

Utilization of wastewater from the recirculating aquaculture system to obtain *Spirulina Platensis* biomass for fish food

Sergiu DOBROJAN¹ , Mircea Nicușor NICOARĂ² , Galina DOBROJAN¹ ,
Gheorghe JIGĂU¹ , Victor MELNIC¹ , Gabriel-Ionut PLAVAN² 

¹ Moldova State University, Chisinau, Republic of Moldova

² Alexandru Ioan Cuza University of Iași, , Iași, Romania

*Correspondence: email: sergiudobrojan84@yahoo.com

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Abstract: The paper presents the experimental results obtained when cultivating the cyanobacterium *Spirulina platensis* on media with the addition of wastewater from the recirculating aquaculture system (RAS) in concentrations of 10%, 30%, 60%, 90% and 100%. Research has shown that the most optimal variant of cultivating cyanobacteria on RAS wastewater is the 10% one, which contributes to obtaining 1.041 ± 0.023 g/l of absolutely dry biomass in 11 days, to accelerating the reproduction rate (0.218 ± 0.002 days⁻¹) and the reproduction coefficient (1.652 ± 0.023 g/l). The pH values showed that, with the increase in the concentration of wastewater in the nutrient cultivation medium, their increase is observed, reaching up to 12.17 in the variant with 100% wastewater. The increase in the concentration of waste water in the nutrient medium for cyanobacteria cultivation causes morphological changes in the filaments, characterized by elongation. Biochemical analyses indicate that the highest amount of biologically active substances is recorded in the biomass cultivated on 10% RAS and that the biomass can be used in fish nutrition.

1. Introduction

The interest in identifying cheap nutrient media for algae/cyanobacteria cultivation for biomass production has been, is and will remain a constant concern of researchers in the field. A cheap source of nutrients, which in most cases generates environmental problems, is wastewater. Depending on their origin, they are loaded with N, P, K, Na, Ca, Mg, Fe, Mn, etc., elements that can be easily used by algae as nutrient sources, thus ensuring, on the one hand, the production of algal biomass, and on the other hand, the purification of these wastewaters. In this context, one of the most popular and important microalgae is *Spirulina platensis*, a species that has attracted the attention of researchers due to its high content of biologically active substances, such as proteins, carbohydrates, lipids, vitamins, chlorophyll and minerals. These compounds have beneficial properties, being exploited in applications for animals, humans and plants (Spinola et al., 2024; Podgorska-Kryszczuk, 2024; Mazo et al., 2007; Rudic et al., 2007). Recent studies have shown that *Spirulina* can be cultivated on various types of wastewater, including water from aquaculture, from zootechnical complexes for raising poultry, pigs, cattle and sheep. This microalga can also be cultivated in wastewater from the food and processing industries, such as those from olive grinding to obtain olive oil, distilleries, confectionery, tapioca processing, rubber industry, paper mills or from activities involving the use of dyes, etc. (Lee et al., 2020; Cardoso et al., 2020; Lu et al., 2020; Markouet al., 2021; Kulkarni et al., 2016; El-Kassas et al., 2015; Hadiyanto et al., 2019; Hadiyanto, 2018; Asthary et al., 2019; Ismail et al., 2020).

In this sense, domestic wastewater is considered a resource rather than a waste, as it contains nutrients and fertilizers, such as N, P, K, B, Cu, Zn, Mo, Fe, Co and Mn, necessary for the growth of microalgae. Cultivation of *Spirulina* on these waters allows both the production of algal biomass and its efficient purification, thus contributing to the sustainable use of resources and the reduction of environmental impact (McCarty et al., 2011; Rodushkin & Odman, 2001).

Research conducted by Chang Y. and his co-authors highlighted that the use of human urine as a nutrient medium for the cultivation of *Spirulina platensis* led to a 97% reduction in ammoniacal nitrogen (N-NH_4), 96.5% in total phosphorus and 85–98% in urea. Also, the biomass obtained registered an exponential increase, with the obtaining of significant amounts of proteins, representing approximately 68.1% of the algal biomass (Changa et al., 2013).

Cultivation of *Spirulina platensis* on wastewater from breweries, with the addition of sodium bicarbonate and sodium chloride, has also become an attractive option. Experiments have demonstrated that this type of cultivation led, first of all, to the efficient removal of nutrients from the water (over 90%, 70.5%, 28%, 55% and 74% for d-COD, N-NO_3^- , P-PO_4^{3-} , N-NH_4^+ and, respectively, TKN), as well as to the accumulation of a significant amount of algal biomass (approximately 950 mg/L), with a high content of carbohydrates and lipids (33.66% and, respectively, 18.64%) (Papadopoulos et al., 2022).

Our previous research has shown that *Spirulina platensis* can be cultivated on nutrient media with the addition of wastewater from poultry, pig and cattle complexes. As a result, a significant increase in algal biomass was observed, with maximum values ranging from 0.95 to 3.77 g/L, as well as the accumulation of significant amounts of biologically active substances, namely proteins (41.74–75.73%), peptides (10.56–19.06%), lipids (4.4–6.41%), carbohydrates (5.33–9.88%), allophycocyanin (6.26–8.47%) and phycocyanin (12.61–16.81%). In addition, the obtained algal biomass generated positive effects when applied in the nutrition of broiler chickens, in the treatment of plant seeds, as well as an adjuvant in the treatment of type 2 diabetes. (Dobrojan, 2011; Dobrojan, 2010; Dobrojan, 2010; Bacalov et al., 2023)

Other research aimed at cultivating *Spirulina platensis* algae on wastewater generated by pig farming, which allowed obtaining a quantity of 0.56 g/L of algal biomass. It presented a protein content of 43.89%, lipids of 9.4%, chlorophyll of 6.41 mg/g and total sugars of 4.18%. At the same time, algae cultivation on these wastewaters led to the significant elimination of the main pollutants from wastewater, namely N, P, COD, Zn and Cu, in proportions of 76%, 72%, 93.1%, 93.5% and, respectively, 82.5% (Liang et al., 2023).

The research conducted indicates the premise of using wastewater as a nutrient medium for cultivating *Spirulina platensis* algae. It is important to mention that the Iasi Research and Development Station for Aquaculture and Aquatic Ecology, of the "Al. I. Cuza" University of Iasi, uses, only for Module 1 of larval and fish growth, a volume of at least 106.76 m³ of water, used in a recirculating system, which represents an important source of nutrients for the cultivation of *Spirulina platensis*. In this context, we proposed to cultivate *Spirulina platensis* algae on wastewater from the recirculating aquaculture system (RAS) of the station.

2. Materials and Methods

Algae strain used

The experiments used the *Spirulina platensis* (Nordst.) Geitl. CAUSM. 26 algae strain, provided by the Microalgae Collection of the Scientific Research Laboratory "Algology V. Salaru", Scientific Research Center for Integrated Natural Sciences, at the Moldova State University.

Experimental conditions

The experiments were carried out under laboratory conditions, at a temperature of 21–25 °C, with natural lighting and without shaking the samples. Cultivation was carried out in Erlenmeyer flasks with a volume of 100 mL, in which the nutrient medium with the algal inoculum constituted 100 mL.

Experimental design

The algae were grown on standard nutrient medium (Zarrouk), used as a control, as well as on media supplemented with wastewater, collected directly from the outlet of the RAS system of the fish farming farm at the Research and Development Station for Aquaculture and Aquatic Ecology Iasi, of the "Al. I. Cuza" University of Iasi, in concentrations of 10%, 30%, 60%, 90% and 100%, as follows:

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

Parameters analyzed

Water quality parameters

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" device. The concentration of dissolved CO₂ by algae was determined by the titrimetric method, using titration with 0.05 N hydrochloric acid (World Health Organization, 2011), and the absorbed CO₂ was established according to the formula Daily growth of algae biomass (g/l BAU)*2.05.

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 method, using the "UV-Vis T80+" spectrophotometer and with the help of high-resolution atomic absorption spectrometry with a graphite furnace electrothermal atomizer and an Argon 5.0 high-purity ion carrier, employing a ContrAA 600 spectrometer (Varghese et al., 1988; Duca et al., 1997; Grigheli et al., 2006; Speiser et al., 2006).

Algae growth indicators

To determine the biomass of *Spirulina platensis* algae, the spectrophotometric method was used, which involves exposing the algal suspension in a cuvette and measuring the optical density at a wavelength of 750 nm. For the quantitative determination of biomass, the optical density values were multiplied by the corresponding coefficient, calculated based on the calibration curve previously constructed for each culture, and the results obtained were expressed in absolute dry biomass (BAU) (Rudic et al., 2002).

The reproduction rate (μ) of the algae was determined according to the formula established by K. M. Hhailov (Hhailov, 1971) using the formula: $\mu = \log 2N_t - \log 2N_0 / t$

Where:

μ - reproduction rate;

N_t – biomass values at the final analyzed time;

N_0 – biomass values at the initial analyzed time.

The algae reproduction coefficient (Cr) was established according to the formula proposed by M. G. Vladimirov and V.E. Semenenko (Vladimirov & Semenenko, 1962).

using the formula: $Cr = (B_f / B_i)$

Where:

Cr- reproduction coefficient;

B_f – biomass values specific to the analyzed period;

B_i - initial biomass values.

Biochemical composition

The protein content was determined spectrophotometrically by the Lowry method (1910), carbohydrates were quantified by applying the Filipovici method (1975), using the anthrone reagent, and lipids were determined according to spectrophotometric methods described in the literature (Rudic et al., 2002).

Algal morphology

The morphological parameters (length and width) of *Spirulina platensis* filaments were determined according to specific methods, using the "Optica B-350" microscope, equipped with a micrometer eyepiece (Minicheva et al., 2003).

3. Results

For the cultivation of algae, as well as other aquatic plant organisms, it is imperative to ensure the adequate supply of nutrients. Nutrients constitute the material and vital basis of the existence of algae, being indispensable for the metabolic processes carried out at the cellular level and for the formation of the structural components of the cell.

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

Residual water addition	Parameters analyzed									
	pH	T °C	O ₂ , mg/l	TDS, g/l	Dissolved CO ₂ , mg/l	Turbidity, NTU	Alkalinity, mg/l	Organic matter, mg/l	Cu (µg/l)	Mn (µg/l)
10%	9,50	21,0	7,13	4,41	3	2	197,7	2	11,07	8,01
30%	9,40	21,0	7,38	4,10	9	6	185,1	6	33,21	24,03
60%	9,0	21,0	7,76	3,50	18	12	161,8	12	66,42	48,06
90%	8,60	21,0	8,19	3,15	27	18	138,7	18	99,63	72,09
100%	8,40	21,0	8,26	0,48	30	20	123,0	20	110,7	80,01

It is known that the wastewater from the RAS contains biogenic elements, microelements and other substances that can be exploited by using them as a nutrient medium for algae. However, the direct use of these waters in the cultivation of algae necessarily requires the preliminary determination of the concentrations of the main water quality parameters (tab. 1).

Table 2. Results of monitoring the biomass of *Spirulina platensis* algae cultivated on wastewater from the recirculating system, g/l BAU

Analysis days	Concentrations experienced					
	10%	30%	60%	90%	100%	Control
1	0,225±0,006	0,225±0,006	0,225±0,006	0,225±0,006	0,225±0,006	0,225±0,006
2	0,343±0,013	0,367±0,048	0,288±0,008	0,243±0,002	0,250±0,010	0,300±0,006
3	0,431±0,005	0,442±0,010	0,383±0,016	0,208±0,013	0,208±0,234	0,398±0,014
4	0,514±0,010	0,523±0,004	0,435±0,009	0,276±0,014	0,277±0,010	0,499±0,008
5	0,587±0,005	0,594±0,006	0,542±0,013	0,348±0,082	0,345±0,026	0,566±0,006
6	0,970±0,024	0,899±0,023	0,781±0,041	0,440±0,008	0,556±0,031	0,793±0,002
7	0,970±0,023	0,899±0,023	0,662±0,023	0,447±0,004	0,438±0,065	0,899±0,023
8	1,018±0,024	0,899±0,047	0,734±0,023	0,497±0,008	0,431±0,068	0,932±0,071

9	1,065±0,041	0,946±0,062	0,757±0,047	0,478±0,012	0,442±0,080	0,970±0,047
10	1,041±0,023	0,994±0,041	0,852±0,041	0,551±0,010	0,639±0,108	0,973±0,063
11	1,041±0,023	0,899±0,062	0,876±0,023	0,473±0,085	0,509±0,059	1,089±0,024

*- BAU – absolutely dry biomass

Biomass monitoring was carried out over 11 days, a period in which the “exponential growth phase” of *Spirulina platensis* was manifested. Starting with the 12th day, the “growth stabilization phase” is observed, and subsequently, starting with the 15th day, the initiation of the “decline phase” of the culture is observed. It is important to analyze these growth parameters especially during the “lag” and “exponential” phases, because during this period the culture registers the highest rate of cell multiplication, which ensures the obtaining of biomass. In the algae cultivation process, an essential indicator of the efficiency of the culture is the rapid accumulation of a significant biomass in a short period of time.

As we can see from the data presented in (table 2) on the first day, the initial biomass was identical for all variants, 0.225±0.006 g/l, which reflects the uniformity of the initial inoculation conditions. During days 2–5, a progressive increase in biomass is observed, the most pronounced being in the variants with 10% and 30% residual water. On day 2, the values vary between 0.243±0.002 g/l (90%) and 0.367±0.048 g/l (30%), the control presenting 0.300±0.006 g/l. On day 5, the biomass reaches 0.587±0.005 g/l for 10%, 0.594±0.006 g/l for 30%, 0.542±0.013 g/l for 60%, 0.348±0.082 g/l for 90%, 0.345±0.026 g/l for 100%, and 0.566±0.006 g/l for the control. This indicates that lower concentrations of wastewater (10–30%) favor biomass accumulation at a faster rate compared to high concentrations (90–100%). On days 6–11, distinct trends are observed: the variant with 10% wastewater reaches a maximum biomass of 1.065±0.041 g/l on day 9, maintaining high values until the end of the period. The 30% variant reaches 0.994±0.041 g/l on day 10, and 60% reaches 0.876±0.023 g/l on day 11. In contrast, the 90% and 100% concentrations show a lower accumulation and a slower growth, with maximum values of 0.551±0.010 g/l and 0.639±0.108 g/l, respectively, at the end of the monitored period. The control shows a constant evolution, reaching 1.089±0.024 g/l on day 11. The most representative results of the biomass of *Spirulina platensis* algae cultivated on media with the addition of RAS, obtained at the end of the exponential phase, were as follows: 10% - 1.041±0.023 g/l BAU; 30% - 0.899±0.062 g/l BAU, 60% - 0.876±0.023 g/l BAU, 90% - 0.473±0.085 g/l BAU; 100% - 0.509±0.059 g/l BAU (tab. 2).

Table 3. Monitoring of growth indicators of *Spirulina platensis* algae cultivated on wastewater from the recirculating system

Analyzed period, days	Concentrations experienced											
	10%		30%		60%		90%		100%		Control	
	μ, zile ⁻¹	Cr, g/l BAU	μ, zile ⁻¹	Cr, g/l BAU	μ, zile ⁻¹	Cr, g/l BAU	μ, zile ⁻¹	Cr, g/l BAU	μ, zile ⁻¹	Cr, g/l BAU	μ, zile ⁻¹	Cr, g/l BAU
2	0,183	1,524	0,212	1,631	0,107	1,28	0,033	1,080	0,046	1,111	0,125	1,333
3	0,099	1,256	0,081	1,204	0,124	1,330	0,068	0,856	0,080	0,832	0,123	1,327
4	0,076	1,192	0,073	1,183	0,055	1,136	0,123	1,327	0,124	1,332	0,098	1,254
5	0,058	1,142	0,055	1,136	0,096	1,246	0,101	1,261	0,095	1,245	0,055	1,134
6	0,218	1,652	0,180	1,513	0,159	1,607	0,102	1,264	0,207	1,611	0,146	1,401
7	0,000	1,00	0,000	1,00	0,072	0,848	0,007	1,016	0,104	0,788	0,054	1,134
8	0,021	1,049	0,000	1,00	0,045	1,109	0,046	1,112	0,007	0,984	0,016	1,037

9	0,020	1,046	0,022	1,052	0,013	1,031	0,017	0,962	0,011	1,025	0,017	1,041
10	0,010	0,977	0,021	1,051	0,051	1,125	0,062	1,153	0,160	1,446	0,001	1,003
11	0,000	1,000	0,044	0,904	0,012	1,028	0,066	0,858	0,099	0,796	0,049	1,119

* Note - The calculation was made based on biomass expressed in g/l BAU; μ - algae reproduction rate; Cr - algae reproduction coefficient.

To select the optimal nutrient media for cultivating *Spirulina platensis* on the investigated wastewater, the algae growth process was analyzed by daily analysis of the reproduction coefficient and the reproduction rate. The reproduction coefficient results attested when cultivating *Spirulina platensis* on media with the addition of wastewater ranged within the limits: 10% - 0.977- 1.524 g/l BAU; 30% - 0.904-1.631 g/l BAU; 60% - 0.848-1.330 g/l BAU; 90% - 0.856-1.327 g/l BAU; 100% -0.796-1.611 and control - 1.003-1.333 g/l BAU (tab. 3). The results of this indicator reflect the fact that for the cultivation of *Spirulina platensis* algae the highest values of this indicator were attested in the nutrient medium with the addition of 10% and 30% residual water, which suggests favorable conditions for the multiplication of algal cells. The values of the algae growth rate attest major results in the sample with the addition of 10% and 30% residual water in the cultivation of *Spirulina platensis* algae, which confirms favorable conditions for the active multiplication of cells.

Table 4. Results of monitoring CO₂ assimilated by *Spirulina platensis* algae cultivated on wastewater from the recirculating system, g/l

Analysis days	Concentrations experienced					
	10%	30%	60%	90%	100%	Control
1	0,242	0,291	0,129	0,037	0,051	0,154
2	0,180	0,154	0,195	0,072	-0,086	0,201
3	0,170	0,166	0,107	0,139	0,141	0,207
4	0,150	0,145	0,219	0,148	0,139	0,137
5	0,785	0,625	0,490	0,189	0,432	0,465
6	0,00	0,00	-0,244	0,014	-0,242	0,217
7	0,098	0,0	0,148	0,103	-0,014	0,077
8	0,096	0,096	0,047	0,04	0,023	0,078
9	-0,050	0,098	0,195	0,150	0,404	0,006
10	0	-0,195	0,049	-0,160	-0,260	0,238
TOTAL	1,671	1,380	1,335	0,732	0,588	1,780

The amount of CO₂ absorbed by algae is an important indicator of their growth, as it reflects how active cellular photosynthesis is and how intensively the algae use the nutrients in the water, thus identifying the suitable nutrient medium for algae cultivation and the growth rate of algal biomass. The values of this indicator fluctuated from one day to the next, being specific to the photosynthesis and respiration processes of *Spirulina platensis*. As the culture evolves, the absorption of CO₂ gradually decreases. The total quantitative analysis of carbon dioxide assimilated by *Spirulina platensis*, cultivated on nutrient media with the addition of wastewater from the RAS system, indicates the following values: 10% – 1.671 g/L; 30% – 1.380 g/L; 60% – 1.335 g/L; 90% – 0.732 g/L; 100% – 0.588 g/L; control – 1.780 g/L (Table 4). CO₂ assimilation is maximum in the nutrient medium with 10% residual water, which indicates that this medium is optimal for growing the algae.

The results shown in (figure 1) show that on day 1, the pH values are relatively uniform between the variants, oscillating between 9.21 (10%) and 9.67 (100%), with the

control located at 9.22. These high values from the beginning show the specific alkaline environment necessary for the development of *Spirulina*. In the following days, the pH registers a progressive increase in all variants, but the amplitude of the changes differs depending on the concentration of the residual water.

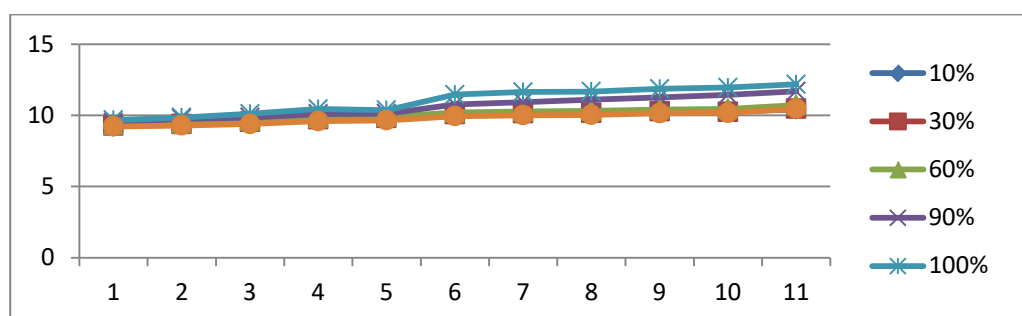


Figure 1. Changes in pH values of nutrient media with the addition of wastewater used in the cultivation of *Spirulina platensis* algae

At low and moderate concentrations (10%, 30% and 60%), the increase is slow and constant, the pH reaching at the end of the analysis period 10.42 (10%), 10.46 (30%) and 10.73 (60%). These values are maintained in the range favorable for the development of *Spirulina*, without indicating major risks of inhibition. For high concentrations (90% and 100%), pH values increase significantly, reaching very high levels on day 11- 11.68 (90%) and 12.17 (100%). This pronounced alkalization of the environment is most likely the consequence of population stress caused by wastewater. The control variant shows a slow and stable increase in pH, from 9.22 to 10.45 during the 11 days, which confirms that wastewater additions accelerate changes in the nutrient environment. The data obtained show that variants with moderate wastewater additions (10–60%) provide stable and adequate conditions for the cultivation of *Spirulina*, while high concentrations (90–100%) lead to excessive alkalization, which can limit growth and reproduction in the long term.

During the experiments, the morphological and dimensional parameters of *Spirulina platensis* (filament length and width) were evaluated, which provide important information regarding the morpho-physiological state of the culture. The results of these parameters, obtained on the 11th day of cultivation, are presented in (Tab. 5).

Table 5. Morphological aspects of *Spirulina platensis* cultivated on wastewater from the RSA system, μ

Morphological parameters analyzed	Experimental nutrient media					
	10%	30%	60%	90%	100%	Control
Filament length	520,00±51,00	574,00±35,00	458,00±41,21	597,00±56,00	630,00±60,00	490,20±48,90
Filament width	5,69±0,37	6,46±0,50	5,46±0,22	6,00±0,52	5,42±0,42	5,47±0,42

The presented results indicate that the filaments of *Spirulina platensis* react dimensionally to the increase in the concentration of nutrient media. Thus, it was found that with the increase in the concentration of wastewater administered in the nutrient medium, the length of the filaments increases: when cultivated on medium with 10% addition of wastewater, the filaments reached $520.00 \pm 51.00 \mu\text{m}$, while in the variant of cultivation on concentrated waters, the values of this indicator reached $630.00 \pm 60.00 \mu\text{m}$. The increase in the length of the filaments of the algae represents its response to the conditions of insufficient nutrient salts and their unbalanced presence in the medium,

being characteristic of the species. In the variants with 10% wastewater, no morphological abnormalities of the filaments and algal cells were found, the same being true for the variants with 30%, except that at this concentration the filaments tended to settle on the bottom of the cultivation vessel. When cultivated on media with 60–100% wastewater, the culture acquired a light green color, and with increasing wastewater concentration, the light green shades intensified; the filaments settled at the bottom of the cultivation vessel and stuck together.

At the same time, when cultivating the algae on media with the addition of wastewater in high concentrations, starting from the 3rd day, the culture was infected with green algae and some invertebrates. The width of the cells does not undergo significant changes depending on the concentration of wastewater administered, the changes not even reaching 1 μm . This indicates the tendency of the culture to preserve the width of the filaments and the strength of the cell wall. Overall, the results suggest that nutrient media with high concentrations favor filament elongation, without producing significant changes in their width, and that they are not favorable for the cultivation of *Spirulina platensis*.

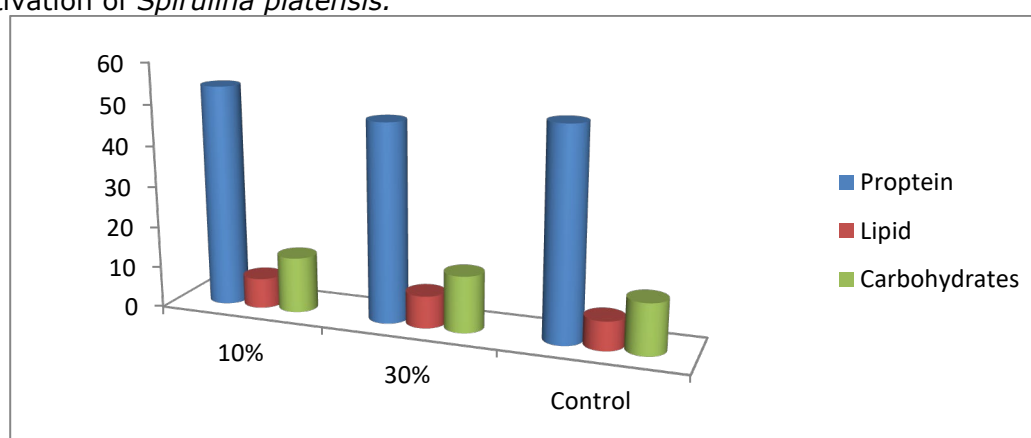


Figure 2. General biochemical composition of *Spirulina platensis* biomass cultivated on media with the addition of wastewater from the RAS system, %

In order to determine the nutritional potential of *Spirulina platensis* cultivated on wastewater from the RAS system, a general biochemical analysis of the algal biomass, cultivated on media with optimal concentrations, was performed, the data being presented in (Figure 2).

The results presented indicate that the cultivation of *Spirulina platensis* on media with the addition of wastewater from the RAS system leads to the accumulation of significant amounts of biologically active substances in the algal biomass. For example, in the medium with the addition of 10% RAS, the total protein content is slightly higher than in the case of cultivation on Zarrouk medium, which emphasizes its importance as a feed for fish nutrition. Cultivation of the alga on media with the addition of wastewater from RAS led to an insignificant increase in the lipid content, the highest amounts being recorded in the variant with 30% wastewater from RAS, which was 0.8% more than in the biomass cultivated on Zarrouk medium. At the same time, cultivation on these media determined the increase in the carbohydrate content in the algal biomass, which considerably increases the interest for its application in the nutrition of aquaculture fish. Overall, the data demonstrate that the cultivation of *Spirulina platensis* on nutrient media with optimal addition of wastewater from RAS influences the biochemical composition of algal biomass, favoring the accumulation of proteins, lipids and carbohydrates and creating the prospect of its application as a nutritional supplement in fish feed.

4. Discussion

There is a lot of research that indicates that *Spirulina platensis* can be successfully cultivated on wastewater from fish farming. Investigations conducted by Sousa Nogueira S. M. followed the cultivation of *Spirulina platensis* on wastewater generated by the rearing of Nile Tilapia (*Oreochromis niloticus*) fry, with the addition of seawater until reaching a salinity of 10‰. As a result, it was found that the maximum cell density of *S. platensis* was 0.22 g/L of dry biomass, with a maximum productivity of 0.03 g/L/day. At the same time, a reduction of over 94.8% in the concentrations of ammonia, nitrites, nitrates and phosphates was observed, suggesting that cultivating the algae on these wastewaters has the effect not only of purifying them, but also of obtaining algal biomass with potential for use in animal nutrition and as a food supplement for humans (Sousa Nogueira et al., 2018).

Other research has followed the cultivation of *Spirulina* sp. LEB 18 on media with wastewater from fish farming, with the addition of 25% nutrients from the Zazzouk medium. As a result, it was found that a significant amount of biomass (3.33 g/L) and biologically active substances were obtained, namely carbohydrates – 69.77%, lipids – 12.77% and carotenoids – 9,68 µg/mL. At the same time, it was observed that *Spirulina* contributed to the elimination of phosphorus from wastewater (99.97%), COD (89.34%) and nitrates (81.10%), as well as the elimination of heavy metals, while a slight increase in the concentration of Al, Fe, Ca and Mg was recorded. These results demonstrate that *Spirulina* can be applied both as a bioremediation agent for aquaculture wastewater and for obtaining biomass rich in biologically active substances (Cardoso et al., 2020).

Another study followed the cultivation of *Spirulina* sp. on wastewater generated by fish farming, the species *Cromileptes altivelis*. Research has shown that the algae can grow efficiently on nutrient media with the addition of 25% wastewater, and as a result, a significant reduction of nitrates and phosphorus was found by up to 89.78% and 84.34%, respectively, suggesting that this can contribute to maintaining water quality according to fish farming standards (Hudaïdah et al., 2022).

Our results also indicate that *Spirulina platensis* can be efficiently cultivated on Zarrouk nutrient medium, supplemented with 10% wastewater from the RAS system, which leads to the accumulation of an algal biomass with a favorable content of biologically active substances (proteins, lipids and carbohydrates), without the occurrence of morphological and dimensional anomalies.

5. Conclusions

As a result of the research carried out, it was found that *Spirulina platensis* algae can be cultivated on nutrient media with the addition of wastewater from the RAS of the Research and Development Station for Aquaculture and Aquatic Ecology Iași, of the "Al. I. Cuza" University of Iași. The results obtained demonstrate that the use of RAS wastewater in reduced concentration represents a favorable condition for the efficient cultivation of the algae, ensuring an optimal balance between biomass growth, reproduction dynamics and stability of physiological parameters. Higher concentrations of wastewater determine a sharp increase in pH, reduced CO₂ absorption and morphological changes of the filaments, without providing additional advantages in terms of biomass accumulation or biologically active substances. The biomass obtained under conditions of 10% RAS is distinguished by a higher biochemical content, which supports its potential for valorization in fish nutrition and confirms the feasibility of integrating wastewater into sustainable biotechnological systems.

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Author contributions:

Sergiu Dobrojan, Galina Dobrojan, Victor Melnic, and Gheorghe Jigau contributed to the algal cultivation experiments, determination of algal productivity, growth indices, and pH of the nutrient media, algae morphology and CO₂ assimilation. Gabriel-Ionut Plava and Mircea Nicușor NICOARĂ contributed to the determination of the biochemical composition of the algal biomass and the chemical composition of nutrient media for algae cultivation.

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