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Project code: **ROMD00586**

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Beneficiary: **Moldova State University**

Project title: **Pilot Project for Environmentally Friendly Algal Biomass Production for Fish Nutrition - Algae4FishRo-Md**

Reports on the determination of the physicochemical parameters of the water in RAS-II (second)

Chisinau, 2025

Introduction

Within the project "Pilot project for the production of environmentally friendly algal biomass for fish nutrition - Algae4FishRo-Md", Code: ROMD00586, financing contract no. 109279/27.05.2025, and in the context of activity A1.1, which is part of the work package "Recycling wastewater from RAS by using algae and integrating algal biomass into the fish diet", water samples were taken to perform the initial analysis, necessary for the preparation of the first reports on the determination of the physicochemical parameters of water from RAS. After a predetermined period, additional samples were taken for the comparative follow-up analysis.

The purpose of collecting water samples was to establish the initial parameters of the nutrient media with the addition of wastewater. The analyses were distributed between the partners: LP1 examined pH, dissolved oxygen (DO), temperature, nitrogen and phosphorus compounds, alkalinity, heavy metals and trace elements, while PP2 evaluated parameters such as pH, TDS, conductivity, dissolved carbon dioxide, organic matter, temperature, as well as the metals Cr, Cu, Pb and Ni. Some of the parameters were analyzed by both partners, as the results were interdependent and necessary to complete the other determinations.

The nutrient media used for algae cultivation represent one of the most important factors determining the growth, development and accumulation of biologically active substances in algal cells. The quantity and composition of nutrient media directly influence the biomass productivity and long-term stability of cultures.

An adequate nutrient medium for algae cultivation must contain optimal amounts of macroelements (nitrogen, phosphorus, potassium, magnesium, calcium, sulfur), microelements (iron, manganese, copper, zinc, cobalt, molybdenum, etc.), sufficient carbon sources and respect optimal physicochemical parameters (pH, salinity, turbidity, temperature), which must be maintained within the limits tolerated by the cultivated species.

Thus, the nutrient medium has the role not only of providing the substances necessary for the vital processes of algal cells, but also of creating balanced conditions that favor both the accumulation of biomass and the biosynthesis of biologically active compounds (proteins, amino acids, lipids, carbohydrates, pigments, phytohormones, etc.).

According to the conclusions presented in Report No. 1 on the analysis of the quality of wastewater from the RAS, these waters cannot fully provide the necessary macro- and microelements for the development of *Chlorella vulgaris* and *Spirulina platensis* algae, and it is necessary to supplement them with mineral salts added to the culture medium.

Based on this, optimization calculations were performed and it was found necessary to carry out experiments, under laboratory conditions, to cultivate both species on standard nutrient media (control) and on media supplemented with wastewater in concentrations of 10%, 30%, 60%, 90% and 100%.

The supplementation was done as follows:

- 10% waste water: 10 ml RAS waste water + 90 ml standard nutrient medium;
- 30% waste water: 30 ml RAS waste water + 70 ml standard nutrient medium;
- 60% waste water: 60 ml RAS waste water + 40 ml standard nutrient medium;
- 90% waste water: 90 ml RAS waste water + 10 ml standard nutrient medium;
- 100% waste water: only RAS waste water, without addition of standard nutrient medium.

Subsequently, physicochemical analyses of the quality of the nutrient media used to cultivate *Chlorella vulgaris* and *Spirulina platensis* algae were performed, the data being presented in the tables below. The project execution team of the State University of Moldova performed the analysis of wastewater from the RAS system by determining the following parameters: pH, TDS, conductivity, dissolved CO_2, organic matter, temperature, , and the content of trace elements/heavy metals (Cr, Cu, Pb, Ni) was determined jointly with the team of the lead project partner LP1.

Working method and equipment used

A potentiometer equipped with a pH electrode, model ”Consort C933” was used to determine the pH. The TDS content was determined using the ”Adwa AD 331” apparatus. The dissolved CO_2 concentration was determined by the titrimetric method, using the titration with 0.05 N hydrochloric acid.

Organic matter was determined by the gravimetric method, by burning the samples at a temperature of 550 °C. The water temperature was measured using an electrode with a temperature sensor. The content of trace elements (Cr, Cu, Pb, Ni, Mn) was determined spectrophotometrically, using the ”UV-Vis T80+” spectrophotometer and using the high-resolution atomic absorption spectrometry apparatus with a graphite furnace electrothermal atomizer and an Argon 5.0 high-purity ion carrier, employing a ContrAA 600 spectrometer.

The mathematical processing of the results was carried out by calculating the arithmetic mean and standard error, using the Microsoft Excel program.

Results:

Tab. 1. Initial chemical composition of the nutrient media with the addition of wastewater from the recirculating system used in the cultivation of *Spirulina platensis* algae

Nutrient media with the addition of waste water	Parameters analyzed						
	pH	T, °C	TDS, g/l	Conductiviti, µs/cm	Dissolved CO_2, mg/l	Turbidity, NTU	Organic matter, mg/l
10%	9,22±0,01	21,0	4,41±0,02	8882,10±10,12	3,10±0,10	2,21±0,05	10,00±0,07
30%	9,28±0,01	22,0	4,10±0,01	8200,00±11,13	9,00±0,12	6,01±0,10	30,10±0,10
60%	9,34±0,01	21,5	3,50±0,01	7000,01±10,11	18,11±0,12	12,10±0,15	60,12±0,10
90%	9,56±0,05	21,2	3,15±0,01	6300,12±11,11	27,01±0,13	18,21±0,21	90,02±2,25

When analyzing the initial chemical composition of the nutrient media with the addition of wastewater from the recirculating system, used to cultivate the *Spirulina platensis* algae, the following aspects are found:

The pH of the media is maintained within alkaline limits, with values ranging between 9.22±0.01 for the variant with the addition of 10% and 9.56±0.05 for the variant with the addition of 90%. This suggests that, with the increase in the proportion of wastewater, the medium becomes slightly more alkaline, a favorable condition for the development of *Spirulina*.

The water temperature varies very little, ranging between 21.0°C and 22.0°C, which demonstrates thermal stability of the nutrient media and good compatibility with the optimal algae cultivation interval.

The concentration of dissolved solids (TDS) shows a gradual decrease, starting from 4.41±0.02 g/l (10%) and decreasing to 3.15±0.01 g/l (90%). The same decreasing trend is observed for the conductivity, which varies from 8882.10±10.12 µS/cm to 6300.12±11.11 µS/cm. This decrease can be correlated with the dilution of certain salts and ions with the increase in the addition of residual water.

In contrast, the dissolved carbon dioxide (CO_2) increases significantly, from 3.10±0.10 mg/l in the variant with 10% residual water, to 27.01±0.13 mg/l in the variant with 90%. This

accumulation of CO_2 can influence both photosynthesis and the carbonic acid balance of the environment.

The water turbidity increases almost ninefold, from 2.21±0.05 NTU to 18.21±0.21 NTU, indicating a higher suspended particle load.

Organic matter shows an evolution directly proportional to the percentage of wastewater added: 10.00±0.07 mg/l (10%), 30.10±0.10 mg/l (30%), 60.12±0.10 mg/l (60%), reaching up to 90.02±2.25 mg/l (90%). This increase suggests a consistent enrichment of the medium with organic substances available for algae metabolism.

Overall, the nutrient media become more alkaline, richer in organic matter and CO_2, but poorer in dissolved mineral salts, which reflects the direct effect of the increase in wastewater addition on the medium composition.

Tab. 2. Initial chemical composition of nutrient media with the addition of wastewater from the recirculating system used in the cultivation of *Chlorella vulgaris* algae

Nutrient media with the addition of waste water	Parameters analyzed						
	pH	T, °C	TDS, g/l	Conductiviti, µs/cm	Dissolved CO_2, mg/l	Turbidity, NTU	Organic matter, mg/l
10%	6,8±0,01	21,0	695,10±11,11	1391,50±10,11	15,01±1,11	2,01±0,11	10,0±0,07
30%	7,03±0,02	21,0	648,31±10,11	1296,30±11,02	18,01±1,01	5,02±0,10	25,01±0,06
60%	7,35±0,03	21,0	578,32±9,11	1157,10±10,13	22,01±1,11	10,01±0,11	50,01±0,05
90%	7,98±0,02	21,0	508,31±7,12	1017,20±8,21	28,01±1,14	15,01±0,12	75,01±0,04

The initial chemical composition of the nutrient media with the addition of wastewater from the recirculating system, used for the cultivation of the algae *Chlorella vulgaris*, shows a series of important changes depending on the proportion of wastewater added. The pH values indicate a transition from a slightly acidic environment to an almost neutral one and subsequently slightly alkaline, increasing from 6.8±0.01 in the variant with 10% wastewater, to 7.98±0.02 in the variant with 90%. This trend reflects the progressive neutralization of the environment and its adaptation to conditions more favorable for the growth of *Chlorella*. The temperature is maintained constant at

21.0°C in all variants, which suggests thermal stability and rigorous experimental control. The content of dissolved solids (TDS) gradually decreases from 695.10±11.11 g/l in the variant with 10% wastewater to 508.31±7.12 g/l in the variant with 90%. The same trend is observed for conductivity, which varies from 1391.50±10.11 µS/cm to 1017.20±8.21 µS/cm. The reduction of these values indicates a dilution of salts and mineral ions available in environments with higher proportions of wastewater. Dissolved carbon dioxide (CO_2) shows a gradual increase, the values being 15.01±1.11 mg/l for 10% wastewater and 28.01±1.14 mg/l for 90%. This accumulation of CO_2 can favor photosynthetic processes, but in high concentrations it can affect the chemical balance of the environment. Turbidity increases constantly, from 2.01±0.11 NTU in the 10% variant to 15.01±0.12 NTU in the 90% variant, which denotes an increased load of suspended solid particles.

Organic matter increases significantly with the increase in the proportion of wastewater, starting from 10.0±0.07 mg/l and reaching 75.01±0.04 mg/l. This increase reflects an additional supply of organic substances, which can constitute sources of nutrients for microalgae.

In conclusion, with the increase in the percentage of wastewater, the nutrient media for *Chlorella vulgaris* become richer in carbon dioxide and organic matter, more turbid, but poorer in dissolved mineral salts. The environment thus undergoes a transformation from slightly acidic to neutral and subsequently alkaline, which suggests increasingly favorable conditions for the growth of this microalgae species.

Tab. 3. Initial chemical composition of trace elements in nutrient media with the addition of wastewater from the recirculating system used to cultivate *Spirulina platensis* algae

Nutrient media with the addition of waste water	Cr, µg/l	Cu, µg/l	Pb, µg/l	Ni, µg/l
10%	1,50±0,02	11,07±0,50	0,3±0,01	2,93±0,03
30%	1,197±0,02	33,21±0,52	0,90±0,01	8,79±0,02
60%	0,743±0,03	66,42±0,50	1,80±0,02	17,58±0,03
90%	0,289±0,02	99,63±0,98	2,71±0,02	26,37±0,03

The analysis of trace elements in the nutrient media reveals distinct changes depending on the concentration of residual water. Chromium (Cr) records a gradual decrease with increasing

residual water addition, from 1.50±0.02 µg/l in the 10% variant to only 0.289±0.02 µg/l in the 90% variant. This reduction can be associated with dilution processes or with possible chemical interactions that limit Cr solubility under these conditions. Copper (Cu) shows a very obvious increase, starting from 11.07±0.50 µg/l (10%), increasing threefold to 33.21±0.52 µg/l (30%), then sixfold to 66.42±0.50 µg/l (60%), and reaching a maximum of 99.63±0.98 µg/l at the 90% addition. This shows a progressive accumulation of copper in wastewater environments. Lead (Pb) also has a constant increase: from 0.3±0.01 µg/l to 2.7 µg/l, which is approximately nine times more in the 90% variant. Nickel (Ni) follows a very pronounced upward trend, with values increasing from 2.93±0.03 µg/l (10%) to 26.37 µg/l (90%), which represents almost a 9-fold increase. In conclusion, the addition of wastewater leads to a significant increase in the concentrations of Cu, Pb and Ni, concomitantly with a reduction in the Cr content. This change can influence both the metabolism of the algae and the quality of the obtained biomass, being important for assessing the safety of using this biomass in fish nutrition.

Tab. 4. Initial chemical composition of trace elements in nutrient media with the addition of wastewater from the recirculating system used in the cultivation of *Chlorella vulgaris* algae

Nutrient media with the addition of waste water	Parameters analyzed				
	Cr, µg/l	Cu, µg/l	Pb, µg/l	Ni, µg/l	Mn, µg/l
10%	0,015±0,00	9,97±0,51	0,00±0,00	0,33±0,01	35,40±0,53
30%	0,045±0,00	29,91±1,01	0,00±0,00	0,98±0,01	106,20±1,62
60%	0,090±0,00	59,91±1,25	0,00±0,00	1,97±0,02	212,40±2,41
90%	0,120±0,00	89,62±2,01	0,00±0,00	2,95±0,01	318,50±3,52

The initial chemical composition of trace elements in nutrient media with the addition of wastewater, used to cultivate the algae *Chlorella vulgaris*, highlights a series of progressive changes depending on the concentration of wastewater introduced. The chromium (Cr) content is very low, but shows a constant increase from 0.015±0.00 µg/l in the variant with 10% wastewater to 0.120±0.00 µg/l in the variant with 90%. This evolution, although numerically small, reflects a gradual accumulation of chromium in the nutrient medium. The copper (Cu) concentration increases significantly, starting from 9.97±0.51 µg/l in the variant with 10% and reaching 89.62±2.01 µg/l in

the variant with 90%. This shows an almost nine-fold enrichment of the medium in copper with the increase in the addition of wastewater. Lead (Pb) was not detected in any nutrient medium variant, all values being 0.00±0.00 µg/l. This indicates the absence of this toxic element, which is an advantage for the quality of the culture media. Nickel (Ni) has a marked upward trend, increasing from 0.33±0.01 µg/l (10%) to 2.95±0.01 µg/l (90%). The values indicate an increase of almost nine times the initial concentration, which can influence both the metabolic processes of the algae and the toxicity of the medium at high levels. Manganese (Mn) is the trace element that registers the most pronounced increase. The values start from 35.40±0.53 µg/l in the variant with 10% waste water and increase progressively to 318.50±3.52 µg/l in the variant with 90%. This represents an increase of almost ten times, which suggests that manganese is brought in significant quantities with the addition of wastewater.

In conclusion, the nutrient media for *Chlorella vulgaris* gradually become richer in chromium, copper, nickel and manganese, while lead is absent. This evolution indicates an additional supply of essential or potentially toxic trace elements, which must be carefully monitored to evaluate both the effect on algae growth and the quality of the biomass obtained.

Carrying out these analyses allowed us to establish the initial composition of the nutrient media used for the cultivation of *Chlorella vulgaris* and *Spirulina platensis* algae, a fact particularly important for predicting biomass production and for evaluating the contribution of algae to the wastewater treatment process. Determining the effectiveness of the treatment process performed by the investigated species will be carried out in the next stage of the project, which will allow us to establish their impact on both the treatment and depollution of the analyzed wastewater.

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