3.11. MICROALGAL BIOMASS PRODUCTION DURING THE PURIFICATION OF THERMOMINERAL WATER

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3.11.1. INTRODUCTION

The south-eastern part of Pannonian plane, which belongs to Serbia, can be treated as relatively perspective resource for obtaining and usage of geothermal and thermomineral water (Milosavljeviæ et al, 1995).

During the thermomineral water treatment with microalgae, high quantity of algal biomass and the mineral salts concentration decrease would be provided in both natural and economic way, since the specific microalgae species can utilize most of the minerals during their growth. Special attention should be given to the cyanobacteria — *Spirulina platensis*, because of its significantly easier cultivation and higher biomass quality comparing with other microorganisms or higher plants (Ciferri, 1983; Ciferri and Tiboni, 1985; Richmond, 1986). Green algae *Scenedesmus* and *Dunaliella* are also actual worldwide producers of biomass in biotechnology (Soong, 1980).

In order to racionalize the thermomineral waters exploatation, the main aim of this research was to examine possible cyanobacteria (*Spirulina platensis*) and green algae (*Scenedesmus quadricauda* and *Dunaliella* sp.) usage as both biomass producers and thermomineral waters purificators. Biomass could be considered as an alternative high calorie food and source of different industrialy importamt compounds. At the same time, high concentration of the minerals, as limithing factor in of thermomineral waters usage, would be reduced during the microalgal cultivation.

3.11.2. MATERIAL AND METHODS

Microalgal strains (*Spirulina platensis, Scenedesmus quadricauda* and *Dunaliella* sp.) are part of the Microbiology laboratory collection (Institute of Biology, University of Novi Sad). The analyses of the total samples mineralization have been made after 3, 5, 7, 9 and 14 days during the thermomineral water purification (demineralization) by microalga *Spirulina platensis*.

Cultivation of microalgae in diluted and undiluted thermomineral water (20%, 40%, 60%, 80%, 100%) and in undiluted water (100%) enriched with 5% liquid swine manure lasted for 15 days. Liquid pig manure was added in order to provide higher nitrogen and phosphorus concentrations. Cultures incubation occurred in Erlenmeyer flasks, at the temperature of 30^0C, with fluorescent light intensity of 850 LUX.

Spirulina platensis biomass was performed indirectly by chlorophyll a concentration analysis (Mackinney, 1941). The biomass concentrations of *Scenedesmus quadricauda* and *Dunaliella* sp. were measured by direct absorbency analyses on spectrophotometer. All results are the average values of four repeated measurements.

3.11.3. RESULTS AND DISCUSSION

Cultivation of microalgal strains has been done in water samples taken from three geothermal drill-holes, in order to examine possible exploitation of Vojvodinian thermomineral waters. According to our results, examined microalgal strains showed specific growth in diluted thermomineral water, as in undiluted water samples enriched with 5% liquid pig manure. However, the growth was very characteristic for different microalgal species applied.

The increase of *Spirulina platensis* biomass (μg chl a/ml) was directly correlated to increase of thermomineral water concentration (Table 1).

Table 1. The increase of *Spirulina platensis* biomass grown in different dilutions of thermomineral water comparing with the control medium

THERMOMINERAL WATER (%)	Pb-1/H	Mk-1/H	Sr-1/H
20%	1.105	1.298	1.162
40%	1.421	2.055	1.374
60%	1.484	2.911	2.621
80%	2.210	3.476	2.637
100%	2.386	3.941	**4.020**
100% + 5% pig manure	**3.473**	**4.357**	2.116
CONTROL (SOT)	3.156	4.124	3.500

Spirulina biomass was extremely high in the water samples taken from Pb-1/H and Mk-1/H (+ 5% liquid swine manure). It has been shown that cultivation of *Spirulina platensis* should be carried out in undiluted thermomineral water. Comparing the biomass quantity in control mineral nutritive medium (SOT), water samples from Sr-1/H demonstrated even better growing conditions for Spirulina cultivation (Table 1). Water samples from Mk-1/H and Pb-1/H showed to be less corresponding media, but enriched by swine manure as additive substance, the deficiency of important elements would be provided.

Green alga *Scenedesmus quadricauda* manifested very good biomass increase in water samples diluted up to 80% (Sr-1/H and Pb-1/H) and in all samples taken from Mk-1/H, with exception of the samples enriched with swine manure (Table 2).

Table 2. *Scenedesmus quadricauda* growth in different dilutions of thermomineral water comparing with the control medium

THERMOMINERAL WATER (%)	Pb-1/H	Mk-1/H	Sr-1/H
20%	0.511	0.345	0.418
40%	0.714	0.719	0.694
60%	**1.149**	1.362	1.217
80%	0.538	1.567	**1.414**
100%	0.406	**1.722**	1.109
100% + 5% pig manure	0.321	1.595	0.823
CONTROL („148")	1.760	1.983	1.814

Comparing these results with the growth of control culture in „148" mineral medium, the water sample Mk-1/H made this green alga cultivation the most efficacious.

In the case of *Dunaliella* sp., all thermomineral water samples inhibited green alga growth (Table 3).

Table 3. *Dunaliella sp.* growth in different dilutions of thermomineral water comparing with the control medium

THERMOMINERAL WATER (%)	Pb-1/H	Mk-1/H	Sr-1/H
20%	0.314	0.294	0.318
40%	**0.389**	0.349	0.308
60%	0.286	0.364	0.339
80%	0.311	0.401	**0.618**
100%	0.305	0.411	0.406
100% + 5% pig manure	0.319	**0.447**	0.433
CONTROL („148")	2.680	2.735	2.842

Generally speaking, *Dunaliella* sp. biomass quantity (μg chl a/ml) increases when undiluted water is enriched with pig manure. Much lower quantities of biomass has been produced in all water samples comparing to control („148" medium).

The aims of microalgal cultivation in thermomineral waters were both biomass production of microalgae and thermomineral waters purification in order to decrease minerals concentrations, which could cause the high level of eutrofication in water recipients. Accordingly, *Spirulina platensis* cultivation in thermomineral water samples has been followed by significant reduction of total minerals. The biomass increase after the seventh day of cultivation has been demonstrated during the *Spirulina* cultivation in Pb-1/H and Sr-1/H undiluted water samples (Figs 1 and 2).

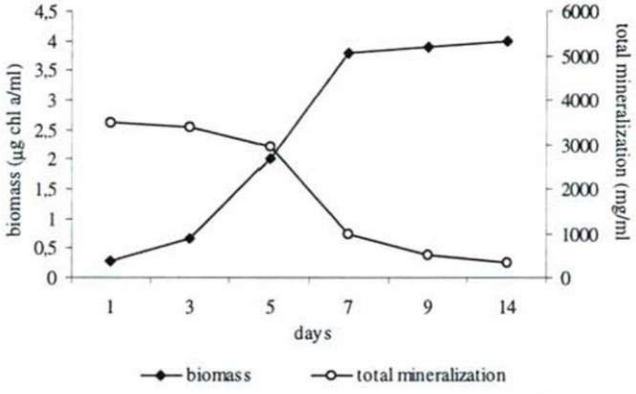

Fig. 1. Comparison of *Spirulina platensis* biomass increase and total mineralization decrease in Sr-1/H water sample.

Until the fourteenth day, water demineralization lasted unperceptibly. *Spirulina platensis* biomass growth stagnated in Mk-1/H water sample, as well as the minerals concentration until seventh day. Sudden minerals decrease and biomass production have been measured after the seventh day (Fig. 3), which can be connected with eventual bacterial occurrence in algal culture.

Even very encouraging results about Vojvodinian thermomineral waters exploitation in order to produce Spirulina biomass were published ten years ago in Yugoslavia (Obreht, 1988), no practical use of experimental experiences exists until today. Vietnam is the only

country with commercial production of *Spirulina platensis* in thermomineral waters (Nguen hun Thuoc et al, 1989) and the chemical composition of those waters is quite similar to the Vojvodinian. The cultivation of *Spirulina platensis* in thermomineral water could be followed by incredible benefits (Ciferri and Tiboni, 1985; Ciferri, 1983): it can be used in purification of thermomineral water, there are no inapplicable parts during the biomass production process (as like higher plants), the growth is extremely fast (in wet or dry climate), *Spirulina* is not competitive with other microorganisms, *Spirulina* is source of some chemical compounds in medicine and industry (great percentage of proteins and vitamins) (Richmond, 1986).

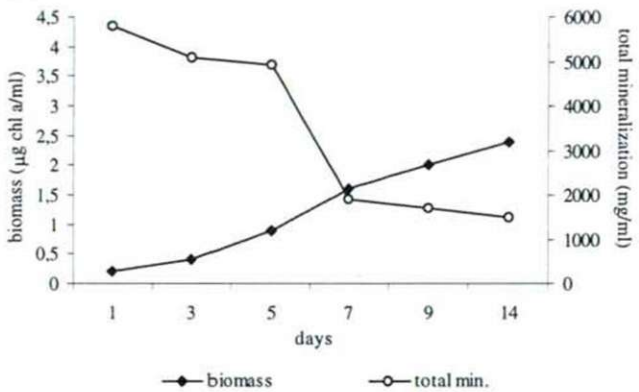

Fig. 2. Comparison of *Spirulina platensis* biomass increase and total mineralization decrease in Pb-1/H water sample.

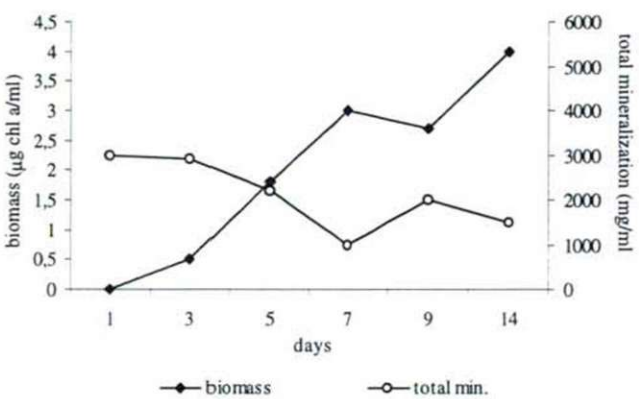

Fig. 3. Comparison of *Spirulina platensis* biomass increase and total mineralization decrease in Mk-1/H water sample.

According to our results, *Spirulina platensis* demonstrated significant reduction of total minerals and the biomass increase after the seventh day of cultivation, which can be considered as indication of future *Spirulina* usage in thermomineral waters purification.

3.11.4. SUMMARY

The growth of the microalgal species *Spirulina platensis, Scenedesmus quadricauda* and *Dunalliela* sp., cultivated in water from three Vojvodinian geothermal drill-holes, were examined during 15 days. Microalgae were grown in thermomineral water, in different dilutions (0%, 20%, 40%, 60%, 80%), as well as in the thermomineral water enriched with 5% liquid swine manure. However, the growth was characterized by different biomass production for every microalgal species: *Spirulina platensis* showed very significant biomass increase in all treatment, specially in one with 5% liquid pig manure. Green alga *Scenedesmus* quadricauda grew only in diluted water samples (<80%). The growth of *Dunalliela* sp. was inhibited during the cultivation in thermomineral water. In the case of *Spirulina platensis*, the effect of microalgal growth on the thermomineral water demineralization was noticed after 7 days of cultivation.

3.11.5. REFERENCES

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