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WATER PRODUCTION FOR AWARE (Virus)

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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:

Viruses not included in any normative.

Contemplated in the EU proposal of a new directive on the Treatment of urban wastewater

Materials

Material: Rexeed 25A filtration units (Asahi Kasei Medical); other molecular biology consumables.

Safety warnings



Virus:

1d 21h

- 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

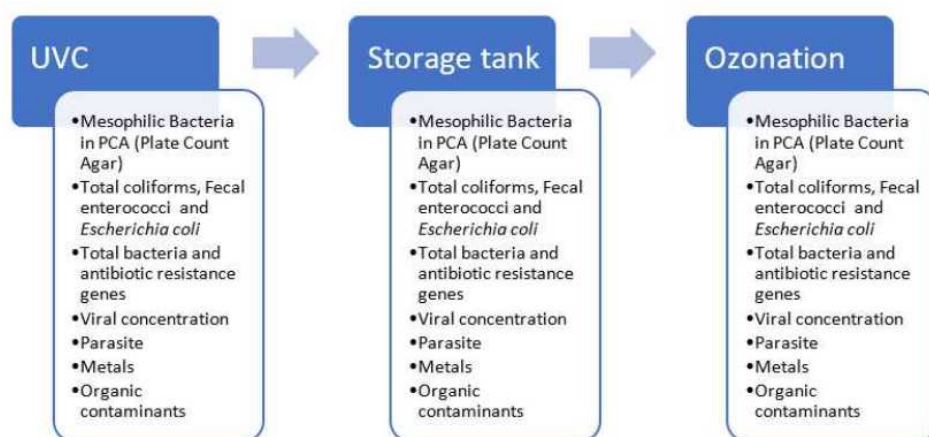


Figure 1. Treatment and storage of municipal treated wastewater used for integrated aquaponics and an indication of the comprehensive quality and risk assessment.

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 O₃) - MITO3X technology - (G)

- Effluent of the vacuum UV oxidation (VUV) (H)
- Effluent of reactive storage / Influent of the recirculation aquaculture system (RAS) (I)

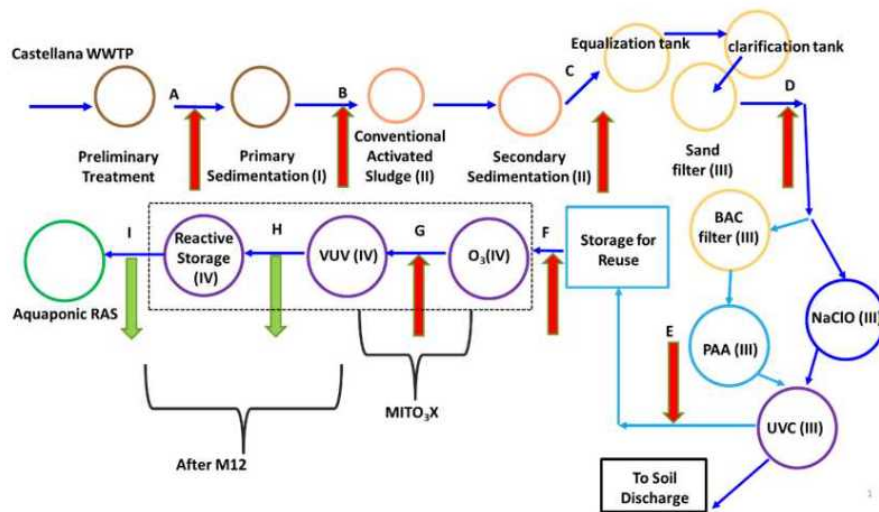




Figure 2. Diagram representing the wastewater treatment plant (WWTP), advanced treatment and sampling points.

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

6h

2 Analysis: Detection and enumeration of virus.

2.1 Method: Virus capture by filtration with Rexeed dialysis units, concentration with aluminium chloride, nucleic acid extraction and detection by RT-qPCR.

2.2 **Observations:** Samples were filtered within  06:00:00 after collection the filtering membranes were immediately frozen and stored at  -80 °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 / 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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