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WATER PRODUCTION FOR AWARE (Total Bacteria and Antibiotic Resistance Genes):

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Celia Manaia¹

¹Universidade Católica Portuguesa



AWARE Project

Horizon Europe 101084245

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Protocol status: Working

We use this protocol and it's working

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Abstract

The protocol summarises the procedures used for analytical control. The protocol describes the Standard Operating Procedure (SOP) for the optimization of advanced tertiary treatment of water, based on a comprehensive quality and risk assessment.

Guidelines

RECOMMENDED/ACCEPTED VALUE:

No legal recommendation available.

16S rRNA geneAmount<3 log units/ mL ; other faecal and antibiotic resistance genes < limit of detection (LOD)
inAmount2000 mL

Materials

	A	B	C	D	E	F	G	H
	Parameter	V (mL) x R	S	Processing	Analytical method	Result	LOD / LOQ	Goal value
	Total bacteria and antibiotic resistance genes	2000 x 3	On ice	Membrane filtration 0.22 µM polycarbon ate membranes	DNA-based analysis quantitative PCR (e.g. 16S rRNA;intl1, uidA, sul1, qacE1, tetX, ermB, crassphage, mefC, ermF, aph(3'')-ib	Gene abundance per volume of water (Log-unit / mL) Removal values (if adequate) (Log-unit)	Total bacteria: 0.7 Other genes: intl1 – 0.01 uidA – 0.01 sul1 – 1.22 qacEΔ1 – 0.2 tetX – 0.2 ermB – 0.2 crassphage – 1.0 mefC – 0.1 ermF – 0.01 aph(3'')-ib – 1.20	Total bacteria (3 log-units / mL) Other genes (

Material: Membrane filtration (nitrocellulose 0.22 µm pore); DNA extraction (QIAGEN, Power Water); Quantitative PCR of genes such as 16S rRNA gene (total bacteria), uidA, marA (bacterial contaminant indicators), crassphage (human fecal contamination), intl1 / incF / sul1 / qacEΔ1 / tetX / ermB / mefC / ermF / aph(3'')-ib (antibiotic resistance indicators); metagenomics analysis of selected samples.

Safety warnings

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Total Bacteria and Antibiotic Resistance Genes):

- 1 The water production for AWARE main activities includes three stages – disinfection by ultraviolet C radiation (UVC), storage for ⌚ 24:00:00 - ⌚ 24:00:00 (according to water load and season) and ozonation. The water quality is monitored at these three stages, for the parameters indicated in Figure 1 below.

2d

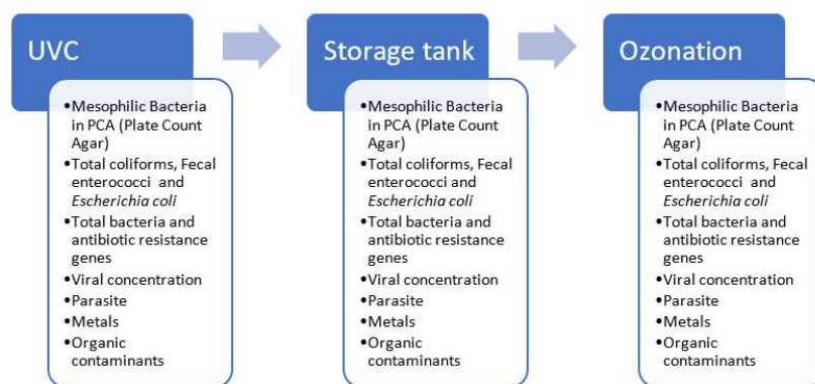


Figure 1. Treatment and storage of municipal treated wastewater used for integrated aquaponics and an indication of the comprehensive quality and risk assessment.

1.1 Sampling, Processing, and Analyses

9h

Water samples are collected (see Figure 2) and processed within a ⌚ 06:00:00 interval, before being shipped for the partner responsible for the analyses (Table 1). In case no processing is needed, samples are frozen and stored at Temperature 🌡️ -80 °C within ⌚ 03:00:00 .

For each sampling event, the date, day of the week and hour; the temperature and rain. Sampling points, indicated in Figure 2 were designated from A to I:

- Influent of primary treatment (A)
- Influent of biological treatment (activated sludge) (B)
- Treated secondary effluent (C)
- Sand filter effluent (D)
- UVC effluent (E)
- Storage for reuse tank effluent (F)
- Ozonation effluent (1 dos, O3) - 🧪 5 mg ITO3X technology - (G)
- Effluent of the vacuum UV oxidation (VUV) (H)

- Effluent of reactive storage / Influent of the recirculation aquaculture system (RAS) (I)

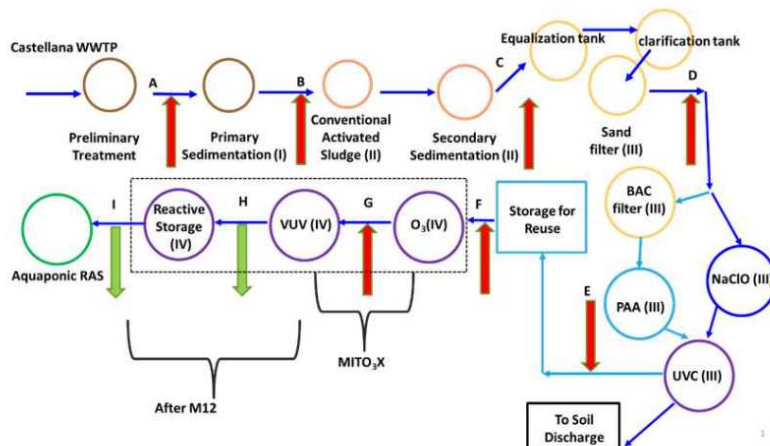


Figure 2. Diagram representing the wastewater treatment plant (WWTP), advanced treatment and sampling points.

Methods: The section below summarises the procedures used for analytical control – detailed protocols are annexed to this protocol.

6h

2 Total Bacteria and Antibiotic Resistance Genes:

2.1 **Analysis:** Culture-independent detection and/or quantification of bacteria and bacterial contaminants.

2.2 **Observations:** Samples were filtered within 06:00:00 after collection the filtering membranes were immediately frozen and stored at -8 °C till shipping in dry ice to the respective partner who proceeded for DNA extraction.

6h

Parameters framed by Legal and Regulatory Requirements:

3 Using the EU Drinking Water Directive:

Mesophilic Bacteria in PCA (Plate Count Agar) – 0 CFU/ 100 mL



Total coliforms and Escherichia coli –Number /Amount 100 mL (0 MPN/  100 mL)

Fecal enterococci –Number/  100 mL (0 MPN/  100 mL)

Viral concentration - There are no legal requirements for viruses. They are not included in any regulation now.

Parasite - EU legislation (2020/741)

Metals - DIRECTIVE 2008/105/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on environmental quality standards in the field of water policy

Organic contaminants - DIRECTIVE 2008/105/EC OF THE EUROPEAN PARLIAMENT AND THE COUNCIL of 16 December 2008 on environmental quality standards in the field of water policy.

Protocol references

- Teixeira et al., 2023 (<https://doi.org/10.1016/j.watres.2023.120761>).
- Rocha et al., 2020 (<https://doi.org/10.1016/j.jece.2018.02.022>).