*Environmental Engineering and Management Journal* *July 2018, Vol.17, No. 7, 1575-1581*
*http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu*

*"Gheorghe Asachi" Technical University of Iasi, Romania*

# INTEGRATED USE OF TWO MICROALGAL SPECIES FOR THE TREATMENT OF AQUACULTURE EFFLUENT AND BIOMASS PRODUCTION

**Katya Velichkova, Ivaylo Sirakov*, Yordan Staykov**

*Department of Biology and Aquaculture, Agricultural Faculty, Trakia University, 6000 Stara Zagora, Bulgaria*

## Abstract

One of the main challenges for the development of biomass algal system production is the high operational and capital costs for these technologies. A great opportunity to overcome these challenges may however exist in the integration of wastewater treatment with algal biomass production. The algae are used for treatment of industrial wastewater, for purification of effluent originating from livestock production, while the studies concerning the use of algae species for the treatment of aquaculture effluent are very few. The aim of our research was to compare the nitrogen and phosphate removal efficiency and the growth of two microalgae species – *Scenedesmus dimorphus* and *Botryococcus braunii,* cultivated in wastewater, originating from freshwater aquaculture production. A laboratory bioreactor was used for algae cultivation. It consisted of 500 mL Erlenmeyer flasks, containing wastewater from semi closed recirculation aquaculture system. Light regime was adjusted at 15:9 h light:dark cycle, the air was enriched with $CO_2$ up to 1% and the water temperature was kept between 25 and 27°C. Samples for water chemical analysis and growth measurement were taken at the beginning of the trial, at the 24th, 96th and the 168th hour after the start of the experiment. A better removal efficiency of nitrogen compound from wastewater originate from aquaculture was determined for *B. braunii* and the concentration of ammonia, nitrite, nitrate and total nitrogen decreased at the end of trial by 50%, 84.4%, 63.1% and 61.5% respectively. A better phosphate removal efficiency (77.8%) and better growth rate were found for *Sc. dimorphus* when aquaculture effluent was used as a growing media.

*Key words:* aquaculture, *Botryococcus braunii, Scenedesmus dimorphus,* wastewater treatment

*Received: March, 2014; Revised final: February, 2014; Accepted: September, 2014; Published in final edited form: July 2018*

## 1. Introduction

Protected areas have many benefits such as the energy consumption of our planet is increasing continuously, because of the rise in industrialization and Earth's population. Natural energy sources are limited and cannot meet the high demand for power consumption of our modern society. New energy sources must be found and introduced to solve the so-called "global energy crisis". A possible solution of this problem could be renewable energy sources, whose importance has increased in these last years. Biofuels are among the major competitive alternatives of fossil fuels.

Biodiesel is a biodegradable and non-toxic alternative fuel obtained from renewable sources such as waste cooking oil, palm, soy-bean, canola, rice bran, sunflower, coconut, corn, fish oil, chicken fat and algae (Pezzela et al., 2016; Sharif et al., 2007). The microalgae are one of the most promising resources, which could be used for biodiesel production.One of the main advantages of algae's cultivation is connected with the amount of oils and lipids per unit area. This difference can be as high as

* Author to whom all correspondence should be addressed: e-mail: ivailo_sir@abv.bg; Phone: +359896669829

30 times in comparison to oilseed crops which are cultivated at land (Sheehan et al., 1998).

*Botryococcus braunii* is a green microalga that produces hydrocarbons up to 75% from its dry biomass and it has already been proven as a renewable source of fuel (Brown et al., 1969; Banerjee et al., 2002; Casadevall et al., 1983; Dayananda et al., 2005; Velichkova et al., 2012). Many researches have proven that *Scenedesmus dimorphus* is one of the most promising algae species for biomass and biofuel production as well (Becker, 1994; Goswami and Kalita, 2011; Varsharani and Geeta, 2011; Velichkova et al., 2013).

One of the main challenges for the development of biomass algal system for biofuels production is the high operational and capital costs for these technologies (Alabi et al., 2009; Benemann, 2008). A great opportunity to overcome these challenges may however exist in the integration of wastewater treatment with algal biomass production.

The microalgae are used for treatment of industrial wastewater: soya sauce effluent (Shirai et al., 1998), brewery industries (Simate et al., 2011), dairy wastewater (Danalewich et al., 1998; Woertz et al., 2009), as well as for purification of effluent, originating from livestock production – poultry (Mahadevaswamy and Venkataraman, 1986; Murugesan et al., 2010), pig farms (Aguirre et al., 2011; Po Chung et al., 1978), cattle farms (Ayala and Vargas, 1987; Lincoln et al., 1996). Until now few studies concerning the exploitation of algae species for the treatment of effluent in aquaculture have been made (Borges et al., 2005; Dumas et al., 1998; Hussenot et al., 1998; Laliberté et al., 1994; Lefebvre et al., 1996). Nevertheless, these studies are ignored (Wang, 2003) and the science publications concerning them are highly limited. The aim of our research was to compare the nitrogen and phosphate removal efficiency as well as the growth of two microalgae species - *Scenedesmus dimorphus* and *Botryococcus braunii* cultivated in wastewater, originating from freshwater aquaculture production to answer whether it is possible to integrate the treatment of an aquaculture effluent with algal biomass production.

## 2. Material and methods

### 2.1. Algae species and media

Two microalgae species were used in the study:
- *Scenedesmus dimorphus* (SKU: AC-1002)
- *Botryococcus braunii* (SKU: AC-1006)

Both microalgae strains were supplied by Algae depot (USA) (www.algaedepot.com). The microalgae cultures were kept in glass flasks of 5 mL volume with reseeding for their preservation in a good physiological condition.

The wastewater originating from the semi closed recirculation aquaculture system (semi – closed RAS) was used as a growing medium for tested microalgae. Effluent treatment in the semi – closed RAS included a mechanical filter and a biofiltration (nitrifying filter with bio balls) for water purification. Daily, 40% of the water volume in semi - closed RAS was replaced with fresh water. The wastewater, used as medium for the tested microalgae was taken after mechanical and biological filtration from the pumping tank, before daily fresh water replacement (Fig. 1). The total biomass of raised in the recirculation aquaculture system common carps was 150 $kg^{-1}$.

### 2.2. Description of the Bioreactor for microalgae cultivation

A bioreactor consisting of 500 mL Erlenmeyer flasks and filled with wastewater from the semi closed RAS was used in the current trial. Three luminescent lamps Sylvania Aqua Star – 18w, 10 000 K, were placed at a distance of 30 mm from the flasks. Light regime was adjusted at 15:9 h light:dark cycle in an illumination incubator until the end of the experiment. The air was enriched with $CO_2$ up to 1%, regulated by manometric control and injected to the bottom of the flasks at a constant flow rate. The water temperature was kept between 25 and 27ºC, pH varied between 6.5 and 7.5 and for this reason was not adjusted.

Samples for water chemical analysis were taken at the beginning of the trial and at the 24th, 96th and 168th hour after the start of the experiment. They were centrifuged at 300 rpm for 10 min, for precipitation and removal of algal cells (Lee and Lee, 2002). Every variant of the experiment was conducted in six replications (Fig. 2). Other analyzed water chemical parameters were measured spectrophotometrically with spectrophotometer DR 2800 (Hach Lange). The methods and range of tests conducted during the experiment are shown in Table 1.

### 2.3. Growth measurement, chlorophyll and carotenoid content of microalgae culture

Optical densities of microalgal cultures were measured at 0, 24, 96 and 168 hours after the start of the experiment, in three repetitions.

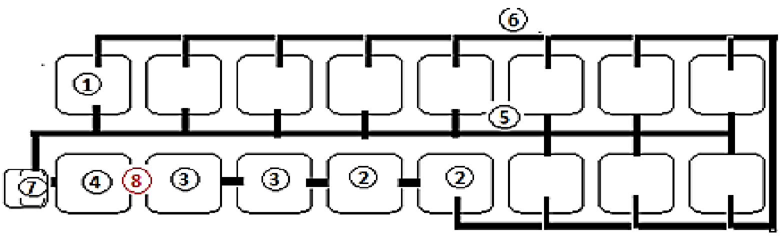


**Fig. 1.** Semi-closed RAS and sampling point: 1. Fish farming tank; 2. Mechanical filter; 3. Biological filter; 4. Pumping tank; 5. Inlet water; 6. Outlet water; 7. Pump; 8. Sampling point

**Table 1.** Methods and ranges of tests, used for monitoring of hydrochemical parameters during experimental period

| ***Parameters*** | ***Determination method*** | ***Measuring range (mg $L^{-1}$)*** |
|---|---|---|
| Ammonia | Indophenol blue | 0.015 – 2 |
| Nitrite – nitrogen | Diazotization | 0.015 – 0.6 |
| Nitrate - nitrogen | 2.6 dimethylphenol | 0.23 – 13.5 |
| Total nitrogen | Koroleff digestion + 2.6 dimethylphenol | 5 - 40 |
| Phosphorus (ortho + total) | Phosphomolybdenum blue | 0.05 – 1.5 $PO_4$-P<br>0.15 – 4.5 $PO_4$ |

The samples with a volume of 1 mL, were appropriately diluted with deionized water and the average absorbance at 550 nm was recorded with the help of a spectrophotometer DR 2800 (Hach Lange).

The isolation of pigments from algae cells included the following procedures: harvesting 2 mL of microalgae cells by centrifugation at 10000 rpm, two times for 3 min and discarding the supernatant, suspending cells in 2 mL methanol/water 90:10 v/v and Vortex mixing for 1 min, heating of the suspension for half an hour, in a water bath at 60ºC, cooling of the samples at room temperature, centrifugating the suspension (10000 rpm for 3 min) and discarding the supernatant with dissolved pigments. The absorbance of the pigments extract (665, 652 nm for chlorophyll content (a+b) and 470, 666 nm for carotenoids content) was recorded spectrophotometrically. The chlorophyll content (mg $L^{-1}$) was computed according to Porra et al., (1989) method and carotenoids - according to Lichtenthaler (1987) method. Data analyses were conducted by using ANOVA (MS Office, 2010).

## 3. Results

### *3.1. Efficiency of nitrogen compounds and phosphate removal in tested microalgae using aquaculture effluent as a medium for their cultivation*

The values of this parameter in the experiments with *B. braunii* and *Sc. dimorphus* were similar at the end of the trial and the differences were not statistically significant ($P \geq 0.05$).

The measured nitrogen compounds (ammonia, nitrite, nitrate and total nitrogen) in the wastewater used for cultivation of both experimental microalgal species decreased significantly during the experiment (Table 2). The concentration of ammonia in the trial with *B. braunii* decreased from 0.302 to 0.158 mg $L^{-1}$ and for *Sc. dimorphus* from 0.301 to 0.20 mg $L^{-1}$. These results corresponded to 50% and 33.5% reduction of ammonia respectively, but differences were not statistically significant ($P>0.05$) (Table 2). *B. braunii* reduced the concentration of nitrite and nitrate in wastewater, originating from aquaculture production by 84.4% and 63.1% respectively and for *Sc. dimorphus* the according percentages were 75% and 44.4%. The pH of wastewater increased in both tested variants during the trial (Fig. 2).

The differences in nitrite values between the tested variants were statistically significant for the 48th and 168th hour samples ($P \leq 0.05$). For the last measurement differences were highly significant ($P \leq 0.01$) (Table 2). The differences in the quantity of nitrate in wastewater used for the cultivation of *B. braunii* and *Sc. dimorphus* were statistically significant for the 96th and 168th hour samples ($P \leq 0.05$) (Table 2). The total nitrogen decreased in the wastewater containing *B. braunii* and *Sc. dimorphus* from the beginning until the end of the experiment by 61.5% and 54.4% respectively and the differences were statistically significant at hours 96 and 168 ($P \leq 0.05$) (Table 2). The quantities of removed phosphates from aquaculture wastewater were similar for both tested strains (Table 2).

The results varied from 1.4 $mgl^{-1}$ at the start to 0.38 mg $L^{-1}$ at the end of experiment for *Sc. dimorphus* and from 1.3 mg $L^{-1}$ to 0.41 mg $L^{-1}$ for *B. braunii*, corresponding to 77.8% and 68.4% phosphate removal efficiency respectively. The differences concerning phosphate in the wastewater, used as a test medium for both explored microalgae species, were statistically significant for none of the taken wastewater samples ($P>0.05$) (Table 2).

### *3.2. Growth parameters of tested microalgae, using aquaculture effluent as a medium for their cultivation*

The optical densities were higher at the 4$^{th}$ day since the beginning of experiment (96 hour) for both microalgae species cultivated in the wastewater and reached 0.95 for *Sc. dimorphus* and 0.8 for *B. braunii* (Fig. 3). After this day of maximum optical density, the values of this parameter declinedin both tested microalgae strains, as the nutritional compound in wastewater decreased heavily.

The measured optical density in *Sc. dimorphus* strains was higher by 10.5%, compared to the value of *B. braunii* at the end of trial, but the differences were not statistically significant ($P \geq 0.05$). At the end of the experiment the results showed that the contents of pigments - chlorophyll a+b and carotenoids were higher by 8.6% and 5.15% respectively in *Sc. dimorphus*, compared to their quantity in *B. braunii*, when they are cultivated in wastewater originating from freshwater aquaculture. However, the differences were not statistically significant for any of the measurements made concerning these growing parameters ($P \geq 0.05$) (Fig. 4).

## 4. Discussions

Many authors stated that algal species usually increase pH, due to the photosynthetic $CO_2$ assimilation (Borowitzka, 1998; Chevalier et al., 2000; Larsdotter, 2006). These points are confirmed in recent research as well.

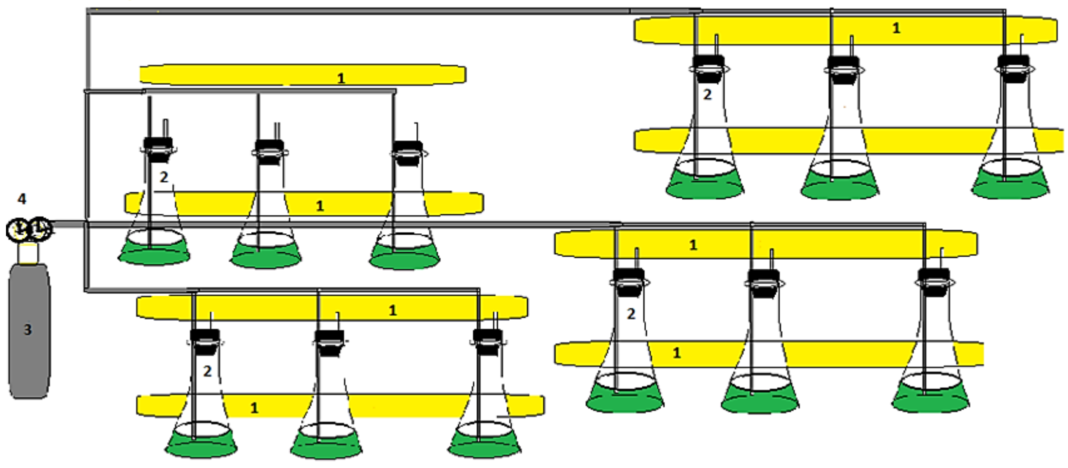


**Fig. 2.** Bioreactor used in conducted trials:
1) Luminiscent lamps; 2). Erlenmeyer flasks; 3). $CO_2$ bottle; 4). Manometer;

**Table 2.** Changes in nitrogen compounds of *Sc. dimorphus* and *B. braunii* in wastewater from aquaculture production during the treatment period (mg $L^{-1}$)

| ***Hours*** | ***Microalgae species*** | ***Hydrochemical parameters (mean ± SEM)*** | | | | |
|---|---|---|---|---|---|---|
| | | ***Ammonia*** | ***Nitrite – nitrogen*** | ***Nitrate - nitrogen*** | ***Total nitrogen*** | ***Phosphate*** |
| 0 | *Sc. dimorphus* | 0.301±0.01$^{ns}$ | 0.08±0.02$^{ns}$ | 1.8±0.2$^{ns}$ | 18.7±0.2$^{ns}$ | 1.4±0.02$^{ns}$ |
| | *B. braunii* | 0.302±0.02$^{ns}$ | 0.09±0.01$^{ns}$ | 1.9±0.1$^{ns}$ | 17.7±0.9$^{ns}$ | 1.3±0.01$^{ns}$ |
| 24 | *Sc. dimorphus* | 0.241±0.01$^{ns}$ | 0.0241±0.02$^{ns}$ | 1.75±0.07$^{ns}$ | 12.25±0.35$^{ns}$ | 1.36±0.11$^{ns}$ |
| | *B. braunii* | 0.228±0.08$^{ns}$ | 0.0218±0.007$^{ns}$ | 1.6±0.1$^{ns}$ | 11.75±0.35$^{ns}$ | 1.17±0.28$^{ns}$ |
| 96 | *Sc. dimorphus* | 0.202±0.09$^{ns}$ | 0.0232±0.007$^{ns}$ | 1.3±0.14$^{*}$ | 11.1±0.28$^{*}$ | 0.75±0.71$^{ns}$ |
| | *B. braunii* | 0.172±0.1$^{ns}$ | 0.0162±0.007$^{ns}$ | 0.95±0.07$^{*}$ | 9.3±0.74$^{*}$ | 0.95±0.03$^{ns}$ |
| 168 | *Sc. dimorphus* | 0.20±0.05$^{ns}$ | 0.0226±0.007$^{*}$ | 1.00±0.014$^{*}$ | 8.5±0.98$^{*}$ | 0.31±0.75$^{ns}$ |
| | *B. braunii* | 0.158±0.12$^{ns}$ | 0.0147±0.02$^{*}$ | 0.7±0.04$^{*}$ | 6.8±0.4$^{*}$ | 0.41±0.02$^{ns}$ |

*Note: *-show statistically significant differences (p<0.05), ns=no significant difference*

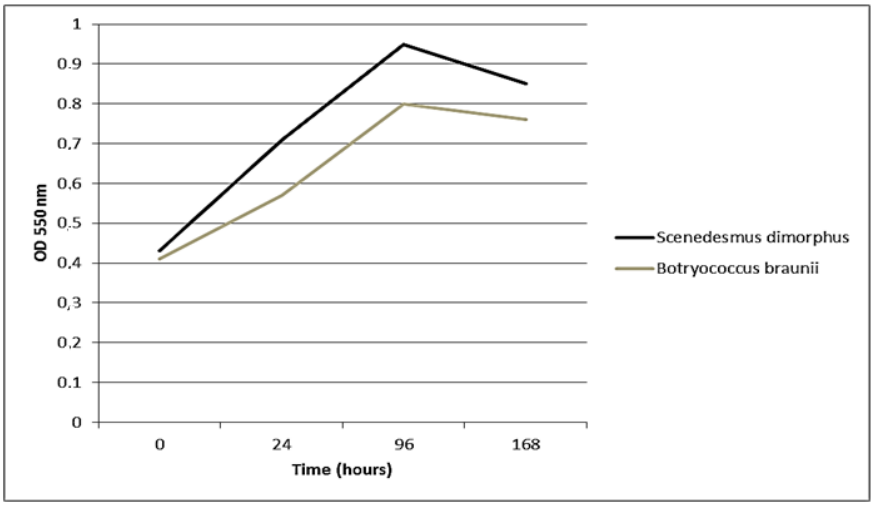


**Fig. 3.** Optical density (OD) of *Sc. dimorphus* and *B. braunii,* cultivated in wastewater from aquaculture production

One of the possible consequences of high pH in media is the induction of flocculation in algal cultures, which could be a reason for reduction of nutrient uptake and growth.

If the pH values increase over 9, most of inorganic carbon is converted to carbonate, which cannot be assimilated by the algae.

In our opinion pH did not suppress microalgae growth in the current trial, because pH values did not reach very high levels and varied between 6.5 and 7.43. Because of the low values of ammonia in wastewater originating from aquaculture, it could be concluded that this parameter had no negative effect on the microalgae growth.

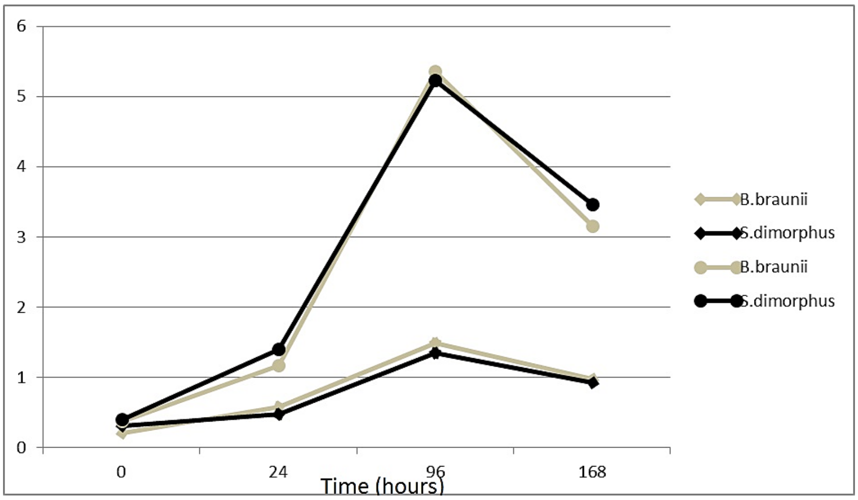


**Fig. 4.** Chlorophyll (a+b) and carotenoids (mg $L^{-1}$) concentrations in *Sc. dimorphus* and *B. braunii*, cultivated in wastewater from aquaculture production

Concentrations higher than 20 mg $NH_4^+N$ per liter in wastewater are not recommended due to ammonia toxicity (Borowitzka, 1998). The preferred nitrogen compound for microalgae growth is ammonia and when it is available, no alternative nitrogen sources will be used (Bhaya et al., 2000). The low ammonia concentration in wastewater used as a medium for both tested microalgae species could explain the advanced utilization of two other nitrogen sources – nitrite and nitrate. The higher concentration of nitrite makes it a less convenient nitrogen source for algae, because of its toxicity (Becker, 1988). During the experiment the values of nitrite were not so high, which probably excluded the possibility of nitrite toxicity for tested microalgae species.

The efficiency in nitrogen removal (ammonium, nitrite, nitrate and total nitrogen) from aquaculture effluent in *B. braunii* was higher compared to the *Sc. dimorphus* strain in all measurements. The results for nitrogen and phosphorus removal by *B. braunii* in the current research were found to be better than thosereported for swine wastewater treatment, by using tubular bioreactor (Lee et al., 1999). In their research removal rates of swine wastewater nitrogen and phosphorus by*B. braunii* were 43.9% and 41.7%, respectively, after 14 days of incubation. The results for chlorophyll density of *B. braunii,* cultivated in wastewater as a growing medium from the current research were similar with those of other authors (Tsukahara and Sawayama, 2005) stating that *B. braunii* could effectively remove nitrogen and phosphorus from secondary sewage treatement (SST) in the continuous bioreactor system.

The main factors affecting *B. braunii's* growth are availability of nitrogen and phosphate, light intensity and pH (Qin, 2005). In the current study, measured growth parameters indicated a better growth of *Sc. dimorphus,* compared to *B. braunii,* when wastewater from aquaculture was used as a growing medium. Among the main disadvantages of *B. braunii* is its slow growth: its doubling time is 7 hours (Qin, 2005). According to Banerjee et al., (2005) the growth of *B. braunii* is 20 times slower, compared to fast-growing algae so they recommended for its cultivation a low-cost investment system, like raceway pond, as economically effective. Our results for the hemical parameters of effluent waters were very similar to those of previous studies (Borges et al., 2005; Hussenot et al., 1998; Tovar et al., 2000). Namely the wastewaters from fish production are distinguished mainly by dissolved inorganic nutrients like nitrogen and phosphorus. This makes aquaculture effluent one possible medium for low cost microalgae biomass production (Laliberté et al., 1994). Ammonia removal efficiency of *Sc. dimorphus* in the current research was lower compared to other reports (Gonzalez et al., 1997), but higher than the results of Chevalier and De la Noüe (1985), which reported a removal efficiency of 23.31%. Ammonia, total nitrogen and phosphate removal efficiency were higher in other algal species from genus *Scenedesmus – Sc. quadricauda* used for swine wastewater treatment after appropriate dilution with tap water (Gantar et al., 1991).

At the 8th day, the percent of ammonia, total nitrogen and phosphate uptake varied between 99.5 - 100, 97.9% - 99.4% and 74.3 - 99.9% respectively at different tested dilutions. The water samples from locations around the Organized Industrial Zone were found to stimulate the growth of green microalgae *Desmodesmus* (=*Scenedesmus*) *subspicatus* (Katalay et al., 2012). Charity et al., (2009) evaluated the growth, removal of nutrients and organic matter of microalgae *Scenedesmus* sp. in wastewater derived from remains of fish for 15 days.

They established 2.24 mg/L chlorophyll and 0.89 mg/L carotenoids, and dry biomass 0.44 mg/mL. In our results a high growth rate of the microalgae culture and high chlorophyll and carotenoid contents were also observed. Volotina et al. (2004) also determined a high biomass after 24 hours cultivation in artificial wastewater.

The two tested microalgae are a good option for the biological treatment of aquaculture effluent. Both species showed a good nitrogen and phosphorus removal efficiency and the current research provides a good alternative technology for *B. braunii* and *Sc. dimorphus* production - integration of effluent treatment from aquaculture and biomass production, which could decrease operational costs and will make the technology for tested microalgae production highly sustainable.

## 5. Conclusions

This research provided a proof of concept for an integrated technology of aquaculture effluent treatment process that combines nutrient removal and microalgae production for potential use as a biofuel feedstock.

A better removal efficiency of nitrogen compound (ammonia, nitrite, nitrate and total nitrogen) was determined for *B. braunii* and better phosphate removal efficiency as well as better growth rate were found for *Sc. dimorphus* when wastewater from aquaculture effluent was used as a growing medium.


## Acknowledgements

This study was supported by the Faculty of Agriculture, Trakia University (project 3E/2012). We also would like to thank Mrs. Maia Andonova and Mr. Alexander Iordanov for their great help inchecking and editing our manuscript.


## References


Aguirre P., Álvarez E., Ferrer I., García J., (2011), Treatment of piggery wastewater in experimental high ratealgal ponds, *Revista Latino-americana de Biotecnologia Ambiental y Algal*, **2**, 57-66.

Alabi A.O., Tampier M., Bibeau E., (2009), *Microalgae Technologies and Processes for Biofuels/Bioenergy Production in British-Columbia: Current Technology, Suitability and Barriers to Implementation*, Seed Science Ltd, British Columbia Innovation Council, Canada.

Ayala F., Vargas T., (1987), Experiments on Spirulina culture on waste-effluent media and at the pilot plant, *Hydrobiologia*, **151**, 91-93.

Banerjee A., Sharma R., Chisti Y., Banerjee U. C., (2002), *Botryococcus braunii*: A renewable source of hydrocarbons and other chemicals, *Critical Reviews in Biotechnology*, **22**, 245-279.

Becker E.W., (1994), *Microalgae: biotechnology and microbiology*, Cambridge University Press, Cambridge, UK.

Becker E.W., (1988), *Micro-algae for Human and Animal Consumption*, In: *Micro-Algal Biotechnology*, Borowitzka M.A., Borowitzka L.J. (Eds.), Cambridge University Press, Cambridge, 222-256.

Benemann J.R., (2008), Opportunities and challenges in algae biofuels production, Algae World 2008, On line at: http://www.futureenergyevents.com/algae/whitepaper/algae_positionpaper.pdf.

Bhaya D., Schwarz R., Grossman R., (2000), *Molecular Responses to Environmental Stress,* In: *The Ecology of Cyanobacteria - Their Diversity in Time and Space*, Whitton B.A., Potts M. (Eds.), Kluwer, Dordrecht, 397-442.

Borges M.T., Silva P., Moreira L., Soares R., (2005), Integration of consumer-targeted microalgal production with marine fish effluent biofiltration–a strategy for mariculture sustainability, *Journal of Applied Phycology*, **17**, 187-197.

Borowitzka M.A., (1998), *Limits to Growth in Wastewater Treatment with Algae*, In: *Inland Saline Aquaculture Workshop*, Wong Y.S., Tam N.F.Y. (Eds.), Springer Verlag., 203-226.

Brown A.C., Knights B.A., Conway E., (1969), Hydrocarbon content and its relationship to physiological state in the green alga *Botryococcus braunii*, *Phytochemistry*, **8**, 543-547.

Casadevall E., Largeau C., Metzger P., Chirac C., Berkaloff C., (1983), Hydrocarbon production by unicellular microalga *Botryococcus braunii*, *Biosciences*, **2**, 129.

Charity E., Andrade R., Alexandra L., Vera B., Carmen H., (2009), Biomass production of microalga Scenedesmus sp. with wastewater from fishery, *Revista Téchnica Zulia*, **32**, 126-134.

Chevalier P., De la Noüe J., (1985), Wastewater nutrient removal with microalgae immobilized in carrageenan, *Enzyme and Microbial Technology*, **7**, 621-624.

Chevalier P., Proulx D., Lessard P., Vincent W. F., De la Noüe J., (2000), Nitrogen and phosphorus removal by high latitude mat-forming cyanobacteria for potential use in tertiary wastewater treatment, *Journal of Applied Phycology*, **12**, 105-112.

Danalewich J.R., Papagiannis T. G., Belyea R. L., Tumbleson M. E., Raskin L., (1998), Characterization of dairy waste streams, current treatment practices, and potential for biological nutrient removal, *Water Research*, **32**, 3555-3568.

Dayananda C., Sarada R., Bhattacharya S., Ravishankar G., (2005), Effect of media and culture conditions on growth and hydrocarbon production by *Botryococcus braunii*, *Process Biochemistry*, **40**, 3125-3131.

Dumas A., Laliberté G., Lessard P., De la Noüe J., (1998), Biotreatment of fish farm effluents using the cyanobacterium *Phomidium bohneri*, *Aquacultural Engineering*, **17**, 57-68.

Gantar M., Obreht Z., Dalmacija B., (1991), Nutrient removal and algal succession during the growth of *Spirulina platensis* and *Secenedesmus quadricauda* on swine wastewater, *Bioresource Technology*, **36**, 167-171.

Gonzalez L. E., Cañizares R. O., Baena S., (1997), Efficiency of ammonia and phosphorus removal from Colombian agro industrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*, *Bioresourse Technology*, **60**, 259-262.

Goswami R., Kalita M., (2011), *Scenedesmus dimorphus* and *Scenedesmus quadricauda*: two potent indigenous microalgae strains for biomass production and CO2 mitigation – A study on their growth behavior and lipid productivity under different concentration of urea as nitrogen source, *Journal of Algal Biomass Utilization*, **2**, 42-49.

Hussenot J., Lefebvre S., Brossard N., (1998), Open-air treatment of wastewater from land- based marine fish farms in extensive and intensive systems: Current technology and future perspectives, *Aquatic Living Resources*, **11**, 297-304.

Katalay S., Boyacioglu M., Cakalarslan O., Parlak H., Karaaslan M., (2012), Phytotoxicity of water and sediment from Nif Brook (Izmir, Turkey) on green algae *Desmodesmus (=Scenedesmus) subspicatus*, *Ekoloji*, **21**, 25-31.

Laliberté G., Proulx D., De Pauw N., De la Noüe J., (1994), Algal technology in wastewater treatment, *Archiv fur Hydrobiologie- Beiheft Ergebnisse der Limnologie*, **42**, 283-302.

Larsdotter K., (2006), Wastewater treatment with microalgae - a literature review, *Vatten*, **62**, 31-38.

Lee S.J., Kim S.B., Kim H.S., Kwon G.S., Yoon B.D., (1999), Mass cultivation of *Botryococcus braunii* for advanced treatment of swine waste water and lipid production in photobioreactor, *Sanop Misaengmul Hakhoechi*, **27**, 166.

Lee K., Lee C.G., (2002), Nitrogen removal from wastewaters by microalgae without consuming organic carbon sources, *Journal of Microbiology and Biotechnology*, **12**, 979-985.

Lefebvre S., Hussenot J., Brossard N., (1996), Water treatment of land-based fish farm effluents by outdoor culture of marine diatoms, *Journal of Applied Phycology*, **8**, 193-200.

Lichtenthaler H. K., (1987), Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes, *Methods in Enzymology*, **148**, 350-382.

Lincoln E.P., Wilkie A.C., French B.T., (1996), Cyanobacterial process for renovating dairy wastewater, *Biomass Bioenergy*, **10**, 63-68.

Mahadevaswamy M., Venkataraman L.V., (1986), Bioconversion of poultry droppings for biogas and algal production, *Agricultural Waste*, **18**, 93-101.

Murugesan S., Venkatesh P., Dhamotharan R., (2010), Phycoremediation of poultry wastewater by microalga, *Bioscience Biotechnology Research Communications*, **3**, 142-147.

Pezzella C., Vastano M., Casillo A., Corsaro M.M., Sannia G., (2016), Production of bioplastic from waste oils by recombinant *Escherichia coli*: a pit-stop in waste frying oil to bio-diesel conversion race, *Environmental Engineering and Management Journal*, **15**, 2003-2010.

Po Chung W.G., Pond J.M., Kingsbury E.F., Walker J., Krook L., (1978), Production and nutritive value of *Arthrospira platensis*, a spiral blue-green alga grown on swine wastes, *Journal of Animal Science*, **47**, 319-330.

Porra R.J., Thomson W.A., Kriedemann P.E., (1989), Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy, *Biochimica et Biophysica Acta*, **975**, 384-394.

Qin J., (2005), *Bio-hydrocarbons from algae: impacts of temperature, light and salinity on algae growth*, Rural Industries Research and Development Corporation, On line at: http://www.rirdc.gov.au/reports/EFM/05-025.pdf.

Sharif A., Nasrulhaq A., Majid H., Chandran S., Zuliana R., (2007), *Biodiesel Production from Waste Cooking Oil as Environmental Benefits and Recycling Process: A Review*, Asia Biofuel Conference Book, Singapore, 28.

Sheehan J., Dunahay T., Benemann J., Roessler P., (1998), A look back at the US Department of energy’s aquatic species program-biodiesel from algae, National Renewable Energy Laboratory (NREL) Report: NREL/TP-580-24190, Golden, Colorado, On line at: https://www.nrel.gov/docs/legosti/fy98/24190.pdf.

Shirai F., Kunii K., Sato C., Teramoto Y., Mizuki E., (1998), Cultivation of microalgae in the solution from the desalting process of soy sauce waste treatment and utilization of the algal biomass for ethanol fermentation, *World Journal of Microbiology and Biotechnology*, **14**, 839-842.

Simate G. J., Cluett J., Iyuke S., Musapatika E. T., Ndlovu S., (2011), The treatment of brewery wastewater for reuse: state of the art, *Desalination*, **273**, 235-247.

Tovar A., Moreno C., Manuel-Vez M. P., Garcia-Vargas M., (2000), Environmental impacts of intensive aquaculture in marine waters, *Water Research*, **34**, 334-342.

Tsukahara K., Sawayama S., (2005), Liquid fuel production using microalgae, *Journal of the Japan Petroleum Institute*, **48**, 251-259.

Varsharani H., Geeta G., (2011), Isolation of microalgae with biodiesel productivity prospects, *Karnataka Journal of Agricultural Sciences*, **24**, 585-588.

Velichkova K., Sirakov I., Georgiev G., (2012), Cultivation of *Botryococcus braunii* strain in relation of its use for biodiesel production, *Journal of Bio Science and Biotechnology*, 157-162.

Velichkova K., Sirakov I., Georgiev G., (2013), Cultivation of *Scenedesmus dimorphus* strain for biofuel production, *Agricultural Science and Technology*, **5**, 181-185.

Wang J.K., (2003), Conceptual design of a microalgae-based reticulating oyster and shrimp system, *Aquacultural Engineering*, **28**, 37-46.

Woertz I., Feffer A., Lundquist T., Nelson Y., (2009), Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock, *Journal of Environmental Engineering*, **135**, 1115-1122.