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WATER PRODUCTION FOR AWARE (Mesophilic Bacteria in PCA (Plate Count Agar)) V.3

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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:

According to drinking water EU legislation

Materials

	A	B	C	D	E	F	G	H
	Parameter	V (mL) x R	S	Processing	Analytical method	Result	LOD / LOQ	Goal value
	Mesophilic Bacteria in PCA (Plate Count Agar)	0.1 x 3	No	Spread method	Spread plate; 48 h incubation at 37 °C	Number of Colony Forming Units (CFU/mL)	10	0 CFU/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: Culture medium Plate Count Agar (PCA); Microbiological incubator; other microbiology consumables.

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

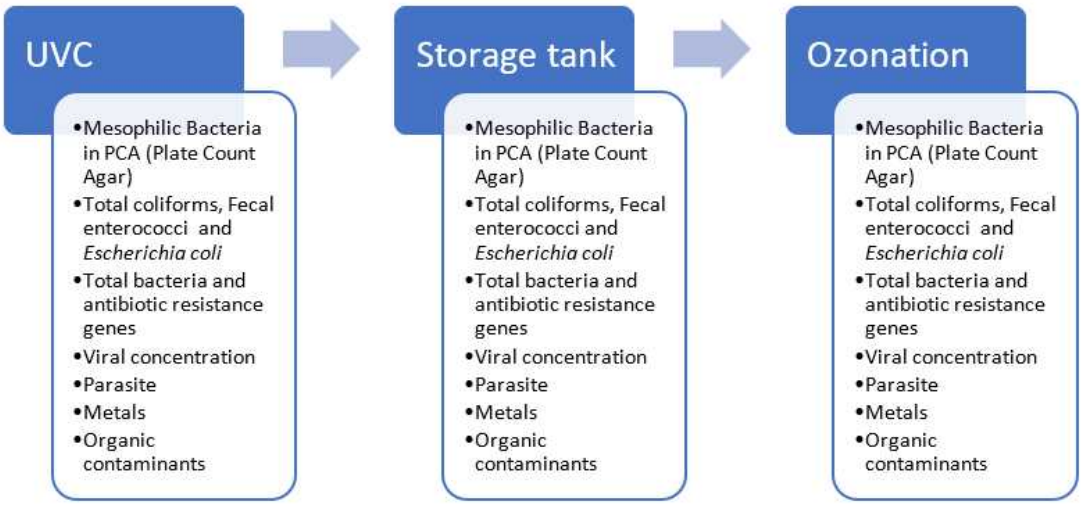

Figure 1. Treatment and storage of municipal treated wastewater used for integrated aquaponics and an indication of the comprehensive quality and risk assessment.

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)

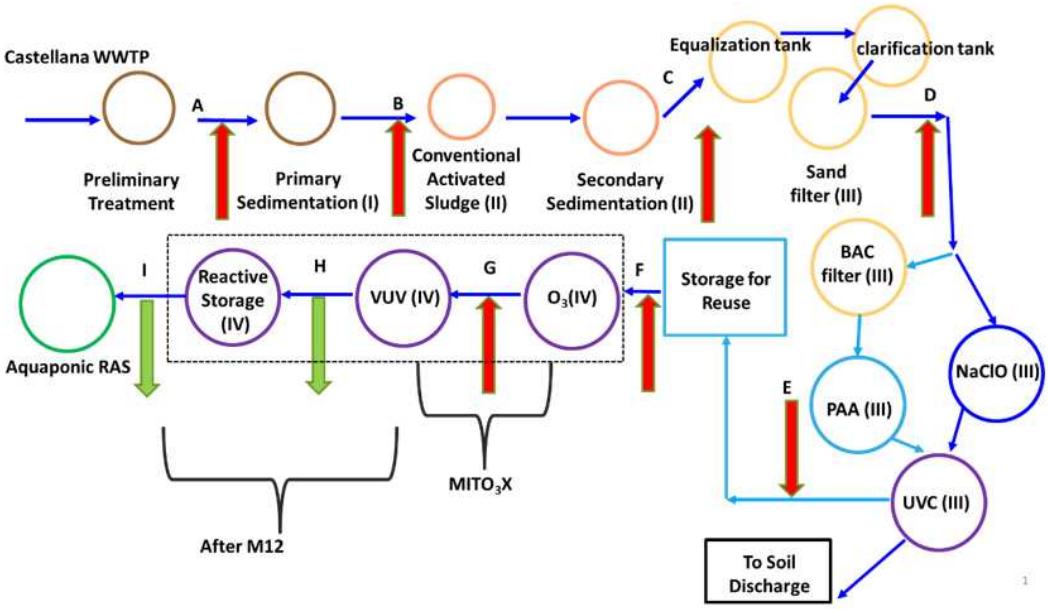

Figure 2. Diagram representing the wastewater treatment plant (WWTP), advanced treatment and sampling points.

Methods: The section below summarises the procedures used for analytical control – detailed protocols are annexed to this protocol. 5d

2 **Mesophilic Bacteria in PCA (Plate Count Agar):**

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

2.2 **Method:** 2d

Spread plate; units 48:00:00 a incubation at 37 °C ; emuneration of colony forming (CFU/ mL):

2.3 **Observations:** Samples processed and analysed within 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

- American Public Health Association (APHA). (2009). Standard Methods for the Examination of Water and Wastewaters. APHA – AWWA - WPCF (Eds.), Pennsylvania, Washington.
- ISO 4833:2003 - Microbiology of food and animal feeding stuff – Horizontal method for enumeration microorganisms – Colony count technique at 30 °C .
- EN ISO 11133:2014 - Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.