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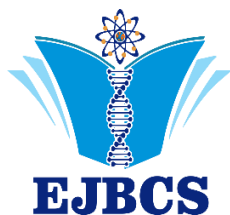
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Utilization of hydrothermal process water for microalgal growth

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Abstract: Microalgae are one of the most effective biological sources for renewable energy production. They can be produced at rates that can be 50 times more than that of the conventional crops. They have a production capacity throughout the year. Comparing with other biomass sources such as terrestrial, agricultural and solid waste, algal biomass provides a more stable and manageable energy production system. However, there are some constraints for efficient microalgae production such as the need for large quantities of nutrients, high cost of installations and operation of production systems. For these reasons, using wastewaters obtained from different processes as a medium to grow microalgae has attracted new research interest. In the present study, the aqueous phase obtained from hydrothermal carbonization of orange pomace was utilized as a nutrient source in *Chlorella minutissima* growth. Different dilution rates (50x, 100x, 200x and 400x) were used to observe the effect of aqueous phase concentration on algal growth during 30 days. According to the results of microalgae cultivation, the medium with the lowest dilution rate was determined as the optimum medium because of giving the best growth value compared to other dilution rates.

Keywords: Microalgae, *Chlorella minutissima*, hydrothermal carbonization, HTC process water, algal cultivation

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1. Introduction

Microalgae have extensive application potential in different areas such as nutraceutical industries, renewable energy, and biopharmaceutical and because of this potential, they are considered as important resources all around the world. Researchers investigate several microalgae species for their potential to produce specific value-added products that have remarkable biological and pharmacological qualities. Also, they have the specific ability for the conversion of atmospheric CO₂ to several products such as lipids, carbohydrates, protein, and different bioactive metabolites. Although microalgae usage in different application areas is a feasible way to obtain value-added products in the biopharmaceutical and bioenergy fields, researchers have identified some challenges and limitations that must be tackled to improve algal biotechnology systems for industrial application such as enhancing and stabilization of microalgae growth rate, efficient product synthesis, and pretreatment of biomass (Khan et al. 2018). To overcome these challenges and limitations, large scale microalgae cultivation must be provided by cost-effective systems and high-value products. According to the studies, different species of microalgae require different media depending on their needs but in general almost all species need major

requirements that are nitrogen, iron, phosphorous, inorganic and organic carbon sources for their growth (Grobbelaar et al. 2004). This situation is a key to provide a feasible, sustainable and economically viable algal biotechnology system with the help of successful cultivation processes for efficient algae production. Nowadays, pollution related problems originated from human being are the main concerns of the society. Especially pollution of freshwater is a huge problem to ensure the continuity of life. This problem motivates the researchers to investigate novel techniques to prevent pollution. As a result of the investigations, they realized that microalgae species provide an elegant way to solve water pollution problems with tertiary and quinary treatment methods because they have specific abilities to use inorganic nitrogen and phosphorous during their growth processes and also they can remove some toxic organic compounds and heavy metals without secondary pollution (Richmond et al. 1986; Oswald et al. 1988a; Tam et al. 1995; Rai et al. 1981). While the search for new sustainable and clean energy sources and wastewater treatment systems are continuing, society has recognized novel sustainable resources such the utilization of waste organic materials providing to reduce the potential risk and amount of greenhouse gas emissions (Hastings et

al. 2009; Hillier et al. 2009). As a result, hydrothermal processes have been accepted as environmentally friendly and economically viable methods. In 1913, Bergius discovered hydrothermal carbonization (HTC) that is a specific process mimicking the natural process of coal formation with the help of cellulose conversion into coal-like materials. During the HTC process, lignite-like solid products and an aqueous phase (AP) are obtained from a raw biomass material (Marinovic et al. 2015). At the beginning of the HTC studies, researchers focused on the solid product from treatment, but in recent years the unwanted liquid part has received increased attention because of its potential. Current studies showed that aqueous phase has nitrogen, high amounts of organic carbon and several toxic components such as cyclic oxygen and heavy metals. AP from HTC may support microorganisms and plant growth with several essential nutrients in its composition. If AP is utilized as the only nutrient source, the dilution rate of AP is important to provide efficient microalgae growth, while high AP dilution causes low nutrient concentration in the medium. Low AP dilution leads obviously to growth inhibition because of excessive toxic substances. With a suitable dilution rate, AP may be used as nutrition sources for algae. For example, *Chlorella vulgaris* were grown in AP from HTC of *Nannochloropsis oculata*. Growth rates in AP were determined higher than synthetic growth medium (BG-11) and no inhibition was observed (Du et al. 2012). Biller et al. (2012) reported that *Chlorella vulgaris*, *Spirulina platensis* and *Scenedesmus Dimorphous* were successfully cultivated in AP. HTC process water from activated sludge was also used as a media for *Chlorella sp.* and *Coelastrella sp.* and it was reported that growth rates in BG-11 and AP were similar and no inhibition in algae growth was observed (Belete et al. 2019). If the challenges and problems about safe disposal of AP in hydrothermal treatment and necessary nutrient requirements for mass microalgae cultivation are considered simultaneously, the utilization of the liquid phase from HTC for microalgal growth is an excellent idea. This process can provide a cost-effective close-loop system that includes the recovery of nutrients from waste AP from HTC and efficient microalgal growth with the help of these nutrients in an integrated system. The present study aimed to utilize the process water from HTC of orange pomace for efficient microalgal cultivation and high productivity rate and to treat HTC process water by using this cost-effective and environmentally friendly method. Firstly, HTC was performed for orange pomace and the obtained process water was characterized. Subsequently, unicellular marine algae "*Chlorella minutissima*" were cultivated in HTC process water diluted in different ratios. Finally, microalgae growth in AP, characterization of algae cultivated in synthetic medium and for various dilutions of the HTC process water were evaluated and discussed.

2. Materials and Method

2.1 Microalgae Culture and HTC Process Water

Wild type *Chlorella minutissima* obtained from the Culture Collection of Algae at Göttingen University (Goettingen, Germany) was used as the algal strain in this study. HTC

experiments with orange pomace (Valencia-Spain) were carried out in a 300 ml batch reactor (Top Industries, France) made of a nickel-base alloy (Inconel 718). For each experiment, 30 g of dried orange pomace and 180 g of distilled water were used as 1/6 of biomass to water weight ratio. HTC was conducted at 200 °C for 1 h (Ozcimen et al. 2019). Büchner filtration was used in the separation of solid and liquid products. The hydrochar product was dried in an oven at 105 °C for 24 h and stored for other studies. The liquid phase was weighed, stored and protected from light in a refrigerator at 4 °C until the use in this study.

2.2 Cultivation of Algae in AP from HTC

The algal strain was cultivated as 10% of microalgae to medium volume ratio in total 150 ml including BG-11 medium and 2 g/L glucose to provide enough starting cultures for this study. 50x, 100x, 200x and 400x dilution rates were used for AP medium and dilution of AP was performed with distilled water. BG-11 medium was used in the control group to understand the growth efficiency of *Chlorella minutissima* in the aqueous phase diluted in a different ratio. After the inoculation of microalgae, 250 ml Erlenmeyer flasks that include 150 ml autoclaved medium and *Chlorella minutissima* were incubated in an illuminated incubator–shaker at 150 rpm, 25 ± 3 °C under continuous cool-white fluorescent light (8000 lx) for 30 days. To observe algal growth simultaneously, samples were taken from the culture media day by day and algal growth was determined with optical density measurement at 680 nm (OD₆₈₀) using a UV-Vis spectrophotometer (PG Instruments T60V) and the growth rate was calculated from the following relationship: $GR = (\ln OD_t - \ln OD_0) / t$ where OD₀ is the optical density at the initial day, OD_t is the optical density measured on day t (Wang et al., 2010).

2.3 Characterization Analysis

After the 30 days cultivation period, algae were analyzed for the total carbohydrate, protein, lipid content and dry weight determination. Total carbohydrate, protein, and lipid content were determined with the phenol-sulfuric acid method (Dubois et al. 1965), modified Lowry method (Lowry et al. 1951) and modified Bligh and Dyer (1959) method, respectively. The functional groups of microalgae cultivated in different media were analyzed by Fourier transform infrared spectroscopy (FTIR) using FT-IR spectrometer (Bruker Alpha). Each spectrum was recorded in a wavenumber range from 4000 cm⁻¹ to 500 cm⁻¹. For the analysis of the aqueous phase, pH and electrical conductivity were measured by M1000 Benchtop pH and conductivity meters (labForce). The acidity value analysis of the process water was determined with a wet analysis method (ASTM D1067–16). Total organic carbon (TOC) was measured with a TOC analyzer (VarioTOC) with 0.2 mL injection volume and the aqueous phase was analyzed for chemical oxygen demand (COD) using COD Cell Test Tube C4/25 tube in S6 photometer (photoLab). Formic acid, lactic acid, acetic acid, hydroxymethylfurfural (HMF), and furfural analysis were conducted with HPLC (Spectra Physic).

3. Results and Discussion

3.1 Characterization of Hydrothermal Process Water

Hydrothermal carbonization of orange pomace was performed at 200°C for 1 h to analyze. The AP from HTC was used for algal cultivation. Lower temperature and shorter treatment time were applied to prevent the formation of toxic compounds limiting algal growth and loss of energy. To determine the most suitable dilution rate of AP for algal growth, AP was analyzed and physicochemical properties were revealed as it is seen in Table.1. The pH of AP from hydrothermal treatment may vary from 3.7 to 10.0 depending on biomass composition. In this study, this value was determined as 4.2. Its acidic structure and high carbohydrate/low protein content of biomass may lead to organic acid release to AP and also insufficient ammonia derived low protein content can be the reason of low pH of AP. Orange pomace is a complex source including pectins, carotenoids, polyphenols and flavonoids (Benelli et al. 2010) and it was reported that those compounds mostly dissociated at 175°C in water treatment (Hoshino et al. 2009; Wang et al. 2014; Wijngaard et al. 2012; Wijngaard and Brunton, 2009). Also, orange pomace consists of lignin in small quantities, cellulose, and hemicellulose. Hemicellulose decomposes at 180°C and affects AP composition. Total organic carbon (TOC) provides the determination of organic molecules or pollutants in the sample, chemical oxygen demand (COD) is the amount of oxygen needed for oxidizing all organic carbon completely and the COD value is used for the interpretation of inorganic material in the sample. In the study of Erdogan et al. (2015), TOC value was determined as 22.79 g/l and COD value was 0.90 g/l for orange pomace. For the HTC treatment performed at 175°C for 60 min, TOC and COD values were reported as ~25 g/l and ~65 g/l, respectively. For different treatments conducted at 190°C for 60 min, values were reported as ~22g/l and ~58g/l. TOC (22.79 g/l) was much more than COD value (0.90 g/l) and this shows that the amount of organic substances was higher compared with inorganic substances in AP. Generally, the carbon content calculated from the percentage of TOC is referred as the yield of AP, and high yield of AP was obtained from lignocellulosic biomasses (Panisko, Wietsma, and Lemmon et al. 2015) and food wastes (Maddi, Panisko, and Wietsma et al. 2017). Madsen et al. (2016) showed that decrease in the TOC values of AP from hydrothermal treatment was observed in the order of protein, carbohydrate and lipid, which means that TOC concentration of AP is mostly affected by the protein content of biomass. In our study, formic acid, lactic acid and acetic acid content of AP were analyzed using HPLC and determined as 2193.5, 1823 and 1646.5 ppm, respectively. According to studies of Erdogan et al. (2015) for HTC treatment performed at 175°C for 60 min, those values were reported as 0.9, 4.1, 1.7 g/l. For different treatments conducted at 190°C for 60 min, formic acid, lactic acid, acetic acid contents of AP were reported as 1.9, 5.2 and 2.5 g/l respectively. Short-chain organic acids such as acetate or acetic acid that are mainly formed with the decomposition of proteins and carbohydrates are mostly abundant organic acids in AP from hydrothermal treatment.

It is a known fact that acetate or acetic acid promotes mixotrophic growth rate of algae that increases recycling carbon and productivity as acting substrate (Bhatnagar et al. 2011). Also, the acid content in AP is affected by the decomposition of hemicellulose derivatives. 9.99% hemicellulose content in biomass reported by Rivas-Cantu et al. (2013) may have affected the AP content and increased the acid content and acidity (Li, Zhang, and Zhu et al. 2017). In our study, the concentration of HMF and furfural were also analyzed and found as 2617.9 and 794.7 ppm, respectively. In the study of Erdogan et al. (2015), the concentrations of HMF and furfural were reported as 312.1, 679.2 for HMF and 1585.2, 1180.1 for furfural ppm for two different temperature conditions (175 and 190°C). The degradation of polyphenol and organic acid derived from sugars leads to the formation of furfurals (Wijngaard and Brunton 2009). The concentration of HMF and furfural that was higher than 1 mM caused a decrease in the algal growth rate and the time needed to achieve maximum population density was prolonged. If those values were greater than 7 mM, complete inhibition was observed for algal growth due to direct inhibition of photosynthesis system. However no inhibitory effect was observed at concentrations lower than 1 mM (Yu et al. 1990). To decrease the inhibitory effect of these toxic compounds and observe the effect of nutrient concentrations on algal growth, different dilution rates (50x, 100x, 200x, 400x) were used in AP for the growth medium of algae.

Table 1. Physicochemical characteristics of AP from HTC

pH	Acidity	Conductivity (mS/cm)	TOC (g/l)	COD (g/l)	Formic acid (ppm)	Lactic acid (ppm)	Acetic acid (ppm)	HMF (ppm)	Furfural (ppm)
4.2	0.135	3.6	22.79	0.90	2193.5	1823	1646.5	2517.9	794.7

3.2 Algal Growth and Characterization of Algae

Algal growth in terms of optical density was observed during 30 days at 680 nm and the growth curve was plotted using these data as it is seen in Figure 1. *Chlorella minutissima* directly adapted to the diluted AP medium besides synthetic growth medium (BG-11). The shorter adaptation time provides a time-efficient growth process to obtain maximum population density in a short time interval. The growth rate of microalgae was calculated for different dilution rates and synthetic medium. For BG-11, 50x, 100x, 200x and 400x diluted AP, those values were determined as 0.0607, 0.054, 0.05, 0.047, 0.046 g l⁻¹ day⁻¹ and final biomass concentration were 1.313, 1.06, 0.887, 0.871 and 0.848 g/l; respectively. The growth rate of *Chlorella minutissima* in BG11 is compatible with the result of Jena et al. (2011). The high final biomass concentrations and productivity rate were observed with diluted AP medium. Among the four dilutions of the aqueous phase from HTC, both concentrations of final biomass and biomass productivity were in the following order: 50x > 100x > 200x > 400x. These results show that nutrient amount in AP decreased with the increasing dilution rate. In higher concentrations growth inhibition of toxic substances was not observed. However the maximum biomass productivity (0.054) among different dilutions was lower than synthetic medium with minor deviation. The most possible reason for

this decline may be the extremely low COD value of AP. As mentioned before, COD is an indirect measure of inorganic compounds in the sample. Especially in low concentrations of AP, COD was in extremely low concentration and this situation may have caused inorganic nutrient deficiencies in algae.

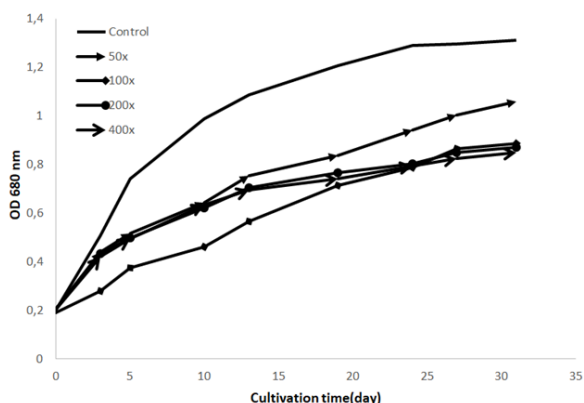


Figure 1. Algal growth curves in controlled media and different dilutions of AP

Dry weight, carbohydrate, protein and lipid contents of *Chlorella minutissima* cultivated in different dilution ratios and BG-11 medium were analyzed. Total carbohydrate, protein and lipid content based on dry weight are shown in Table 2. Among all nutrients accumulated in the algae structure, carbohydrate was detected with the highest amount. Microalgae may be used for different uses depending on their structural composition. Thus, it is very important to determine carbohydrate, protein and lipid contents of algae cultivated in AP from HTC. Also, analyzing the effect of different dilution rates of AP on the algal content is important to determine the efficiency of the AP medium. The lipid content of algae is an important parameter because it may be utilized in biodiesel production. It is seen in Table 2 that microalgae cultivated in all dilutions of AP had higher lipid content compared to that cultivated in BG-11. However, *Chlorella minutissima* grown in BG-11 had higher lipid content than the reported results (Sharma et al. 2016; Khan et al. 2018). The algal biomass after cultivation can be utilized as protein-rich high quality aquacultural or animal feed additives (Molino et al. 2018; Beneman et al. 2013).

Table 2. Dry weight, carbohydrate, protein and lipid content analysis of microalgae cultivated in BG-11 and different dilutions of AP

	BG-11	50X	100X	200X	400X
Dry weight (g/l)	0.345	0.279	0.233	0.229	0.223
Carbohydrate (%)	36.33	35.22	34.80	34.13	34.73
Protein (%)	33.08	32.56	33.14	32.32	31.80
Lipid (%)	23.19	25.13	23.60	24.06	24.68

Fourier transform infrared spectroscopy (FTIR) was performed in a wavenumber range from 4000 cm⁻¹ to 500 cm⁻¹. FTIR spectra of microalgae cultivated in various dilutions of AP and BG-11 are shown in Figure 2 which shows that spectra for microalgae cultivated in AP and synthetic medium are similar.

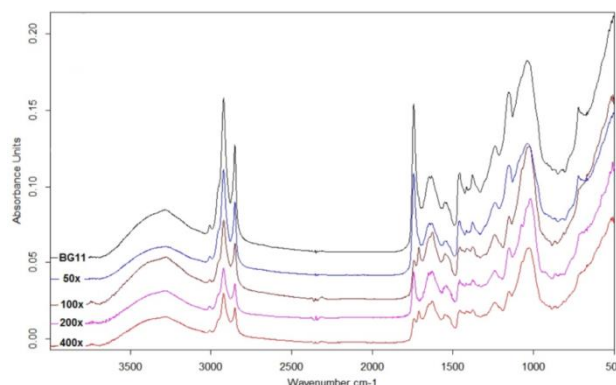


Figure 2. The FTIR spectra of microalgae cultivated in different media

The FTIR spectrum of *Chlorella minutissima* showed different characteristic peaks around 3300 cm⁻¹, 2900- 2800 cm⁻¹, 1750-1500 cm⁻¹ and 1085-1050 cm⁻¹. The absorption at 3300 cm⁻¹ can be assigned to the stretching vibrations of OH groups (Radhika and Mohaideen 2015) and derived moisture content of algae (Gibbons et al. 1968; Karbowiak et al. 2011). The weak peak around 2900-2800 cm⁻¹ is typical for C-H stretching derived vibration of CH₂. The peaks around 1750 cm⁻¹ and 1085-1050 cm⁻¹ are related with C=O and C-O stretching, respectively (Yang et al. 2015). According to Giordano et al. (2010) the typical band around 1750-1500 cm⁻¹ can be assigned to lipids bands, 1160-1540 cm⁻¹ were characteristics of proteins and the bands at 1085-1050 cm⁻¹ were characteristics of carbohydrates.

5. Conclusion

The concentration of AP from HTC of orange pomace was optimized for higher productivity of *Chlorella minutissima*. The highest productivity was observed for 50x dilution of AP. Content analysis of microalgae cultivated in dilutions of AP and BG-11 showed that microalgae may be utilized for different usages such as agricultural, animal feed or biodiesel sources because of their high protein and lipid contents. These results proved that the aqueous phase obtained as a waste from the HTC process can be used in the cultivation of *Chlorella minutissima*. It is then demonstrated that the waste HTC liquid can be used as an alternative growth medium instead of using the high-cost media required for the production of algae. In addition, the HTC process water can be disposed with a cost-free and environmentally friendly method as well.

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Conflict of interest disclosure:

There was no conflict of interest.

References

- Belete YZ, Leu S, Boussiba S, Zorin B, Posten C, Thomsen L, Wang S, Gross A, Bernstein R. 2019. Characterization and utilization of hydrothermal carbonization aqueous phase as nutrient source for microalgal growth. *Bioresour Technol.* 290: 121758.
- Benelli P, Riehl CAS, Jr AS, Smânia EFA, Ferreira SRS. 2010. Bioactive extracts of orange (*Citrus sinensis* L. Osbeck) pomace obtained by SFE and low pressure techniques: mathematical modeling and extract composition. *J Supercrit Fluids.* 55: 132–141.
- Benemann J. 2013. Microalgae for biofuels and animal feeds. *Energies.* 6: 5869–5886.
- Bhatnagar A, Chinnasamy S, Singh M, Das KC. 2011. Renewable biomass production by mixotrophic algae in the presence of various carbon sources and wastewaters. *Appl Energy.* 88(10): 3425-3431.
- Biller P, Ross AB, Skill SC, Lea-Langton A, Balasundaram B, Hall C, Riley R, Llewellyn CA. 2012. Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process. *Algal Res.* 1: 70–76
- Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol.* 37(8): 911-917.
- Du Z, Hu B, Shi A, Ma X, Cheng Y, Chen P, Liu Y, Lin X, Ruan R. 2012. Cultivation of a microalga *Chlorella vulgaris* using recycled aqueous phase nutrients from hydrothermal carbonization process. *Bioresour Technol.* 126: 354-357.
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. 1956. Colorimetric method for determination of sugars and related substances. *Anal Chem.* 28: 350-356.
- Erdogan E, Atila B, Mumme J, Reza MT, Toptas A, Elibol M, Yanik J. 2015. Characterization of products from hydrothermal carbonization of orange pomace including anaerobic digestibility of process liquor. *Bioresour Technol.* 196: 35-42.
- Gibbons G, Goad L, Goodwin T. 1968. The identification of 28-isofucosterol in the marine green algae *Enteromorpha intestinalis* and *Ulva lactuca*. *Phytochemistry.* 7: 983–988.
- Giordano M, Kansiz M, Heraud P, Beardall J, Wood B, McNaughton D. 2001. Fourier transform infrared spectroscopy as a novel tool to investigate changes in intracellular macromolecular pools in the marine microalga *Chaetoceros muellerii* (bacillariophyceae). *J Phycol.* 37: 271–279
- Grobbelaar JU. 2004. Algal nutrition. In: Richmond A (ed) *Handbook of microalgal culture: biotechnology and applied phycology.* Blackwell, Oxford, pp. 97–115.
- Hastings A, Clifton-Brown J, Wattenbach M, Mitchell CP, Stampfl P, Smith P. 2009. Future energy potential of Miscanthus in Europe. *GCB Bioenergy.* 1: 180–96.
- Hillier J, Whittaker C, Dailey G, Aylott M, Casella E, Richter GM, Riche A, Murphy R, Taylor G, Smith P. 2009. Greenhouse gas emissions from four bioenergy crops in England and Wales: integrating spatial estimates of yield and soil carbon balance in life cycle analyses. *GCB Bioenergy.* 1: 267–81.
- Hoshino M, Tanaka M, Terada A, Sasaki M, Goto M. 2009. Separation and Characterization of Pectin from Juice Processing Residue Extracted By Sub- Critical Water The 5th ISFR.
- Jena U, Vaidyanathan N, Chinnasamy S, Das KC. 2011. Evaluation of microalgae cultivation using recovered aqueous co-product from thermochemical liquefaction of algal biomass. *Bioresour Technol.* 102(3): 3380-3387.
- Karbowiak T, Ferret E, Debeaufort F, Andree V, Philippe C. 2011. Investigation of water transfer across thin layer biopolymer films by infrared spectroscopy. *J Membr Sci.* 370: 82–90.
- Khan SA, Malla FA, Malav LC, Gupta N, Kumar A. 2018. Potential of wastewater treating *Chlorella minutissima* for methane enrichment and CO₂ sequestration of biogas and producing lipids. *Energy.* 150: 153-163.
- Li K, Zhang L, Zhu L, Zhu X. 2017. Comparative study on pyrolysis of lignocellulosic and algal biomass using pyrolysis-gas chromatography/mass spectrometry. *Bioresour Technol.* 234: 48-52.
- Lowry OH, Rosebrough NJ, Lewis Farr A, Randall RJ. 1951. Protein measurement with Folin phenol reagent. *J Biol Chem.* 193 (1): 265–275.
- Maddi B, Panisko E, Wietsma T, Lemmon T, Swita M, Albrecht K, Howe D. 2017. Quantitative characterization of aqueous byproducts from hydrothermal liquefaction of municipal wastes, food industry wastes, and biomass grown on waste. *ACS Sustain Chem Eng.* 5(3): 2205-2214.
- Madsen RB, Biller P, Jensen MM, Becker J, Iversen BB, Glasius M. 2016. Predicting the chemical composition of aqueous phase from hydrothermal liquefaction of model compounds and biomasses. *Energy & Fuels.* 30(12): 10470-10483.
- Marinovic A, Pileidis FD, Titirici MM. 2015. Hydrothermal carbonisation (HTC): history, state-of-the-art and chemistry. *Porous Carbon Materials from Sustainable Precursors*, 32th edn. Cambridge, UK
- Molino A, Iovine A, Casella P, Mehariya S, Chianese S, Cerbone A, Rimauro J, Musmarra D. 2018. Microalgae characterization for consolidated and new application in human food, animal feed and nutraceuticals. *Int J Environ Res Public Health.* 15: 1–21.
- Oswald WJ. 1988. Large-scale algal culture systems (engineering aspects). In: Borowitzka MA, Borowitzka LJ (eds) *Micro-Algal Biotechnology*, Cambridge Univ. Press, Cambridge, pp. 357–394.
- Ozcimen D, Missaoui A, Bostyn S, Belandria V, Gökalp I. 2019. Characterization of solid and aqueous phase products from hydrothermal carbonization of orange pomace. 2nd International Symposium on Hydrothermal Carbonization, Berlin, Germany.
- Panisko E, Wietsma T, Lemmon T, Albrecht K, Howe D. 2015. Characterization of the aqueous fractions from hydrotreatment and hydrothermal liquefaction of lignocellulosic feedstocks. *Biomass and Bioenergy.* 74: 162-171.
- Radhika D, Mohaideen A. 2015. Fourier transform infrared analysis of *Ulva lactuca* and *Gracilaria corticata* and their effect on antibacterial activity. *Asian J Pharm Clin Res.* 8: 209–212.
- Rai LC, Gour JP, Kumar HD. 1981. Phycology and heavy metal pollution. *Biol Rev.* 56: 99–151.
- Richmond A. 1986. *Handbook of microalgal mass culture*, CRC Press, Florida, 528 pp.
- Rivas-Cantu RC, Jones KD, Mills PL. 2013. A citrus waste-based biorefinery as a source of renewable energy: technical advances and analysis of engineering challenges. *Waste Manage Res.* 31(4): 413-420.
- Sharma AK, Sahoo PK, Singhal S, Patel A. 2016. Impact of various media and organic carbon sources on biofuel production potential from *Chlorella* spp. *3 Biotech.* 6(2): 116.
- Tam NFY, Wong YS. 1995. *Wastewater treatment with microorganisms.* The commercial Press (H.K.) Ltd. 2D Finnie St. Quarry Bay, Hong Kong.
- Wang L, Min M, Li Y, Chen P, Chen Y, Liu Y, Ruan R. 2010. Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Appl Biochem Biotechnol.* 162(4): 1174-1186.

- Wang X, Chen Q, Lü X. 2014. Pectin extracted from apple pomace and citrus peel by subcritical water. *Food Hydrocoll.* 38: 129–137.
- Wijngaard H, Brunton N. 2009. The optimization of extraction of antioxidants from apple pomace by pressurized liquids. *J Agric Food Chem.* 57: 10625–10631.
- Wijngaard H, Hossain MB, Rai DK, Brunton N. 2012. Techniques to extract bioactive compounds from food by-products of plant origin. *Food Res Int.* 46: 505–513.
- Yang J, Cao J, Xing G, Yuan H. 2015. Lipid production combined with biosorption and bioaccumulation of cadmium, copper, manganese and zinc by oleaginous microalgae *Chlorella minutissima* UTEX2341. *Bioresour Technol.* 175: 537-544.
- Yu S, Forsberg Å, Kral K, Pedersen M. 1990. Furfural and Hydroxymethylfurfural inhibition of growth and photosynthesis in *Spirulina*. *Br Phycol J.* 25(2): 141-148.