Chemical Data Collections 35 (2021) 100754

Contents lists available at ScienceDirect

Chemical Data Collections

journal homepage: www.elsevier.com/locate/cdc

Data Article

Tofu wastewater-derived amino acids identification using LC-MS/MS and their uses in the modification of chitosan/TiO_2 film composite

Haya Fathana [a,b], Muhammad Iqhrammullah [a,b], Rahmi Rahmi [b,*], Muhammad Adlim [a,c], Surya Lubis [b]

[a] Graduate School of Mathematics and Applied Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
[b] Chemistry Department, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
[c] Department of Chemistry Education, Faculty of Teacher Training and Education, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia

ARTICLE INFO

Keywords:
Chitosan
Amino acid
TiO_2
Simple additive weighting
Methylene blue
L-tyrosine

ABSTRACT

Amino acid is an important ingredient for material modification in the wide spectrum of fields, starts from pollutant removal up to biomedical application. Tofu wastewater has been used as a source of protein containing various amino acids. Herein, we qualitatively and quantitatively analyzed the amino acid contents of tofu wastewater by means of liquid chromatography with tandem mass spectrometry (LC-MS/MS). There were 20 amino acids identified from the tofu wastewater, dominated by L-Tyrosine (25.93%), by L-Cystine (15.75%), L-Glutamic Acid (14.36%), and L-Aspartic Acid (10.38%). The modification of chitosan using the tofu wastewater hydrolysate increased the tensile strength from 1.52 to 1.74 kgf/mm^2 and adsorption capacity from 1.06 to 2.27 mg/g. Based on the simple additive weighting analysis, the best composite was obtained with composition of 0.975 g, 0.5 mL, and 0.025 g for chitosan, amino acid, and TiO_2, respectively. In conclusion, amino acid contents in tofu wastewater could be used to improve the performance of chitosan-based film composite in removing methylene blue from water.

Specifications table

Subject area	*Physical Chemistry*
Compounds	*Chitosan-amino acid-TiO2 film*
Data category	*physicochemical and Chromatogram*
Data acquisition format	*LC-MS/MS, Spectrophotometer UV-Vis*
Data type	*Raw and analyzed,*
Procedure	*Amino Acid Hydrolysis, Adsorption process to obtain adsorption capacity and Tensile tested*
Data accessibility	

* Corresponding author.
E-mail addresses: m.iqhram@oia.unsyiah.ac.id (M. Iqhrammullah), rahmi@fmipa.unsyiah.ac.id (R. Rahmi).

https://doi.org/10.1016/j.cdc.2021.100754
Received 20 March 2021; Received in revised form 6 June 2021; Accepted 16 July 2021
Available online 18 July 2021

1. Rationale

As a soy bean derivative product, tofu contains a rich level of protein comprised of wide range of amino acids. The wastewater, or often called soy whey, resulted from the tofu production process also contains the remaining protein, making it as a potential source of amino acids [1]. The amino acids are nutrients for the growth of bacteria, leading some researchers to employ the wastewater as probiotic medium [2,3]. The growth of red spinach [4] and microalgae [5] have also been reported to be enhanced by the treatment using tofu wastewater. Other functionality of the amino acids derived from tofu wastewater is for the modification of adsorbent; as in our case, we used the amino acids for chitosan modification.

A mixture of chitosan and amino acid can yield a conjugate that is useful in controlling the properties of the material surface. Chitosan modification using glycine, for instance, could elevate the piezoelectric properties of a sensor material used in wearable biomedical diagnostics [6,7]. In the case of separation technology, the employment of chitosan modification using amino acids is even much wider, covering enzyme purification [8], drugs immobilization and specific target release [9,10], and pollutant removal [11,12]. The selection of amino acid is important, as it determines the final properties of the modified material. Therefore, data provided in our work are necessary, informing the type of amino acids contained in the tofu wastewater. Further, we used these amino acids to modify TiO_2-filled chitosan films to improve their ability in the adsorptive removal of cationic dye – methylene blue from water medium.

2. Procedure

2.1. Amino acid preparation

Amino acids were hydrolyzed from tofu wastewater using the method described in our previous work [13]. Briefly, 200 mL of tofu wastewater (TW, pH 5.6, 27 °C), obtained from Tahu Sumedang Timbul Jaya (T. Umar street, Bandar Raya, Banda Aceh, Aceh, Indonesia) was combined with NaOH 2 N to reach a pH of 10. This mixture was heated at 50℃ for 1 h and subsequently filtered. The resulted filtrate was acidified with HCl 2 N until it reached pH 4.5 (isoelectric pH). On the next day, it was centrifuged at - 4℃ for 20 min. The obtained pellets were washed using distilled water and dried at 50℃ for 5 h. Dried protein pellets were combined with 0.5 mL 6 N HCl per mg protein, followed by heating and stirring at a temperature of 70℃ for 4 h. Finally, the excess HCl was evaporated at 55℃ (vacuum; 45 mbar) for 20 min. The amino acids obtained were then characterized using LC-MS/MS for qualitative and quantitative analysis.

2.2. LC-MS/MS characterization

Characterization using LC-MS/MS was carried out at the Regional Health Laboratory, Jakarta, Indonesia. The operating mode of MS/MS used in study was *Multiple Reaction Monitoring* (MRM). As much as 1 mL sample was vortexed for one minute and then centrifuged for 5 min (13,000 rpm at 4 °C). The supernatant obtained was then injected in the liquid chromatograph, coupled with a tandem mass spectrometer. The system used a non-ion-pairing reagent, heptafluorobutyric acid, to yield good sensitivity, repeatability, and recovery of the analysis [14]. The details of the operating system could be found below:

The type of instrument	: LC-MS/MS Quatromicro Triple Quadrupole (From Waters, Milford, MA)
Column	: C8 Xbridge 3.5 mm (2.1 × 100 μm) (From Waters, Milford, MA)
Mobile phase (gradient)	: 0.1% Heptafluorobutyric Acid in Water (90) 0.1% Heptafluorobutyric Acid in Acetonitril (10)
Injection volume	: 30 μL
Flow rate	: 0.15 ml/min
Source Temperature	: 150 °C
Desolvation Temperature	: 450°C Gas
Flow Desolvation	: 600 L/h

2.3. Preparation of composites

In order to prepare the composite, 0.9 g chitosan was dissolved into 100 mL acetic acid 2% for 2 h at 250 rpm. Next, 0.5 mL of protein hydrolysate was added and stirred for another 1 h. After the mixture was homogeneous, the priorly dispersed 0.1 g TiO_2 (in distilled water) was added and stirred for another 2 h. The homogeneous mixture was poured into a 17 × 12 cm^2 film mold and oven-dried at 40℃ for 48 h. The procedure was repeated for various compositions of chitosan and TiO_2; (0.925:0.075), (0.950:0.05) and (0.975:0.025).

2.4. Tensile strength characterizations

The tensile strength of the films was obtained using Universal Testing Machine HT8503 (Hung Ta Instrument Co., Ltd, Taichung, Taiwan). Samples were cut into a standard shape (ASTM D638- TYPE IV). Tensile strength characterization was carried out at the Material Physics Laboratory of Mathematics and Natural Sciences Faculty, Universitas Syiah Kuala. The tip of the film clamped to the

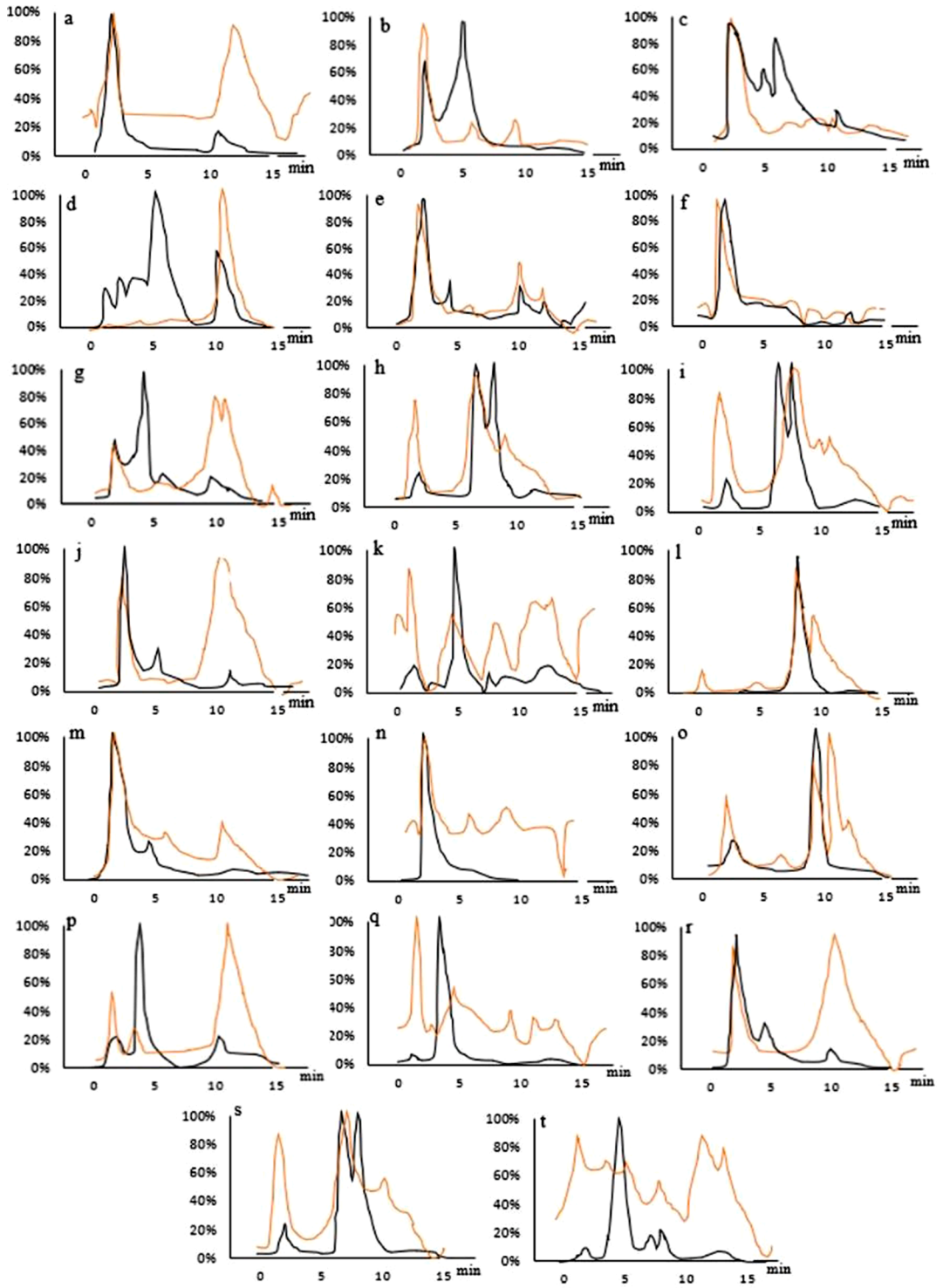

Fig. 1. Reference (black line) and sample (orange line) chromatograms of Tryptophan (a), L-Arginine (b), L-Aspartic Acid (c), L-Cystine (d), L-Glutamic Acid (e), Glycine (f), L- Histidine (g), L-Isoleucine (h), L-Leucine (i), L-Lysine (j), L-Methionine (k), L- Phenylalanine (l), L-Proline (m), L-Serine (n), L-Threonine (o), L-Tyrosine (p), L- Valine (q), L-Glutamine (r), L-Hydroxyproline (s), and L-Asparagine (t). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Table 1
Amino acid concentration results in protein hydrolysate of tofu wastewater.

	Area Reference	Sample	Amino acid Concentration (μg/mL)	(%)
L- Tryptophan	129895	1779	0.70	0.297
L-Arginine	27283	9625	15.36	6.527
L-Aspartic Acid	6180	4538	24.43	10.382
L-Glutamic Acid	5810	5339	33.79	14.359
Glycine	23872	7553	5.94	2.524
L-Histidine	10544	1058	3.88	1.648
L-Cystine	37026	35374	37.07	15.753
L-Isoleucine	54026	15526	9.43	4.007
L-Leucine	86023	44920	17.13	7.279
L-Lysine	34598	3274	3.46	1.470
L-Methionine	43250	4755	4.10	1.742
L-Phenylalanine	76364	5596	3.03	1.287
L-Proline	49223	8426	4.93	2.095
L-Serine	437325	2481	0.15	0.063
L-Threonine	32439	3060	2.81	1.194
L-Tyrosine	5914	7966	61.02	25.931
L-Valine	22915	829	1.06	0.450
L-Glutamine	33004	3281	3.63	1.542
L-Hydroxyproline	83097	5933	2.34	0.994
L-Asparagine	19385	625	1.05	0.446

testing machine. The clamp was pulling the sample at a rate of 20 mm/min until break.

2.5. Adsorption study

As much as 0.025 g composite film that had been cut 1 × 1 cm^2 was added into an Erlenmeyer containing 20 mL methylene blue (30 ppm). The Erlenmeyer flask was put on the shaker for 30 min. The leftover methylene blue was filtered and analyzed using a spectrophotometer UV-Vis (λ_{max} = 660 nm). The results were expressed as adsorption capacity at equilibrium (qe (mg/g)) and the removal percentage (%R), see Eqs. (1) and (2).

$$Qe = \frac{Co^{-}Ce}{wx} \cdot V_y \tag{1}$$

$$\%R = \left(\frac{Co^{-}Ce}{Co}\right)x \quad 100 \tag{2}$$

Where Co, Ce, wx, and V_y are initial concentration (μg/mL), concentration at equilibrium (μg/mL), weight of x adsorbent (g), and volume of y adsorbate (mL), respectively.

2.6. Simple additive weight (SAW) method

SAW method is a weighted addition method by determining the weighted summation of the performance rating of each alternative on all criteria [15,16]. From the results obtained, it is known that the highest preference value for each alternative (Npi) is the best composite. This result was obtained from the weighted sum of criterion I (tensile strength) of 40% and criterion II (adsorption capacity) of 60%. The value of criterion II was set higher than criterion I because the main purpose of making this composite film is to obtain adsorbent with a large adsorption capacity. Therefore, the percentage of adsorption capacity should be higher than the tensile strength value. However, the tensile strength value still affected the mechanical strength of the composite film as an adsorbent. Each of the criteria was calculated using the following Eq. (3). Then, Npi was calculated based on weighted percent using Eq. (4).

$$R_{ij} = \left(\frac{X_{ij}}{MAX(X_{ij})}\right) \tag{3}$$

$$Np_i = \sum_{j=1}^{n} W_j R_{ij} \tag{4}$$

Where,

R_{ij}: Normalized matrix
X_{ij}: Rows and columns of the matrix
Max X_{ij}: Maximum value of each criterion
V_i: Alternative final value

Table 2
Film composites with different composition ratios and their SAW score based on tensile strength and adsorption capacity at $t = 30$ min.

Composition				Mechanical Properties		Methylene Blue Uptake		
Label	Chitosan (g)	Amino Acid (mL)	TiO_2 (g)	Tensile Strength (kgf/nm^2)	Elongation (%)	q_{30} (mg/g)	Removal (%)	Npi
a	1	-	-	1.52	38.32	1.0615	4.43	0.351
b	1	0.5	-	1.74	74.12	2.2665	9.45	0.5114
c	9.975	0.5	0.025	2.53	24.24	5.753	23.97	1
d	9.950	0.5	0.05	1.86	56.8	3.458	14.41	0.6547
e	9.925	0.5	0.075	1.87	64.88	3.299	13.75	0.6397
f	9.900	0.5	0.10	1.07	13.00	4.118	17.16	0.5986

W_j: Specified weight

3. Data result, value and validation

The amino acids were obtained by hydrolyzing the suspended protein from the tofu wastewater using excessive amount of the hydrolyzing agent to prevent the readsorption of the liberated amino acids onto carbohydrate and other particles existing in the wastewater. Chromatograms of the reference amino acids showed more than one peak because of impurities. Amino acid standards obtained from Sigma with a concentration of 0.5 μmol/mL ±4% in 0.2 N lithium citrate buffer (pH 2.2) containing thiodiglycol (2% w/v) and phenol (0.1% w/v) as antioxidant and preservative. The identification and quantification of the isolated amino acids by LC-MS/MS was carried out by external references (Fig. 1). The results of the quantitative analysis were presented in Table 1. L-Tyrosine (25.93%) was found to dominate the amino acid contents in the tofu wastewater, followed by L-Cystine (15.75%), L-Glutamic Acid (14.36%), and L-Aspartic Acid (10.38%). These data were obtained from the Regional Health Laboratory, Jakarta, Indonesia. Based on their data (unpublished), the relative standard deviation (RSD) was around 2.5–4.48%. In regards of the amino acids' stability, the qualitative and quantitative data might be affected by the storage temperature. For instance, a study found that amino acids were stable when stored below -20 °C [17]. In this study, we did not conduct the stability analysis.

The amino acids containing hydrolysate of tofu wastewater was used to modify chitosan matrix. Since we used dark condition, the methylene blue removal was mostly influenced by the variation of tofu wastewater-derived amino acids attributed to the modified functional groups [13]. Herein, we added photocatalyst TiO_2 to improve the methylene blue removal. Further studies on the effect of TiO_2 under the UV-irradiated condition would be reported separately. In order to select the best composition, we employed SAW method with 40 and 60% priorities for the tensile strength and adsorption capacity, respectively (Table 2). Addition of amino acids in the chitosan adsorbent may allow the material to possess higher negative affinity, which can attract more cationic particles *viz.* methylene blue. Several reports have addressed the importance of O- and N-containing functional groups for the adsorptive removal process [18–21]. In our case, these groups were correlated with the addition of amino acids in chitosan.

4. Conclusions

L-Tryptophan, L-Arginine, L-Aspartic Acid, L-Glutamic Acid, Glycine, L-Histidine, L-Cystine, L- Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Phenylalanine, L-Proline, L-Serine, L-Threonine, L-Tyrosine, L-Valine, L-Glutamine, L-Hydroxyproline, and L-Asparagine were recorded in the tofu wastewater obtained from LC-MS/MS analysis with different compositions. The amino acids with a percentage of more than 10%, from the highest to the lowest, were L-Tyrosine L-Cystine, L-Glutamic Acid, and L-Aspartic Acid. The chitosan-based composite with the modification using tofu wastewater hydrolysate yielded higher mechanical properties and adsorption capacity compared to those without. Tofu wastewater could be used as a feed ingredient for amino acids in increasing the material properties and adsorption capacity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

The authors wish to thank Universitas Syiah Kuala for its financial support under Grant Number: 292/UN11/SPK/PNBP/2020 through PRUUPD scheme.

References

[1] J.Y. Chua, S.Q. Liu, Soy whey: more than just wastewater from tofu and soy protein isolate industry, Trends Food Sci. Technol. 91 (2019) 24–32, https://doi.org/10.1016/j.tifs.2019.06.016.
[2] J.Y. Chua, S.Q. Liu, Effect of single amino acid addition on growth kinetics and flavor modulation by Torulaspora delbrueckii in soy (tofu) whey alcoholic beverage fermentation, Food Res. Int. 135 (2020), 109283, https://doi.org/10.1016/j.foodres.2020.109283.

[3] I. Rukmi, S. Pujiyanto, N.S. Mulyani, N. Faidah, L. Ayu, The effect of coconut water and tofu wastewater as nitrogen source on the production of alkali protease from Aspergillus flavus DUCC K225, J. Phys. Conf. Ser. 1217 (2019), 012127, https://doi.org/10.1088/1742-6596/1217/1/012127.
[4] W. Anggraini, M. Zulfa, N.N. Prihantini, F. Batubara, R. Indriyani, Utilization of tofu wastewater for the growth of red spinach (Alternanteraamoenavoss) in floating raft hydroponic cultures, J. Phys. Conf. Ser. 1467 (2020), 012005, https://doi.org/10.1088/1742-6596/1467/1/012005.
[5] Widayat, J. Philia, J. Wibisono, Cultivation of microalgae chlorella sp on fresh water and waste water of tofu industry, E3S Web Conf. 31 (2018) 04009. 10.1051/e3sconf/20183104009.
[6] E.S. Hosseini, L. Manjakkal, D. Shakthivel, R. Dahiya, Glycine–chitosan-based flexible biodegradable piezoelectric pressure sensor, ACS Appl. Mater. Interfaces 12 (2020) 9008–9016, https://doi.org/10.1021/acsami.9b21052.
[7] E. Safitri, H. Humaira, M. Murniana, N. Nazaruddin, M. Iqhrammullah, N.D. Md Sani, C. Esmaeili, S. Susilawati, M. Mahathir, S. Latansa Nazaruddin, Optical pH sensor based on immobilization anthocyanin from Dioscorea alata L. onto polyelectrolyte complex pectin–chitosan membrane for a determination method of salivary pH, Polymers 13 (2021) 1276, https://doi.org/10.3390/polym13081276.
[8] M.J. Boggione, C.R.A. Mahl, M.M. Beppu, B. Farruggia, Synthesis and characterization of chitosan membranes functionalized with amino acids and copper for adsorption of endoglucanase, Powder Technol. 315 (2017) 250–257, https://doi.org/10.1016/j.powtec.2017.04.014.
[9] M. Motiei, S. Kashanian, A. Taherpour, Hydrophobic amino acids grafted onto chitosan: a novel amphiphilic chitosan nanocarrier for hydrophobic drugs, Drug Dev. Ind. Pharm. 43 (2017) 1–11, https://doi.org/10.1080/03639045.2016.1254240.
[10] J. Fernandes, M.V. Ghate, S. Basu Mallik, S.A. Lewis, Amino acid conjugated chitosan nanoparticles for the brain targeting of a model dipeptidyl peptidase-4 inhibitor, Int. J. Pharm. 547 (2018) 563–571, https://doi.org/10.1016/j.ijpharm.2018.06.031.
[11] D. Hu, H. Huang, R. Jiang, N. Wang, H. Xu, Y.-G. Wang, X. Ouyang, Adsorption of diclofenac sodium on bilayer amino-functionalized cellulose nanocrystals/chitosan composite, J. Hazard. Mater. 369 (2019) 483–493, https://doi.org/10.1016/j.jhazmat.2019.02.057.
[12] Q. Zia, M. Tabassum, Z. Lu, M.T. Khawar, J. Song, H. Gong, J. Meng, Z. Li, J. Li, Porous poly(L–lactic acid)/chitosan nanofibres for copper ion adsorption, Carbohydr. Polym. 227 (2020), 115343, https://doi.org/10.1016/j.carbpol.2019.115343.
[13] H. Fathana, Rahmi, S. Lubis, Adlim, The effect of amino acids extracted from tofu wastewater on chitosan performance for methylene blue removal from water, IOP Conf. Ser. Mater. Sci. Eng. 1087 (2021), 012057, https://doi.org/10.1088/1757-899X/1087/1/012057.
[14] K.E.L. Hawari, Z. Daher, E. Verdon, M.A.L. Iskandarani, Impact of ion-pairs for the determination of multiclass antimicrobials residues in honey by LC-MS/MS, Food Addit. Contam. Part A 34 (2017) 2131–2143, https://doi.org/10.1080/19440049.2017.1372641.
[15] L. Rahmi, R. Nurfatimah, Preparation of polyethylene glycol diglycidyl ether (PEDGE) crosslinked chitosan/activated carbon composite film for Cd2+ removal, Carbohydr. Polym. 199 (2018) 499–505, https://doi.org/10.1016/J.CARBPOL.2018.07.051.
[16] Rahmi, M. Iqhrammullah, U. Audina, H. Husin, H. Fathana, Adsorptive removal of Cd (II) using oil palm empty fruit bunch-based charcoal/chitosan-EDTA film composite, Sustain. Chem. Pharm. 21 (2021), 100449, https://doi.org/10.1016/j.scp.2021.100449.
[17] C.F. Chiang, J.A. Larsen, M. Sahtout, R.E. Horoschak, Z.S. Yu, A.J. Fascetti, Impact of storage temperature, storage duration, and deproteinization on plasma amino acid concentrations in dogs, Res. Vet. Sci. 136 (2021) 416–421, https://doi.org/10.1016/j.rvsc.2021.03.022.
[18] M. Iqhrammullah, Marlina, S. Nur, Adsorption behavior of hazardous dye (Methyl Orange) on cellulose-acetate polyurethane sheets, IOP Conf. Ser. Mater. Sci. Eng. 845 (2020), 012035, https://doi.org/10.1088/1757-899X/845/1/012035.
[19] Marlina, M. Iqhrammullah, S. Saleha, Fathurrahmi, F.P. Maulina, R. Idroes, Polyurethane film prepared from ball-milled algal polyol particle and activated carbon filler for NH3–N removal, Heliyon 6 (2020) e04590, https://doi.org/10.1016/j.heliyon.2020.e04590.
[20] A. Alni, K. Puspita, M.A. Zulfikar, Biosorbent from Chinese cabbage (Brassica pekinensia L.) for phenol contaminated waste water treatment, Key Eng. Mater. 811 (2019) 71–79, https://doi.org/10.4028/www.scientific.net/KEM.811.71.
[21] R.A. Fitri, A. Wirakusuma, A. Fahrina, M.R. Bilad, N. Arahman, Adsorption performance of low-cost java plum leaves and guava fruits as natural adsorbents for removal of free fatty acids from coconut oil, Int. J. Eng. 32 (2019) 1372–1378, https://doi.org/10.5829/ije.2019.32.10a.06.