperature constant (Q_{supply}) as reported in the following equation (Eq. 1):

$$Q_{biota} = Q_{loss} - Q_{supply} = \sum_{i}^{t}(Q_{c,reactor,i} + Q_{c,boiler,i} + Q_{c,pipes,i} + Q_{evap,i} + Q_{conv,i}) - Q_{supply} \quad (1)$$

where t represents the monitoring time. $Q_{c,reactor}$, $Q_{c,boiler}$, and $Q_{c,pipes}$ are the contribution of heat lost from the envelope of the reactor, the boiler, and the pipes, respectively. Q_{evap} and Q_{conv} represent the losses due to the evaporation flow rate and convection, respectively, from the free surface of the reactor.

Temperature of thermophilic sludge was monitored by a probe (Endress+Hauser Oxymax W COS31) submerged into the reactor. Data about temperature and relative humidity of the external air were collected from a control unit located less than 2 km from the pilot-scale plant (ARPA Lombardia, 2021). For the energy balance the geometrical parameters and material thermal properties have also been considered. Conductivity of steel and rock wool were assumed equal to 17 W m^{-1}

Table 2
Management strategies for imposed anoxic conditions in the thermophilic reactor.

Conditions	Number per day and duration [h] of:		Weeks																	
	Non aeration	Aeration	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15	#16	#17	#18
A	0	24			X	X		X												
B	1 × 2 = 2	1 × 22 = 22	X	X			X		X	X	X	X								
C	2 × 2 = 4	2 × 10 = 20											X	X						
D	3 × 2 = 6	3 × 6 = 18													X	X	X	X	X	X

K^{-1} and 0.04 W m^{-1} K^{-1}, respectively (Incropera et al., 1995). The internal convection heat transfer coefficient (α) for pipes has been evaluated assuming a laminar flow with Eq. (2):

$$\alpha_i = 900 \bullet (1 \quad + \quad 0.001 \bullet T_1) \tag{2}$$

where T_1 represents the average sludge temperature. The convection heat loss from the free surface has been evaluated as presented in (Shah, 2022), according to Eqs. (3, 4):

$$Q_{conv,i} \quad [W] = h_i \bullet A \bullet (T_S - T_a)_i \tag{3}$$

$$h_i \left[\frac{W}{m^2K}\right] = 0.22 \bullet (T_S - \quad T_a)^{\frac{1}{3}}{}_i \tag{4}$$

where h represents the convection heat transfer coefficient and A is the exposed area. T_S and T_a are the temperature of the sludge and the external air, respectively. As proposed by (Asdrubali, 2009), the evaporative heat loss was determined on the basis of the evaporative flow rate (Eqs. 5, 6):

$$Q_{evap,i} \quad [W] = G_i \bullet r_0 \tag{5}$$

$$G_i \left[\frac{kg}{s}\right] = k \bullet A_{open} \bullet [P_S \bullet (T_S) - \Phi \bullet P_S \bullet (T_a)] \tag{6}$$

where G_i is the evaporative flow rate. r_0 represents the latent heat of vaporization and P_S is the saturation pressure of the sludge. Due to the composition of the sludge (composed for more than 90% by water), r_0 was assumed equal to 2260 kJ kg^{-1} and P_s was calculated as a function of sludge temperature with the Buck equation for the vapour pressure of water, that is the most accurate for the sludge temperature range according to (Lide and Kehiaian, 2020). The mass transport coefficient (k) was considered equal to 3.4×10^{-8} kg m^{-2} Pa^{-1} s^{-1} as proposed by (Incropera et al., 1995). T_a and T_S are the air and sludge temperatures, respectively. Φ represents the relative humidity while A_{open} is the free surface area where the evaporation occurs.

2.4. Data analysis and elaboration

To reduce data fluctuations and to obtain more stable results, all balances were made on weekly basis. Thermal energy released weekly by ThBio and estimated with the model described in Section 2.3. was compared with VS in the reactor and COD load removed, calculated as follows (Eq. 7):

$$\mu COD_{rem} \quad [\%] = \frac{LCOD_{in} - LCOD_{out} - LCOD_{acc}}{LCOD_{in}} * 100 \tag{7}$$

Where $LCOD_{in}$ and $LCOD_{out}$ represent the weekly load of COD in the fed mesophilic sludge and in the permeate, respectively. $LCOD_{acc}$ express the load of COD accumulated weekly in the thermophilic reactor.

3. Results and discussion

3.1. Monitoring of pilot-scale system

The OLR was in the range 0.1–0.2 kg_{COD} kg_{VS}^{-1} d^{-1} for the entire duration of the experimental activities (Fig. 2a). However, COD removal rates varied being significantly influenced by anoxic conditions with

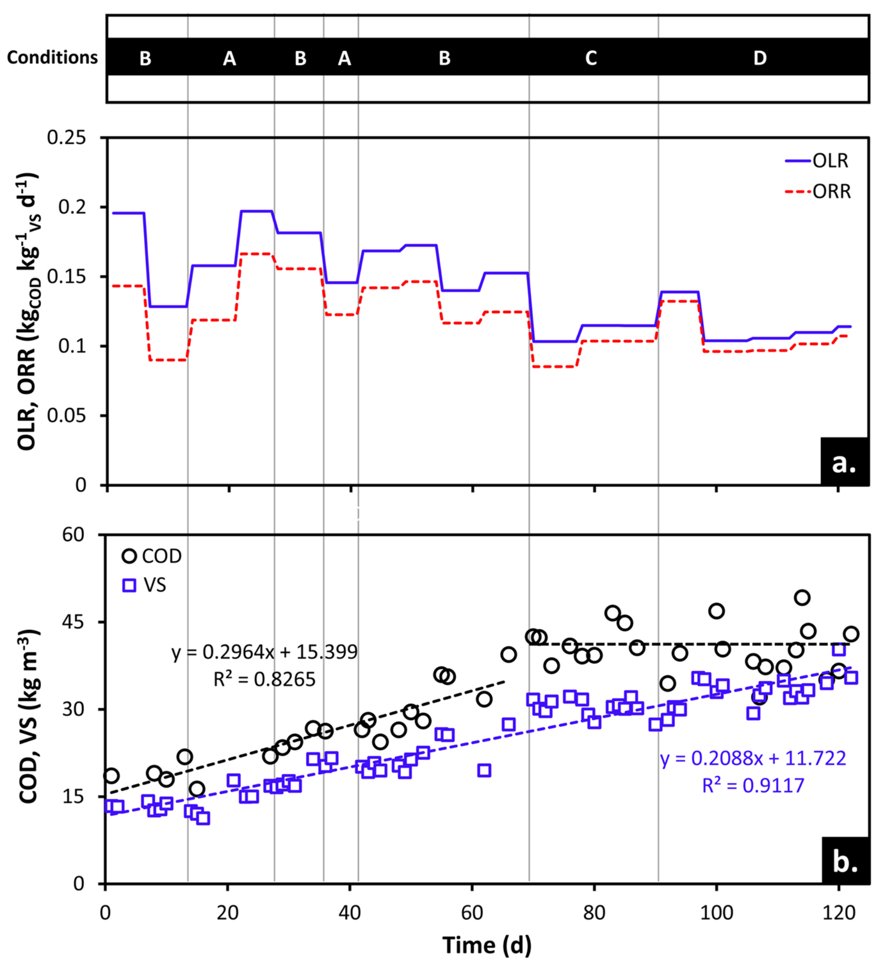

Fig. 2. Continuous monitoring of (**a**) organic loading rate (OLR) and organic removal rate, (**b**) COD and VS measured in the reactor.

higher values reached in 6 h of daily anoxic time (92.7 ± 1.3 %), followed by 4 h (86.3 ± 5.3 %). In case of lower daily anoxic time, COD removal yields remained almost the same: 81.3 ± 4.9 % vs. 80.5 ± 4.3 % with 0 h and 2 h, respectively. Also, in our previous tests of thermophilic degradation, mesophilic sewage sludge was subjected to alternate aerobic/anoxic conditions to enhance organic matter removal (Collivignarelli et al., 2017, 2015). In fact, organic substance removal was stimulated by stronger alternate aerobic/anoxic conditions, due to the combined effect of solubilization of particulate COD during anoxic phases and mineralization of dissolved organic substance during aerobic conditions (Collivignarelli et al., 2017; Wei et al., 2003). Moreover, despite in these tests the initial N-NO_x concentration is very limited (1–3 mg L^{-1}), in presence of marked anoxic conditions, higher denitrification phenomena with organic substance consumption is expected (Li et al., 2022).

In terms of COD removal, Visvanathan et al. (2007) obtained similar results (up to 79%) treating a landfill leachate with thermophilic membrane system but without providing anoxic periods. Also, paper mill wastewater were treated effectively without providing alternate conditions (Suvilampi and Rintala, 2003). However, in case of BSS, the presence of a significant part of COD in particulate form necessitates the alternate conditions of aerobic and anoxic phases (Bougrier et al., 2005; Tomei et al., 2008).

Focusing on the thermophilic reactor, during 0 h and 2 h of anoxic conditions, COD tended to accumulate reaching 40 kg m^{-3} of concentration from initial 18.5 kg m^{-3} (Fig. 2b). On the contrary, when daily anoxic conditions increased, COD in thermophilic reactor remains almost constant 40–45 kg m^{-3}. This result can be related with two factors: (i) the higher concentration of VS in the second phase of experimental activities which help to remove organic matter faster (Fig. 2b), and (ii) the solubilization of particulate organic matter during anoxic periods subsequently mineralized by ThBio in aerobic conditions (Collivignarelli et al., 2017; Wei et al., 2003).

VS concentration continue to increase since start of monitoring periods from 13 kg m^{-3} to 35 kg m^{-3}. Although it may seem like a substantial increase, it should be noted that on average 1–1.5 kg_{VS} d^{-1} were fed for a total of about 122–183 kg during the entire monitoring phase. Therefore, the overall removal of VS was approximately 82–88 %, in agreement with what has been highlighted in our previous works (Collivignarelli et al., 2021b, 2021a).

3.2. Thermal balance

The thermal energy balance was based on a set of measured parameters, daily monitored on the pilot-scale system for more than four months. External temperature and relative humidity ranges in − 3.8–19.8 °C, and 9.4 – 100 %, respectively (Fig. S1). Temperature of sludge and daily operating time of heating system were also collected and used in the model. In this last case, during the monitoring period the daily activity of the boiler varied depending on the external conditions and the organic matter degradation activity of the sludge (5.0 ± 2.2 kWh d^{-1}).

The wide range of variation means a relevant thermal activity occurs in the rector. Looking at the outcomes of Fig. 3a, indeed, the results of the daily energy balance show different peaks in the energy supplied by the boiler. As expected these peaks are related to a lower energy release by ThBio, as no clear correlation occurs with the conduction and evaporation heat losses. The contribution of heat lost by conduction phenomenon in the reactor and the boiler (5.6 ± 0.1 kWh d^{-1} and 2.3 ± 0.1 kWh d^{-1}, respectively) were lower than pipes contribution (41.1 ± 0.5 kWh d^{-1}) due to the presence of thermal insulation. The evaporation from the free surface at the top of the reactor was almost half of the conduction from the pipes (19.5 ± 0.6 kWh d^{-1}), while the convection phenomenon was negligible (0.2 ± 0.1 kWh d^{-1}).

A reported in Fig. 3b, the amount of total thermal energy released daily by ThBio seems to be influenced by anoxic conditions with 6 h > 2 h > 0 h > 4 h (65.1 ± 3.2 kWh d^{-1}, 64.7 ± 3.7 kWh d^{-1}, 63.0 ± 5.1 kWh d^{-1}, and 61.2 ± 7.5 kWh d^{-1}, respectively). However, evaluating data on a weekly basis (Table S1), values remained almost unchanged being 420.0 ± 101.0 kWh w^{-1} in condition A and 412.2 ± 101.0 kWh w^{-1} in condition D.

The release of thermal energy by the microbial metabolism was sufficient to guarantee the self-thermal conditions in all tested conditions (A, B, C, and D), with the activation of the auxiliary energy supply system for a limited period during tests (6.4 d out of 122 d). This result broads the knowledge about the possibility of granting autothermal thermophilic conditions feeding a high amount of organic substance (COD ≥ 20,000–40,000 mg L^{-1}) not only in fully aerobic conditions (Kambhu and Andrews, 1969; LaPara and Alleman, 1999; Popel and Ohnmacht, 1972) but also in aerobic/anoxic alternate conditions. Data establish how the energy input from the boiler is very low compared to the heat released by the ThBio: reducing the heat losses from the system, through isolation and control of boundary thermal conditions, definitely

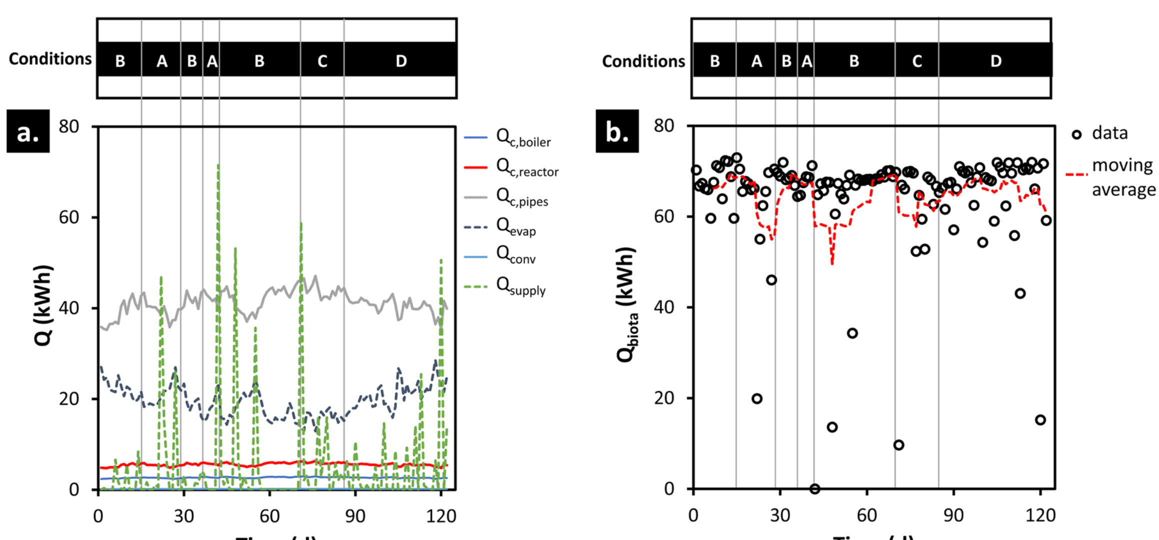

Fig. 3. (**a**) Thermal energy contributions as a function of operating time. (**b**) Thermal energy released by ThBio during mineralization of mesophilic sewage sludge. Moving average period was set equals to 7 d.

help achieving the auto-thermal conditions.

3.3. Specific thermal energy production

Thermal energy released by ThBio in diverse management phases was compared with COD removed and VS present in the reactor (Fig. 4). Considering mean values for each conditions tested, increasing anoxic time (from A to D conditions), data analysis revealed an almost constant total energy released by ThBio and an increase in organic substance removed. This agrees with Layden et al. (2007b, 2007a) who showed that, from a microbiological point of view, a higher amount of organic substance removed matches with a higher amount of energy released by ThBio during their metabolic reactions.

Moving from conditions A to D, the concentration of VS increased as results of microbial growth and mesophilic VS fed to the reactor (Section 3.1.). Therefore, this aspect has been taken into account when specific coefficient of thermal energy released by ThBio ($k_{T,biota}$) was determined.

Based on results (Fig. 5a), the anoxic conditions strongly influenced the specific energy released during organic carbon degradation by biota. Increasing anoxic conditions from 0 h d^{-1} to 6 h d^{-1}, $k_{T,biota}$ reduced from 1.84 ± 1.26 Mcal kg_{VS}^{-1} kg_{COD}^{-1} to 0.51 ± 0.14 Mcal kg_{VS}^{-1} kg_{COD}^{-1}. From an initial analysis, this result could be related to the amount of organic substance fed to the reactor which acted as limiting factor being almost totally removed by the increasing concentration of ThBio in the reactor, on average higher in periods C and D than in A and B.

However, focusing on thermal energy released by ThBio based on COD removed ($k_{T,COD}$), an evident reduction increasing daily anoxic time has been showed dropping from 26.6 ± 13.7 Mcal kg_{COD}^{-1} (aerobic conditions) to 15.6 ± 4.2 Mcal kg_{COD}^{-1} (6 h d^{-1} of anoxic time) (Fig. 5b). Therefore, the crucial result is that for the same quantity of organic substance removed, the ThBio produce less energy if subjected to more marked anoxic conditions.

However, in literature, no data about thermal energy released by ThBio during aerobic/anoxic conditions are available since most of studies focused on energy balance of thermophilic fully aerobic or anaerobic systems or specific energy released was not provided (Lawrence and McCarty, 1970; Liu et al., 2011; Nosrati et al., 2007; Leite et al., 2016) and therefore, make a direct comparison is not possible. For instance, Liu et al. (2011) studied the application of an autothermal thermophilic aerobic reactor for sewage sludge digestion, highlighting

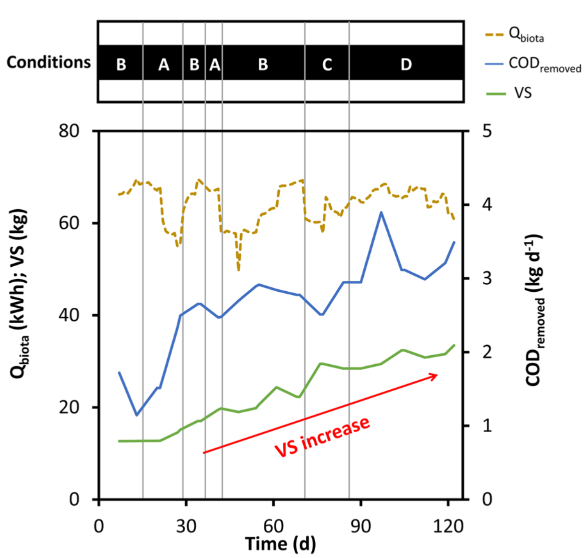

Fig. 4. Thermal energy released by ThBio (Q_{biota}) compared with organic substance removed ($COD_{removed}$) and ThBio present in the reactor (VS). Data are represented as moving average (period: 7 d).

stable conditions were reached up to 61.5 °C with high performance of VS removal (up to 41.2% in 15 d), but no data about the specific energy released by biota were provided. Leite et al. (2016) compared the total amount of energy produced in single- and two-phase thermophilic anaerobic digester of secondary sludge but also in this case they did not study the specific amount of thermal energy released by ThBio as a function of VS and/or COD in the reactor.

A lower release of thermal energy in case of stronger daily anoxic conditions could be due to the alteration of metabolic activity of microorganism during alternate aerobic/anoxic conditions. For instance, several authors proved that low concentration of oxygen stimulates ThBio to a maintenance metabolism with limited adenosine triphosphate (ATP) production while, on the contrary, aerobic conditions stimulate the replenishment of cells' energy stores degrading organic substance (Chudoba et al., 1992; Semblante et al., 2014). These results were in agreement with those reported by Chen et al. (2003) and Yağcı et al. (2018) which found also that the reduction in the excess sludge production is related with the cyclic aerobic/anoxic phases due to the alteration of catabolism and anabolism produced by the change of ATP concentration in the sludge. However, most of the studies were carried out in mesophilic conditions and a definitive explanation of the phenomenon and the responsible biological mechanisms in ThBio remains still unclear requiring further studies.

These results represent an interesting point since in future the optimization of operative conditions for support thermal energy release by ThBio during organic substance degradation in alternate aerobic/anoxic conditions could be exploited in terms of energy recovery.

3.4. Possible limitations of the study and tips for future research

This study tries to understand the complex phenomenon of thermal energy release from ThBio, specifically quantifying the amount of energy as a function of daily aerobic/anoxic time.

The difficulties to maintain stable VS concentration in the system could represent a possible limit due to the different average concentration of solids in the reactor during initial phases (A and B) compared to the final ones (C and D). This was due to the different daily aerobic/anoxic time tested. In conditions A and B, anoxic time was absent or not enough to completely solubilize organic matter and reduce, with consequential VS increase.

Longer duration of phases tested, and the scale-size factor should be considered to better study the statistical difference between diverse conditions and to foresees results in large-scale applications, respectively. The authors also suggest to deeply investigate the optimal operational point in term of daily anoxic time, between organic substance removed and thermal energy released, considering several aspects such as economic and environmental impacts.

4. Conclusions

Thermal energy released by ThBio during the metabolic reactions of degradation of mesophilic sewage sludge proved to be strongly influenced by daily anoxic conditions. Starting from aerobic conditions and increasing anoxic conditions up to 6 h d^{-1}, COD removal grown from 81.3 ± 4.9 % to 92.7 ± 1.3 %, but at the same time $k_{T,biota}$ and $k_{T,COD}$ consistently reduced from 1.8 ± 1.3 Mcal kg_{VS}^{-1} kg_{COD}^{-1} and 26.6 ± 13.7 Mcal kg_{COD}^{-1} to 0.5 ± 0.1 Mcal kg_{VS}^{-1} kg_{COD}^{-1} and 15.6 ± 4.2 Mcal kg_{COD}^{-1}, respectively. This suggest that the optimal operational point in term of daily anoxic time, between organic substance removed and thermal energy released, should be investigated taking into account several aspects such as costs, VS reduction, and environmental impacts. Moreover, further studies are suggested to better evaluate biological mechanisms responsible of this behaviour and to optimize conditions for the potential recovery thermal energy release from ThBio during organic substance degradation in alternate aerobic/anoxic conditions.

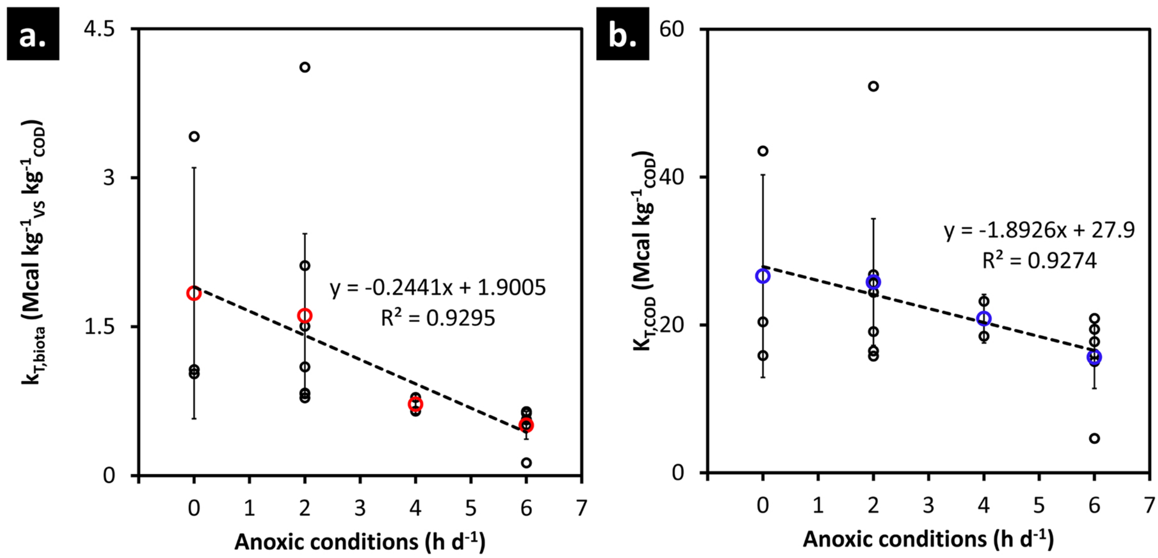

Fig. 5. (**a**) Specific thermal energy released by ThBio ($k_{T,biota}$) and (**b**) thermal energy released by ThBio based on COD removed ($k_{T,COD}$) as a function of anoxic conditions. Black and coloured dots represent raw data and mean, respectively. Black lines consist in the linear interpolation of mean values.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psep.2022.08.034.

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CONCLUSIONS AND FUTURE OUTLOOKS

The Ph.D. thesis concerned a technology aimed at reducing biological sewage sludge production. The core of the system is a thermophilic aerobic/anaerobic membrane biological reactor (TAMR). The target could be obtained by two different approaches:

(i) prevention in the water line (treating industrial wastewater or aqueous waste with low production of thermophilic sludge),

(ii) minimization in the sludge line (directly treating biological sludge already produced).

Regardless of the application, TAMR produces two residues:

(i) an aqueous permeate abundant in carbon and nitrogen,

(ii) a surplus thermophilic biological sludge characterized by small quantities.

The final focus of this Ph.D. research was to study and propose various recovery alternatives for these process residues.

This thesis consists of a collection of some research articles and chapters published during my Ph.D. studies and the structure of the work is based on four Work Steps (WSs) on TAMR, each divided into two sub-steps. For each WSs, the main scientific conclusions found are reported below:

- **WS1 - Monitoring and performance study**
  - **WS1.1 - Monitoring and performance of full-scale TAMRs for aqueous waste treatment**

    The focus is on the routinary monitoring of two full-scale TAMRs treating high-strength industrial wastewater, so located in two different water lines. Results highlighted the strong performance of an advanced biological system in treating high loads of different chemical pollutants (COD: 79-82%, N-NO_x: 97-99%, TAS: 95%, MBAS: 98%) and the low thermophilic sludge production (0.052 kg_{VS} kg_{COD}^{-1}) compared to conventional activated sludge systems. Thanks to an advanced molecular technique, the presence of active nitrifying bacteria in the thermophilic biological reactor was excluded and the marked ammonia removal was linked to the stripping caused by pure oxygen injection.
  - **WS1.2 - Monitoring and performance of a pilot-scale TAMR for sewage sludge minimization**

    The application of TAMR in the sludge line to minimize sewage sludge already produced by a CAS system was studied thanks to a 9 months-experimentation with a pilot scale. The monitoring results revealed high performance on COD (up to 90%) and volatile solids (up to 92%), and a low specific sludge production (0.0004–0.05 kg_{VS} kg_{COD}^{-1}). The versatility of the technology was demonstrated for the first time by testing a double location, both downstream of a thickener and downstream of an anaerobic digestor. The cost analysis highlighted the economic feasibility of the process, with a saving of 73–96% on current operating costs through the location of TAMR after pre-thickening.

- **WS2 - Process optimization**
  - **WS2.1 - Statistical analysis of monitoring data**

    Based on the monitoring data, collected for five years, of a full-scale TAMR treating various high-strength aqueous waste, a multivariate statistical analysis (Pearson and Spearman correlation coefficients and multivariate linear regression) was applied highlighting a correlation between several pollutants fed and TAMR performances (COD fed - N-NO_x fed, μ_{COD} - $\mu_{N\text{-}NOx}$, COD fed - total nitrogen fed, etc). This numerical analysis was developed as a functional tool for water utilities and managers of treatment plants to predict removal yields of the TAMR system in the presence of a limited amount of data or to decide the correct mixture of aqueous waste to feed to the biological reactor.
  - **WS2.2 - Rheological behavior of thermophilic sludge**

    Considering the high concentration of heavy metals in aqueous waste fed to a full-scale TAMR, the strong influence of some heavy metals (Fe^{3+}, Cu^{2+}, Al^{3+}) on the rheological properties of thermophilic sludge was demonstrated. That depended on the initial consistency of the sludge, and the types and dosage of heavy metals.

Furthermore, calcium carbonate, sand, and sodium bentonite were separately added to the thermophilic sludge to evaluate the influence of non-volatile solids and results suggested that the thermophilic sludge consistency significantly enhanced increasing sodium bentonite. On the contrary, calcium carbonate and sand showed a relatively small influence on the rheological properties. The thixotropic behavior of thermophilic sludge, more pronounced at high shear rates, was also demonstrated. The study of thermophilic sludge rheology represents a crucial point considering that sludge consistency affects hydrodynamic behaviour in reactors and therefore the performance of the process.

- **WS3: Residues and energy recovery**
  - **WS3.1 - Reuse of residues**

The possible recovery of the permeate was investigated by oxygen uptake rate (OUR) and nitrogen uptake rate (NUR) tests. An excellent biological treatability of the permeate by the mesophilic biomass (> 31 mg_{O2} g_{VSS}^{-1} h^{-1}) was observed and the denitrification kinetics reached with the diluted permeate (4.0 $mg_{N\text{-}NO3\text{-}}$ g_{VSS}^{-1} h^{-1}) were found comparable to those of methanol (4.4 $mg_{N\text{-}NO3\text{-}}$ $g_{VSS}^{-1}h^{-1}$). The results highlighted the feasibility of reusing the aqueous residue of TAMR as an alternative carbon source for denitrification processes in CAS systems to improve the kinetics of nitrate removal, in place of external sources of carbon of synthetic origin. After stripping in an acid tower for ammonia recovery, the permeate could be recirculated in the WWTP water line hosting TAMR, confirming the complementarity between mesophilic and thermophilic processes for the biodegradation of organic substances.

In the case of TAMR application in the sludge line, the reduced surplus of thermophilic sludge extracted from the biological reactor was analysed and compared with the treated mesophilic sludge fed to TAMR. The results showed the higher chemical, chemical-physical, and microbiological quality of the thermophilic sludge and demonstrated the classification as "high-quality sludge" for reuse in agriculture, as required by the Lombardy legislation. Moreover, 92% of P-PO_4^{3-} and P-org precipitated in inorganic form and accumulated in the thermophilic sludge. In basic soils with chelating activity, the phosphorus contained in the thermophilic sludge presented a good bioavailability for crops (84.0 ± 25.3%), in the case of agricultural reuse.

- **WS3.2 - Thermal energy production**

  The study focused on exothermic degradation of the biodegradable organic substance in the thermophilic bioreactor (1 m^3 pilot plant) operating in aerobic/anoxic alternate conditions for sewage sludge minimization. The thermal energy released by thermophilic biota (to date, no data are available in the literature) was estimated thanks to the application of a thermophysical model, and a strict dependence between thermal energy and anoxic hours was detected. Results suggested that the increase of daily anoxic time from 0 h d^{-1} to 6 h d^{-1} (i) stimulated COD removal (81% vs. 93%, respectively), but at the same time, (ii) $k_{T,biota}$ (specific coefficient of thermal energy released by thermophilic biomass) and $k_{T,COD}$ (thermal energy released by thermophilic biomass based on COD removed) reduced from 1.8 Mcal kg_{VS}^{-1} kg_{COD}^{-1} and 26.6 Mcal kg_{COD}^{-1} to 0.5 Mcal kg_{VS}^{-1} kg_{COD}^{-1} and 15.6 Mcal kg_{COD}^{-1}, respectively. This suggested that the optimal operational point in terms of daily anoxic time, between organic substance removed and thermal energy released, should be investigated considering several aspects such as costs, volatile solids reduction, and environmental impacts. Moreover, this study represented a starting step for future research on the possible recovery of the thermal energy release from TAMR.

- **WS4: Dissemination of results**
  - **WS4.1 - Scientific publications**

    Based on the results of WS1, WS2, and WS3, 9 documents were published in international peer-reviewed Journals and Books, indexed in Scopus and/or Web of Science.
  - **WS4.2 – Ph.D. thesis**

    Thanks to the collaboration with the Supervisor and the research group ($TEC_{2.0}$, Laboratory of Water and Waste Treatment Processes and Technologies "Elisa Gazzola", Department of Civil Engineering and Architecture, University of Pavia), I was able to write and compose this thesis which concerned the activity of my Ph.D. experience.

For future research and experimentation, the application of the technology to industrial sludge from the treatment of industrial wastewater and aqueous waste could be tested, comparing the results with conventional biological sludge treatment. Furthermore, a possible toxic and chronic effect of these industrial substrates on thermophilic sludge should be investigated.

Process upgrades could also be further developed, such as the introduction of mobile support material with a high specific surface area into the reactor, which could promote the development of a biofilm. The traditional suspended biomass already present and the new attached biomass would represent a hybrid biomass process. Furthermore, the support materials introduced into the reactor could be derived from recovery and recycling operations.

As mentioned above, the mechanism of thermal energy production by the thermophilic reactor should be further clarified and explored; at the same time, process upgrades should be designed to recover the heat produced, avoiding waste of resources.

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