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Short Communication: Antioxidant activity of ethanol extract of *Chlorella sorokiniana* cultured in tofu wastewater

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Abstract. *Mursandi H, Susanty D, Nurhayati L, Okasari AA. 2022. Short Communication: Antioxidant activity of ethanol extract of* Chlorella sorokiniana *cultured in tofu wastewater. Nusantara Bioscience 14: 155-159.* Microalgae are microorganisms that grow quickly and produce secondary metabolites with antioxidant activity. Antioxidants of microalgae can be utilized in various aspects such as cosmetics, pharmaceuticals, supplements, and feed. Microalgae utilization will be more profitable if the microalgae can be cultured on waste media. This study aims to determine the concentration of a suitable medium for the growth of *Chlorella sorokiniana* Shihira & R.W.Krauss, total flavonoids, total phenolics, and the potential of ethanolic extract of *C. sorokiniana* as an antioxidant. This study cultured the microalgae *C. sorokiniana* on tofu liquid waste media at various concentrations (15, 20, 25, and 30%). The growth of *C. sorokiniana* on the media was observed using a spectrophotometer at 680 nm wavelength. *C. sorokiniana* biomass was collected on the 7th day. The biomass was extracted using ethanol as a solvent. Phytochemical analysis was performed using the standard method, Total phenolic content, total flavonoid content, and antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) were conducted to determine the IC_{50} value. The results showed that the best growth of *C. sorokiniana* was on TLW media at a concentration of 30%. The ethanolic extract of *C. sorokiniana* showed the presence of alkaloids, flavonoids, steroids, tannins, and saponins. The total phenolic content in the ethanolic extract of *C. sorokiniana* was 18.39 ± 0.29 mgGAE/g, and the total flavonoid content was 31.93 ± 5,60 mgQE/g. The IC_{50} of the ethanolic extract of *C. sorokiniana* was 288.95 mg/L, which shows this extract has a potent antioxidant.

Keywords: Antioxidant, *C. sorokiniana,* flavonoid, phenolic

INTRODUCTION

Microalgae are photosynthetic microorganisms with varied cell morphology, i.e., unicellular and multicellular. Microalgae have advantages, namely high photosynthetic efficiency, high mass production, fast-growing, and can use certain wastes as a source of nutrients. The diversity of microalgae globally is estimated to reach millions of species, most of which are not yet identified and cannot be cultivated. As many as 200,000-800,000 microalgae live in nature, 35,000 species have been identified, and 15,000 chemical components from microalgae have been identified (Hadiyanto and Azim 2012). Microalgae have been applied in various fields, including energy (Li et al. 2011; Makareviciene et al. 2011) and health as antioxidants (Li et al. 2007). Microalgae that have been known to have antioxidant activity, including *Botryococcus* (Rao et al. 2006), *Dunaliella* (Herrero et al. 2006), and *Haematococcus* (Cerón et al. 2007), *Chlorella* (Lai, 2017; Napitupulu 2019), and 32 selected microalgae (Goiris et al. 2012). The antioxidant activity of these microalgae is related to the produced metabolites. One of the microalgae capable of antioxidants is *Chlorella sorokiniana* Shihira & R.W.Krauss.

The *C. sorokiniana* can grow in mixotrophic conditions with various carbon and nitrogen sources, making it ideal for the cultivation of raw waste materials (Ramanna et al. 2014). The *C. sorokiniana* has a high growth rate and can absorb the nutrients contained in the media (Lizzul et al. 2018). The optimum growth temperature of *C. sorokiniana* is 35°C-45°C (de-Bashan et al. 2008) with less than 4-6 hours of light (Janssen et al. 1999). The *C. sorokiniana* produced carotenoids at 0.69% dry weight under extremophilic conditions (Matsukawa et al. 2000). A previous study by Lai (2017) showed that the ethanolic extract of *C. sorokiniana* had an IC_{50} of 7.36 mg/mL.

The *C. sorokiniana* can utilize waste for culture medium. Waste as microalgae culture reduces production costs and the solutions for handling waste problems. Previous studies showed that *C. sorokiniana* grew well in wastewater, such as domestic waste (Ramanna et al. 2014) and livestock waste (Chen et al. 2020; Susanty and Oksari 2020). Tofu wastewater can also be used as a medium for cultivating *Chlorella*. In Indonesia, tofu industries contribute about 20 million cubic meters of liquid waste annually (Widayat and Hadiyanto 2016). Utilization of tofu wastewater for microalgae culture media can be an alternative to waste treatment. Tofu wastewater contains large amounts of carbon, nitrogen, and phosphorus that can be used as a nutrient source for microalgae (Syaichurrozi and Jayanudin 2016). In this study, *C. sorokiniana* was cultured on tofu wastewater media at various concentrations (15, 20, 25, and 30%), and the antioxidant activity was conducted on the ethanolic extract of *C. sorokiniana*.

MATERIALS AND METHODS

Materials

The microalgae used in this study was *C. sorokiniana* (InaCCM 38). Tofu wastewater was obtained from the Ciriung area, Bogor, West Java, Indonesia. Chemicals and equipment: universal pH indicator, distilled water, filter paper, 70% alcohol, NaOH 2N, gallic acid, hydrochloric acid, sulfuric acid, acetic anhydride, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (SIGMA-ALDRICH), 96% ethanol (SMART LAB), $FeCl_3$, Folin Ciocalteu (MERCK), chloroform, quercetin (HIMEDIA), Na_2CO_3, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, Mg band, $AlCl_3$ 10%, potassium acetate 1 M. Equipment: glass jars, analytical balance, TL lamp, hemocytometer, laminar airflow, autoclave, oven, centrifuge (Hettich Zentifugen EBA 20), water bath, rotary evaporator, Ultra Violet spectrophotometer. -Visible (UV-Vis) (OPTIZEN™ POP-SPECTROPHOTOMETER UV-VIS SMART) and laboratory glassware.

Preparation of tofu wastewater media

The tofu wastewater was taken from the tofu pressing process unit. The N, P, and K concentration in tofu wastewater was then measured. The nitrogen content was determined using the Kjeldahl method (IKLab-102-188), while phosphorus and potassium levels were determined using the spectrophotometric method (IKLab-105-191). The microalgae culture media used four concentrations of tofu wastewater (15, 20, 25, and 30%). Culture media were sterilized before being used as growth media of *C. sorokiniana*.

The measurement of *Chlorella sorokiniana* growth by optical density (Haneda 2015)

The growth of *C. sorokiniana* in various concentrations of tofu wastewater was observed using a UV-VIS spectrophotometer at 684 nm wavelength at 10 days of cultivation.

Biomass production of *Chlorella sorokiniana*

The biomass of *C. sorokiniana* was collected at optimum growth by separating the media with microalgae using a centrifuge, then air-dried at room temperature to obtain dry biomass.

Extraction of secondary metabolic compounds of *Chlorella sorokiniana*

The *C. sorokiniana* biomass with the best growth was extracted with ethanol 96% (1:10 (w/v)), using a shaker for 3 days. The ethanol extract was evaporated using a rotary evaporator at a temperature of 60°C to obtain a concentrated extract.

Phytochemical testing of *Chlorella sorokiniana* extract

Alkaloids, flavonoids, terpenoids/steroids, tannins, and saponins content were analyzed based on Harbone's (1996) methods.

Determination of total phenolic content of *Chlorella sorokiniana* extract

Total phenolic content was analyzed using the Folin Ciocalteu reagent, referring to the method of Azaman et al. (2017). Fifty (50) mg of microalgae ethanolic extract was dissolved into 50 mL of distilled water. One ml of the solution was taken and added with 2.5 mL of Folin Ciocalteu reagent, allowed to stand for 5 minutes at room temperature. After that, 2.5 mL of 7.5% Na_2CO_3 was added and allowed to stand again for 45 minutes. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 760 nm. Standard gallic acid was used at 5, 10, 15, 20, and 25 mg/L concentrations. Each concentration of standard gallic acid has the same treatment as the extract solution. Therefore, the total phenolic extract of the sample is expressed in mg gallic acid equivalent /g sample (mg GAE/g sample).

Determination of total flavonoid content of *Chlorella sorokiniana* extract (Ukieyanna 2012)

Fifty mg of extract of *C. sorokiniana* was dissolved into 50 mL of solution with ethanol. One (1) mL of extract solution was added with 0.2 mL of $AlCl_3$ 10%, 0.2 mL of sodium acetate, and 3 mL of ethanol and calibrated with distilled water. The absorption was measured at the maximum wavelength. The resulting absorbance was entered into the regression equation of the quercetin standard curve.

Analysis of antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Jerez-Martel et al. 2017; Kusumah 2019)

The *C. sorokiniana* extract and quercetin (as positive control) were put into a test tube with 2 mL of 0.1 mg/L DPPH solution, shaken with a vortex until homogeneous, and incubated in a dark room for 30 minutes. The absorption was measured at a wavelength of 515 nm. The sample concentration and the percent inhibition are plotted on the x and y axes of the linear regression equation, respectively, and that equation determines the IC_{50} (Nurjana et al. 2011).

RESULTS AND DISCUSSIONS

The cell density of *Chlorella sorokiniana*

The density of microalgae cells was determined by the optical density (OD) method, which describes the growth of microalgae cells per unit of time and can be used as a benchmark to determine the carrying capacity of media or nutrients for cell growth and division (Istirokhatun et al. 2017). The best growth of *C. sorokiniana* was obtained at the treatment of 30% tofu wastewater (Figure 1).

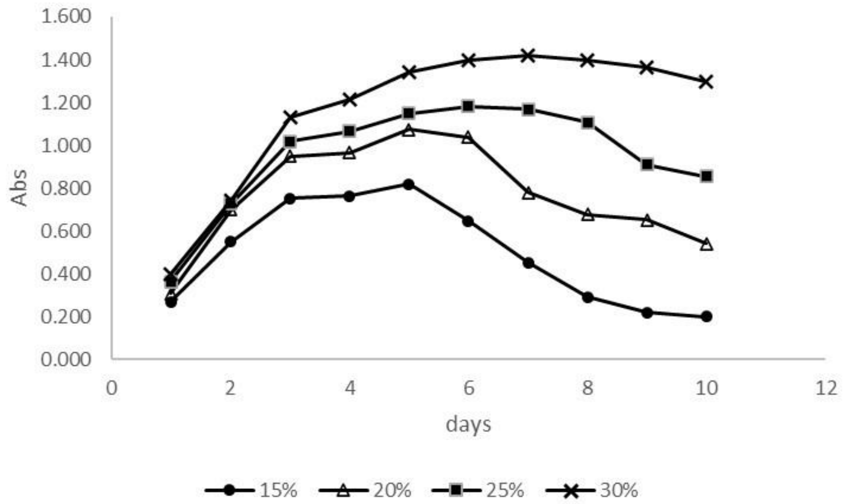

Figure 1. Optical density (OD) of *C. sorokiniana* in various concentrations of tofu wastewater media

The *C. sorokiniana* quickly adapted to tofu waste media and utilized the nutrients in the media. It was shown by the rapid adaptation/lag phase. The length of the lag phase depends on the amount and age of the inoculum and the substrate used as the medium. The availability of nutrients is the limiting growth factor and affects the adaptability of microalgae. Nutrient imbalance causes inhibition of the reduction process of organic compounds and the growth of microalgae (Rini 2012).

The results showed that *C. sorokiniana* could be cultivated in tofu liquid waste media because it contains many organic substances, such as proteins, carbohydrates, and fats. The growth of microalgae *C. sorokiniana* is influenced by various factors, i.e., the availability of macro and microelements in the medium. The limited amount of nutrients can eliminate the ability of cells to build functional structures (Chrismadha et al. 2006). Nitrogen, phosphorus, and potassium are the macro elements that play a role in C. sorokiniana growth. The tofu wastewater used in this study contains 0.02% nitrogen, 0.01% phosphor, and 0.07% potassium.

Nitrogen is essential in the growth and formation of essential compounds such as proteins, chlorophyll, lipids (Yusandi 2010), amino acids, nucleic acid amides, coenzymes, and many essential compounds. Phosphorus in the growth media could be used as an energy reserve and a constituent of the energy-rich compound; it is also used for genetic information systems, cell membranes, and phosphoproteins (Intaglietta 1977). The limited availability of phosphorus influences the production of nucleic acids. It can reduce protein formation, cell formation, or cell division (Komarawidjaja 2011) and microalgae growth rate (Ji and Sherrell 2008). Meanwhile, potassium plays a role in carbohydrate metabolism and is a cofactor for several coenzymes (Munir et al. 2017).

Secondary metabolites in ethanol extract of *C. sorokoniana*

The results of phytochemical screening showed that the ethanolic extract of *C. sorokiniana* contained alkaloids, flavonoids, steroids, saponins, and phenolics (Table 1).

The alkaloid test of the ethanolic extract of microalgae *C. sorokiniana* showed positive results in all three reagents. There are three alkaloid biosynthesis, i.e., the mevalonic acid, phenylpropanoid, and poly acetic acid pathways. Furthermore, based on GC-MS analysis, chlorella extract contained several secondary metabolites, especially alkaloid compounds (Olasehinde et al. 2019) with antioxidant activity (Bariyyah et al. 2013).

The total phenolic content of the ethanolic extract of *C. sorokiniana* was 18.39 ± 0.29 mgGAE/g. Phenolic compounds can scavenge DPPH free radicals, and the OH group in phenolic compounds strongly influenced the ability to scavenge DPPH (Nakiboglu et al. 2007). The chemical structure, number, and position of hydroxy and methyl groups on the ring determine the difference in the antioxidant activity of phenolic. The more substituted hydroxyl groups in the molecule, the stronger the free radical scavenging ability because more hydrogen atoms can be donated (Lin et al. 2009). Phenolic compounds have several biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects (Cotta et al. 2012).

Table 1. Phytochemical test results of ethanol extract of *Chlorella sorokiniana*

Test	Results
Alkaloid	+++
Flavonoids	+++
Steroids	+++
Saponins	+
Tannins	+++

Note: (-) Negative; (+) Not Concentrated; (++) Concentrated; (+++) Very Concentrated

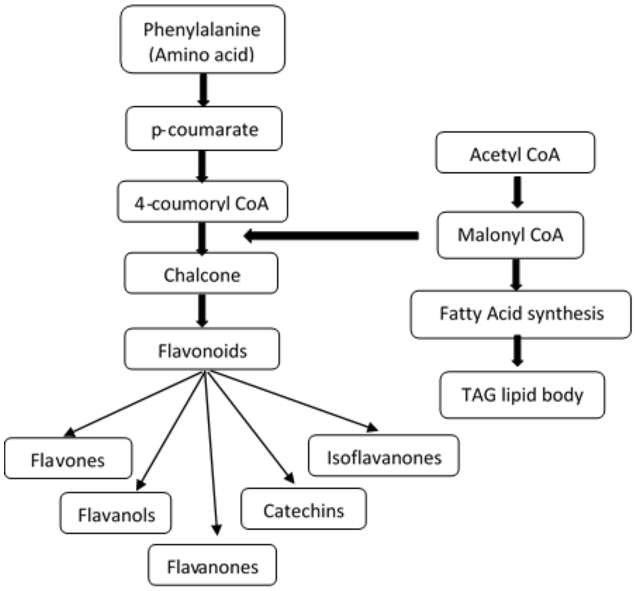

Figure 2. Flavonoid biosynthetic pathway (Yadavalli et al. 2020)

The total flavonoid content of the ethanol extract of *C. sorokiniana* was 31.93 ± 5.60 mgQE/g. Flavonoids produced by the phenylpropanoid metabolism pathway lead to nine main sub-groups: colorless chalcone, aurone, isoflavonoids, flavones, flavonols, flavandiols, anthocyanins, condensed tannins and phlobaphene pigments (Winkel-Shirley 2001; Yadavalli et al. 2020). Algae produce polyketides by condensing acetyl-CoA, leading to flavones and flavonols catalyzed by chalcone synthase (CHS). Phenylalanine produced 4-coumaroyl CoA (Figure 2) (Yadavalli et al. 2020).

Antioxidant activity of ethanol extract of *C. sorokoniana*

Based on the results, the ethanolic extract of *C. sorokiniana* had an IC_{50} value of 288.95 mg/L, which was classified as weak antioxidant activity (Jun et al. 2003), while quercetin, as a positive control, had an IC_{50} value of 7.74 mg/L which was classified as very strong antioxidant activity. The antioxidant activity of *C. sorokiniana* ethanol extract cultured in tofu waste (7,360 µg/mL) was higher than that of *C. sorokiniana* cultured in Seuoka Culture Medium, which was 2,062 µg/mL (de Carvalho et al. 2020). Therefore, tofu wastewater could be used as a culture medium for *C. sorokiniana*, and further research should be done to isolate bioactive compounds to get strong antioxidant activity.

Secondary metabolites in the ethanolic extract of *C. sorokiniana* contributed to the antioxidant activity. Flavonoids are compounds that act as antioxidants. The antioxidant mechanism of flavonoids is to scavenge reactive oxygen species (ROS) directly, prevent regeneration and indirectly increase the antioxidant activity of cellular antioxidant enzymes (Akhlaghi and Bandy 2009). There are several ways to prevent ROS formation by flavonoids, namely by inhibiting xanthine oxidase and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase enzymes and chelating metals (Fe^{2+} and Cu^{2+}) to inhibit redox reactions that produce free radicals (Atmani et al. 2009).

In conclusion, microalgae *C. sorokiniana* cultured in tofu wastewater had the best growth at a concentration of 30% at 7 days of cultivation. The ethanol extract of *C. sorokiniana* contained phenolic compounds (18.39 ± 0.29 mgGAE/g) and flavonoids (31.93 ± 5.60 mgQE/g). The IC_{50} of the ethanolic extract of *C. sorokiniana* was 288.95 mg/L, categorized as a weak antioxidant.

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REFERENCES

Akhlaghi M, Bandy B. 2009. Mechanisms of flavonoid protection against myocardial ischemia-reperfusion injury. J Mol Cell Cardiol 46 (3): 309-317. DOI: 10.1016/j.yjmcc.2008.12.003.

Atmani D, Chaher N, Berboucha M, Ayouni K, Lounis H, Boudaoud H, Debbache N, Atmani D. 2009. Antioxidant capacity and phenol content of selected Algerian medicinal plants. Food Chem 112 (2): 303-309. DOI: 10.1016/j.foodchem.2008.05.077.

Azaman SN, Nagao N, Yusoff FM, Tan SW, Yeap SK. 2017. A comparison of the morphological and biochemical characteristics of *Chlorella sorokiniana* and *Chlorella zofingiensis* cultured under photoautotrophic and mixotrophic conditions. Peer J 5 (1): e3473. DOI: 10.7717/peerj.3473.

Bariyyah SK, Hanapi A, Fasya AG, Abidin M. 2013. Uji aktivitas antioksidan terhadap DPPH dan identifikasi golongan senyawa aktif ekstrak kasar mikroalga *Chlorella* sp. hasil kultivasi dalam medium ekstrak tauge. Alchemy 2 (3): 195-204. DOI: 10.18860/al.v0i0.2890. [Indonesian]

Cerón MC, García-Malea MC, Rivas J, Acien FG, Fernandez JM, Del Río E, Guerrero MG, Molina E. 2007. Antioxidant activity of *Haematococcus pluvialis* cells grown in continuous culture as a function of their carotenoid and fatty acid content. Appl Microbiol Biotechnol 74 (5): 1112-1119. DOI: 10.1007/s00253-006-0743-5.

Chen CY, Kuo EW, Nagarajan D, Ho SH, Dong C, Di Lee, DJ, Chang JS. 2020. Cultivating *Chlorella sorokiniana* AK-1 with swine wastewater for simultaneous wastewater treatment and algal biomass production. Bioresour Technol 302: 122814. DOI: 10.1016/j.biortech.2020.122814.

Chrismadha T, Panggabean L, Mardiati Y. 2006. Pengaruh konsentrasi nitrogen dan fosfor terhadap pertumbuhan, kandungan protein, karbohidrat dan fikosianin pada kultur *Spirulina fusiformis*. J Berita Biologi 8 (3): 163-169. [Indonesian]

Cotta SR, da Mota FF, Tupinambá G, Ishida K, Rozental S, e Silva DO, da Silva AJR, Bizzo HR, Alviano DS, Alviano CS, Seldin L. 2012. Antimicrobial activity of *Paenibacillus kribbensis* POC 115 against the dermatophyte *Trichophyton rubrum*. World J Microbiol Biotechnol 28 (3): 953-962. DOI: 10.1007/s11274-011-0893-1.

de Carvalho EM, Ramos MM, Ansilago M, Mussury RM, Velasques J. 2020. Enhancing secondary metabolites in *Chlorella sorokiniana* using alternative medium with vinasse. Preprints 2020: 2020080541. DOI: 10.20944/preprints202008.0541.v1.

de-Bashan LE, Trejo A, Huss VAR, Hernandez JP, Bashan Y. 2008. *Chlorella sorokiniana* UTEX 2805 a heat and intense, sunlight-tolerant microalga with potential for removing ammonium from wastewater. Bioresour Technol 99 (11): 4980-4989. DOI: 10.1016/j.biortech.2007.09.065.

Goiris K, Muylaert K, Fraeye I, Foubert I, De Brabanter J, De Cooman L. 2012. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. J Appl Psychol 24 (6): 1477-1486. DOI: 10.1007/s10811-012-9804-6.

Hadiyanto, Azim M. 2012. Mikroalga Sumber Pangan dan Energi Masa Depan. Edisi Pertama. UPT Universitas Diponegoro Press, Semarang. [Indonesian]

Haneda RN. 2015. Biochemical composition of *Chlorella sorokiniana* grown in a novel design of hybrid photobioreactor. J Microbial Biochemical Technol 07 (02): 76-82. DOI: 10.4172/1948-5948.1000185.

Harbone JB. 1996. Metode Fitokmia Penuntun Cara Modern Menganalisis Tumbuhan. Institut Teknologi Bandung, Bandung. [Indonesian]

Herrero M, Jaime L, Martín-Álvarez PJ, Cifuentes A, Ibáñez E. 2006. Optimization of the extraction of antioxidants from *Dunaliella salina* microalgae by pressurized liquids. J Agric Food Chem 54 (15): 5597-5603. DOI: 10.1021/jf060546q.

Intaglietta M. 1977. Measurement of flow dynamics in the microcirculation. Med Instrum 11 (3): 149-52.

Istirokhatun T, Aulia M, Utomo S. 2017. Potensi *Chlorella* sp. untuk menyisihkan COD dan nitrat dalam limbah cair tahu. J Presipitasi: Media Komunikasi dan Pengembangan Teknik Lingkungan 14 (2): 88. DOI: 10.14710/presipitasi.v14i2.88-96. [Indonesian]

Janssen M, Kuijpers TC, Veldhoen B, Ternbach MB, Tramper J, Mur LR, Wijferls RH. 1999. Spesific growth rate of *Chlamydomonas reinhardtii* and *Chlorella sorokiniana* under medium duration light/dark cycles; 13-87s. J Biotechnol 70: 323-333. DOI: 10.1016/S0168-1656(99)00084-X.

Jerez-Martel I, García-Poza S, Rodríguez-Martel G, Rico M, Afonso-Olivares C, Gómez-Pinchetti JL. 2017. Phenolic profile and antioxidant activity of crude extracts from microalgae and cyanobacteria strains. J Food Qual 2017 (4): 1-8. DOI: 10.1155/2017/2924508.

Ji Y, Sherrell RM. 2008. Differential effects of phosphorus limitation on cellular metals in *Chlorella* and *Microcystis*. Limnol Oceanogr 53 (5): 1790-1804. DOI: 10.4319/lo.2008.53.5.1790.

Jun M, Fu HY, Hong J, Wan X, Yang CS, Ho CT. 2003. Comparison of antioxidant activities of isoflavones from kudzu root (*Pueraria lobata* Ohwi). J Food Sci 68 (6): 2117-2122. DOI: 10.1111/j.1365-2621.2003.tb07029.x.

Komarawidjaja W. 2011. Industri pengolahan rumput laut sebagai media kultur mikroalga *Chlorella* sp. Teknologi Lingkungan 12 (3): 241-250. DOI: 10.29122/jtl.v12i3.1232. [Indonesian]

Kusumah RR. 2019. Total Flavonoid dan Aktivitas Antioksidan dari Ekstrak Metanol Buah Bisbul (*Dyospyros discolor)*. [Skripsi]. Universitas Nusa Bangsa. Bogor. [Indonesian]

Lai PF. 2017. Optimizing extraction process and characterization of antioxidant ingredients from *Chlorella sorokiniana*. MOJ Food Process Technol 5 (1): 202-210. DOI: 10.15406/mojfpt.2017.05.00114.

Li Y, Lian S, Tong D, Song R, Yang W, Fan Y, Qing R, Hu C. 2011. One-step production ofbiodiesel from *Nannochloropsis* sp. on solid base Mg-Zr catalyst. Appl Energ 88: 3313-3317. DOI: 10.1016/j.apenergy.2010.12.057.

Li Y, Zhao ZK, Bai F. 2007. High-density cultivation of oleaginous yeast Rhodosporidium toruloides Y4 in fed-batch culture. Enzyme Microb Technol 41: 312-317. DOI: 10.1016/j.enzmictec.2007.02.008.

Lin HY, Kuo YH, Lin YL, Chiang W. 2009. Antioxidative effect and active components from leaves of lotus (*Nelumbo nucifera*). J Agric Food Chem 57 (15): 6623-6629. DOI: 10.1021/jf900950z.

Lizzul AM, Lekuona-Amundarain A, Purton S, Campos LC. 2018. Characterization of *Chlorella sorokiniana*, UTEX 1230. Biology 7 (2): 1-12. DOI: 10.3390/biology7020025.

Makareviciene PDV, Andrulevičiūtė V, Skorupskaitė V, Kasperovičienė J. 2011. Cultivation of microalgae *Chlorella* sp. and *Scenedesmus* sp. as a potential biofuel feedstock. Environ Res Eng Manag 57 (3): 21-27.

Matsukawa R, Hotta M, Masuda Y, Chihara M, Karube I. 2000. Antioxidants from carbon dioxide fixing *Chlorella sorokiniana*. J Appl Psychol 12 (3-5): 263-267. DOI: 10.1023/a:1008141414115.

Munir F, Hariyati R, Wiryani E. 2017. Pengaruh limbah cair tahu terhadap pertumbuhan populasi *Chlorella pyrenoidosa* H. Chick dalam skala laboratorium. J Biologi 6 (2): 84-92. [Indonesian]

Nakiboglu M, Urek RO, Kayali HA, Tarhan L. 2007. Antioxidant capacities of endemic *Sideritis sipylea* and *Origanum sipyleum* from Turkey. Food Chem 104 (2): 630-635. DOI: 10.1016/j.foodchem.2006.12.012.

Napitupulu HP. 2019. Pengaruh Perbedaan Fotoperiode Pada Kultur *Chlorella* sp. dengan Sistem Fotobioreaktor Kuntinu. [Thesis]. Universitas Riau, Pekanbaru. [Indonesian]

Nurjana, Abdullah A, Apriandi A. 2011. Aktivitas antioksidan dan komponen bioaktif keong ipong-ipong (*Fasciolaria salmo*) antioxidant activity and bioactive compound of ipong-ipong snail (*Fasciolaria salmo*). J Pengolahan Hasil Perikanan Indonesia 14 (1): 22-29. [Indonesian]

Olasehinde TA, Odjadjare EC, Mabinya LV, Olaniran AO, Okoh AI. 2019. *Chlorella sorokiniana* and *Chlorella minutissima* exhibit antioxidant potentials, inhibit cholinesterases and modulate disaggregation of β- amyloid fibrils. Electron J Biotechnol 40: 1-9. DOI: 10.1016/j.ejbt.2019.03.008.

Ramanna L, Guldhe A, Rawat I, Bux F. 2014. The optimization of biomass and lipid yields of *Chlorella sorokiniana* when using wastewater supplemented with different nitrogen sources. Bioresour Technol 168: 127-135. DOI: 10.1016/j.biortech.2014.03.064.

Rao AR, Sarada R, Baskaran V, Ravishankar GA. 2006. Antioxidant activity of *Botryococcus braunii* extract elucidated in vitro models. J Agric Food Chem 54 (13): 4593-4599. DOI: 10.1021/jf060799j.

Rini IS. 2012. Pengaruh Konsentrasi Limbah Cair Tahu Terhadap Pertumbuhan dan Kadar Lipid *Chlorella* sp. [Skripsi] Universitas Islam Negeri Maulana Malik Ibrahim, Malang. [Indonesian]

Susanty D, Oksari AA. 2020. Growth and secondary metabolites content of chloroform extract of *Chlorella* sp. and *Chlorella sorokiniana* cultured on chicken broiler waste media. Nusantara Biosci 12 (1): 28-32. DOI: 10.13057/nusbiosci/n120105.

Syaichurrozi I, Jayanudin Jayanudin. 2016. Kultivasi *Spirulina platensis* pada media bernutrisi limbah cair tahu dan sintetik. JBAT 5 (2): 68-73. DOI: 10.15294/jbat.v5i2.7398. [Indonesian]

Ukieyanna K. 2012. Aktivitas Antioksidan Kadar Fenolik dan Flavonoid Total Tumbuhan Suruhan (*Peperomia pellucid* L. Kunth). [Skripsi]. Institut Pertanian Bogor, Bogor. [Indonesian]

Widayat W, Hadiyanto H. 2016. Pemanfaatan limbah cair industri tahu untuk produksi biomassa mikroalga *Nannochloropsis* sp. sebagai bahan baku biodiesel. Reaktor 15 (4): 253. DOI: 10.14710/reaktor.15.4.253-260. [Indonesian]

Winkel-Shirley B. 2001. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol 126: 85-493. DOI: 10.1104/pp.126.2.485.

Yadavalli R, Ratnapuram H, Motamarry S, Reddy CN, Ashokkumar V, Kuppam C. 2020. Simultaneous production of flavonoids and lipids from *Chlorella vulgaris* and *Chlorella pyrenoidosa*. Biomass Conversion and Biorefinery. Springer. DOI: 10.1016/j.crcon.2019.10.003.

Yusandi F. 2010. Pengaruh Nitrogen Terhadap Kandungan Essensial Biomassa *Chlorella vulgaris* Buitenzorg. [Hon. Thesis]. Universitas Indonesia, Jakarta. [Indonesian]