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WATER PRODUCTION FOR AWARE (Total Coliforms, Fecal Enterococci and Escherichia coli:) V.2

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Celia Manaia[1]

[1]Universidade 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:

Total coliforms: Amount0 MPN/ mL
E.coli: Amount0 MPN/ mL
Fecal enterococci: Amount0 MPN 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 coliforms, Fecal enterococci and Escherichia coli	100 x 3	No	None	Colilet 18 system (IDEEX) method – Total coliforms and E.coli Enterolert E system (IDEEX) method – Fecal enterococci	Most Probable Number (MPN/mL)	1	0 MPN /100 mL

Table 1: Samples, Processing and Analysis of the different parameters analysed.
V, volume; R, Replicates; S, Shipment conditions; LOD / LOQ, Limit of Detection / Quantification

Materials: Sterile bottles, IDEEX kit, Colilert-18 reagent, Microbiological incubator.

Safety warnings

WATER PRODUCTION FOR AQUAPONICS

1 The water production for AWARE main activities includes three stages – disinfection by ultraviolet C radiation (UVC), storage for 12:00:00 - 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. 1d 12h

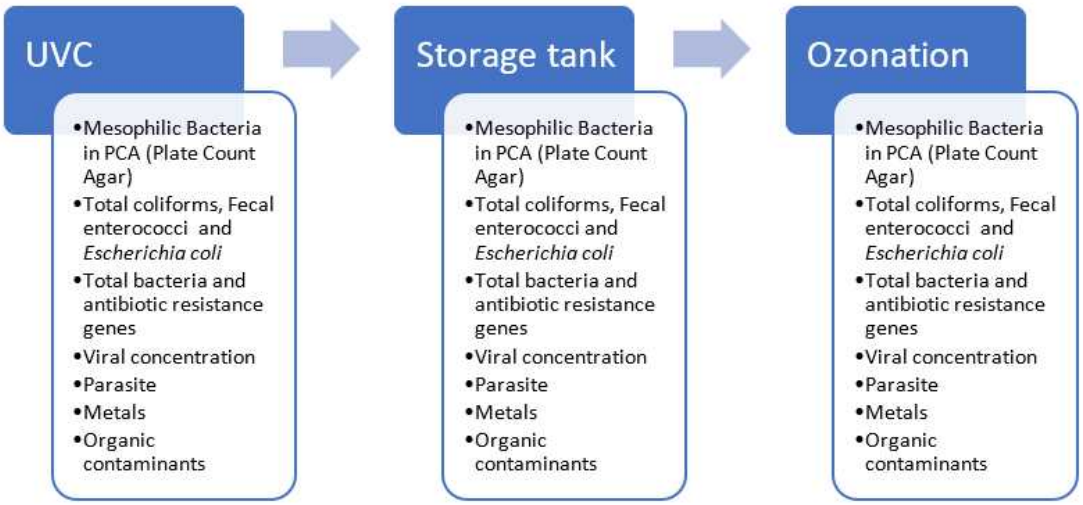

Figure 1. Treatment and storage of municipal treated wastewater used for integrated aquaponics and an indication of the comprehensive quality and risk assessment.

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 -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 dose, e.g., 5 mg O_3) - MITO3X technology - (G)
- Effluent of the vacuum UV oxidation (VUV) (H)

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

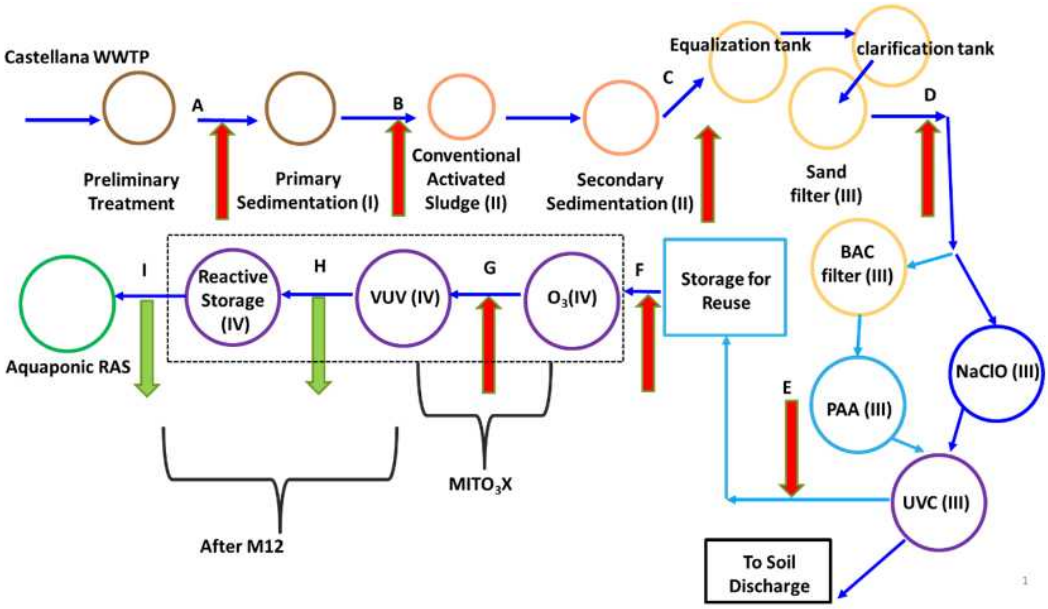

Figure 2. Diagram representing the wastewater treatment plant (WWTP), advanced treatment and sampling points.

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

5d

2 **Total Coliforms, Fecal Enterococci and Escherichia coli:**

2.1 **Analysis:** Enumeration of culturable mesophilic bacteria at 37 °C

2.2 **Method:**

	A	B	C
	1	Total coliforms E.coli	**Colilet 18 (IDEEX) method**
	2	**1**	100 mL of each water sample poured in sterile bottles from IDEEX kit
	3	**2**	Adding of Colilert-18 reagent
	4	**3**	Mix sample + reagent poured into the QuantiTray/2000 System
	5	**4**	Incubation 18 hours, 35 °C ± 0.5 °C

	A	B	C
	6	**5**	Results analysis

Jspreadsheet CE

Reference:Go togo to step #2.3

– UNI EN ISO 9308-2:2014 Water quality - Count of Escherichia coli and coliform bacteria - Part 2: Most probable number method.

– EN ISO 11133:2014 - Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

	A	B	C
	1	Fecal enterococci	Enterolert E system (IDEEX)
	2	**1**	100 mL of each water sample poured in sterile bottles from IDEEX kit
	3	**2**	Adding of reagent
	4	**3**	Incubation 24 hours, 41 °C ± 0.5 °C
	5	**4**	Results analysis

Jspreadsheet CE

Reference:Go togo to step #2.3

– UNI EN ISO 7899-1:2001 - Water quality - Research and enumeration of intestinal enterococci - Miniaturized method (Most Probable Number) for surface and wastewater.

– EN ISO 11133:2014 - Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

2.3 **Observations:** Samples were processed and analysed withinDuration 12:00:00 after collection. 12h

Parameters framed by Legal and Regulatory Requirements: 9h

3 Using the EU Drinking Water Directive:

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

Total coliforms and *Escherichia coli* –Number / 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

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