Influence of salinity on the growth and development of *Spirodela polyrrhiza* biomass and the possibility of using it for the production

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INTRODUCTION

The multidirectional possibilities of using *Lemnaceae* plants, among others, in the production of biofuels, phytoremediation, bioindication and rapid growth have aroused very significant interest in this group of plants in recent years. The aim of the study was to assess the effect of salt stress on the growth and development of *Spirodela polyrrhiza*. Plants were grown on a diversified culture medium including: standard "Z" medium, tap water, 1% digestate from biogas plant in Piaszczyna, which were supplemented with various NaCl concentrations (25 to 100mM). The plants were cultivated under phytotron conditions at 24°C. After 10 days of culture, plant growth, fresh and dry biomass, as well as physicochemical parameters, i.e. chlorophyll content index, gas exchange parameters (net photosynthesis, transpiration, stomata conductivity and intercellular concentration of CO_2), measurement of chlorophyll fluorescence were analysed. On the last day of the experiment, the percentage of starch was determined in *Spirodela* shoot segments using the Starch Assay Kit from Sigma-Aldrich, product Code STA20. The analysis of individual Lemnaceae plant growth and development parameters in the experiment indicates new possibilities of using this group of plants in biotechnology and confirms that macrophytes can be successfully used in the production of biofuels - bioethanol.

MATERIAL & METHODS

The experiment was conducted under laboratory conditions on the model water plant *Spirodela polyrrhiza*, derived from the collection of *in vitro* cultures of the Department of Plant Ecophysiology of the Faculty of Bioscience of the University of Lodz. They were cultured in the presence of different concentrations of NaCl and in control variants. Morphological observation was carried out daily throughout the experiment. There were three control variants (I) standard "Z" liquid medium (pH 6.4), (II) tap water (pH 7.0) and (III) tap water supplemented with 1% post-fermentation effluent from a biogas plant in Piaszczyna (pH 8.1). Medium “Z” is a standard medium for growing in vitro culture plants containing in all necessary micro and macro elements. Plants were cultivated was for 10 days in a phytotron room at 24°C, under constant lighting with PHILIPS MASTER TL-D lamps with a power of 2x18W / 840. The pH of the liquid medium "Z" was determined using a SevenCompact™ S210 pH meter. *Spirodela* sp. macrophytes were grown in 250 ml Erlenmayer flasks with 100 ml liquid medium.

The experimental medium was prepared on the basis of previous results from a macrophyte culture obtained at the Department of Plant Ecophysiology at the University of Lodz. The experiment was carried out according to the following experimental variants:

Control series:

- ✓ Control 1 - 100 ml standard "Z" medium
- ✓ Control 2 - 100 ml of tap water
- ✓ Control 3 - 99 ml of tap water + 1 ml of fermentation effluent from a biogas plant (1% leachate - the most optimal concentration obtained on the basis of previous experiments. This leachate concentration does not require preliminary plant adaptation).

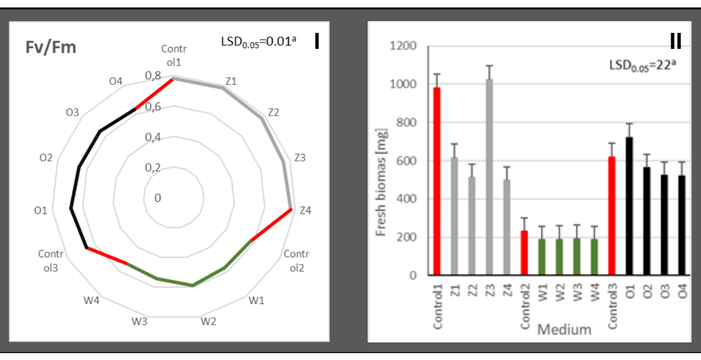

Figure 1. Chlorophyll fluorescence (I) and fresh biomass (II) of *Spirodela polyrrhiza* plants grown on different variants of NaCl supplemented medium. Control series: Control 1 - 100 ml of "Z" medium; Control 2 - 100 ml of tap water; Control 3 - 99 ml tap water + 1 ml post-fermentation effluent; Experimental variants: "Z" medium supplemented with 25mM NaCl (Z1), "Z" medium supplemented with 50mM NaCl (Z2), "Z" medium supplemented with 75mM NaCl (Z3), "Z" medium supplemented with 100mM NaCl (Z4); tap water supplemented with 25mM NaCl (W1), tap water supplemented with 50mM NaCl (W2), tap water supplemented with 75mM NaCl (W3), tap water supplemented with 100mM NaCl (W4); 1% leachate supplemented with 25mM NaCl (O1), water 1% leachate supplemented with 50mM NaCl (O2), 1% leachate supplemented with 75mM NaCl (O3), 1% leachate supplemented with 100mM NaCl (O4). Vertical bars denote ± SE. LSD at alpha level of 0.05.

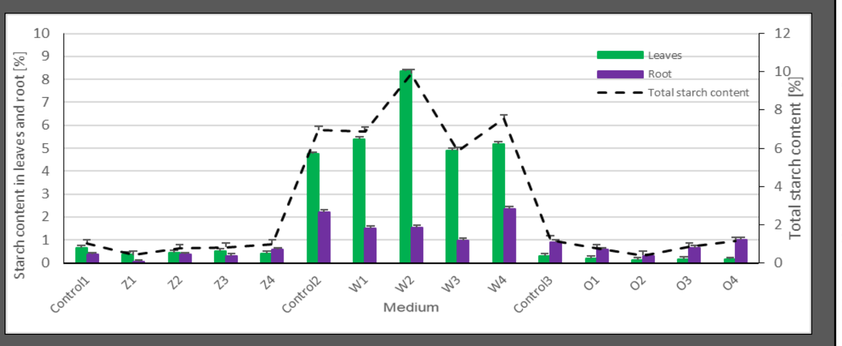

Figure 3. Total starch content in leaves and roots of *Spirodela polyrrhiza* plants grown on different variants of NaCl supplemented medium. Control series: Control 1 - 100 ml of "Z" medium; Control 2 - 100 ml of tap water; Control 3-99 ml tap water + 1 ml post-fermentation effluent; Experimental variants: "Z" medium supplemented with 25mM NaCl (Z1), "Z" medium supplemented with 50mM NaCl (Z2), "Z" medium supplemented with 75mM NaCl (Z3), "Z" medium supplemented with 100mM NaCl (Z4); tap water supplemented with 25mM NaCl (W1), tap water supplemented with 50mM NaCl (W2), tap water supplemented with 75mM NaCl (W3), tap water supplemented with 100mM NaCl (W4); 1% leachate supplemented with 25mM NaCl (O1), water 1% leachate supplemented with 50mM NaCl (O2), 1% leachate supplemented with 75mM NaCl (O3), 1% leachate supplemented with 100mM NaCl (O4).

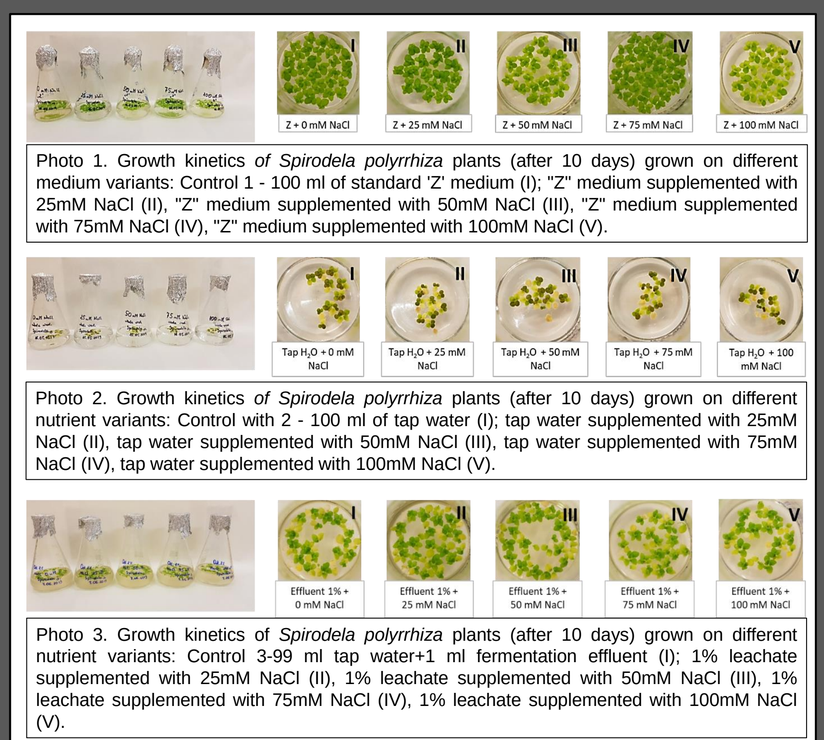

Photo 1. Growth kinetics *of Spirodela polyrrhiza* plants (after 10 days) grown on different medium variants: Control 1 - 100 ml of standard 'Z' medium (I); "Z" medium supplemented with 25mM NaCl (II), "Z" medium supplemented with 50mM NaCl (III), "Z" medium supplemented with 75mM NaCl (IV), "Z" medium supplemented with 100mM NaCl (V).

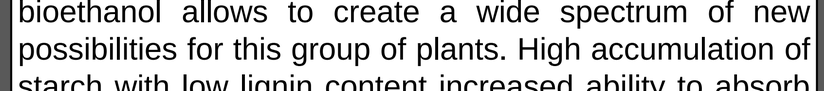

Photo 2. Growth kinetics *of Spirodela polyrrhiza* plants (after 10 days) grown on different nutrient variants: Control with 2 - 100 ml of tap water (I); tap water supplemented with 25mM NaCl (II), tap water supplemented with 50mM NaCl (III), tap water supplemented with 75mM NaCl (IV), tap water supplemented with 100mM NaCl (V).

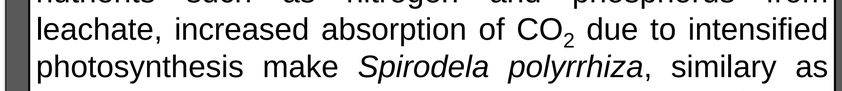

Photo 3. Growth kinetics of *Spirodela polyrrhiza* plants (after 10 days) grown on different nutrient variants: Control 3-99 ml tap water+1 ml fermentation effluent (I); 1% leachate supplemented with 25mM NaCl (II), 1% leachate supplemented with 50mM NaCl (III), 1% leachate supplemented with 75mM NaCl (IV), 1% leachate supplemented with 100mM NaCl (V).

RESULTS & CONCLUSION

The obtained results indicated that *Spirodela* sp. in response to abiotic stress such as high salinity of the environment was able to increase starch production in comparison with other popular energy plants. The use of duckweed as a source of starch for the production of bioethanol allows to create a wide spectrum of new possibilities for this group of plants. High accumulation of starch with low lignin content increased ability to absorb nutrients such as nitrogen and phosphorus from leachate, increased absorption of CO_2 due to intensified photosynthesis make *Spirodela polyrrhiza*, similary as other water duckweeds, a promising raw material for the production of biofuels. The conducted experiment indicates the effective use of aquatic plants in the production of biofuels and creates opportunities for the development of innovative, alternative and cost- effective sources of energy.

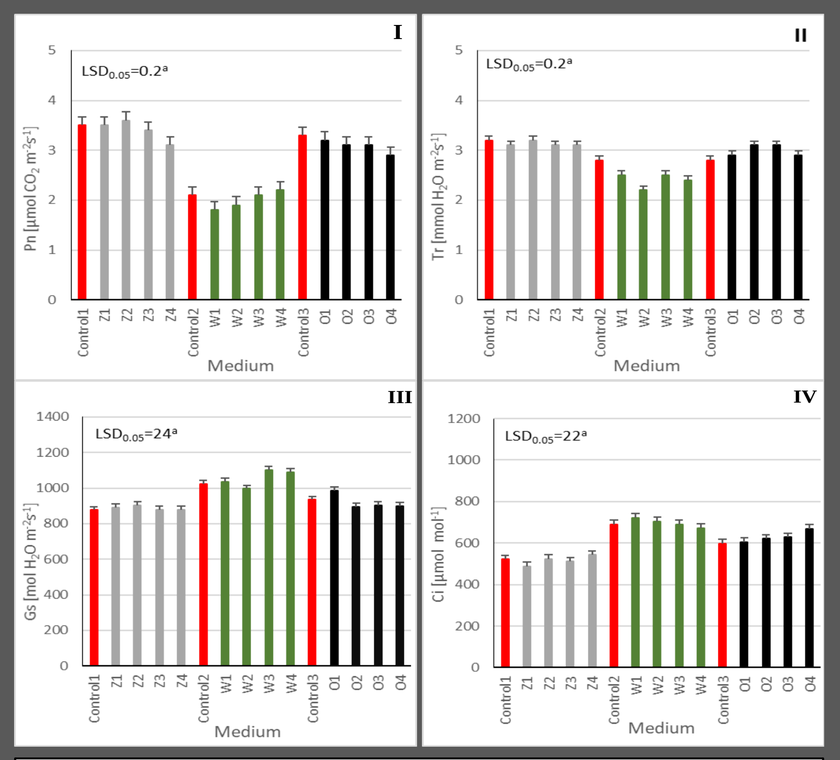

Figure 2. Net photosynthesis (I), transpiration (II), stomatal conductance (III) and intercellular concentration of CO_2 (IV) in leaves of *Spirodela polyrrhiza* plants grown on different variants of NaCl supplemented medium. Control series: Control 1 - 100 ml of standard "Z" medium; Control of 2 - 100 ml of tap water; Control 3 - 99 ml of tap water + 1 ml of digestate; Experimental variants: "Z" medium supplemented with 25mM NaCl (Z1), "Z" medium supplemented with 50mM NaCl (Z2), "Z" medium supplemented with 75mM NaCl (Z3), "Z" medium supplemented with 100mM NaCl (Z4); tap water supplemented with 25mM NaCl (W1), tap water supplemented with 50mM NaCl (W2), tap water supplemented with 75mM NaCl (W3), tap water supplemented with 100mM NaCl (W4); 1% leachate supplemented with 25mM NaCl (O1), water 1% leachate supplemented with 50mM NaCl (O2), 1% leachate supplemented with 75mM NaCl (O3), 1% leachate supplemented with 100mM NaCl (O4). Vertical bars denote ± SE. LSD at alpha level of 0.05.

Photos biogas plant in Piaszczyna (Poland). K.Piotrowski

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