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Treatment of Wastewater Originating from Aquaculture and Biomass Production in Laboratory Algae Bioreactor using Different Carbon Sources

(Rawatan Air Sisa daripada Pengeluaran Akuakultur dan Biojisim di Makmal Alga Bioreaktor Menggunakan Punca Karbon yang Berbeza)

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ABSTRACT

The aim of present study was to explore the effect of different carbon sources on biomass accumulation in microalgae Nannochloropsis oculata *and* Tetraselmis chuii *and their ability to remove N and P compounds during their cultivation in aquaculture wastewater. Microalgae cultivation was performed in laboratory bioreactor consisted from 500 mL Erlenmeyer flasks, containing 250 mL wastewater from semi closed recirculation aquaculture system. The cultures were maintained at room temperature (25-27ºC) on a fluorescent light with a light: dark photoperiod of 15 h: 9 h. The microalgae species were cultivated in wastewater with different carbon sources: glucose, lactose and saccharose. The growth of strains was checked for 96 h period. In the present study,* N. oculata *and* T. chuii *showed better growth in wastewater from aquaculture with saccharose carbon source during the experiment. The most effective reduce of nitrate and total nitrogen was proved in* N. oculata *cultivated in wastewater with glucose as carbon source.* T. chuii *cultivated in wastewater containing glucose showed 8.27% better cleaning effect in ammonium compared with* N. oculata. T. chuii *grew in wastewater with glucose as carbon source showed 19.5% better removal effect in phosphate compared with* N. oculata *strain.*

Keywords: Biomass; Nannochloropsis oculata*;* Tetraselmis chuii*; wastewater*

ABSTRAK

Tujuan kajian ini dijalankan adalah untuk mengkaji kesan punca karbon yang berbeza terhadap pengumpulan biojisim pada mikroalga Nannochloropsis oculata *dan* Tetraselmis chuii *serta keupayaan mereka untuk mengeluarkan sebatian N dan P semasa penanaman di dalam akuakultur air sisa. Penanaman mikroalga dijalankan dalam bioreaktor makmal yang terdiri daripada 500 termos mL Erlenmeyer, yang mengandungi 250 mL air sisa daripada sistem edaran semula akuakultur separuh tertutup. Kultur dikekalkan pada suhu bilik (25-27ºC dengan cahaya lampu neon: fotokala gelap 15 h: 9 h. Spesies mikroalga telah ditanam dalam air sisa dengan punca karbon berbeza: glukosa, laktosa dan sakarosa. Pertumbuhan strain telah dipantau untuk tempoh 96 jam. Dalam kajian ini* N. oculata *dan* T. chuii *menunjukkan pertumbuhan yang lebih baik dalam air sisa oleh akuakultur, dengan punca karbon sakarosa semasa eksperimen. Paling berkesan mengurangkan nitrat dan jumlah nitrogen telah dibuktikan dalam* N. oculata *yang ditanam di dalam air sisa dengan glukosa sebagai punca karbon.* T. chuii *yang ditanam dalam air sisa mengandungi glukosa menunjukkan kesan pembersihan 8.27% lebih baik dalam ammonium berbanding dengan* N. oculata. T. chuii *yang membesar dalam air sisa dengan menggunakan glukosa sebagai punca karbon menunjukkan kesan penyingkiran 19.5% lebih baik dalam fosfat berbanding dengan strain* N. oculata.

Kata kunci: Air sisa; biojisim; Nannochloropsis oculata; Tetraselmis chuii

INTRODUCTION

The production of microalgae presents scientific interest because they are widely used like a sources of metabolic products, which could be applied as food supplements and pharmaceutical purposes (lipids, enzymes, polymers, pigments) as well as for the treatment of wastewater and biofuel production (Perez-Garcia et al. 2011). The content of metabolic products in algae strongly depends on cultivation media - nutrition media, synthetic or real wastewaters (Bashan et al. 2004, 2002; Harun et al. 2010; Lebeau & Robert 2006; Pulz 2001; Pulz & Gross 2004). This wide application of algae explains the interest in controlling their growth ability and capacity.

Tertiary wastewater treatment by microalgae is an old idea that so far has very limited application (Perez-Garcia et al. 2011). Studies on nutrient removal from wastewater by immobilized microalgae are limited (Ruiz-Marin et al. 2010; Sousa et al. 2014).

The primary focus of new work is on the ability of algae to remove residual nitrogen (N) and phosphorus (P) from wastewater (Kim et al. 2007; Hodaifa et al. 2008; Li et al. 2010a; Martinez et al. 2000; Munoz & Guieysse

2006; Voltolina et al. 2005) while near-total removal of N and P has been reported in batch systems (Li et al. 2010a; Michels et al. 2014), under continuous conditions the rate of removal is explored significantly lower (Voltolina et al. 2005).

Carbon is the most important element found in algal biomass and it constitutes over 50% in typical algal biomass (Becker 1994; Kargupta et al. 2015). According to Cid et al. (1992), high productivity of algae culture is possibly due to the synergistic effect of light and the organic carbon. Sugars, mainly monosaccharides, have been the most used organic substrates (Cerón et al. 2006; Chandra et al. 2014).

The aim of present study was to explore the effect of different carbon sources on biomass accumulation in microalgae *Nannochloropsis oculata* and *Tetraselmis chuii* and their ability to remove N and P compounds during their cultivation in aquaculture wastewater and to establish the influence of the studied algal species on the number of some sanitary indicator-microorganisms and pathogen microbial species in these waters.

Materials and Methods

MICROALGAE STRAIN, MEDIUM AND CULTIVATION

N. oculata (SKU: 100-NOC00-50) and *T. chuii* (SKU: 100-TCH00-50) was supplied from Algae depot – USA (www.algaedepot.com).

N.oculata and *T.chuii* were cultivated in wastewater from semi closed recirculation aquaculture system (RAS), before cleaning at mechanical and biological filter (Figure 1). The biomass of cultivated fish in RAS was 120 kg. The two microalgae species were cultivated in wastewater with different carbon sources: glucose, lactose, saccharose (1.125 g.l^{-1}). The wastewater with microalgae strain was used as a control without carbon source.

The samples of wastewater were filtered through a 25 mm, 3 µm glass microfiber filters (GF/C) mounted on a Millipore filtration unit. The cells in exponential period were inoculated (10% v/v) in a liquid medium.

The laboratory bioreactor used for microalgae cultivation consisted from 500 mL Erlenmeyer flasks, containing 250 mL wastewater from semi-closed recirculation aquaculture system (Figure 2). The cultures were maintained at room temperature (25-27ºC) with air condition on a fluorescent light with a light: dark photoperiod of 15 h: 9 h. The strains were checked for 96 h growth period.

OPTICAL DENSITY AND HYDRO CHEMICAL ANALYSIS

The growth of *N. oculata* and *T. chuii* were measured via spectrophotometer DR 2800 (Hach Lange, Germany). Optical density (OD) for biomass factor was determined at wavelength 650 nm. One mL of sample was diluted with deionized water and the absorbance of the sample was read at 650 nm.

The samples for hydrochemical analysis were taken in the start of the trial, at 24, 48, 72 and 96 h after the start of the experiment. The samples were centrifuged at 300 rpm for 10 min for sedimentation and for freeing them from

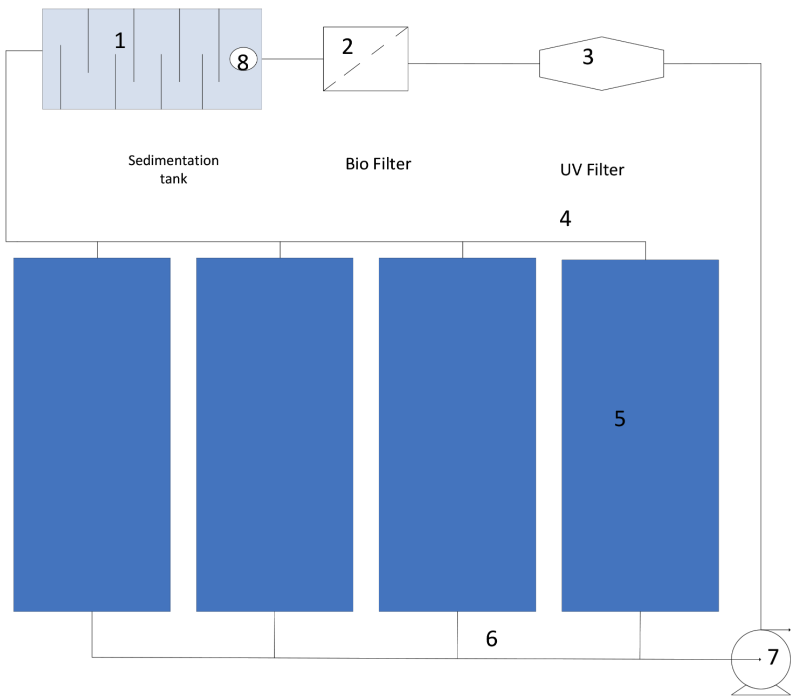

FIGURE 1. Recirculation aquaculture system (general view): 1 - sedimentation tank; 2 - bio filter; 3 - UV filter; 4 - inlet water; 5 - tank; 6 - outlet water; 7 - pump; 8 - sampling point

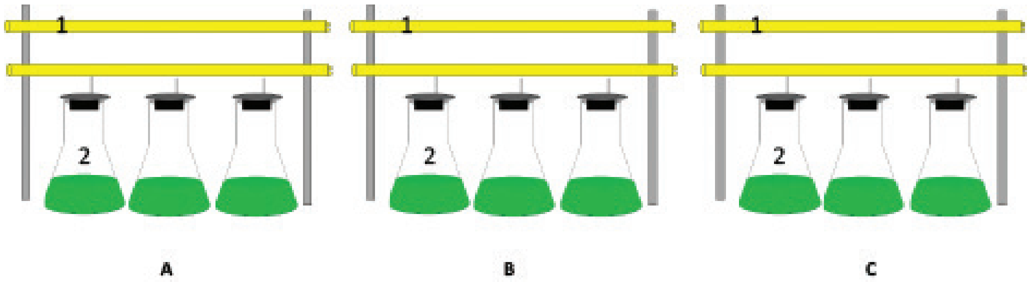

FIGURE 2. Laboratory bioreactor for microalgae cultivation: A - with glucose; B - with lactose; C - with saccharose; 1 - Erlenmeyer flasks; 2 - Luminescent lamp

algae cells (Lee & Lee 2002). The measurement of pH was made with a portable combined meter and with a pH probe (Hach Lange, Germany). Other analyzed hydro chemical parameters were measured spectrophotometrically with spectrophotometer DR 2800 (Hach Lange, Germany). The methods and range of tests which were used during the experiment are shown in Table 1.

MICROBIOLOGICAL ANALYSIS

For the quantitative detection of some sanitary indicator-microorganisms (Coliforms, Enterobacteriaceae) and pathogens (*Salmonella* sp.) in the treated water, medium sheets (Rida®Count Coliform; Rida®Count *Salmonella*/Enterobacteriaceae, R-Biopharm AG, Germany) coated with dry culture medium were used. One mL of the sample had been applied onto the fabric of the medium sheet. The sheets were incubated at 35°C for 24-48 h and the colonies were counted. The results are expressed in colony forming units (CFU/mL). Specific microorganisms are forming colony with different colors on the specific test cards.

STATISTICAL ANALYSIS

Data analyses were conducted by using t-test, one-way Analysis of Variance ANOVA (MS Office 2010) and Stat Soft 7 for Windows.

RESULTS AND DISCUSSION

Microalgae need carbon, nitrogen, phosphorus and other micronutrients to grow. They can fix carbon dioxide from air through photosynthesis while taking up nitrogen and phosphorus from wastewaters.

In present study *N. oculata* and *T. chuii* showed good growth in wastewater from RAS with different carbon source during the experiment and the differences were statistically significant at $p \leq 0.05$; $p \leq 0.01$ (Table 2). The

TABLE 1. Methods and range of tests used for monitoring the water quality parameters during experiment

Quality parameters	Determination method	Measuring range (mg.l^{-1})
Nitrite – nitrogen	Diazotization	0.015 – 0.6
Nitrate - nitrogen	2.6 dimethylphenol	5 - 35
Total nitrogen	Koroleff digestion + 2.6 dimethylphenol	5 - 40
Phosporus (ortho + total)	Phosphormolybdenum blue	0.05 – 1.5 0.15 – 4.5

TABLE 2. Hydro chemical (mg.l^{-1}) and growth parameters during experiment

x±Sx / Parameters	*N. oculata* glucose	*N. oculata* lactose	*N. oculata* saccharose	*T. chuii* glucose	*T. chuii* lactose	*T. chuii* saccharose
Nitrate	6.37±15.6*	10.19±0.39*	8.03±5.29*	7.06±8.3*	7.49±6.04*	7.18±6.6ns
Amonium	0.23±0.008**	0.31±0.001**	0.28±0.003**	0.23±0.009*	0.27±0.004*	0.29±0.002ns
Total nitrogen	7.18±11.6*	8.05±4.9ns	8.63±3.9**	7.47±8.7*	8.25±5.6*	8.31±5.29ns
Total phosphorus	6.5±18.8ns	7.3±11.2*	7.83±8.7*	6.4±19.1ns	7.07±12.7ns	7.29±8.8ns
pH	6.5±0.17ns	6.5±0.04**	6.5±0.03ns	6.41±0.07ns	6.36±0.06*	6.48±0.03ns
Optical density	0.656±0.15**	0.824±0.24*	0.954±0.28*	0.614±0.11**	0.73±0.17**	0.99±0.31**

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

growth observed in wastewater without carbon source was 50% lower compared to those with carbon source. The optical density of *N. oculata* and *T. chuii* was higher in wastewater enriched with saccharose (1.68 OD), compared to the samples cultivating with additional lactose and glucose (Figure 3).

Usharani and Lakshmanaperumalsamy (2010) studied bio-treatment of phosphate from wastewater using *Pseudomonas* sp. and the effect of different carbon sources - glucose, starch, saccharose and lactose. The maximum growth of *Pseudomonas* sp. they observed in glucose (0.9886 OD) followed by starch (0.9456 OD), saccharose (0.9095 OD) and lactose (0.8407 OD). In the present study *N. oculata* cultivated in wastewater with glucose, the algal growth was 18.3% higher than the results of Usharani and Lakshmanaperumalsamy (2010) and 42% higher for lactose, respectively.

Cerón et al. (2006) investigated the potential use of various carbon sources (fructose, glucose, mannose, lactose and glycerol) for culturing *Phaeodactylum tricornutum*. The maximum biomass productivities have been established in cultures with glycerol, fructose and glucose.

Bastos et al. (2011) evaluated cyanobacteria *Aphanothece microscopica* growth on agro-industrial effluents with glucose, lactose and saccharose. High biomass productivity for lactose suggests rapid assimilation of this sugar. Our results indicated that the two microalgae species cultivated in wastewater with lactose also showed a better growth rate compared to glucose.

Many microalgae species are unlikely to grow in extreme pH environments (such as pH ≥10) (Goldman et al. 1982). During wastewater treatment with algal systems, several factors could influence the pH. Nitrification process and ammonium consumption would result in pH decrease due to H^+ releasing (Gonzalez et al. 2008; Li et al. 2010b).

For *N. oculata* cultivating in wastewater with different carbon sources pH varied from 7.0 to 6.0 and for *T. chuii* - from 6.8 to 6.0 (Bio-treatment of phosphate from synthetic wastewater using *Pseudomonas* sp. YLW-7. 4). Usharani and Lakshmanaperumalsamy (2010) also observed the similar changes in pH value - the initial pH7.0 after 72 h was found to be 6.0 in culture medium with glucose and lactose.

At the beginning of the experiment the concentration of nitrate in used waters was 11 mg.l^{-1}. At the end the most effective reduce of nitrate was detected in *N. oculata* cultivated in wastewater from RAS with glucose (1.44 mg.l^{-1}) and the differences were statistically significant at $p \leq 0.05$ (Table 2). The results for *N. oculata* were 60.2% higher than the same of *T. chuii* (Figure 5(a)). With lower purifying ability were the both microalgae species cultivated in wastewater with lactose and saccharose.

Lowrey (2011) cultivated *Tetraselmis* sp. in 33% dairy wastewater and observed decreasing concentration of total nitrogen (TN) 51%, nitrate 55% and total phosphorus (TP) 40%. Lee and Lee (2002) concluded that various forms of nitrogen, such as nitrate and ammonium, in urban wastewaters can be taken up by most algae. Over 98% of initial 127 mg NO_3-N/l was removed after 10 days of algae culturing (Lee & Lee 2002). However, the total nitrogen removal efficiency at 96 h was lower (85%). This was rather expected, since large amounts of ammonium produced protons reduce the pH. The low pH condition must have had a harmful effect on algae growth and thus nitrogen consumption.

According to Hii et al. (2011), *Nannochloropsis* sp. preferred ammonia rather than nitrate when both ammonia and nitrate were available. At the end of the experiment cultivation of *Nannochloropsis* sp. in wastewater, 50 % of ammonia and 33.24% of nitrate were removed from the medium.

The preferential uptake of ammonia and the suppression of nitrate uptake by ammonia availability is well studied (Admiraal et al. 1987; Blasco & Conway 1982; Cresswell & Syrett 1979; Flynn 1999; Flynn et al. 1997; Herrera et al. 1972; Losada et al. 1970; Maguer et al. 2007; Paasche & Kristiansen 1982; Parker 1993; Syrett & Morris 1963).

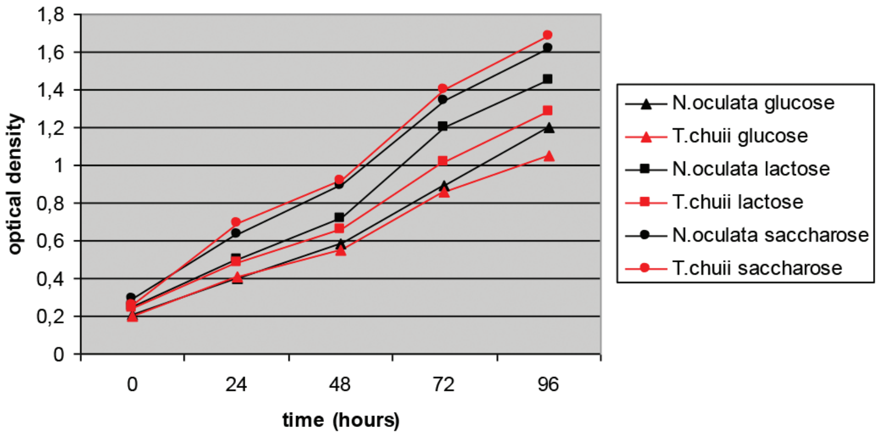

FIGURE 3. Optical density of *N. oculata* and *T. chuii* in wastewater with different carbon sources

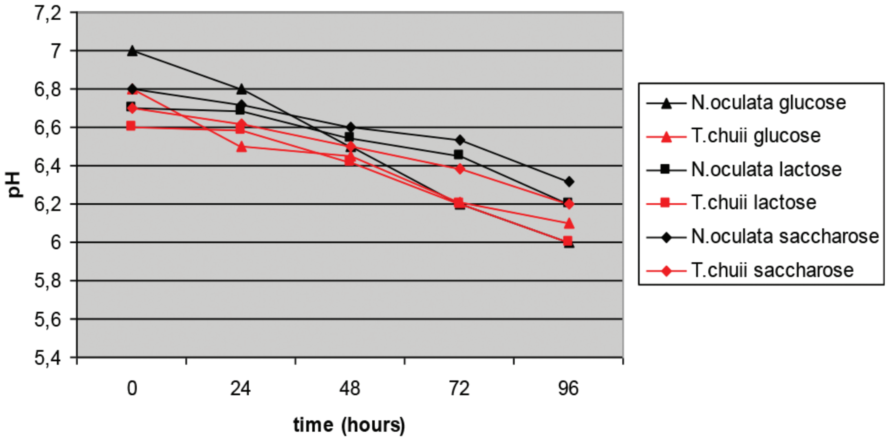

FIGURE 4. pH of *N. oculata* and *T. chuii* in wastewater with different carbon sources

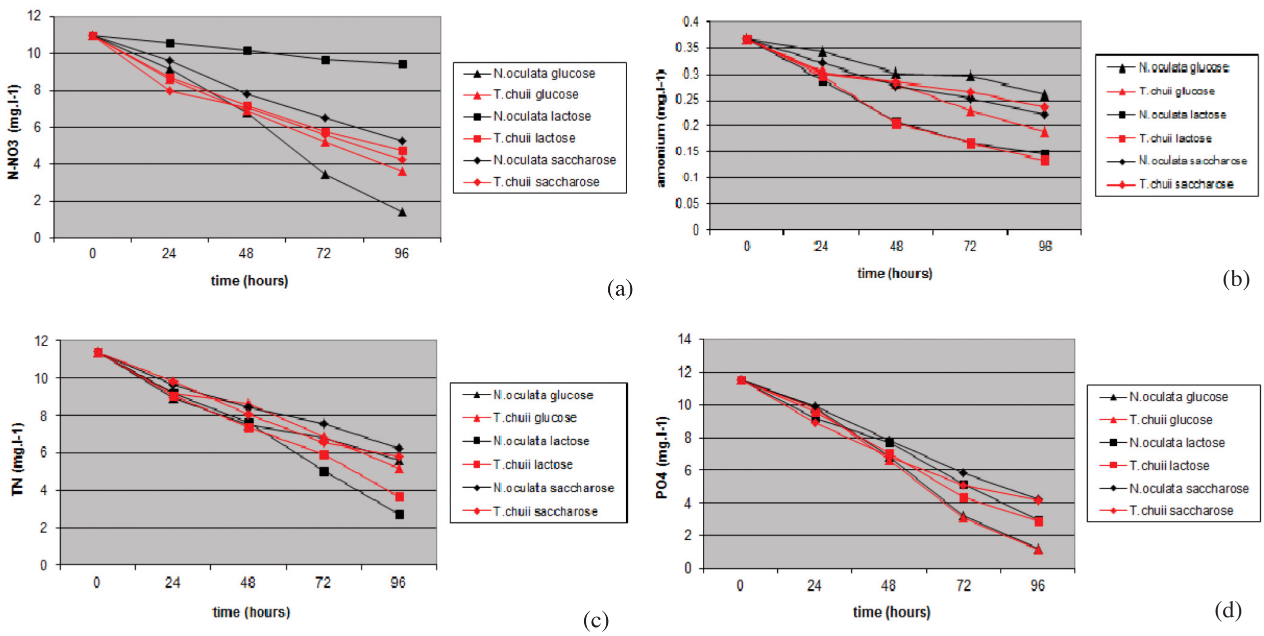

FIGURE 5. Hydro chemical parameters in wastewater of *N. oculata* and *T. chuii* cultivated with different carbon sources: (a) - nitrates; (b) - ammonium; (c) - total nitrogen; (d) - phosphorus compounds

In this trial, the ammonium concentration started from 0.366 mg.l^{-1}. After 96 h, it was reduced to 0.13 mg.l^{-1} for *T. chuii*, with glucose as additive (Figure 5(b)), which is 8.27% higher activity compared to *N. oculata*. The differences between the ammonium concentrations in wastewater with glucose as carbon source used for cultivation of the two microalgae species were statistically significant (Table 2).

The similar results were obtained in respect to total nitrogen removal where *N. oculata* showed higher absorbance activity growing in wastewater with glucose and lower with lactose and saccharose (Figure 5(c)). *T. chuii* cultivated with glucose showed 25.6% less absorption compared to *N. oculata* and the differences were statistically significant at $p \leq 0.05$ (Table 2).

In terms of phosphorus compounds uptake, *T. chuii* growing in wastewater with glucose is 19.5% more effective, compared to *N. oculata* grown under the same condition (Figure 5(d)). For both explored microalgae species the differences concerning phosphates in control wastewater and with glucose as carbon source were not statistically proven (Table 2).

Usharani and Lakshmanaperumalsamy (2010) used synthetic wastewater polluted with phosphates and with addition of different carbon sources for cultivation of *Pseudomonas* sp. have established maximum phosphate removal of 68% for glucose carbon source followed by starch (66%), sucrose (65%) and lactose (62%).

A scientific publications related to microbiological analyzes of cultivation of microalgae at various carbon supplements are very limited. Statistical analysis indicated that the type of applied carbon sources had no significant effect on the effectiveness of the biofiltration activities of the two microalgae species. The influence of the various carbon supplements on the bacterial growth

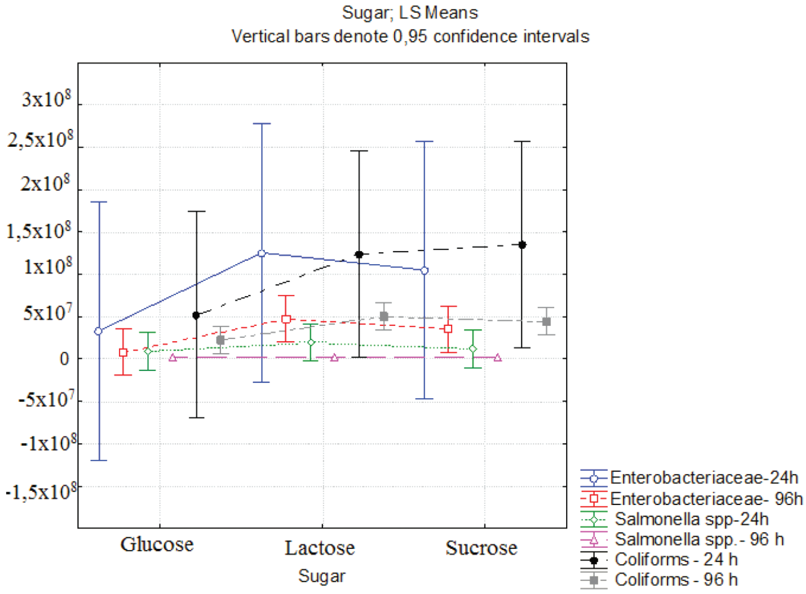

FIGURE 6. Influence of different carbon sources on the bacterial growth

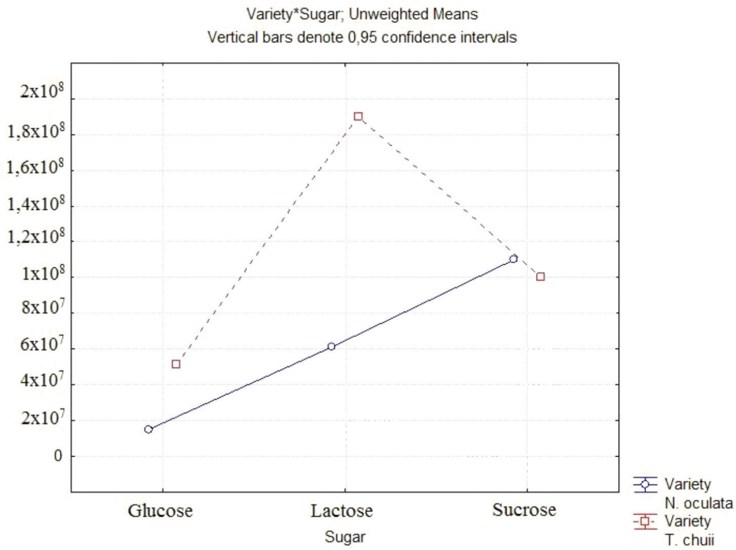

FIGURE 7. Influence of the microalgae species on the bacterial growth

shows reduction in bacterial growth after 96 h incubation in all treatments performed (Figure 6). The saccharose showed the highest potential to decrease the number of Enterobacteriaceae and Coliforms. It was determined that *N. oculata* has more reduction potential against Enterobacteriaceae when cultivated with glucose and lactose in wastewater, while *T. chuii* is more effective cultivated with saccharose (Figure 7).

Conclusion

The highest influence of the different carbon sources, added to wastewater from RAS, on the tested hydrochemical parameters was obtained in the treatments with glucose as carbon source for both microalgae species *N. oculata* and *T. chuii*. The best algal growth was observed in microalgae cultivation in wastewater from RAS with carbon sources saccharose. The use of glucose as an additional carbon source in wastewater decreases in the highest level the number of sanitary indicator-microorganisms compared to lactose and saccharose.

Acknowledgements

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