Revista Latinoamericana de Biotecnología Ambiental y Algal
Vol. 10 No. 1 p. 1-12.

Artículo Original

Cultivation of the microalgae *Parachlorella kessleri* using wastewater from a fishmeal & oil industry and its application for nitrogen removal

Cristina Viriato[1,2*], Cristian B. da Silveira[1], Maiara P. de Souza[3], Rosana de C. de Souza Schneider[3,4], Everton Skoronski[5]and Fábio de F. Neves[1]

[1]Departamento de Engenharia de Pesca, Universidade do Estado de Santa Catarina, Laguna, Brasil. ([*]Corresponding author: cristinavf@outlook.com)
[2]Programa de Pós-Graduação em Aquicultura e Pesca, Instituto de Pesca, APTA, SAA, São Paulo, Brasil
[3]Programa de Pós-graduação em Tecnologia Ambiental, Universidade de Santa Cruz do Sul, Santa Cruz do Sul, Brasil
[4]Departamento de Química e Física, Universidade de Santa Cruz do Sul, Santa Cruz do Sul, Brasil
[5]Departamento de Engenharia Ambiental e Sanitária, Universidade do Estado de Santa Catarina, Lages, Brasil

Abstract

The cultivation of algae using an alternative medium, mainly residues, is promising and has been intensively studied. The industrial or agro-industrial wastewaters are alternatives as they contain high concentrations of micro and macronutrients. At the same time, the major interest is the cultivation of the alga combined with the removal of nutrients from the aqueous medium. The aim of this study was to evaluate the growth and biochemical composition of the microalgae *Parachlorella kessleri* LCBA001. The algae grown has conducted associated with the wastewater treatment of a fishmeal& oil industry. Different conditions of medium were evaluated, varying the proportion of TAP (tris acetate phosphate) medium and the wastewater. Parameters as growth indicators, biochemical analyses of algal biomass, nutrient analyses before and after cultivation and the nitrogen removal yield were assessed. Microalgae growth was lower in the tests involving the wastewater than control test. However, there were no significant changes in the biochemical composition of the biomass. The removal of total Kjeldahl nitrogen (*TKN*) ranged from 83 to 90%. The results obtained demonstrated that the wastewater from a fishmeal & oil industry can be valorised as an alternative medium for algae growth.

Keywords: *Parachlorella kessleri; wastewater; nitrogen removal; algae growth.*

Resumen

El cultivo de algas utilizando un medio alternativo, principalmente residuos, es prometedor y se ha estudiado intensamente. Las aguas residuales industriales o agroindustriales son alternativas, ya que contienen altas concentraciones de micro y macronutrientes. Al mismo tiempo, el

principal interés es el cultivo del alga combinado con la eliminación de nutrientes del medio acuoso. El objetivo de este estudio fue evaluar el crecimiento y la composición bioquímica de la microalga *Parachlorella kessleri* LCBA001. El cultivo algal ha sido conducido, mientras se asociaba a un tratamiento de efluentes de una industria de harina y aceite de pescado. Se evaluaron diferentes condiciones de medio, variando la proporción de medio TAP (tris acetato fosfato) y el agua residual. Se evaluaron los parámetros como indicadores de crecimiento, análisis bioquímicos de biomasa de algas, análisis de nutrientes antes y después del cultivo y el rendimiento de eliminación de nitrógeno. El crecimiento de microalgas fue menor en los tratamientos que involucraron los efluentes que en los tratamientos de control. Sin embargo, no hubo cambios significativos en la composición bioquímica de la biomasa. La eliminación del nitrógeno total de Kjeldahl (TKN) osciló del 83 al 90%. Los resultados obtenidos demostraron que los efluentes de una industria de harina y aceite de pescado pueden ser valorados como un medio alternativo para el crecimiento de algas.

Palabras chave: *Parachlorella kessleri; aguas residuales; eliminación de nitrógeno; crecimiento de algas.*

1. Introduction

The fishmeal is the main source of protein used in the feed for most of the species in the world aquaculture. This is one of the most expensive ingredients considered for aquatic animal diets (Naylor *et al.*, 2000). Three main types of wastewater are generated in the fishmeal production: bail water, blood water and stickwater (Roeckel *et al.*, 1996; López and Lechuga, 2001; Miller *et al.*, 2001). The stickwater accounts for the highest wastewater generation. This residue reaches up to 60% of the processed fish weight (García-Sifuentes *et al.*, 2011). The stickwater is composed of a variety of solids which must be removed before its discharge into the environment (Fernández *et al.*, 2003). The total solids represent from 6 to 10% of stickwater content and consist of proteins, lipids and inorganic ions (García-Sifuentes *et al.*, 2009).

Large amounts of nutrients and harmful metals are a potential threat to natural ecosystems and human well-being (Cai *et al.*, 2013). These substances are commonly presented in industrial and agro industrial wastewater. Microalgae have been promising to be used for the removal of nitrogen, phosphorus and other inorganic substances from different wastewaters. They play a fundamental role in wastewater bioremediation, as demonstrated in several studies (Shimura *et al.*, 2012; Zhu *et al.*, 2013; Pacheco *et al.*, 2015). The microalgae *Parachlorella kessleri* is one of the species used for this purpose (Koutra *et al.*, 2017). This species is characterized by a considerable growth rate, extreme temperature tolerance, resistance to shear stress, low adhesion to bioreactor surfaces and negligible tendency to form aggregates (Li *et al.*, 2013). In addition, Caporgno *et al.* (2015) stated that this species is of interest for the production of biofuels when grown in domestic wastewater. Otherwise, other applications may be given to algal biomass, depending on the composition and production methods, leading to new industrialization strategies leveraged by alternative cultivation methods (Souza *et al.*, 2018).

Therefore, considering the adaptability of microalgae and the abundance of industrial effluents with adequate nutritional content, the objective of this study was to evaluate the

growth and biochemical composition of the microalga *Parachlorella kessler*i cultivated in wastewater from oil and fishmeal factory and its effect on the removal of nitrogen.

2. Material and Methods

2.1. Wastewater sample

The wastewater sample was collected at the fishmeal & oil industry located on the seacoast of Santa Catarina State, in Southern Brazil. The wastewater is generated after condensation of the evaporator, joining the water drained by the unloading of the fish and then flows to the treatment plant. The system is constituted by the following equipment: static sieve, dissolved air flotation system, aerated lagoons and clarifier. In this research, we evaluate the microalgae performance as a tertiary treatment for wastewater from fishmeal & oil industry. This treated wastewater has a light brown colour and organic matter odour. The total Kjeldahl nitrogen (TKN) concentration was 1,174.6 mg L^{-1}. The chemical oxygen demand (COD) was 962 mg L^{-1}. Moreover, the sample contained the presence of total phosphorus of 7.44 mg L^{-1} and negligible concentrations of suspended solids, oils & greases and dissolved oxygen. The wastewater samples were autoclaved for sterilization before the experiments using microalgae.

2.2. Experimental design

The tests were performed in Erlenmeyer flasks of 1000 mL containing 900 mL of culture medium. Four treatments were evaluated as follow: the control (C) which was used only synthetic culture medium TAP (tris acetate phosphate); the water W (tap water was used) and wastewater (WW) at a 50:50 ratio; the control and wastewater at a 50:50 ratio; and, the wastewater without any addition. The different proportion of culture medium was inoculated with samples from an exponentially growing culture to result in a cell density of 10^7 cells mL^{-1}. The flasks were randomly exposed in the culture room with continuous illumination (200 μmol photons m^{-2} s^{-1} PFD). The experiments were kept for 10 days at 29 °C under gently turbulence provided by an air blower (BOYU 007). The tests were performed in triplicate. The pH was daily monitored using a pH-meter (CienlaB) and the salinity was measured by a portable refractometer.

2.3. Determination of microalgae growth parameters

The cell density of the microalgae was daily monitored, in each experimental unit, using a Neubauer chamber. The data was used to obtain the growth curve for the cultures of *Parachlorella kessleri* LCBA001, regarding each treatment. Maximum Cell Density (*MCD*) and the cultivation time (*T*) were analysed. The parameter *T* measures, in days, the time interval within the inoculation and the instant which *DCM* is reached. The Specific Growth Rate (μ) is also obtained in this interval (Lourenço, 2006). The Growth Rate (k) and the Duplication Time (T_2) were calculated according to Derner (2006). The Productivity (P) was calculated according to Arredondo-Vega and Voltolina (2007).

2.4. Analytical methods

The biomass of *P. kessleri* was separated from each treatment by centrifugation for 10 minutes, 4000 rpm and 20º C (G Force Centrifuge), after the end of the cultivation. The supernatant was separated for water analysis and the wet biomass was scattered in Petri dishes and dried in an oven for 18 h at 50 °C.

2.4.1. Proteins

The protein composition of the biomass was determined using a CHNS elemental analyser (Perkin Elmer PE-2400). The analyses were carried out in triplicate. The result for the nitrogen was multiplied by the conversion factor of 4.78, commonly used for microalgae. This procedure converts the nitrogen content obtained into protein, which allows expressing the results as protein concentration (Templeton and Laurens, 2015).

2.4.2. Lipids

Lipid extraction was performed by the Bligh and Dyer method (Bligh and Dyer, 1959) and derivatization was performed with BF_3-methanol according to Souza *et al.* (2017). The extracts obtained were analysed using a gas chromatography coupled to a mass spectrometer (GC-MS) (Shimadzu GC 2010 MS-QP 2010 Plus) and a ZB-WAX capillary column. Helium was used as carrier gas at a constant flow of 1.0 mL min^{-1}. The injector temperature was 250 °C and the volume injected was 1 μL, using a split injection mode (1:10). The following temperature programming condition was used: temperature of oven at 70 °C; heating gradient of 4°C min^{-1}; constant temperature of 240 °C for 5 min and then 250 ° C totalling 49.50 minutes of analysis. The interface temperature was 260 °C and the electron impact ionization source temperature was 270 °C.

2.4.3. Total Kjeldahl nitrogen (TKN)

The *TKN* analysis was carried out for each treatment before they undergone inoculation, at the beginning of the experiment, and after the separation of the biomass at the end of the test. The *TKN* was determined according to the methodology SM 4500-N_{org} C recommended in the standard methods for the examination of water and wastewater (APHA, 2005).

2.6. Statistical analysis

The results of growth and biochemical composition were submitted to analysis of variance (ANOVA, $\alpha<0.05$). Tukey's test was used to compare the means of treatments when the significant differences were detected (Zar, 1996). The PAST software was used for the analyses.

3. Results and Discussion

3.1. Microalgae growth parameters

The average values of cell density were calculated over the days of cultivation for each experimental treatment (Fig. 1). The difference between the results for W:WW, C:WW and WW was not statistically significant ($p>0.05$). However, the obtained data from the control test showed the difference when compared to the results collected from other conditions. Additionally, the control condition was responsible for the highest cellular density results. The growth curve resulted similar among the treatments that included wastewater from the fishmeal & oil industry, which were lower than control test and it supposes that even 50% of this effluent is already enough to inhibit the *P. kessleri* growth. Meanwhile, the addition of nutrients did not solve this problem.

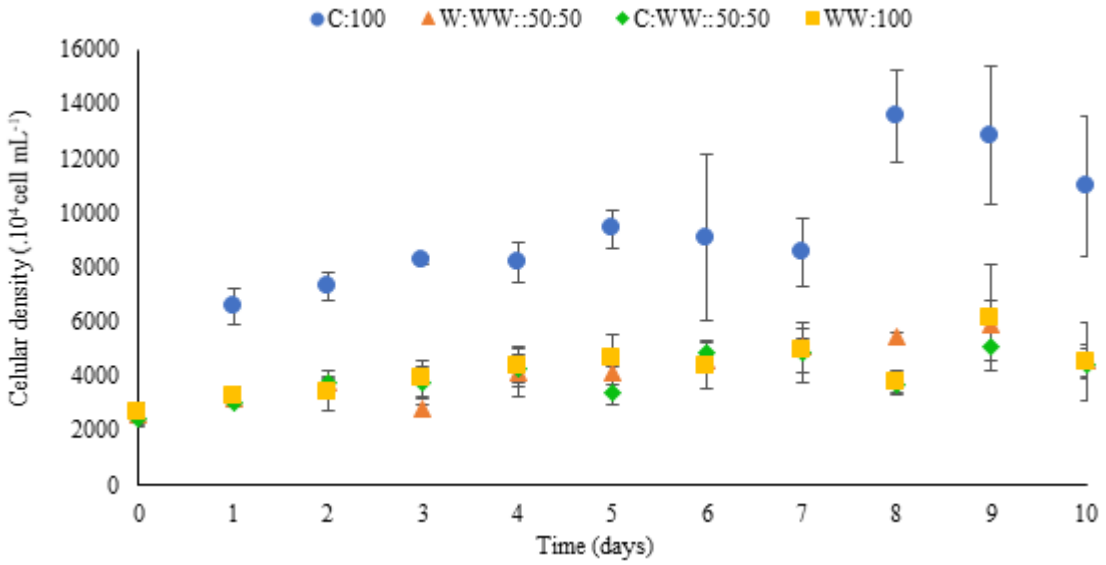

Figure 1. Effect of the growth medium on the cellular density of the algae *P. kessleri* LCBA001.

The difference between the cultivation time (*T*) for all tests was not statistically significant (p>0.05) (Table 1). The same results were also observed to the specific growth rate (μ) and the productivity (*P*). By contrast, the statistically significant difference was observed for the results regarding MCD, growth velocity (*k*) and doubling time (T_2), between the control and the other tests. Nevertheless, there was no statically significant difference between the parameters obtained from W:WW, C:W and WW conditions. It is noteworthy that the species *P. kessleri* LCBA001 showed a slow growth when cultivated in the wastewater from the fishmeal & oil industry. The dark colour of the wastewater could have caused a shading effect in the cultures and consequently a reduction of the photosynthesis. Also, the high toxicity of the residue should be considered another factor that could influenced the inhibition of growth. For example, ammonia toxicity is a factor that could limit the application of algae technology in wastewater treatment. Ammonia toxicity was observed in *Chlorella* sp. when concentration reached around 0,5 mg L^{-1} in artificial wastewater (Lu *et al*., 2018). In this study the ammonia concentration was not analysed. However, as the total nitrogen in the beginning of the cultivation was around 1,174.6 mg L^{-1}, there is a probability that the ammonia concentration was higher than that reported by these authors.

On the other hand, the difference between the conditions was not statically significant (p>0.05) regarding the productivity (*P*) and the growth rate (μ). In this case, the parameter μ was calculated using the productivity data. Possibly, the bacterial growth inhibited the growth of the microalgae in the tests using wastewater, since the inoculum used were not axenic. Additionally, the wastewater contained a high organic load (*COD* of 962 mg/L). These conditions create a competitive environment for both, the microalgae and bacteria growth. However, the growth rate (μ) values determined in this study were similar to the results obtained by Álvarez-Días *et al.* (2017) using a domestic wastewater (1.59 d^{-1}).

Regarding the control condition, the duplication time (T_2) values were similar to those obtained by Bauer *et al.* (2017), whose microalgae species and luminosity were the same. Moreover, these authors carried out the tests in a different culture medium (BG11) and in a vertical tubular photo bioreactor. Alternatively, the T_2 was around 7 days for the tests with the addition of the wastewater to the cultivation medium. This effect corroborates with the low growth rate (k) due to the wastewater usage.

Table 1. Results for the maximum cell density (*MCD*), cultivation time (T), growth rate (k), duplication time (T_2), Specific Growth Rate (μ) and Productivity for the different cultivation of *P. kessleri* grown.

Parameter of growth	C:100	W:WW::50:50	C:WW::50:50	WW:100
MCD (10^4 cell mL^{-1})	13613 ± 1739 a	6030 ± 639 b	5413 ± 635 b	6176 ± 1881 b
Cultivation time (days)	8.33 ± 0.58 a	8.67 ± 0.58 a	7.33 ± 1.53 a	8.33 ± 1.15 a
Growth rate - k	0.28 ± 0.02 a	0.14 ± 0.02 b	0.15 ± 0.01 b	0.14 ± 0.01 b
Duplication time (days)	3.52 ± 0.26 a	7.16 ± 0.77 b	6.51 ± 0.60 b	7.05 ± 0.91 b
Specific growth rate - μ (day^{-1})	1.12 ± 0.06 a	0.94 ± 0.04 a	1.05 ± 0.13 a	0.98 ± 0.10 a
Productivity ($g\ L^{-1}\ day^{-1}$)	2.59 ± 0.51 a	1.34 ± 0.14 a	2.22 ± 0.37 a	2.65 ± 0.29 a

Values expressed as mean ± standard deviation.
Different lowercase letters represent statistically significant differences.

The pH values varied throughout the cultivation tests (Fig. 2). As expected, the control started with the lowest pH value (7.62 ± 0.08). This value has a statistically significant difference when compared to the results obtained from W:WW (8.75 ± 0.06), C:WW (8.54 ± 0.03) and WW (8.84 ± 0.03). The WW test accounted for the highest pH value because of the wastewater proportion in the cultivation medium (100%). The mean pH at the end of the culture was 6.53 ± 0.75 for the control, 6.58 ± 2.15 for the W:WW, 7.58 ± 1.07 for the C:WW and 6, 14 ± 2.86 for WW. There was no statistically significant difference between the results ($p>0.05$). The lowest variations of the pH values were in the control test (from 5.67 to 7.07) and C:WW (from 6.43 to 8.55). As these tests involved the growth in TAP culture medium containing buffer solution (BASE TRIS), this may be the reason for higher pH stability in these cultures. According to Caporgno *et al.* (2015), the ideal pH for *P. kessleri* is around 7.5 and this value is compatible with the conditions observed in the tests using medium TAP. On the other hand, the reduction of pH may be related to bacterial growth and its incorporation of CO_2 by the heterotrophic metabolism, which reduces the pH of the water. This fact corroborates to the equivalent productivity for all the experimental conditions.

The salinity changed according to the amount of effluent and TAP culture medium. The increasing order of the average salinity for each condition of cultivation was C < W:WW<C:WW<WW and the values obtained were 2 ± 1, 4 ± 1, 6 ± 1 and 8 ± 2, respectively. It is worth to mention that a high salinity may inhibit cell growth and affect the shape and structure of

microalgae cells due to the osmotic pressure difference (Zhu *et al.*, 2016). The osmotic stress caused by the salinity can affect the physiological and biochemical properties of microalgae (Alkayal *et al.*, 2010). Consequently, the salinity may have been another factor contributing to the slower growth of *Parachlorella kessleri* LCBA001 compared with control test.

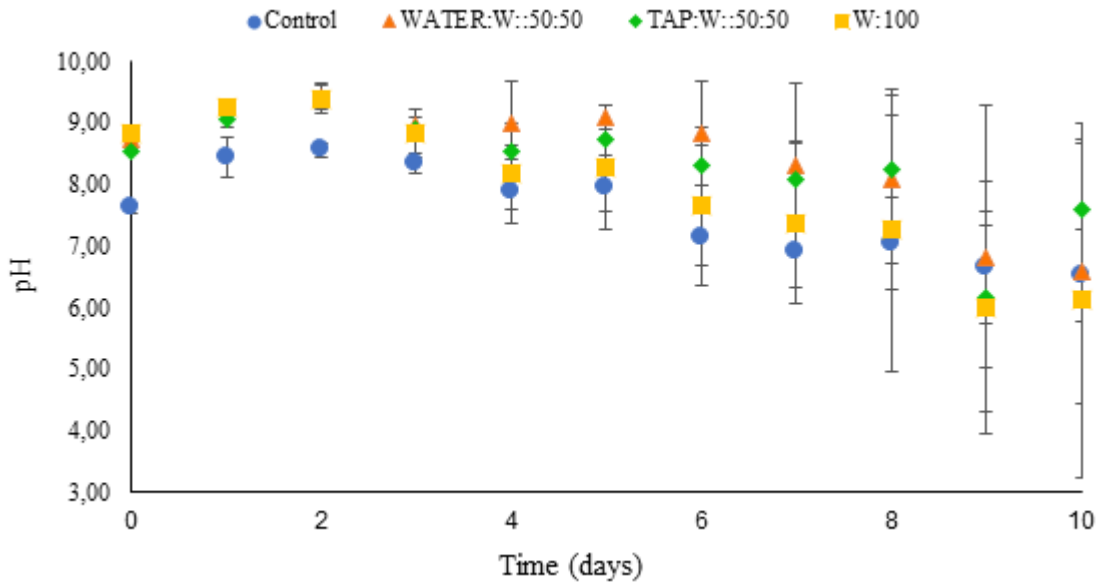

Figure 2. Effect of the cultivation conditions on the pH during the development of *P. kessleri* cultures.

3.2. Biochemical analysis of microalgae

The difference between the results for the total protein was not statistically significant (Table 2). Salati *et al.* (2017) characterized protein content in the *Chlorella* sp. cultivated mixotrophically using agro-industrial by-products as supplementary culture medium. The values determined were up to 50%, whereas, in this present study, our tests were able to reach 57% in terms of total protein. The high protein content present in *P. kessleri* biomass can be used as feed for growing animals, or even as a substitute for the fishmeal (mainly protein source in aquaculture feeds). For example, Raji *et al.* (2018) using a ration including *Chlorella* biomass as ingredient instead of a ration whole constituted of fishmeal as protein source, increase the growth performance of the cultivated African catfish.

Table 2. Effect of cultivation medium on the results for protein and lipid content of *P. kessleri* biomass.

Parameter	C:100	W:WW::50:50	C:WW::50:50	WW:100
Protein (%)	57.1 ± 0.2a	52.4 ± 0.6 a	54.9 ± 0.1 a	53.4 ± 0.6 a
Lipid content (%)	14.4 ± 0.7 a	9.9 ± 1.6 b	9.1 ± 1.55 b	9.5 ± 1.0 b

Values expressed as mean ± standard deviation.
Different lowercase letters represent statistically significant differences.

The difference between the lipid content for the control and the tests were statistically significant. However, the conditions involving the application of wastewater showed no statistically significant difference and the values in these conditions were smaller than that one obtained in the control test. The use of wastewater decreased the lipid content of the microalgae and it could be associated with the high amount of nitrogen. Liu *et al.* (2018) found values of lipid content less than 10% in the mixotrophic culture of *Chlorella pyrenoidosa* using concentrations of ammonia greater than 50 mg L^{-1}. The researchers concluded that the high biomass and lipid production rate of microalgae is only reached under low concentrations of ammonia, compared with the results of the present study.

The fatty acid profile found in the different test is shown in Fig. 3 by the overlap of the chromatograms. From the chromatogram presented, it was observed that for all the tests, the profile of the fatty acids was similar in qualitative terms. It should be noted that the predominant fatty acids were palmitic (C16:0), palmitoleic (C16:1 n-7), stearic (C18:0), oleic (C18:1 n-9), linoleic (C18:2 n-6) and γ-linolenic acid (C18:3 n-3).

The results obtained are consistent with those found in the literature for the microalga *Parachlorella kessleri*. Li *et al.* (2013) demonstrated in his work that palmitic, stearic, oleic and linoleic fatty acids, with limited nitrogen conditions in the culture medium, were the predominant fatty acids. Meanwhile, Gao *et al.* (2017) obtained with this same microalga a predominant profile of palmitic, oleic and linoleic fatty acids, which corresponded to 88.15% of the total fatty acids.

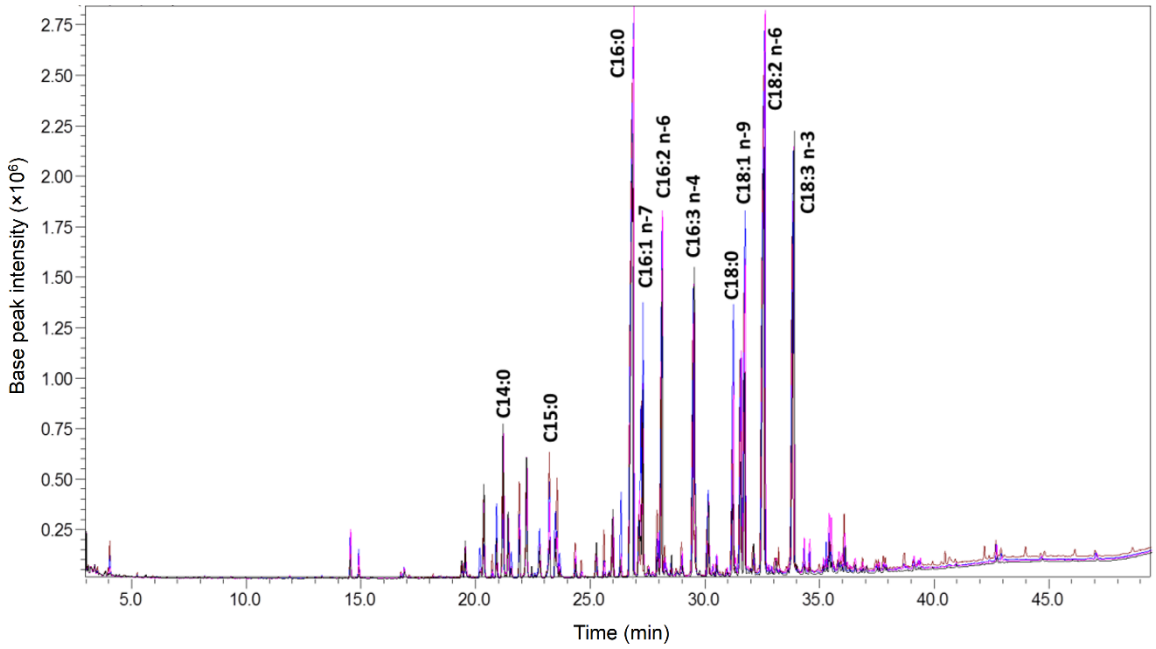

Figure 3. Chromatograms of the methyl esters related to the fatty acids present in the different tests.

3.3. Total Kjeldahl nitrogen removal

The total Kjeldahl nitrogen (*TKN*) concentration decreased in the tests with the addition of wastewater. The reduction of *TKN* concentration varied from 83% in C:WW test to 90% in W:WW test. These reductions is more expressive than observed in the control test (76%) (Fig. 4). The removal of nitrogen by microalgae occurs mainly by the assimilation of inorganic nitrogen, especially ammonia, a process that does not waste energy. Nitrate via also is assimilated but with energy consumption, therefore, nitrate is assimilated when the concentrations of ammonia are low (Gonçalves *et al.*, 2017). The decreasing of the *TKN* is likely related to the assimilation of inorganic nitrogen, as well as the decomposition of organic nitrogen by the bacteria. This observation is supported by the utilization of non-axenic culture. Some studies have reported positive associations between microalgae and bacteria during the cultivation (Gonçalves *et al.*, 2017). Further studies using *P. kessleri* LCBA001 should evaluate the concentration of different forms of nitrogen in order to elucidate the nitrogen transformations that occurred in the cultures.

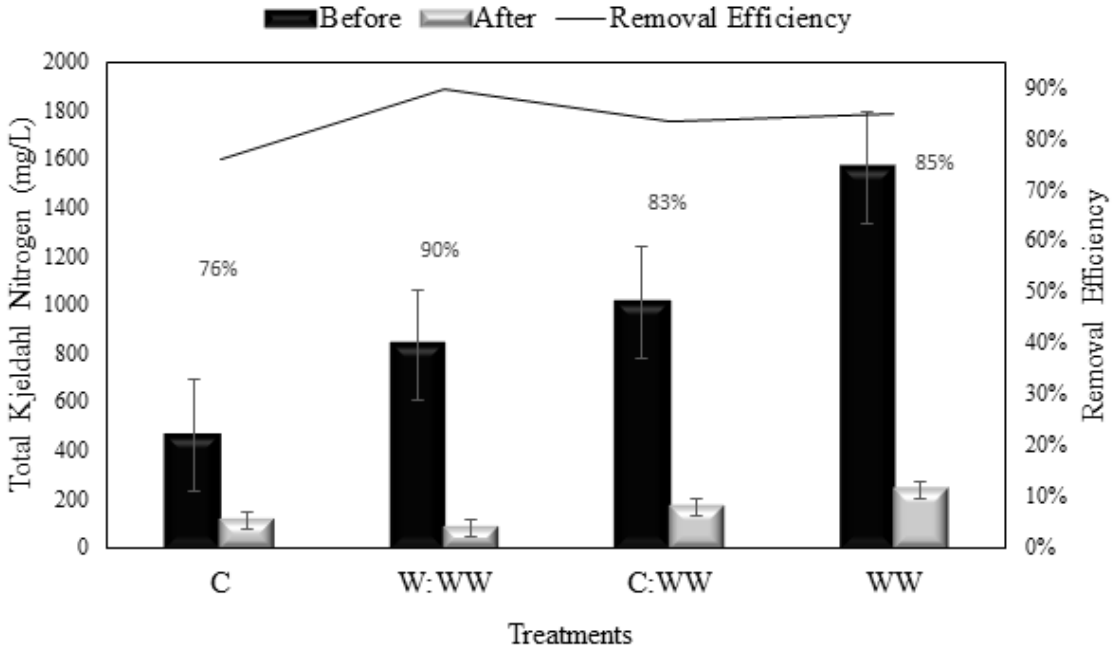

Figure 4. Effect of cultivation conditions on the TKN concentration.

Caporgno *et al.* (2015), obtained results of total nitrogen removal yield above 96% for *Parachlorella kessleri* cultivation using domestic wastewater. The present study applied a wastewater with higher concentrations of total Kjeldahl nitrogen and our results were above 83%. There was no statistically significant difference in the growth of the microalga, among the treatments using wastewater. The same was observed for the biochemical composition of the biomass, as well as in the *TKN* reduction. Another hypothesis is that the wastewater contains some nutrient limitation or some toxic element which, even when diluted by 50%, do not differ from treatment using 100% wastewater. Therefore, it is suggested to use directly the

treated wastewater from the fishmeal & oil industry for tertiary wastewater treatment assisted by the production of microalgae *Parachlorella kessleri* LCBA001. In other words, no water or culture medium is needed for dilution. This practice contributes to the reduction in the water and fertilizer demand and provides sustainability aspects for the process.

4. Conclusion

The microalga *Parachlorella kessleri* LCBA001 is able to be cultured using wastewater from fishmeal & oil industry as nutrient source. However, the growth rate is slower compared with control test. The wastewater did not influence the biomass protein content, but the lipid content was lower. The reduction of the total nitrogen concentration present in the wastewater was above 83%. The microalga strain *Parachlorella kessleri* LCBA001 demonstrated potential to be used as a tertiary treatment process of wastewater from fishmeal & oil industry.

5. Acknowledgements

This research was made possible by a financial support from the umbrella project: *"Bioplásticos de microalgas cultivadas em efluente: uma alternativa sustentável para a indústria catarinense"* (Grant number NPP2015010002656).

6. References

Alkayal, F. R. L., Albion, R. L., Tillett, L. T., Hathwaik, M. S., Lemos, J. C. Cushman. 2010. Expressed sequence tag (EST) profiling in hypersaline shocked *Dunaliella salina* reveals high expression of protein synthetic apparatus components. *Plant Sci.* 179(5):437–449.

Álvarez-Días, P. D., Ruiz, J., Arbib, Z., Barragán, J., Garrido-Perez, M. C., Perales, J. A. 2017. Freshwater microalgae selection for simultaneous wastewater nutrient removal and lipid production. *Algal Res.*24:477-485.

Arredondo-Vega, B. O., Voltolina, D. 2007. Métodos y herramientas analíticas en la evaluación de la biomasa microalgal. Centro de Investigaciones del Noroeste –CIBNOR. La Paz, México. 97 p.

APHA. Standard methods for the examination of water and wastewater. 2005. Washington D. C. American Public Health Association. 20th ed.

Bauer, L. M., Costa, J. A. V., Rosa, A. P. C., Santos, L. O. 2017. Growth stimulation and synthesis of lipids pigments and antioxidants with magnetic fields in *Chlorella kessleri* cultivations. *Bioresour. Technol.* 244:1425-1432.

Bligh, E. G., Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37(8):911-917.

Cai, T., Park, S.Y., Li, Y. 2013. Nutrient recovery from wastewater streams by microalgae: status and prospects. Renew. Sustain. *Energy Rev.* 19:360–369.

Caporgno, M. P., Taleb, A., Olkiewicz, M., Font, J., Pruvost, J., Legrand, J.,

Bengoa, C. 2015. Microalgae cultivation in urban wastewater: Nutrient removal and biomass production for biodiesel and methane. *Algal Res.* 10:232-239.

Derner, R.B. 2006. Efeito de fontes de carbono no crescimento e na composição bioquímica das microalgas *Chaetoceros muelleri* e *Thalassiosira fluviatilis*, com ênfase no teor de ácidos graxos poliinsaturados. Tese (Doutorado em Ciência dos Alimentos). Centro de Ciências Agrárias, Universidade Federal de Santa Catarina. Florianópolis, SC. Brasil. 140p.

Duan, X. G. Y., Ren, L. L., Liu, and W. X., Zhu. 2012. Salt-induced osmotic stress for lipid overproduction in batch culture of Chlorella vulgaris. *Afr. J. Biotechnol.* 11(27):7072–7078.

Fernández, D. M. D., Montero, P. Y., Gómez, G.M.C. 2003. Effect of freezing fish skins on molecular and rheological properties of extracted gelatin. *Food Hydrocolloid* 7:281-286.

Gao, Y., Lv, J., Feng, J., Liu, Q., Xie, S. 2017. Morphology, phylogeny and lipid components of an oil-rich microalgal strain. *J. Appl. Bot. Food Qual.* 90:298-305.

García-Sifuentes, C.O., Pacheco-Aguilar, R., Lugo-Sánchez, M.E., Garcia-Sánchez, G., Ramirez-Suárez, J.C. Y., García-Carreño, F.L. 2009. Properties of recovered solids from stick-water treated by centrifugation and pH shift. *Food Chem.* 114:197-203.

García-Sifuentes, C.O., Pacheco-Aguilar, R., Ramirez-Suárez, J.C., Ruíz, G. C. 2011. Stickwater multi-step treatmnent: effect on organic material removal. *Ciencias Biológicas y de la Salud* 13:10-16.

Gonçalves, A. L., Pires, J. C. M., Simões, M. 2017. A review on the use of microalgal consortia for wastewater treatment. *Algal Res.* 24:403-415.

Koutra, E., Grammatikopoulos, G., Kornaros, M. 2017. Microalgal post-treatment of anaerobically digested agro-industrial wastes for nutrient removal and lipids production. *Bioresour. Technol.* 224:473–480.

Li, X., Pribyl, P., Bišová, K., Kawano, S., Cepák, V., Zachleder, V., Cizkova, M., Branyikova, I., Vitova, M. 2013. The microalga *Parachlorella kessleri*—a novel highly efficient lipid producer. *Biotechnol. Bioeng.* 110:97–107.

Liu, L., Zhao, Y., Jiang, X., Whang, X., Liang W. 2018. Lipid accumulation of Chlorella pyrenoidosa under mixotrophic cultivation using acetate and ammonium. *Bioresour. Technol.* 262:342-346.

López, R.O., Lechuga, A.M. 2001. Contaminantes en los Cuerpos de Agua del Sur de Sonora. *Salud Pública de México* 43:289-305.

Lourenço, S.O. 2006. Cultivo de microalgas marinhas – princípios e aplicações. RiMa, São Carlos. 606p.

Lu, Q., Chen, P., Addy, M., Zhang, R., Deng, X., Ma, Y., Cheng, Y., Hussain, F., Chen, C., Liu, Y., Ruan, R. 2018. Carbon-dependent alleviation of ammonia toxicity for algae cultivation and associated mechanisms exploration. *Bioresour. Technol*. 249: 99-107.

Miller, J.D., Hupka, J., Niewiadomski, M., Flores, B., Morse, M. 2001. Advanced wastewater treatment for the fish processing industries near Ensenada, Baja California. Final report. Project

Number W-00-3. University of Utah, USA. 1-28.

Naylor, R.L., Goldburg, R.J., Primavera, J.H. 2000. Effects of aquaculture on world fish supplies. *Nature* 5:1017-1024.

Pacheco, M. M., Hoeltz, M. Moraes, M. S. A., Schneider, R. C. S. 2015. Microalgae: Cultivation techniques and wastewater phycoremediation. *J. Environ. Sci. Health* 50:573-589.

Raji, A. A., Alaba, P. A., Yusuf, H., Bakar, N. H. A., Taufek, N. M., Muin, H., Alias, Z., Milow, P., Razak, S.A. 2018. Fishmeal replacement with *Spirulina platensis* and *Chlorella vulgaris* in African catfish (*Clarias gariepinus*) diet: Effect on antioxidant enzyme activities and haematological parameters. *Res. Vet. Sci.* 119:67–75.

Roeckel, M., Aspe, E., Marti, M.C. 1996. Achieving clean technology in the fsh-meal industry by addiction of new process step. *J. Chem. Technol. Biotechnol.* 67:96-104.

Salati, S., D'Imporzano, G., Menin, B., Veronesi, D., Scaglia, B., Abbruscato, P., Mariani, P., Adani, F. 2017. Mixotrophic cultivation of *Chlorella* for local protein production using agro-food by-products. *Bioresour. Technol.* 230:82-89.

Shimura, H., Itoh, K., Sugiyama, A., Ichijo, S., Ichijo, M. et al. 2012. Absorption of radionuclides from the Fukushima nuclear accident by a novel algal strain. *Plos One* 7(9):e44200.

Souza, L. dos S., Simioni, C., Bouzon, Z. L., Schneider, R. C. S., Gressler, P., Miotto, M. C., Rossi, M. J., Rörig, L. R. 2017. Morphological and ultrastructural characterization of the acidophilic and lipid-producer strain *Chlamydomonas acidophila* LAFIC-004 (Chlorophyta) under different culture conditions. *Protoplasma* 254(3):1385-1398.

Souza, M. P. de S., Hoeltz, M., Gressler, P. D., Benitez, L. B., Schneider, R. C. S. 2018. Potential of microalgal bioproducts: general perspectives and main challenges. *Waste Biomass Valor*. 1-18.

Templeton, D. W., Laurens, L. M. L. 2015. Nitrogen-to-protein conversion factors revisited for applications of microalgal biomass conversion to food, feed and fuel. *Algal Res.* 11:359-367.

Zar, J.H. 1996. Biostatistical analysis. 3rd. ed. New Jersey. Prentice Hall. 622p.

Zhu, L. D., Li, Z. H., Hiltunen, E. 2016. Strategies for Lipid Production Improvement in Microalgae as a Biodiesel Feedstock. *BioMed Res. Int.* 2016:1-8.

Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P., Yuan, Z. 2013. Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. *Water Res.* 47:4294–4302.