BIOTECHNOLOGY FOR URBAN GREENING: THE ZERO MILE SYSTEM© FOR THE REUSE OF DISHWASHER WASTEWATER

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ABSTRACT: The increasing demand for tap water and the need for sustainable management of water resource require new solutions including the reuse of domestic greywater. The Zero Mile system proposes an innovative approach based on the integration of biotechnology in architectural design. The core of the system is a biofilter composed of an *ad hoc* engineered microbial consortium, capable of removing nitrogen and phosphorus from dishwasher wastewater. This makes reusable the wastewater, for irrigating edible plants in domestic and urban environments. The system design has been conceived for an efficient integration into living spaces, promoting the concept of circular architecture. In this review, the main results obtained from biological experiments and architectural studies were reported, discussing the potential of the system from a circular economy and environmental sustainability perspective.

Keywords: Autotrophic/heterotrophic consortium; Biofilter design; Dishwasher wastewater upcycling

1. INTRODUCTION

The sustainable management of water resources is one of the main global environmental challenges, as the growing demand for tap water clashes with the progressive depletion of available freshwater reserves (WWAP, 2017). According to the guidelines of the 2030 Agenda, adopting resource reuse solutions is essential to reduce water waste and ensure greater sustainability (Shakir et al., 2017; Bixio et al., 2005). Greywater, originating from household appliances and activities, contains relatively low levels of contaminants and represents an underutilized opportunity for water recovery and reuse at both domestic and urban levels (Raschid-Sally and Jayakody, 2008; Toze, 2006; Barbagallo et al., 2001).

The Zero Mile system (Fig. 1) was developed to address this need, proposing an innovative approach that combines advanced biotechnology and environmental design for the treatment and reuse of dishwasher wastewater (Muñoz and Guieysse, 2006). The idea behind the system is to upcycle dishwasher wastewater transforming it from waste into a valuable resource through the use of an *ad hoc* engineered microbial biofilter, making water a useful asset for the domestic cultivation of edible or ornamental plants (Muñoz and Guieysse, 2006).

Fig. 1. The Zero Mile system project, diagram adapted from the design by Maximiliano Romero

The core of the system is a biofilter based on a microbial consortium composed of selected microorganisms capable of degrading organic matter and removing nutrients, such as nitrogen and phosphorus (Congestri et al., 2020, Alabiso et al., 2024a). This solution offers a dual advantage: on the one hand, it enables the regeneration of wastewater for reuse in domestic and urban environments, reducing the consumption of tap water; on the other hand, it produces nutrient-rich water, ideal for the growth of plants without the need for additional fertilizers (Libutti et al., 2018).

The innovation of the Zero Mile system is not limited to its biological component but extends to its integration with sustainable design and architecture (Walk and Dierich, 2014). While integrating biofiltration systems and vertical gardens into buildings can enhance biodiversity, reduce environmental impact, and promote local food production, such a strategy may overcome a critical issue: the consumption of tap water. This issue is particularly relevant in regions and periods of low rainfall, where water scarcity challenges the sustainability of urban greening initiatives. In many cases, mitigating this problem requires large-scale, centralized water management systems that, although effective, are often costly, complex, and difficult to implement at the individual building level.

The Zero Mile system addresses this limitation by introducing a decentralized biofiltration model designed for integration into individual apartments. Unlike large-scale centralized water treatment systems, which require significant infrastructure investments and complex implementation, this approach allows for on-site wastewater treatment and reuse at apartment/building level. By reclaiming and repurposing wastewater directly where it is produced, the system reduces reliance on tap water, minimizes distribution losses, and enhances water resilience in urban environments. This contributes to the development of more sustainable, self-sufficient, and resource-efficient living spaces (Specht et al., 2014). Additionally, the modular design of the system ensures flexibility, allowing adaptation to different building typologies and a variety of water reuse needs, both at an individual and collective scale (Costa et al., 2021).

This review aims to analyze the main results obtained from experiments on the Zero Mile system, evaluating both the biological efficacy of the biofilter and its impact in terms of design and practical applications (Toze, 2006). The objective is to provide an overview of the potential of this technology in the context of circular water resource management and the development of sustainable housing models (Barbagallo et al., 2001).

2. BIOFILTER DESIGN: BIOLOGICAL FEATURES

2.1. Microbial consortium cultivation and experimental settings

The engineered microbial consortium used in the Zero Mile system was composed of the heterocytous cyanobacterium *Trichormus variabilis* from the Tor Vergata University of Rome Collection (VRUC168; ATCC Strain designation: IUCC 1444; MSU A-37) and three bacterial strains (*Acinetobacter* sp., *Aeromonas* sp., and *Exiguobacterium* sp.) previously isolated from dishwasher wastewater (Congestri et al, 2020). The microbial consortium has been tested in different setup as described in Alabiso et al. (2024a) and cultivated in a semi-batch system under controlled laboratory conditions (25 °C, irradiance of 130 µmol photons $m^{-2}s^{-1}$, 12:12 light-dark cycle). To simulate the operational conditions envisaged for the Zero Mile system, consortium treated DWW (t-DWW) was replenished with untreated DWW (u-DWW) every 4 days.

The tests were conducted at increasing experimental size, from 50 mL (Small size Replenish-test, 25% refill every 4 days, duration 28 days), to 500 mL (Medium size Replenish-test, 25% refill every 4 days, duration 52 days) to 5.0 L (Large size Replenish-test, almost complete replenishment every 4 days, lasting 40 days). The consortium growth was monitored in all test as optical density (OD): at λ 665, measuring *in vivo* chlorophyll *a* content (proxy of the cyanobacterial growth curve) and at λ 730, measuring culture turbidity (proxy of the overall growth of the cultures). At the end of the tests the final biomass was also measured, as dry weight. In Fig. 2 are shown the growth curves of the Small size Replenish-tests.

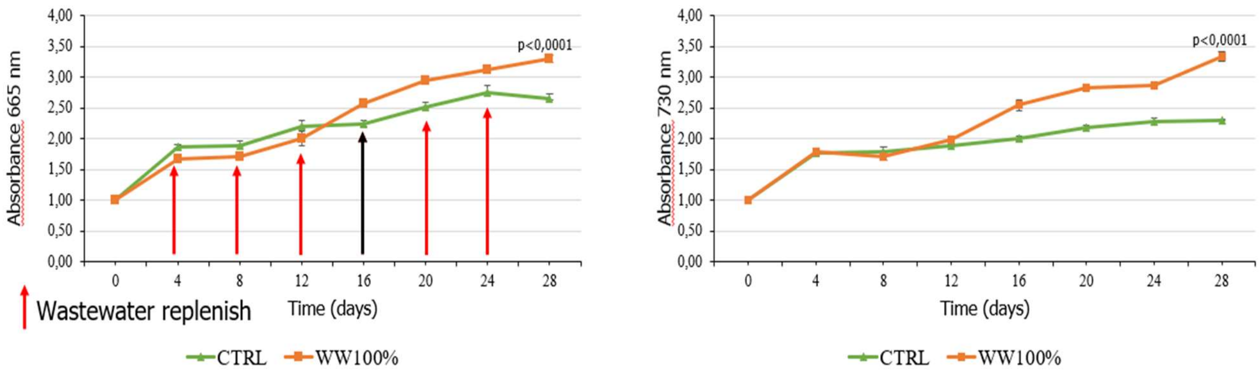

Fig. 2 Consortium growth curve in a Small size Replenish-test, (*left*) absorbance at λ 665, measuring *in vivo* chlorophyll *a* content, proxy of the cyanobacterial growth curve, and (*right*) absorbance at λ 730, measuring culture turbidity, proxy of the overall growth of the culture.

2.2. Microbial consortium features

A distinct emergent property is evident immediately after the consortium assembly: the consortia develop three-dimensional (3D) structures, blue-green suspended aggregates, that do not stick to the containers. Although *T. variabilis* is known to produce biofilms on exposed surfaces (Di Pippo et al., 2012), the consortia never adhere to the container walls, regardless of the material (plastic or glass). A further property of the consortia is that morphology and size of the aggregates depend on the growth vessel shape and, obviously, on the consortium's developmental stage (Fig. 3). The 3D structure is reversible: it can be disaggregated by strong shaking, but it quickly reconstitutes if the container is left to rest. This suggests that the 3D organization provides a selective advantage for all consortium members, likely due to easy cooperative interactions between the cyanobacterium and heterotrophic bacteria while they are in tight topological connection.

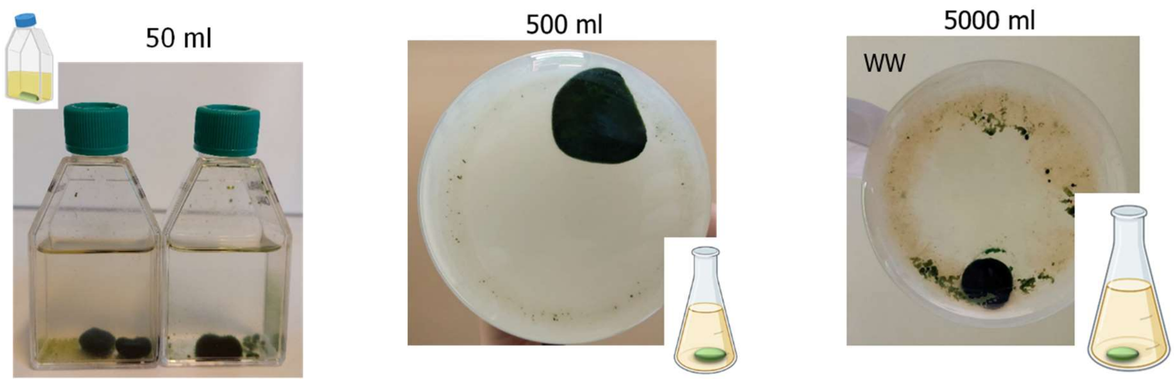

Fig. 3 Different 'shape' of the 3D structure, according to the container in which the consortia thrive: thick and rectangular in small rectangular 50 mL plastic flasks; thin and roundish in the medium/large circular glass flasks. Consortia were grown in dishwasher wastewater.

The cooperative interactions between autotrophic cyanobacterium and heterotrophic bacteria is based on their complementary metabolic activities: the heterotrophic bacteria attack and degrade organic material (food leftovers) producing carbon dioxide and consuming oxygen, while cyanobacteria consume carbon dioxide and produce oxygen through photosynthesis. The exchange of carbon dioxide and oxygen among partners allows to remove food leftover from wastewater, mineralizing their nutrient content which, in inorganic form, can be utilized by all the microbial partners to produce microbial biomass or can be used as plant fertilizer by watering plants with the treated wastewater.

The microbial community composition was evaluated using a targeted Next Generation Sequencing (NGS) approach in the Small size Replenish-test (Fig. 4; Alabiso et al. 2023).

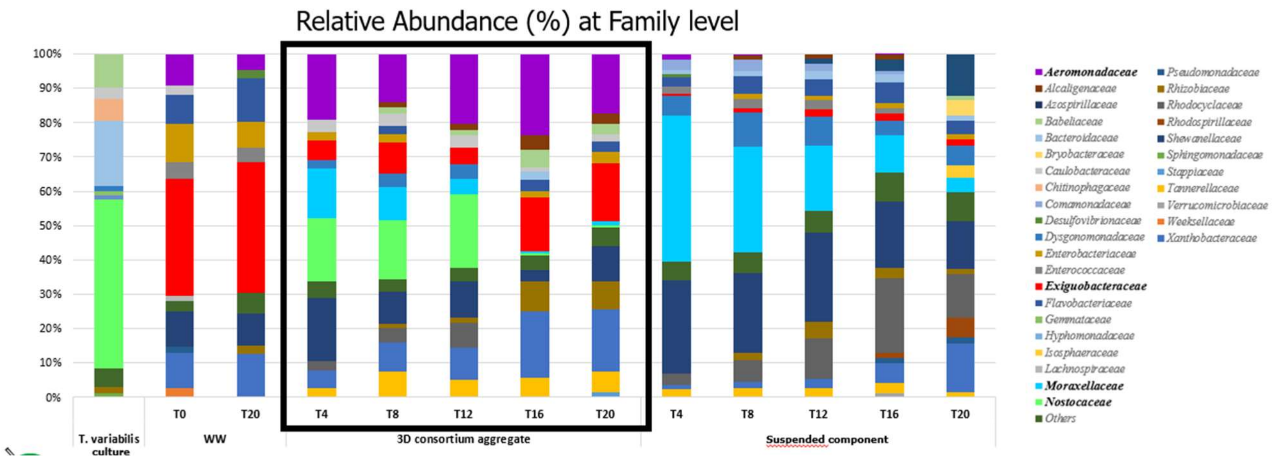

Fig. 4 Taxonomic composition of: i) *T. variabilis* culture before the consortium assemblage; ii) dishwasher wastewater at time 0 and after 20 days; iii) 3D aggregate, from day 4 to day 20; iv) suspended microbial component in the dishwasher wastewater.

Overall, the composition of both the 3D aggregate and the bacteria suspended in the dishwasher wastewater remained quite stable over time, notwithstanding the dishwasher wastewater change in the every four days replenishment, which could had altered their composition.

2.3. Nutrient removal efficiency

In all tests, the efficiency of nutrient removal was evaluated by measuring total nitrogen and total phosphorus concentrations after the treatment. As shown in Fig. 5, the efficiency of treatment remains quite high in all the tests set-up, and the residual amounts of total nitrogen and phosphorus in the treated wastewater lay into the range of the values stated by the urban wastewater dumping Directive 91/271/EEC (Urban Waste Water Treatment Directive), amended by the Directive 98/15/EC49 (requirements of Annex I). Hence, the treated DWW can be used for plant watering.

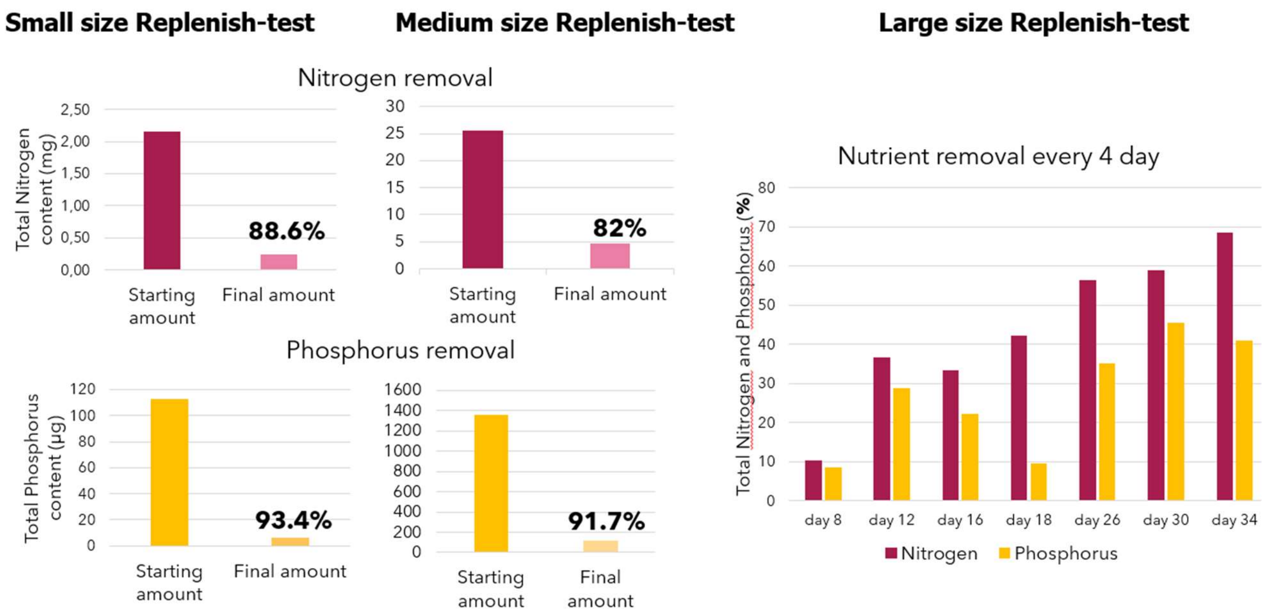

Fig. 5 Nitrogen and phosphorous removal in the three test set-ups

3. CONSORTIUM TREATED WASTEWATER FOR PLANT GROWTH

3.1 Indoor or outdoor cultivation

The efficiency of treated wastewater to water and fertilize plants, was evaluated in two parallel experiments: i) indoor cultivation in the Zero Mile demonstrator, and ii) open-field cultivation at the Botanic Gardens of the Tor Vergata Rome University. Both tests were conducted using *Lactuca sativa* cv. Canasta (Asteraceae).

The Indoor Cultivation test was performed using the plant-dedicated structure of the Zero Mile demonstrator, which may contain twenty-four seedlings. The plants were watered every two days with 40 mL of either treated DWW or tap water (Rome water main), as a control; 12 seedings were used for each experimental batch. The experiment lasted 42 days, under controlled indoor conditions: at the end of the experiments all plants were collected and six plants for each experimental batch, were weighted (fresh weight) and morphometrically analysed by means of the ImageJ platform (version 1.52a; U.S. National Institutes of Health, Bethesda, MD, USA), while the remaining six plants were used to evaluate the biochemical plant descriptors, as reported in the next section (§ 3.2)

The *Open-field Cultivation test* was conducted in May–June 2023 at the Botanic Gardens of the Tor Vergata Rome University. Two groups of fifteen plants, spaced 25 cm apart and divided into 3 subgroups (each representing a replicate) were watered every day with 200 mL of a 1:1 (v/v) dilution of treated DWW (in 100 mL distilled water) or distilled water as a control, respectively. The experiment lasted 56 days, then xxx plants were collected and weighted (fresh weight) and morphometrically analysed by means of the ImageJ platform (version 1.51; U.S. National Institutes of Health, Bethesda, MD, USA)

3.2. Evaluation of plants by biochemical descriptors

To assess the physiological and biochemical impact of treated wastewater on plants, leaf subsamples of both indoor and outdoor cultivation were collected for biochemical analyses: i) photosynthetic pigment concentrations, *i.e.* Chlorophyll *a*, chlorophyll *b*, and carotenoid; ii) total phenols and flavonoids; iii) soluble solid content (Alabiso et al., 2024b).

Table 1. Fresh weight, photosynthetic pigments and quality indices of *Lactuca sativa* cv. Canasta plants watered with consortium treated dishwasher wastewater (t-DWW) or control water (, in indoor or outdoor cultivations. Data represents mean ± SD (n=3); asterisks indicate the significance of the differences between treatments (* p<0.05; ** p<0.001; from Alabiso et al., 2024b)

Descriptor	Indoor		Outdoor	
	t-DWW	Control	t-DWW	Control
Fresh weight (g)	13.51±1.19**	8.12±0.55	631.60±88.23**	471.22±89.57
Total length (cm)	22.53±3.02	19.02±1.24	54.17±5.66*	46.56±5.14
Chlorophyll *a* (μg g^{-1} FW)	120.25±3.93	118.31±5.21	58.22±0.65	59.74±0.87
Chlorophyll *b* (μg g^{-1} FW)	126.81±2.11	123.08±2.36	89.97±6.35	76.58±6.16
Chlorophyll *a* + *b* (μg g^{-1} FW)	247.06±3.21	241.39±4.35	148.20±5.74	136.32±5.37
Carotenoids (μg g^{-1} FW)	67.93±0.61	67.73±1.21	32.13±0.13	32.00±0.12
Soluble Solid Content (%)	10.97±0.15	10.80±0.20	5.75±0.48	5.96±0.83
Total Phenols (mg GA Eq g^{-1} FW)	134.00±5.71	152.97±11.21	44.98±14.99	278.05±14.04**
Total Flavonoids (mg Q Eq g^{-1} FW)	11.65±0.95	14.32±1.82	43.96±22.82	251.08±20.75**

As reported in Table 1, both indoor and outdoor, the plants watered with the treated dishwasher wastewater grown better or, at least comparably, to the control plants and this is confirmed by all the descriptors: an increased production of secondary metabolites was found for carotenoids, but also for phenolic and flavonoid compounds, widely used to evaluate stress or beneficial effects in cultivated plants, and for soluble solids, a reliable descriptor of the overall crop quality.

4. CONCLUSIONS

As a whole, these results confirmed the robustness of the *ad hoc* engineered consortium, in bioremediating dishwasher wastewater and the usefulness of the reclaimed wastewater for plant watering and fertilization. Hence, the Zero Mile system can bring significant benefits in terms of reducing the amount of wastewater and fresh water consumed and permits the production of high quality plant food. A further contribution of the Zero Mile system to the sustainable development, concerns the on-site production of vegetables, with the gratification of ‘grow your own’ food, the improvement of the air/environment quality and the contribution to a sustainable diet.

Finally, the integration of the wastewater treatment system in the context of the built space is a strategy which may improve the efficiency of domestic water use: the proposed system may favour the implementation of urban greening, aimed at the climate neutrality of buildings, with a possible reduction in environmental pollution.

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