May 08, 2024

WATER PRODUCTION FOR AWARE (Parasite)

DOI
dx.doi.org/10.17504/protocols.io.3byl49bdzgo5/v1

Celia Manaia[1]

[1]Universidade Católica Portuguesa

AWARE Project
Horizon Europe 101084245

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.3byl49bdzgo5/v1

Protocol Citation: Celia Manaia 2024. WATER PRODUCTION FOR AWARE (Parasite). **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl49bdzgo5/v1

Protocol status: Working
We use this protocol and it's working

Created: May 08, 2024

Last Modified: May 08, 2024

Protocol Integer ID: 99417

Keywords: water sampling, water processing, water analysis, waste water treatment, advanced tertiary treatment SOP

Funders Acknowledgement:
Horizon Europe
Grant ID: 101084245

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:

Regulation 2020/741 Minimum requirements for water reuse.
Guidelines to support the application of Regulation 2020/741 on minimum requirements for water reuse
Council Directive 93/88/EEC of 12 October 1993 on the protection of workers from risks related to exposure to biological agents at work.
Relevant food-borne parasites are not included in any normative.

Materials

	A	B	C	D	E	F	G	H
	Parameter	V (mL) x R	S	Processing	Analytical method	Result	LOD / LOQ	Goal value
	Parasite	250 x 12	On ice	Membrane Filtration Nitrocellulose /polycarbonate membranes of 0.45 µM	High throughput sequencing	Presence/absence (Amplicon Sequence Variants, ASVs) of hazardous species (potential human pathogens: hypersensitive pneumonitis, asthma, allergy, immunosuppressive clinical symptoms and rhinitis, Legionella spp. and its host; fish pathogen)	Acremonium sclerotigenum - 341 Aspergillus versicolor - 20 Cladosporidium sp. - 1101 Penicillium sp. - 27 Naegleria sp. - 240 Alternaria sp.- 122 Cryptocarion sp. - 56 Legionella sp. - 10 Rhogostoma sp.- 130	ASV < LOD

Material: Filtration ramp; DNA extraction kit, PCR equipment; fluorimeter for DNA quantification; outsourcing of sequencing service; molecular biology consumables.

Safety warnings

Parasite

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

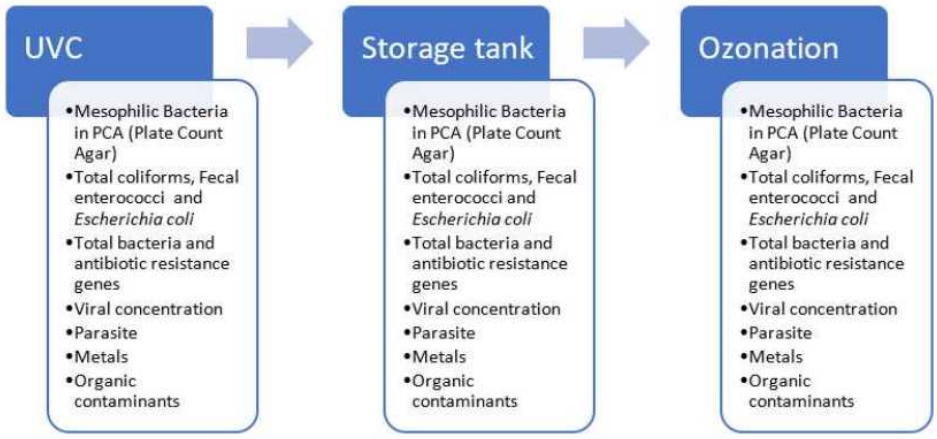

Figure 1. Treatment and storage of municipal treated wastewater used for integrated aquaponics and an indication of the comprehensive quality and risk assessment.

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)

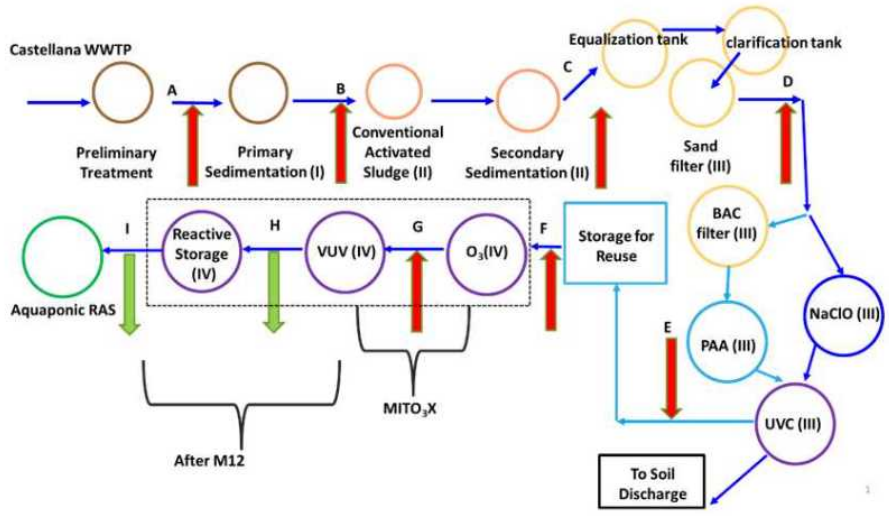

Figure 2. Diagram representing the wastewater treatment plant (WWTP), advanced treatment and sampling points.

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

2 **Parasites:**

Analysis: Detection of parasites.

2.1 **Filtration:**

3 L of water were filtered through **0.45 µm** pore size diameter Nitrocellulose / polycarbonate sterile membranes, using a membrane filter holder (47 mm diameter) apparatus. For every approx. 250 mL of water, a new sterile membrane was used to avoid clogging of membranes. Filters corresponding to each litre of water were separated, inserted into well-sealed tubes (50 mL stored a -80 °C and then transported in dry ice.

2.2 **Centrifugation:** 1d 0h 15m

(1 L) of water was concentrated by centrifugation at 2.500 g for 00:15:00 ususing sterilized tubes. Pellets obtained were resuspended using of 1 mL of 10 % formaldehyde solution and maintained for 24:00:00 . Pellets resuspended in formaldehyde solution were centrifugated at 2.500 g for , the supernatant was discarded and all pellets were pooled in 1 mL of 70 % ethanol.

2.3 **DNA Isolation, Amplification and sequencing:**

DNA extraction was carried out using a commercially available kit (Powerwater kit, Qiagen) in accordance with the manufacturer's guidelines. The V4 and V9 of the 18S rRNA gene hypervariable regions of the eukaryote 18S rRNA gene were amplified using universal primers. Subsequently, PCR products were purified and subjected to external sequencing using Illumina MiSeq platform 300bp paired-end sequencing.

2.4 **Bioinformatic analysis:**

Raw sequence data were processed using QIIME2 software 2023.9. Pairedend reads were subjected to quality filtering (FastQC) and denoising (DADA2 plugin). Subsequently, the taxonomic assignment was conducted using the reference database SILVA 18S release 138 with a clustering threshold of 99 % similarity. Alpha rarefaction analysis, Shannon diversity index, richness estimation, and relative abundance calculations were performed using the Vegan R package to assess microbial community diversity and composition particularly focusing on the detection of genetic material from potential pathogens.

2.5 **Observations:** Samples were filtered within 12:00:00 after collection the filtering membranes were immediately frozen and stored a -80 °C till shipping in dry ice to the respective partner for further analysis.

12h

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

- Mthethwa N.P., Amoah I.D.,
Reddy P., Bux, F., Kumari S. 2021. A review of the application of next-generation sequencing methods for profiling of protozoan parasites in water: Current methodologies, challenges, and perspectives. Journal of Microbiological Methods, 187:106269
- Ríos-Castro R., Romero A., Aranguren R., Pallavicini A., Banchi E., Novoa B., Figueras A. 2021. High Throughput Sequencing of Environmental DNA as a Tool for Monitoring Eukaryotic Communities and Potential Pathogens in a Coastal Upwelling Ecosystem. Frontiers in veterinary science, 8, 765606.
EFSA (2018). Public health risks associated with food-borne parasites. EFSA Journal 16 (12):5495.