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Assessing the long-term efficiency of the MULESL system: a sustainable solution for wastewater treatment and agricultural water reuse

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This study evaluates the long-term performance of the MULESL (MUch LEss SLudge) system for treating municipal wastewater intended for agricultural reuse, with particular focus on contaminants of emerging concern (CECs). The system showed stable removal of conventional pollutants under variable hydraulic loads, achieving 94% and 90% reductions in total and soluble COD, respectively, with residual BOD_5 of 9 mg L^{-1}. High removal efficiencies were also observed for TSS (>94%), VSS (>93%), TN (83%), TKN (91%), and NH_3 (92%), while pH and conductivity remained within regulatory limits. From a database of over 40 000 compounds, 46 CECs were selected as the most abundant in influent wastewater; metformin, caffeine, and 1,7-dimethylxanthine were the most prevalent. The MULESL system achieved up to 85% overall CEC removal. Post-treatment significantly improved microbiological quality: UV disinfection (20 s) reduced *E. coli* to <10 CFU/100 mL, meeting stringent European reuse standards, while peracetic acid (2–3 mg L^{-1}) provided slightly lower but effective results. The high efficiency is attributed to the biofilm–granular structure, long solids retention time, and enhanced sorption and biodegradation processes, highlighting the MULESL system as a compact and resilient solution for safe wastewater reuse.

Environmental significance

This study demonstrates that the MULESL (MUch LEss SLudge) system can simultaneously address conventional pollution control and the growing environmental risks posed by contaminants of emerging concern in reclaimed water. By achieving high and stable removal of organic matter, nutrients, and up to 85% of priority contaminants of emerging concern (CECs) under long-term operation, the system reduces the potential release of persistent pharmaceuticals into soils, crops, and receiving ecosystems. Its low-sludge, compact configuration and compatibility with simple post-disinfection make MULESL a resource-efficient solution for water-scarce regions, supporting safe agricultural reuse while minimizing environmental footprints. Overall, the findings contribute practical evidence toward closing the urban water cycle and advancing sustainable wastewater reuse aligned with European environmental protection goals.

1. Introduction

Water scarcity has become one of the most pressing global challenges, driven by climate change, rapid population growth, and unsustainable patterns of water consumption. As agriculture accounts for almost 70% of global freshwater withdrawals, it is particularly vulnerable to this crisis. Declining water availability directly threatens food security and the livelihoods of rural communities. In this context, wastewater recovery has emerged as a promising and sustainable strategy to alleviate water stress. Unfortunately, more than 80% of wastewater is discharged untreated into the environment, posing significant risks to ecosystems, human health, and economic development.[1,2] Yet, when adequately treated, wastewater can be transformed from a source of pollution into a valuable resource. Its reuse in irrigation not only conserves freshwater but also supports a circular approach to water management by recovering water and nutrients for productive use. Wastewater reuse in agriculture provides multiple benefits: it ensures a reliable water supply in drought-prone regions, reduces the demand on freshwater resources, and can enhance soil fertility by supplying organic matter and essential nutrients. Several regions, including the Middle East, North Africa, and parts of Europe, have already made significant progress in integrating reclaimed water into their agricultural systems.[3,4] However, global adoption remains limited, with only about 11% of wastewater currently reused worldwide,[4] underscoring the need for broader implementation and improved treatment technologies to meet environmental and public health standards.

However, wastewater recovery for irrigation represents both a challenge and an opportunity, while insufficient infrastructure and emerging pollutants remain obstacles. Beyond conventional contaminants, wastewater serves as a primary

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pathway for the release of diverse Contaminants of Emerging Concern (CECs) into the environment.[5,6] In addition, it is known that conventional WWTPs are not specifically designed to remove CECs.[7] Therefore, treated water from WWTPs used in agriculture is strictly regulated to ensure it is free from harmful substances that could leach into the soil and ecosystems and harm human health due to their potential toxicity, persistence, and ability to bioaccumulate, making them a critical focus for ongoing research and regulatory efforts.[8–11] This limitation raises significant concerns about the safety and quality of reclaimed water resources, emphasising the need for sustainable strategies to mitigate wastewater pollution and protect water resources.[12]

In the last decade, numerous advanced technologies and approaches have been tested for treating various pollutants, especially contaminants of emerging concern. For instance, chemical (coagulation–flocculation, electrochemical, photolysis), physical (membrane filtration, ion exchange, adsorption), biological (bacteria-assisted, fungal-assisted, enzyme-assisted), and advanced oxidation process (photocatalytic, ozonation, photo-Fenton, sono catalytic).[13–15] These approaches offer flexible, simple, and eco-friendly solutions, but they also have some drawbacks, such as pH dependence, low efficiency towards CECs, high costs, secondary sludge disposal issues, and longer treatment times.[16,17] Moreover, most of those proposed treatment technologies have yet to be evaluated for full-scale applicability.[16]

An innovative and cost-effective solution like MULESL (MUch LEss SLudge) presents a promising alternative for WWTPs, effectively targeting two main objectives in the water line at full scale: reducing sewage sludge and treating diverse wastewater pollutants, particularly emerging contaminants, due to its extended sludge retention time (SRT), promoting the growth of slow-growing microorganisms.[18] This technology was recently innovated at a demonstrative scale by Di Iaconi and colleagues (Patent no. 102017000130809) to evaluate its effectiveness in reducing sludge production, achieving substantial biomass retention by incorporating a porous plastic material into the biological compartment of the treatment plant.[19] Its granular biomass is a specific type of biofilm with different chemical and physical properties compared to those of activated sludge.[20,21] Besides this, the MULESL system offers several advantages over conventional technologies, primarily due to its unique physical properties, such as high density and a defined shape and volume. These characteristics enable it to occupy most of the voids within porous plastic materials, allowing for extended SRTs without requiring a separate sedimentation stage. This enhances maintenance metabolism and process efficiency. Additionally, MULESL simplifies the overall treatment scheme by combining primary and secondary treatment into a single stage. It can also be easily integrated into existing wastewater treatment plants through retrofitting of the activated sludge process or incorporated into new plant designs.[19]

Literature review reveals that only two studies have investigated the full-scale application of the MULESL plant. Notably, Di Iaconi *et al.*, (2020)[19] evaluated the first full-scale implementation of the MULESL system for the removal of chemical pollutants. The system demonstrated high treatment performance, achieving average removal efficiencies of up to 95% for total suspended solids (TSS), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), ammonia (NH_3), total Kjeldahl nitrogen (TKN), and total nitrogen (TN). Moreover, solid and COD mass balance analyses over the entire operational period indicated a specific sludge production of just 0.13 kg of dry sludge per kg of COD removed, representing a 77% reduction compared to the combined primary and secondary treatment processes at the Putignano wastewater treatment plant during the same timeframe. Similarly, De Sanctis *et al.*, (2022)[22] compared the MULESL system to a conventional WWTP in removing conventional pollutants, emerging contaminants (CECs), and controlling odour emissions. The results indicated that MULESL achieved slightly higher removal efficiencies for traditional pollutants, such as COD, TSS, VSS, BOD_5, TN, NH_3, and TKN, exceeding 95%, compared to the conventional WWTP (>70%). Additionally, MULESL demonstrated significantly higher effectiveness in removing CECs, achieving the elimination of approximately 60% of the identified compounds.[22] Nevertheless, this removal rate remains below the threshold proposed by the new European Directive on municipal wastewater treatment, which requires up to 80% removal of CECs.[23,24] During the initial application, structural deficiencies in the MULESL basin restricted both studies to short-term operation. This period comprised a 7 month start-up phase for biomass development and a subsequent 5 month steady-state phase operated at hydraulic load of 375 m^3 day^{-1}. As a result, the plant's long-term steady-state performance could not be assessed during this first implementation.

In light of these considerations, this study aims to evaluate the long-term efficiency of the MULESL plant in removing chemical pollutants, especially CECs, and determine the feasibility of reusing treated effluent for agricultural purposes.

2. Materials and methods

2.1. Experimental set-up and operational conditions

The MULESL plant was restarted in January 2021, having been shut down in May 2019 for extraordinary maintenance work, which included refurbishing the entire biomass section by building a new concrete section having a volume of 180 m^3 and inoculating it with approximately 180 m^3 of activated sludge taken from the Putignano treatment plant's purge stream (Fig. 1).

The MULESL plant has been upgraded with a pilot refinement system to enable the recovery of its effluent for irrigation purposes. The design phase enabled the inclusion of a refinement plant with a capacity of 30 m^3 day^{-1}, which is divided into parallel lines: (i) line 1: performs chemical disinfection based on peracetic acid, (ii) line 2: performs physical disinfection based on UV radiation.

The effluent from the MULESL plant is sent, by means of a submersible pump located in the liquid phase compartment of the MULESL plant, to a 10 m^3 storage tank (TK-01 in Fig. 1C). In order to retain the fraction consisting of suspended particulate matter, a vacuum-type cartridge filter (FC-01) made of

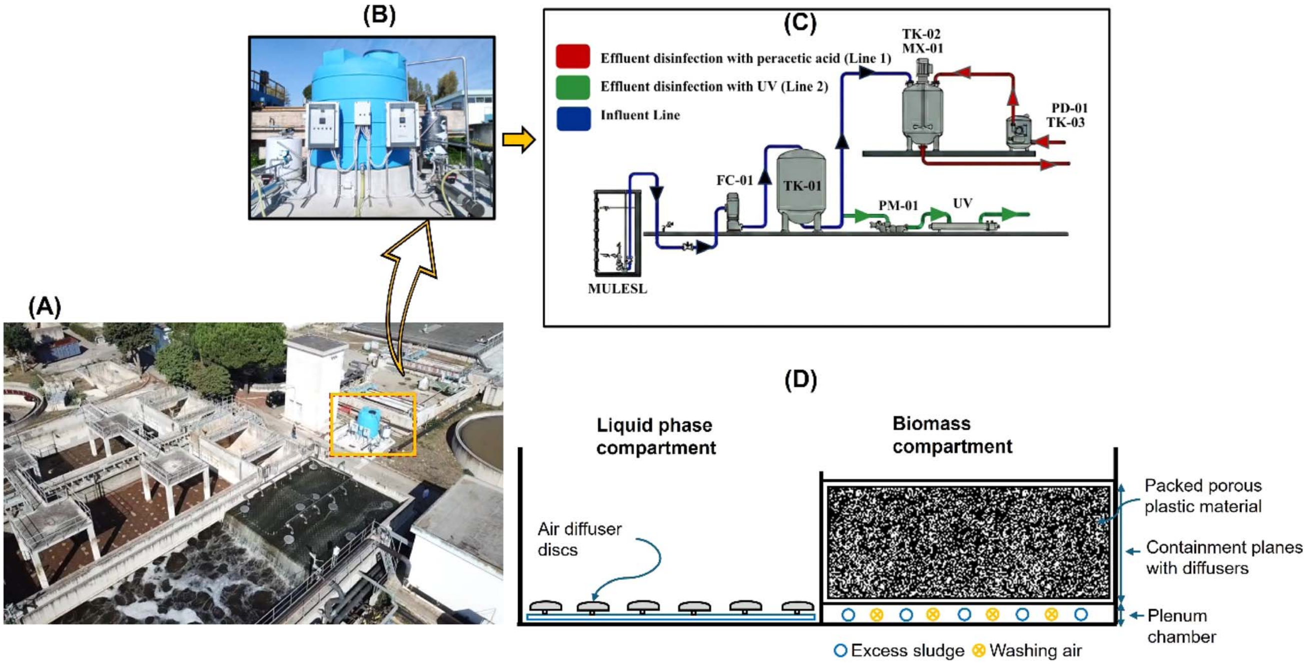

Fig. 1 Aerial photograph of the actual MULESL plant (A) and refinery (B), schematic diagram of the refinery system (C), and MULESL system (D).

stainless steel with suction pads and filter elements has been placed along the pipe at the outlet of the MULESL demonstrator. The TK-01 tank acts as a buffer and starting point for the two refinement lines. Each line is equipped with a special pump that draws the effluent from the tank and sends it to the dedicated treatment.

Line 1 (Fig. 1C), which performs chemical disinfection using peracetic acid, consists of a 1 m^3 reactor (TK-02) made of plastic, equipped with a rapid mixer (MX-01) and a chemical reagent addition system. This reactor is filled by gravity from the TK-01 tank by opening a dedicated valve until the required volume is reached. The TK-02 reactor operates in batch mode.

Line 2 (Fig. 1C), which performs physical disinfection using UV radiation, consists of a centrifugal pump (PM-01) and a UV reactor flow meter. The centrifugal pump draws the effluent from the MULESL plant from the TK-01 storage tank and sends it, at a specific flow rate selected by the operator, to the cylindrical UV reactor in which two mercury vapor UV lamps are arranged (power: 320 W, current: 2.10 A, voltage: 145 V). Further technical details about the operation of the MULESL plant can be found in the SI, as well as in Di Iaconi *et al.*, (2020)[19] and De Sanctis *et al.*, (2022).[22]

The experimental campaign of the demonstration plant was divided into two periods. The activities of the first period (Period A) concerned the plant's start-up phase, while the activities of the second period (Period B) focused on optimizing its treatment capacity. The start-up phase of the MULESL plant is critical because a specific type of biomass, consisting of a mixture of granules and biofilm confined in a plastic filling medium with distinct characteristics, is formed during this period. This makes the system unique and forms the basis for reducing sludge production. Once the activities of Period A were completed with the formation of this biomass, the organic load applied to the MULESL plant was increased by increasing the hydraulic load up to values of 235 m^3 day^{-1}, corresponding to a potential of almost 2000 population equivalents (Period B). During Period B, which lasted 2 years, a portion of the MULESL plant effluent was sent to the refinement plant to improve its quality and make it suitable for agronomic reuse. Concerning refinement, four UV disinfection times were tested (30, 20, 15, and 12 seconds), corresponding to UV doses of 120, 80, 60, and 48 mJ cm^{-2}, respectively. The chemical disinfection was carried out by adding peracetic acid (PAA). This disinfectant is actually a mixture of hydrogen peroxide, peracetic acid, and acetic acid. The presence of acetic acid and the formation of acetic acid following the reaction of peracetic acid with organic matter and microorganisms present in wastewater cause an increase in COD and BOD_5 values in treated water. At this stage, it was therefore decided to evaluate the effect of adding low doses of this disinfectant (1, 2, and 3 mg L^{-1} of PAA). The reaction time chosen was 30 minutes, which is typical for chemical disinfection processes in municipal treatment plants.

2.2. Analytical methods

2.2.1. Conventional pollutants.

The performance of the MULESL plant was monitored during the two periods by analysing influent and effluent composite samples with a frequency of 1–2 times a week.

For the analysis of conventional pollutants, pH and EC were measured by selective probes. Chemical oxygen demand (COD), biochemical oxygen demand over 5 days (BOD_5), total nitrogen (TN), ammonia (NH_3), nitrate (NO_3^-), nitrite (NO_2^-), and total phosphorus (TP) were measured using DR Lange test kits. For the analytical determination of soluble COD (COD_{sol}), samples were pre-filtered using a 0.45 μm polytetrafluoroethylene (PTFE) membrane filter. Total Kjeldahl nitrogen (TKN) was calculated as the difference between TN and oxidized nitrogen (NO_3^- + NO_2^-). Total suspended solids (TSS) and volatile suspended solids (VSS) were determined according to standard procedure.[25] *Escherichia coli* (*E. coli*) and total coliforms were detected and quantified using IDEXX Colilert-18 and Quanti-Trays/2000, according to the company's instructions.[26] This method uses the Most Probable Number (MPN) statistical approach and Defined Substrate Technology (DST) to detect both coliforms and *E. coli* simultaneously.[27] Each analysis was carried out in duplicate for each ten-fold serial dilution tested.

2.2.2. Contaminants of emerging concern analysis, calibration, and sensitivity. During the last 6 months of the experimental period B, the performance of the MULESL plant was also evaluated in terms of CECs removal by analysing influent and effluent composite samples with a frequency of 1–2 times every two weeks. The analysis of the CECs was performed using instrumental ultra-high-performance liquid chromatography (UPLC; Ultimate 3000 System, Thermo Fisher Scientific) coupled with high-resolution mass spectrometry (TripleTOF® 5600+ System, AB Sciex). The samples were analysed using UPLC-QTOF/MS/MS, following the method described elsewhere.[28,29]

Samples were analyzed by UPLC-QTOF/MS/MS using a calibration curve prepared from a stock solution of certified standards available in the laboratory, with concentrations ranging from 0.1 to 1 μg L^{-1} (0.1, 0.25, 0.50, 0.75, and 1 μg L^{-1}). To improve detection sensitivity for a wide array of compounds present at trace levels, each 2000 μL sample was processed using an online solid-phase extraction (SPE) system. To account for matrix effects, internal standards were added to each sample before analysis. The internal standard mix included isotopically labeled compounds: carbamazepine D10, caffeine 13C3, primidone D5, sulfamethoxazole D4, venlafaxine D6, diclofenac 13C6, gabapentin D4, naproxen D3, citalopram D4, and gemfibrozil D6. This mixture was prepared in methanol at a final concentration of 500 μg L^{-1}.

Data from high-resolution mass spectrometry were processed using AB Sciex software (*e.g.*, tools PeakView 2.2, MasterView 1.1, and LibraryView 1.1.0). The data files were processed for suspect screening analysis using the CECs list of the SusDat database (about 58 000 compounds) compiled by the Norman network. Target compounds were identified by matching the retention time and MS/MS spectra with those of the corresponding analytical standards analyzed under the same conditions.

All analytical standards (purity: 96.0–99.0%) were purchased from LabService Analytica (Bologna, Italy). Solvents used in the chromatographic analyses, including acetonitrile, methanol, and formic acid, were of UPLC grade, and ultrapure water was obtained from a Milli-Q Gradient A-10 system (Millipore, Burlington, USA).

3. Results and discussion

As reported in the previous paragraph, the experimental campaign was divided into two periods. The activities of the first period (period A) concerned the start-up phase of the MULESL plant, while those of the second period focused on optimizing its treatment capacity, including effluent refinement (period B). In fact, at the end of period A, the MULESL plant was upgraded with a refinement unit that treated a fraction of the purified effluent in order to recover it for irrigation purposes.

3.1. Start-up period

Fig. 2 shows the profiles of the various monitored parameters at the MULESL plant's inlet and outlet, as well as the relative removal efficiencies, during period A (start-up phase), which lasted approximately 180 days. This phase allowed the microbial community to gradually acclimate to the specific operational conditions, thereby minimizing the risk of cellular stress that might disrupt metabolic activity and compromise overall treatment performance. Furthermore, the operating conditions used during this period play a crucial role in developing the biomass. Specifically, the volumetric organic load (VOL) applied to the biomass compartment must be adjusted so that it falls within a specific range, which varies depending on the operating period (*i.e.*, the first, second, or third month after start-up). During period A, the VOL was kept within a certain range by adjusting the hydraulic load according to the composition of the wastewater to be treated, which was constantly monitored. Fig. 2A shows the VOL value throughout period A.

Regarding the on-site parameters (Fig. 2B and C), the pH and EC profiles exhibited stable trends in both influent and effluent. Following treatment, a slight increase in pH was observed, whereas conductivity showed a decrease. As regards phosphorus evolution, the trend concentrations of the influent and effluent samples exhibited random variability during the start-up phase, possibly due to microbial adaptation and operational adjustments before reaching steady-state conditions (Fig. 2D).

The profiles COD, TSS, and VSS show that very high removal efficiencies were achieved, exceeding 90%, often reaching over 95% (Fig. 2E, J, and K). Regarding nitrogen, the MULESL plant demonstrated ammonia and TKN removal efficiencies in the 70–100% range, with average values exceeding 90% for ammonia and 92% for TKN (Fig. 2G and H), demonstrating a fairly stable nitrification process, even during the start-up period. Total nitrogen profiles show similar trends to TKN profiles (Fig. 2F), confirming the presence of an extensive denitrification process (*i.e.*, the reduction of oxidised nitrogen to gaseous nitrogen that leaves the reactor). On average, approximately 90% of the nitrogen content fed to the plant is removed, despite there being no scheduled anoxic phase (oxygen is dosed in the form of pure oxygen throughout the treatment cycle). The low values of oxidised nitrogen (Fig. 2I) leaving the MULESL plant clearly indicate that this is a simultaneous nitrification–denitrification process (*i.e.*, nitrogen oxidation and reduction occur at the same site as the biomass). This process is typical of this technology and can be attributed to the high biomass concentration and dynamic operating conditions (typical of sequential reactors), which generate adjacent aerobic and anoxic zones within the bed. Nitrification occurs in the former and denitrification in the latter.

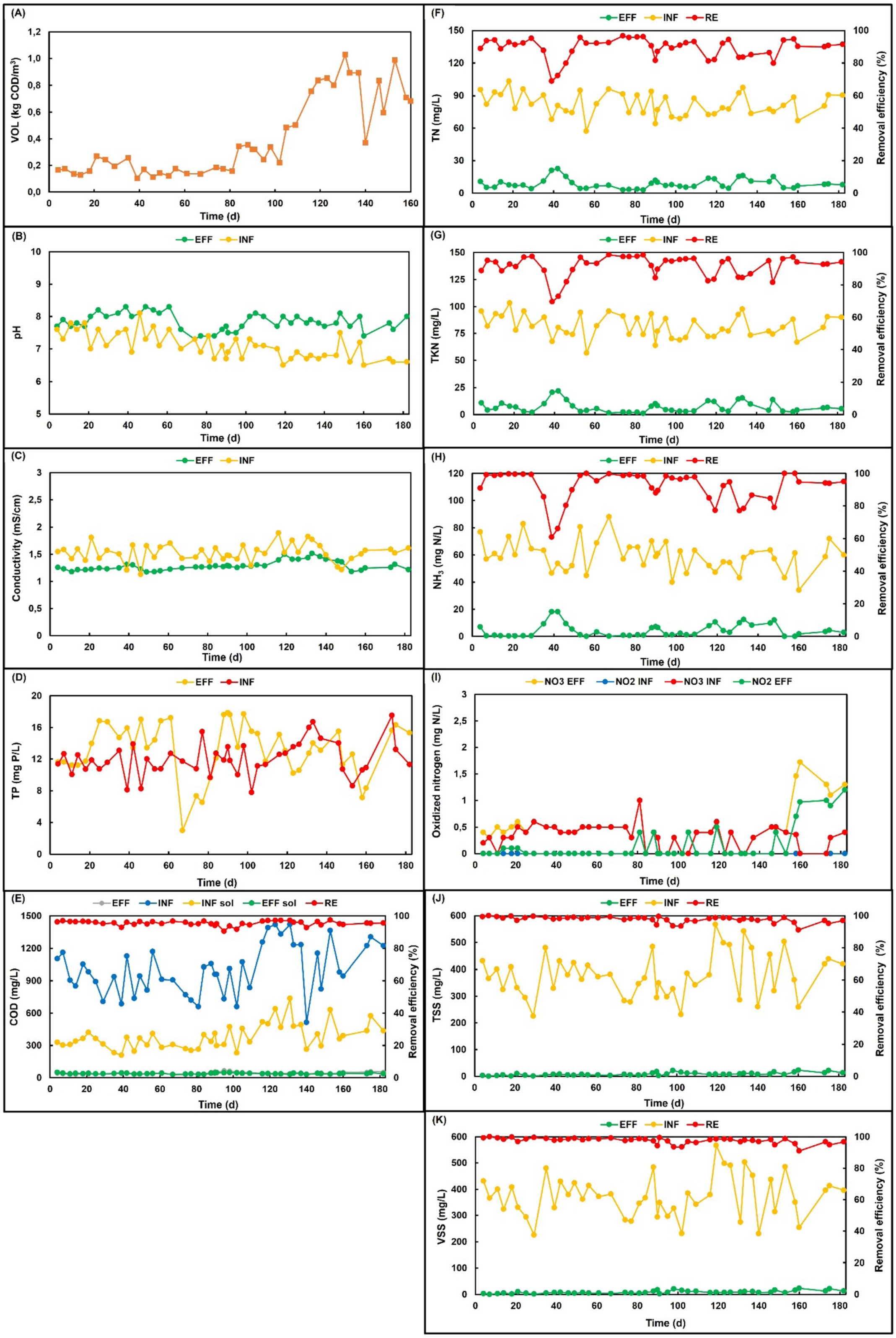

Fig. 2 Variation of conventional water quality parameters in influent (INF) and effluent (EFF) samples along with their corresponding removal efficiencies (RE), over the start-up phase (≈180 days): (A): VOL, (B): pH, (C): conductivity, (D): TP, (E): COD, (F): TN, (G): TKN, (H): NH_3, (I): oxidized nitrogen, (J): TSS, and (K): VSS.

3.2. MULESL performance

3.2.1. Conventional parameters removal. Table 1 provides a summary of the MULESL plant performance in terms of conventional pollutants during two years of period B. Concerning the onsite parameters such as pH and conductivity, the data exhibited a slight increase in the pH value after treatment from 7.1 to 7.9, which may be caused by the biological processes within the bed reactor, as reported in the literature.[30,31] However, conductivity showed a slight decline in the effluent (1589 µS cm^{-1}) compared to the influent (1327 µS cm^{-1}), which is likely related to the marked reduction in total nitrogen concentration, particularly ammoniacal nitrogen.[30] Similar findings were reported by,[19,32] who attributed the limited decrease in conductivity in the SBBGR effluent to salt adsorption onto the biomass. In fact, the effluent exhibited pH and conductivity values well within the regulatory limits for water reuse (6.5–8.4 for pH and <3 mS cm^{-1} for conductivity), as established in several countries, such as Italy, Cyprus, Greece, and Spain.[33]

The data confirm the excellent performance recorded in Period A, even at much higher hydraulic loads (up to 235 m^3 day^{-1}, corresponding to a potential of almost 2000 population equivalents). In particular, the MULESL system achieved a significant reduction in organic matter, with total COD decreasing from 1045 mg L^{-1} to 58 mg L^{-1} and soluble COD from 409 mg L^{-1} to 41 mg L^{-1}, corresponding to average removal efficiencies of 94% and 90%, respectively. The exceptionally high organic matter removal is likely due to the MULESL configuration, which combines granular sludge and attached biofilm confined in a porous plastic carrier. This arrangement increases biomass retention and SRT, promotes high microbial density and functional diversity (fast heterotrophs plus slower degraders), and creates micro-zonation inside granules/biofilms that enables simultaneous aerobic and anoxic degradation pathways. These features enhance resilience to load fluctuations and the conversion of particulate organics to mineralized end-products, explaining the observed reductions in total COD.[19,34,35] Furthermore, the results show complete removal of biodegradable matter, as evidenced by the low BOD$_5$ concentration (9 mg L^{-1}), suggesting that the remaining COD corresponds to non-biodegradable compounds in the influent.[30,31] Consistent with our findings, Di Iaconi *et al.*, (2020)[19] and De Sanctis *et al.*, (2022)[22] achieved COD removals above 94%, which they linked to the distinctive biomass structure, granules, and biofilm confined in porous plastic, ensuring system stability and adaptability under diverse operating conditions. Similar findings were obtained by Rollemberg *et al.*, (2020)[36] using a pilot-scale sequencing batch granular reactor installed in a Brazilian WWTP and fed primarily with pretreated municipal sewage under a shorter HRT (12 hours *vs.* 18 hours) than in our study, achieving a COD removal of up to 92%. Interestingly, COD and BOD$_5$ concentrations in the effluent obtained in our study are well below the discharge limit of 100 mg L^{-1} and 20 mg L^{-1}, respectively, set by national legislation for sewage treatment plants that discharge to the soil.[33]

In parallel, the system achieved excellent removal efficiencies for total and volatile suspended solids (TSS and VSS), exceeding 94% and 93%, respectively. Unfortunately, the effluent concentration of TSS is well above the threshold required by current Italian regulation for water reuse (≤10 mg L^{-1}),[37] despite significant fluctuations in influent concentrations (300–400 mg L^{-1}). The effluent consistently maintained low concentrations, averaging only 22 mg L^{-1}. Furthermore, the influent VSS/TSS ratio, close to unity, confirms that suspended solids were predominantly organic in nature, in line with the total and soluble COD results. This high efficiency can be attributed to the biofilter compartment, which operates as a dense filtration medium where biomass traps suspended material. Once retained, solids are enzymatically hydrolysed and metabolized, ensuring both effective treatment and strong operational robustness.[30] Many studies have

Table 1 Values (mean ± standard deviation) of water quality parameters measured at the inlet and outlet of the MULESL plant monitored during period B and their removal efficiency

Parameter	Influent	Effluent	Removal efficiency (%)
pH	7.1 ± 0.2	7.9 ± 0.2	—
Conductivity [µS cm^{-1}]	1589 ± 243	1327 ± 39	—
COD [mg L^{-1}]	1045 ± 158	58 ± 12	94 ± 1
COD$_{sol}$ [mg L^{-1}]	409 ± 76	41 ± 2	90.2 ± 0.9
BOD$_5$ [mg L^{-1}]	469 ± 151	9 ± 1	98.1 ± 0.5
TSS [mg L^{-1}]	355 ± 39	22 ± 9	94 ± 2
VSS [mg L^{-1}]	338 ± 39	22 ± 9	93 ± 2
TN [mg L^{-1}]	71 ± 9	11 ± 3	83 ± 5
TKN [mg L^{-1}]	70 ± 8	6 ± 2	91 ± 3
NH$_3$ [mg N L^{-1}]	31 ± 7	3 ± 2	92 ± 4
NO$_3^-$ [mg N L^{-1}]	0.4 ± 0.5	5 ± 2	—
NO$_2^-$ [mg N L^{-1}]	0.1 ± 0.2	0.6 ± 0.4	—
TP [mg P L^{-1}]	11 ± 1	6 ± 2	—
PO$_4^{3-}$ [mg P L^{-1}]	7 ± 2	4.6 ± 0.5	—
E. coli [MPN/100 mL]	9.3 ± 1.6 × 10^6	9.1 ± 5.5 × 10^4	—
Total coliforms [MPN/100 mL]	1.1 ± 1.2 × 10^8	2.5 ± 0.4 × 10^5	—

reported similar findings using a biological process, such as the sequencing batch biofilter granular reactor, in treating various types of wastewaters (*e.g.*, leachate, industrial, municipal, textile yarn dyeing effluent), demonstrating a high removal rate towards TSS and VSS up to 80%.[38–42] Conversely, Rollemberg *et al.*, (2020)[36] obtained random performance of TSS varying from 24% to 89% using a pilot-scale sequencing batch granular reactor.

Regarding nitrogen species (TN, TKN, NH_3, NO_3^-, NO_2^-), the results demonstrate the strong efficiency of the MULESL system in nitrogen removal. After treatment, the average concentrations of TN, TKN, and NH_3 decreased to 11 mg L^{-1}, 6 mg L^{-1}, and 3 mg L^{-1}, respectively, compared to their influent concentrations of 71 mg L^{-1}, 70 mg L^{-1}, and 31 mg L^{-1}. Generally, the observed removal trend of nitrogen can be ascribed to the biological mechanisms that govern the nitrogen cycle and should therefore be considered responsible for the conversion of reduced and oxidised nitrogen forms within the biological system.[43,44] The MULESL system demonstrated excellent nitrification performance, achieving TKN and NH_3 removal efficiencies of up to 91% and 92%, respectively. Furthermore, the high elimination of TN (up to 83%), mainly due to effective denitrification, despite the absence of a scheduled final anoxic phase.[40] Indeed, outlet TN concentrations (11 mg L^{-1} on average) were consistently much lower than inlet levels (71 mg L^{-1}), with a huge difference largely explained by conversion to gaseous nitrogen through the denitrification process, given the low sludge production.[19] The nitrification process proceeded effectively, accompanied by a slight release of nitrite and nitrate as intermediate and terminal products,[45] thereby elevating their mean concentrations in the effluent to 0.6 and 5 mg L^{-1}, respectively, relative to influent levels of 0.1 and 0.4 mg L^{-1}. These findings, which highlight the high biomass concentration in the reactor bed, indicate that nitrifying and denitrifying bacteria can coexist within the biomass layers. This observation is consistent with the results reported by Di Iaconi *et al.*, (2020, 2017, 2010),[19,39,40] and had already been demonstrated in suspended biomass systems.[46,47] In relation to agricultural water reuse, the effluent's total nitrogen concentration is below the limit set by Italian legislation, which is less than 15 mg L^{-1}.[33] However, the presence of nitrate in the MULESL effluent should be considered advantageous, as crops utilize nitrogen mainly in the form of nitrate or ammonia.[48]

As regards total and soluble phosphorus (TP and PO_4^{3-}, respectively), the data in Table 1 indicate a moderate decrease in concentration following treatment, with values dropping from 11 and 7 mg L^{-1} in the influent to 6 and 4.6 mg L^{-1} in the effluent, respectively.

The moderate phosphorus removal observed in the MULESL plant may be due to the reduced sludge yield, resulting in lower phosphorus assimilation through biomass growth, and/or microbial uptake during assimilation for growth and development.[22,30,49] A similar finding was reported by De Sanctis *et al.*, (2022, 2020)[22,48] and Lin *et al.*, (2023)[50] in a pilot-scale study where pure oxygen was applied to increase the dissolved oxygen concentration in the liquid phase, reaching a maximum TP removal of up to 58%. This strategy was assumed to reduce the formation of anaerobic zones within microbial aggregates, where biological phosphorus removal typically occurs, as commonly observed in granular reactors.[21] However, considering the different operational approaches (*i.e.*, plant configuration and aeration strategy), and the substantial variability in sewage composition, the studies by Rollemberg *et al.*, (2020)[36] and Yang *et al.*, (2020)[51] demonstrated higher phosphorus removal efficiencies above 80% compared to the MULESL unit. Generally, phosphorus in biological WWTPs is typically removed either through biomass assimilation or by specialized phosphorus-accumulating organisms (PAOs) that require alternating anaerobic and aerobic phases.[21] The presence of PAOs has been extensively reported in granular reactors when operated under conditions favoring phosphorus removal.[21] However, in the present case, the MULESL was not designed for this purpose, nor was it in the earlier study.[22,32,48]

Regarding total coliforms and *Escherichia coli* (*E. coli*), the data shown in Table 1 indicate that the MULESL plant achieves substantial removal efficiencies, expressed in logarithmic units removed (LUR). Specifically, the system removed more than 2.6 LUR of total coliforms, a performance that aligns with values reported in the literature for conventional treatment plants (*e.g.*, constructed wetlands, hybrid multi-soil-layering), where an average removal of approximately 2.5 LUR is achieved after primary and secondary treatment.[52,53] This finding demonstrates that the MULESL plant can match the effectiveness of multi-phase conventional systems in just one treatment stage. The removal performance for *E. coli* was comparable to that for total coliforms, with an average reduction of 2.7 log units (LUR), resulting in effluent concentrations of around 10^4–10^5 most probable number (MPN)/100 mL. These values are consistent with those typically reported for secondary effluents following conventional primary and secondary treatment, which generally achieves a reduction of around 2–3 log units of *E. coli*, leaving residual concentrations in the range of 10^4–10^5 CFU/100 ml.[54] These results demonstrate that, despite operating in a simplified, single-stage configuration, the MULESL plant achieves comparable removal efficiencies of faecal indicators to those of well-established, multi-stage treatment technologies. However, the average residual concentration of *E. coli* in the effluent from the MULESL is too high to allow its direct reuse in agriculture, both according to the current national legislation on wastewater reuse[37] and according to the new European regulation that will come into force in 2023,[55] and consequently it needs a target and supplement treatment processes (*e.g.*, disinfection, polishing steps). However, most European countries have not established concentration limits for coliform indicators in treated water reclaimed for agricultural reuse. Notable exceptions are Portugal and Greece, which set limits of 100–10^4 CFU/100 mL for faecal coliforms and 2 CFU/100 mL for total coliforms, respectively.[33]

3.2.2. CECs removal.

During the experimental period B, the performance of the MULESL plant was also evaluated in terms of CECs removal. Table 2 presents the average concentrations of the selected CECs at the inlet and outlet of the MULESL plant, while Fig. 3 shows their corresponding mean removal efficiencies over a period of 6 months. Out of 40 000 monitored CECs, only 46 were quantified above the detection limit. The influent samples exhibited particularly high concentrations of metformin (152 μg L^{-1}), 1,7-dimethylxanthine (107 μg L^{-1}), and caffeine (102 μg L^{-1}), representing more than 70% of the total CECs load at the inlet (Table 2). This predominance is mainly attributed to their extensive human consumption and widespread excretion in unmetabolized or partially metabolized forms, as well as their continuous discharge from domestic wastewater sources.[56–58] In addition, a significant reduction in the concentration of the majority of CECs, especially those that represent high influent concentration, *e.g.*, metformin, 1,7-dimethylxanthine, caffeine, acetaminophen, and levofloxacin, corresponding to a removal concentration of 132 μg L^{-1} (86%), 101 μg L^{-1} (92%), 99 μg L^{-1} (94%), 41 μg L^{-1} (84%), and 23 μg L^{-1} (69%), respectively. The higher removal efficiency observed in the MULESL system is due to its ability to trap solids and particulate organic matter, enabling hydrophobic micropollutants (*e.g.*, levofloxacin) to be adsorbed.[59] Additionally, the elevated biomass concentration and structured biofilm and granule morphology promote the production of extracellular polymeric substances (EPS), enhancing the sorption of dissolved contaminants. Kang *et al.*, (2018)[60] demonstrated that aerobic granules characterised by protein-rich EPS exhibited a stronger affinity for organic pollutants than activated sludge, which is consistent with the findings of De Sanctis *et al.*, (2022, 2010),[22,61] who reported that amino sugar-rich EPS in biofilter biomass contributed to pollutant adsorption. These mechanisms increase the contact time between pollutants and biomass. The long solids retention time in MULESL also supports the development of slow-growing microorganisms that can degrade or co-metabolise recalcitrant compounds.[62,63] Furthermore, the feast-and-famine operational regime enhances micropollutant removal by stimulating microbial adaptation and co-metabolic activity. This phenomenon has also been observed in activated sludge systems operating at longer hydraulic retention times.[64]

However, an unexpected increase in the concentrations of some compounds after treatment, such as iopromide, metoprolol acid, lamotrigine, and niflumic acid, was observed (Fig. 3), likely due to the deconjugation of human metabolites back to their parent compounds, desorption of previously adsorbed substances from sludge or biofilm, and analytical variations during sampling and analysis.[65,66] Similarly, some studies have reported a similar behavior of some compounds, such as iopromide, metoprolol acid, and lamotrigine, which are detected at higher levels in effluent than in influent, suggesting back-transformation or release processes occurring during treatment.[67,68] Similar findings have been observed for other CECs, including warfarin, indomethacin, and trimethoprim, further confirming that biotransformation and sorption–desorption dynamics can lead to apparent increases in micropollutant concentrations after treatment.[62,63,69]

Throughout the experimental period, the MULESL plant consistently achieved high removal efficiencies for pharmaceuticals, reaching up to 85%, in line with the recent European directive on urban wastewater treatment, which mandates up to 80% removal of contaminants of emerging concern (CECs) for effluent reuse.[70] This efficient removal of CECs was higher compared to that obtained in the first MULESL plant application, reaching up to 60%.[22]

Table 2 Concentration (mean values ± standard deviation) of selected contaminants of emerging concern detected in the inlet and outlet samples

Compounds	Influent (μg L^{-1})	Effluent (μg L^{-1})
Metformin	152 ± 82	19 ± 17
1,7 dimetilxantine	107 ± 51	6 ± 7
Caffeine	102 ± 51	3 ± 4
Acetaminophen	41 ± 19	0.5 ± 1.7
Levofloxacin	30 ± 17	7 ± 4
TBEP	13 ± 9	3 ± 2
Iopromide	12 ± 19	6 ± 7
Diclofenac	6 ± 3	3 ± 1
Cotinine	4 ± 2	0.2 ± 0.1
TCPP	4 ± 2	1.4 ± 0.6
Sitagliptin	3 ± 2	0.7 ± 0.4
Gabapentin	3 ± 2	1.5 ± 0.6
Irbesartan	3 ± 1	1.9 ± 0.5
Sotalol	3 ± 2	1.4 ± 0.5
Fenofibric acid	3 ± 1	0.5 ± 0.4
Ketoprofen	3 ± 2	0.4 ± 0.4
Atenolo	2 ± 1	0.1 ± 0.1
Telmisartan	2 ± 1	1.2 ± 0.6
Mycophenolic acid	2 ± 1	0.08 ± 0.05
Benzotriazole	2 ± 1	1 ± 1
Triisopropanolamine	2 ± 1	0.9 ± 0.9
Metoprolol acid	2 ± 1	2 ± 1
Cocaine	1.3 ± 0.7	0.03 ± 0.05
Flecainide	1.1 ± 0.8	0.8 ± 0.4
Metoprolol	0.9 ± 0.4	0.4 ± 0.2
Trimethoprim	0.9 ± 0.5	0.2 ± 0.2
Lamotrigine	0.8 ± 0.7	0.9 ± 0.4
Carbamazepine	0.8 ± 0.6	0.7 ± 0.2
Clarithromycin	1 ± 1	0.2 ± 0.2
Niflumic acid	0.7 ± 0.6	0.6 ± 0.2
Bisoprolol	0.7 ± 0.2	0.3 ± 0.1
Atorvastatin	0.6 ± 0.4	0.04 ± 0.04
Losartan	0.6 ± 0.2	0.16 ± 0.08
Quetiapine	0.5 ± 0.3	0.02 ± 0.02
Fluconazole	0.4 ± 0.2	0.2 ± 0.1
Sulpride	0.4 ± 0.3	0.2 ± 0.1
TnBP	0.4 ± 0.8	0.08 ± 0.08
Venlafaxine	0.3 ± 0.2	0.17 ± 0.07
EDDP	0.3 ± 0.1	0.13 ± 0.07
Amisulpride	0.3 ± 0.2	0.11 ± 0.05
Cetirizine	0.2 ± 0.2	0.11 ± 0.06
Lidocaine	0.2 ± 0.1	0.15 ± 0.08
Climbazole	0.22 ± 0.08	0.07 ± 0.03
Carbamazepine 10,11-epoxide	0.2 ± 0.1	0.07 ± 0.06
Citalopram	0.2 ± 0.2	0.1 ± 0.1
TPP	0.12 ± 0.06	0.01 ± 0.02
ΣCECs	510 ± 193	66 ± 33

3.2.3. MULESL system integrated with a refinement system for water recovery. Starting from period B, a portion of the effluent from the MULESL plant underwent refinement treatment, allowing for its recovery for irrigation purposes.

Fig. 4 illustrates the removal efficiency of total coliforms and *Escherichia coli* in the analysed samples, expressed as logarithmic units removed (LUR), under different treatment

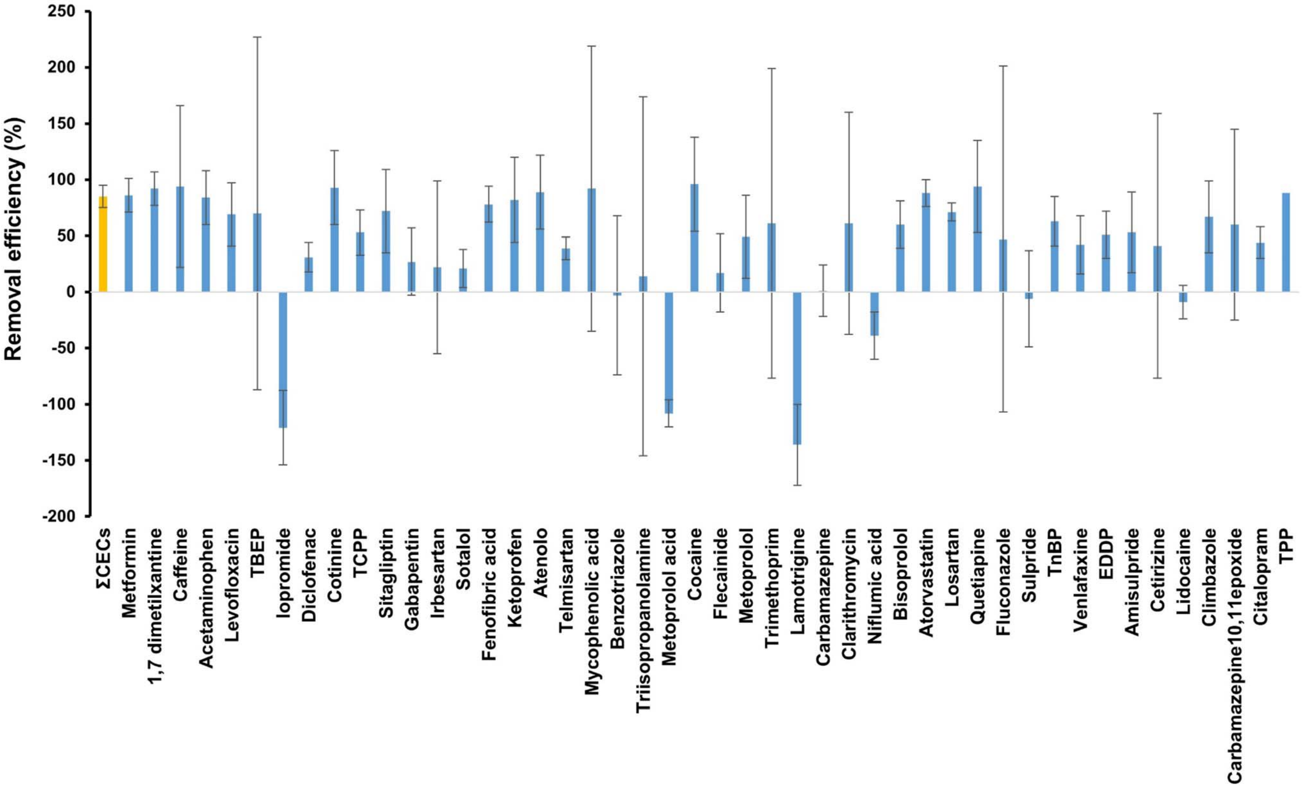

Fig. 3 Removal efficiency of contaminants of emerging concern in the MULESL plant throughout the monitored period.

conditions, whereas the relative residual values are reported in Fig. 5. UV-based disinfection was evaluated at four hydraulic residence times (HRT) in the UV reactor (30, 20, 15, and 12 s). As shown in Fig. 4, UV disinfection significantly enhanced the removal efficiency of coliforms and *E. coli*. As expected, the highest removals were achieved at the longest UV HRT. In detail, MULESL treatment followed by UV disinfection ensured *E. coli* removals up to 6.4 LUR, with residual contents lower than 10 units/100 mL, *i.e.*, the Italian limit for unrestricted water reuse, reflecting the improved clarity of the final effluent. In fact, the presence of residual suspended solids and colour in the liquid directed to the UV disinfection reactor can reduce the effectiveness of this disinfection method because part of the UV radiation is shielded by the coloured compounds present in the liquid itself.[71] Generally, this finding corresponds to the target indicated by current Italian legislation for the agricultural reuse of treated wastewater,[37] which is also one of the most stringent in the world, as well as to the World Health Organisation for the use of wastewater in agriculture.[72] Similarly, previous studies have shown that UV irradiation can achieve multi-log inactivation of *E. coli* and total coliforms in wastewater effluents under appropriate UV doses (>20 mJ cm^{-2}) when turbidity and colour are minimized.[73]

Regarding chemical disinfection, the addition of peracetic acid (PAA) at 2 and 3 ppm showed a high disinfection efficiency against *E. coli* (2.9–3.1 LUR), slightly lower than that achieved by

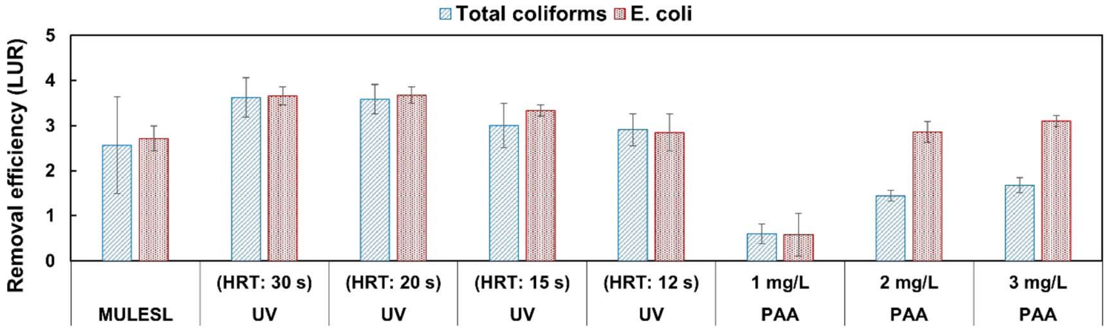

Fig. 4 Removal efficiencies of total coliforms and *Escherichia coli* (*E. coli*) in terms of logarithmic units removed (LUR) under various treatment stages (biological treatment using MULESL, UV disinfection, and chemical disinfection using peracetic acid (PAA)).

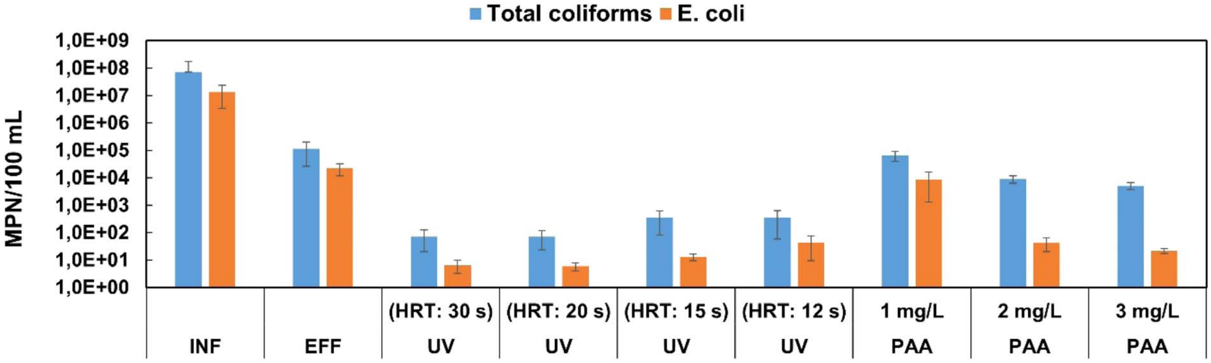

Fig. 5 Residual total coliforms and *Escherichia coli* (*E. coli*) under various treatment stages (biological treatment using MULESL, UV disinfection, and chemical disinfection using peracetic acid (PAA)).

UV treatment, except at 1 mg L^{-1}. The latter dosage appears to have a very limited effect on total coliforms and *E. coli*. This result can be explained by the fact that the reaction of peracetic acid is not selective for microorganisms and that this compound also reacts rapidly with most of the organic matter present in the water undergoing disinfection.[74] The data obtained, therefore, indicate that a dosage of 1 mg L^{-1} of PAA is too low to allow the reuse of treated effluent in agriculture. However, the other PAA concentrations (2 and 3 mg L^{-1}) would therefore easily allow the reuse of treated water according to the European Regulation 2020/741.[55] Likewise, peracetic acid has been reported to achieve approximately 3-log reductions of total coliforms and other bacterial indicators when applied at concentrations ranging from 2 to 7 mg L^{-1} in secondary and tertiary wastewater effluents.[75,76] Furthermore, several review studies emphasize the broad-spectrum antimicrobial activity of PAA and its suitability for treated wastewater reuse, highlighting its effectiveness in meeting regulatory standards while minimizing the formation of harmful disinfection by-products.[77,78]

4. Conclusions

This study demonstrates that the MULESL (MUch LEss SLudge) system is an efficient, compact, biological technology for treating municipal wastewater. It achieved high removal efficiencies for conventional pollutants, such as organic matter (COD and BOD_5), suspended solids (TSS and VSS), and nitrogen species (TN, TKN, and NH_3). Effluent concentrations generally met national and European standards for water reuse. The process also demonstrated stable operation under variable hydraulic conditions, low sludge production, and significant pathogen reduction. However, *E. coli* concentrations exceeded reuse limits after biological treatment, indicating the need for a disinfection step. UV disinfection (80 mJ cm^{-2}, 20 s HRT) effectively reduced *E. coli* concentrations to below the stringent regulatory requirements for agricultural reuse. Peracetic acid (2–3 mg L^{-1}) also improved effluent quality, although with slightly lower efficiency.

In addition, the MULESL system demonstrated significant potential for removing contaminants of emerging concern, achieving an overall removal efficiency of up to 85%. However, slight increases in some compounds after treatment suggest the occurrence of desorption or transformation processes. Overall, these results emphasise the potential of MULESL technology as a sustainable solution for advanced wastewater treatment and water reuse.

Conflicts of interest

The authors confirm that they have no known financial interests or personal relationships that could have influenced the work presented in this paper.

Data availability

The data presented in this study are available on request from the corresponding author.

Supplementary information is available. See DOI: https://doi.org/10.1039/d6va00047a.

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