Materials Today: Proceedings xxx (xxxx) xxx

Contents lists available at ScienceDirect

Materials Today: Proceedings

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

Potential of keratin loaded activated carbon and aqueous extracts of *Allium sativum* for the development of antibacterial wound dressing

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ARTICLE INFO

Article history:
Received 23 April 2021
Accepted 28 April 2021
Available online xxxx

Keywords:
Antibacterial dressing
Activated carbon
Keratin
Allium sativum
Wound

ABSTRACT

Chronic wounds are developed as a consequence of prolonged wound healing and due to bacterial infection and its resistance. An antimicrobial dressing could be fabricated by integration of activated carbon (AC), keratin and homogenized aqueous garlic extract. The constituents were inspected independently for the evaluation of their efficiency as wound dressing component. The activated carbon was observed to adsorb keratin protein efficiently which could act as a carrier. Anti-bacterial tests were performed for the garlic extracts and found that 5% to 10% of extracts could inhibit the bacterial growth effectively. These components were shown to be potential candidates for fabrication of wound dressing material which could be developed into an ideal dressing material after further process optimization.

Selection and peer-review under responsibility of the scientific committee of the 12th National Conference on Recent Advancements in Biomedical Engineering.

1. Introduction

One of the most strenuous tasks in wound care treatment associated with chronic wounds is the selection of type of materials needed for the fabrication of a wound dressing since most of the antibacterial agents doesn't satisfy the clinical requirements [1]. The fabrication of wound dressing implicates the usage of different materials based on their beneficial properties and applied in either individual or combined form [2].

To facilitate the tissue compatibility, polysaccharide or protein biopolymers are often employed owing to their characteristic performance. Among all, Keratin (KER) is a collection of transitional structured protein existing in feathers of birds, skin of snakes, horns, hooves, hair from mammals. These proteins are in surplus amount in body and possess cellular adherence property that stimulates the restoration of skin on wounds [3,4]. Various literatures cited the utility of this protein holding haemostatic traits [5], sustenance in adhesion and multiplication of fibroblasts [6], transportation of keratinocytes and quick tissue granulation on wounds [7]. The keratin protein was also utilized in the development of antimicrobial wound dressings [8,9].

In addition to antimicrobial property, an ideal dressing material should have better adsorption tendency of wound exudates. Usage of activated carbon was greatly preferred for the adsorption of wound fluids and also for the efficient removal of toxins secreted by the bacteria [10]. Many studies have been documented on the utilization of activated carbon for wound healing mainly because of its adsorption property which could purify huge inflammatory proteins and other agents like cytokines, interleukins of blood [11,12]. Israel *et al.* [13] have reported that activated carbon derived from coconut shell is efficient adsorbent than Al_2O_3 and TiO_2 for proteins/ glycoproteins. Most of the studies have used activated carbon derived from viscose, nitrile fibers where characterization of these ACs to identify its potential for biomedical application is less reported.

Broad-spectrum antimicrobials like iodine, silver were employed most of the time for the management of wounds. But their indefinite usage could also serve harmful to the tissues. Garlic (*Allium sativum*), an ancient herb, was exploited for its therapeutic potentials like antioxidant, anti-inflammatory, antimicrobial, antidiabetic and so on [14,15]. Its medicinal benefits were being supplied by an organosulphur bioactive compound called allicin. Studies have revealed the inhibition of bacterial growth in the presence of thiosulfinate allicin by restricting its RNA synthesis [16]. The composite containing keratin, activated carbon, allicin might serve as effective dressing material in healing the wound. Thus, the current research aims to describe the potential benefits of developing a wound dressing using keratin loaded activated carbon supple-

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https://doi.org/10.1016/j.matpr.2021.04.486
2214-7853/
Selection and peer-review under responsibility of the scientific committee of the 12th National Conference on Recent Advancements in Biomedical Engineering.

mented with *Allium sativum* extracts for the performance of antibacterial and wound healing activity.

2. Materials and methods

2.1. Materials

Human hair was obtained from a local hair salon located in the institutional premises of National Institute of Technology, Rourkela (India). Precursor carbonaceous material derived from corn cob was received from Periyar Maniammai Institute of Science and Technology, Tamilnadu (India). Fresh bulbs of garlic were bought from the local vendor. Dialysis membrane (MWCO-12KDa) was procured from Sigma Aldrich (India) and further chemicals utilized in this study were of analytical grade.

2.2. Extraction of proteins from human hair

The keratin containing proteins were extracted from hair using Shindai method [17]. In brief, the hairs were cleaned with shampoo for the elimination of dust particles and then subsequently kept for drying at room temperature. The dried hairs were subjected to delipidization in a mixture of chloroform and methanol (2:1 v/v) ratio for about 24 h which is sufficient enough to remove lipids. The incised hair fragments of 240 mg were dipped into 60 ml buffer solution (pH 8.5) consisting of 25 mM Tris-HCl, 2.6 M thiourea, 5 M urea and 5% β-mercaptoethanol and incubated at 50 °C for 24 h. The hairs were discarded after collecting the filtered liquid with the help of Whatman filter paper no. 1. The filtered liquid obtained was subjected to dialysis for 4 days with repeated change of distilled water at regular intervals until the liquid decolorizes to transparent liquid. The liquid inside the membrane was obtained after dialysis and quantified for protein in UV–Vis spectrophotometer at 595 nm using Bradford assay. Further, the dialyzed solution was then lyophilized using a freeze dryer to obtain human hair protein in a pure powdered form.

2.3. Production of activated carbon and characterization

The precursor carbon material was post-activated by the method of chemical activation using the phosphoric acid [13]. Briefly, 3 g of carbon material was added with 3 ml of 0.1 M H_3PO_4 solution and kept at 800 °C in a muffle furnace for 5 min. Then the sample was washed with 0.5 M acetic acid and further washed with distilled water until the pH of the filtered sample became neutral and dried at 50 °C for overnight. The pH value of activated carbon was determined via 20:1 ratio (water: activated carbon) where the suspension was stirred, centrifuged and the supernatant was examined using pH probe. The percentage of Carbon (C), Hydrogen (H), Nitrogen (N), Sulphur (S) elements of the adsorbent was estimated by means of an elemental analyser. For field emission scanning electron microscopy (FESEM) examination, the samples were fixed to a carbon tape and positioned on the sample in a high-pressure compartment of Carl-Zeiss FESEM (Carl-Zeiss Supra 55) system.

2.4. Adsorption of protein onto activated carbon

The process associated with the adsorption of protein (adsorbate) by the adsorbent was followed as presented by Israel *et al.* [13]. In concise, a stock solution containing 1 mg/ml crude hair protein solution was taken and divided into aliquots of 1 ml in a centrifuge tube. Each aliquot was added with 50 mg of activated and non-activated carbon sample separately and was shaken in a shaker at 25 °C for intervals of 10, 30, 60, and 90 min. The samples were then centrifuged at 6000 rpm for 5 min. The supernatant collected from each sample was then quantified for the presence of protein by performing Bradford assay. The adsorbed protein content was assessed by the difference in the mass of unadsorbed protein in supernatant to that of the protein's mass without supplementation of activated carbon.

2.5. Development of aqueous garlic extracts (AGE)

Aqueous garlic extracts were prepared from the procedure similar to Johnson *et al.* [18]. The procedure could be briefed as follows: Fresh bulbs of garlic were stripped of their outer skin and washed with distilled water. 50 g of this garlic was minced with a disinfected knife and squashed in a blender by supplementing 100 ml of sterile water. The mixture was then filtered through a filter paper. The extract concentration prepared was rated as 50% (w/v). Serial dilutions were also carried out with the use of the extracts to make 2%, 5% and 10% concentration. Storage of these extracts was maintained at −20 °C for later usage.

2.6. Antibacterial assessment of AGE extracts

The antibacterial assessment was done with 2%, 5% and 10% AGE concentration against two gram-positive bacteria (*S. aureus, B. cereus*) and two gram-negative bacteria (*S. flexneri, E. coli*). The extracts in different concentrations were added into well made on streaked agar plate and incubated overnight at 37 °C. Agar well diffusion assay was carried out in duplicates and the results (in mm) were recorded.

3. Results and discussion

3.1. Extraction of keratin protein from hair

As no lipids were detected through Bligh and Dyer method [19] after delipidization, the dried hair was subjected to protein solubilization using Tris- HCl buffer. The dark orange-colored supernatant was obtained and subjected to dialysis. The dialysis was performed for 4 days where the decolorization of supernatant was observed with increased time. After 4 days, the protein solution was analysed using Bradford assay. The concentration of protein in the dialysed solution was found to be 0.225 g/ml obtained by extrapolating from the standard graph.

3.2. Chemical characterization of non-activated and activated carbon

The pH of the pristine carbon material was found to be 9.1. This elevated alkalinity relates to the existence of inorganic molecules which could be beneficial in boosting up the adsorption capacity [20]. Table 1 shown below displays the percentage of elements that existed in activated and non-activated carbon. It could be observed that the C content increases while H and O content decreases after activation. The protons (H^+) dissociated from H_3PO_4 mainly attack the carboxyl bond of the ether/ alcohol groups at higher temperatures. During this time, low molecular fractions of gases and steam are liberated out decreasing the hydrogen and oxygen content on

Table 1
Elemental composition of non-activated and activated carbon.

Elements	Non-activated carbon (%)	Activated carbon (%)
Carbon	70.79	75.03
Sulphur	1.52	1.41
Nitrogen	0.78	0.85
Hydrogen	2.07	1.64
Oxygen	24.84	21.07

the surface which in turn create internal pores due to gas liberation from the particle. The H/C ratio for modified and pristine biochar is 0.029 and 0.021 which shows that the hydrophobic nature has been increased after activation owing to formation of aromatic rings. Similar observations on changes in elemental composition was earlier reported by Chu *et al.* [21], Peng *et al.* [22] that the activation process has changed the elemental composition of activated carbon which could enhance the adsorption efficiency owing to increased hydrophobic interaction between protein and AC

3.3. Morphological characterization of non-activated and activated carbon

The surface topographical features of non-activated carbon were perceived to be uniform with minimum number of pores, whereas the activated carbon exposed pores of diverse range of sizes consisting of mesopores and micropores. The FESEM images of non-activated and activated carbon have been given in Fig. 1. It could be visualized that maximum number of pores are present in the activated carbon in comparison to that of non-activated carbon. The magnitude of pores that existed in non-activated carbon was measured to be 8.5 ± 2.65 μm and for activated carbon as 10.23 ± 1.99 μm. The results obtained were concordant with Villota *et al.* [23] and Liu *et al.* [24]. The events might have befallen as a consequence of dehydration due to the behavior of acid on the activated carbon leading to the formation of cavities. The escalation in the number of pores ensued as a result of carbonization concurrently lead to the development of carbon dioxide vapors on the supplementation of the activating chemical reagent. The results obtained were similar to Ates *et al.* [25]. The produced activated carbon containing a wide range of micro and mesopores could act as carrier for keratin and adsorb the wound exudates efficiently.

3.4. Protein adsorption study of activated carbon

In order to use activated carbon as a carrier for keratin function, the keratin protein has to be adsorbed on its surface and pores. The mass of protein adsorbed on the surface for a respective time of contact has been given in Table 2. It could be noted that with increase in time, the mass of protein adsorbed is increasing for activated carbon compared to non-activated carbon. The formation of more binding sites on the activated carbon and increasing contact time facilitates more amount of protein to get adsorbed on the surface. The mass of 1.094 mg to 1.530 mg of protein has been adsorbed per gram of non-activated carbon whereas 1.738 mg to 1.968 mg of protein has been adsorbed per gram of activated carbon. Also, the saturation time has been increased from 60 min to 90 min for activated carbon compared to non-activated carbon. As evident from the elemental composition and FESEM images, activation process increased the hydrophobic nature and micropores of AC which in turn favours hydrophobic interaction and pore filling mechanism for the adsorption of keratin protein to retain on activated carbon. Hassan *et al.*, [26] also reported that AC containing high aromaticity is suitable for sorption of organic compounds where hydrophobic interaction, pore filling, precipitation are dominant sorption mechanisms.

3.5. Agar well diffusion assay

Agar well diffusion assay was carried out to determine the antibacterial activity of the AGE against four different bacterial strains. The agar plates showing the zone of inhibition can be seen in Fig. 2. The zone of inhibition caused by the extracts (Table 3) revealed that the zone of inhibition is increasing significantly with increasing concentration of aqueous garlic extracts. The growth of all the bacterial strains used in this assay was hindered at 10% AGE

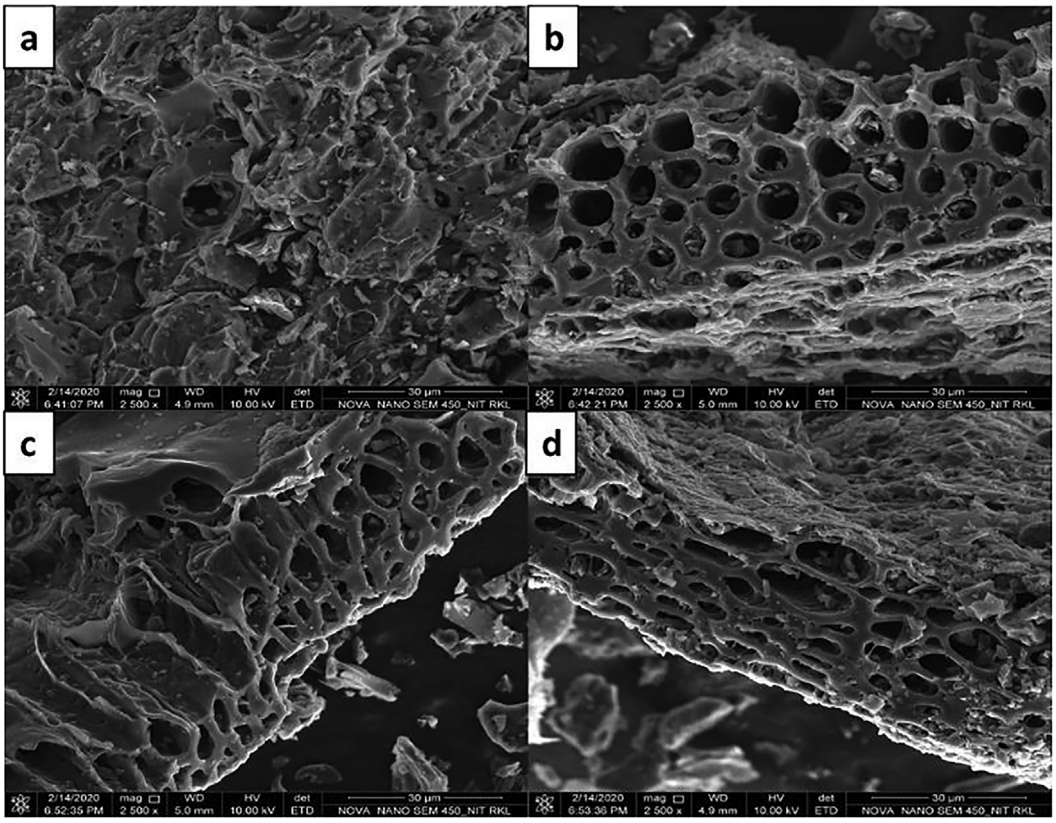

Fig. 1. FESEM images of (a, b) non-activated carbon and (c, d) activated carbon.

Table 2
The amount of protein adsorbed in non-activated and activated carbon at different time intervals.

Time of contact (min)	Mass of adsorbed protein in non-AC (mg)	Mass of adsorbed protein in AC (mg)
10	0.0547	0.0869
30	0.0535	0.0996
60	0.0765	0.0990
90	0.0512	0.0984

concentration showing maximum zone of inhibition. It could be noted that *S. aureus* was found to be lesser resistant amidst bacterial strains used in testing. It proves that AGE retained inherent broad spectrum attributes associated with both types of bacteria since a notable distinction in the sizes of the inhibition zones could be witnessed among the different groups of bacteria. This might be linked to the lipid content in their membrane prevailing in diverse bacterial strains and their absorptivity to allicin and additional garlic allied components intake [27]. The conclusion achieved demonstrated comparable results of the zone of inhibition as reported by Chand *et al.* [28].

4. Conclusion

Keratin or hair protein, activated carbon and garlic extracts were studied individually to check their effectiveness to be used as a component for wound dressing. Hair protein sequestered from delipidised hair fragments was estimated using Bradford Assay and was measured to be 0.225 mg/ml. The quantified hair protein was investigated for adsorption on activated and non-activated carbon. Results acquired in activated carbon were rewarding owing to the greater number of pores. This ascertained the fact that activated carbon possessed the innate ability to act as a carrier for keratin and also could aid in the adsorption of wound discharges efficiently after process optimization. On the assessment of antibacterial ability of AGE extracts, the results displayed maximum value of the zone of inhibition against bacterial growth on 10% AGE concentration. AGE substantiated its intrinsic antibacterial trait which could be an alternative to current antimicrobials used on determining the optimal concentration. Further studies could be conducted on fabricating a wound dressing and assess its cytotoxicity, haemocompatibility and *in-vivo* experimentation. Therefore, the components such as hair protein, activated carbon, and AGE extracts could be considered as potential candidates, while on integration with optimized conditions, an ideal antibacterial wound dressings could be fabricated that enhances the wound healing process.

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.

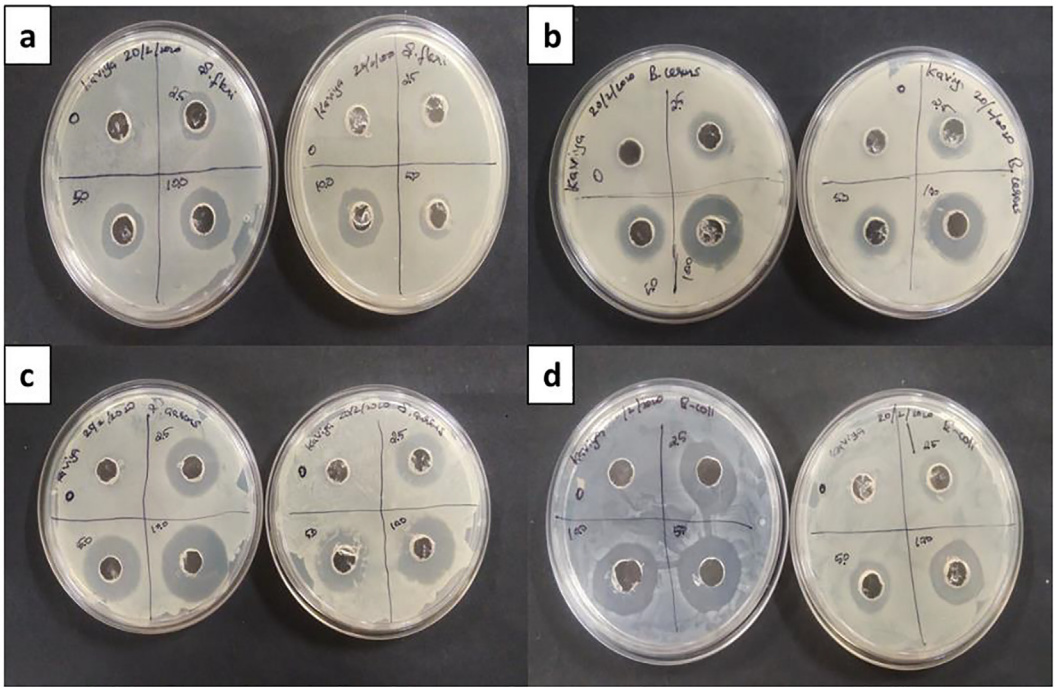

Fig. 2. Zone of inhibition observed at 2%, 5%, 10% AGE in (a) *S. flexneri,* (b) *B. cereus,* (c) *S. aureus,* and (d) *E. coli.*

Table 3
Values of zone of inhibition (in mm) at concentrations of 0, 2, 5 and 10% of aqueous garlic extracts.

Sample tested	Concentration of AGE (%)	Zone of inhibition (mm)			
		E. coli	*B. cereus*	*S. aureus*	*S. flexneri*
Aqueous garlic extract (AGE)	0	0	0	0	0
	2	17.60 ± 3.21	16.00 ± 1.00	19.50 ± 0.70	13.00 ± 1.40
	5	20.00 ± 4.36	16.60 ± 1.53	22.50 ± 1.30	14.50 ± 1.29
	10	21.75 ± 1.70	22.70 ± 0.50	24.30 ± 2.50	18.00 ± 1.00

Acknowledgment

The authors thank the Department of Biotechnology and Medical Engineering of National Institute of Technology Rourkela for providing the research facility. The authors thank the Science and Engineering Research Board (SERB) of Department of Science and Technology (DST) of Government of India for the research grant [File No: ECR/ES/2017/003397].

References

[1] H. Cheng, Z. Shi, K. Yue, X. Huang, Y. Xu, C. Gao, Z. Yao, Y.S. Zhang, J. Wang, Acta Biomater. 124 (2021) 219–232.
[2] J.G. Powers, L.M. Morton, T.J. Phillips, Dermatol Ther. 26 (2013) 197–206.
[3] Y. Chen, Y. Li, X. Yang, Z. Cao, H. Nie, Y. Bian, G. Yang, Acta Biomater. 125 (2021) 208–218.
[4] S. Sivakumar, R. Murali, D. Arathanaikotti, A. Gopinath, C. Senthilkumar, S. Kesavan, B. Madhan, Int. J. Biol. Macromol. 177 (2021) 463–473.
[5] T. Aboushwareb, D. Eberli, C. Ward, C. Broda, J. Holcomb, A. Atala, M. Van Dyke, J. Biomed. Mater. Res. Part B Appl. Biomater. 90 (2009) 45–54.
[6] S. Wang, F. Taraballi, L.P. Tan, K.W. Ng, Cell Tissue Res. 347 (2012) 795–802.
[7] P.M. Pechter, J. Gil, J. Valdes, M. Tomic-Canic, I. Pastar, O. Stojadinovic, R.S. Kirsner, S.C. Davis, Wound Repair Regen. 20 (2012) 236–242.
[8] M. Zhai, Y. Xu, B. Zhou, W. Jing, J. Photochem. Photobiol. B Biol. 180 (2018) 253–258.
[9] M.E. Villanueva, M.L. Cuestas, C.J. Pérez, V.C. Dall, G.J. Copello, J. Colloid Interface Sci. 536 (2019) 372–380.
[10] M.H. Kim, D. Cho, O.H. Kwon, W.H. Park, J. Alloy. Compd. 735 (2018) 2670–2674.
[11] Y. Liu, Q. Li, H. Zhang, S. Yu, L. Zhang, Y. Yang, New Carbon Mater. 35 (2020) 323–335.
[12] W.Y. Huang, C.L. Yeh, J.H. Lin, J.S. Yang, T.H. Ko, Y.H. Lin, J. Mater. Sci. - Mater. Med. 23 (2012) 1465–1478.
[13] O.K. Israel, Afr. J Pure Appl. Chem. 3 (2009) 6–10.
[14] H.A. Saleh, G.S.A. El-Aziz, H.N. Mustafa, A.H.A. Saleh, A.O. Mal, A.H.S. Deifalla, M. Aburas, Folia Morphol. 77 (2018) 1–15.
[15] J. Ansary, T.Y. Forbes-Hernández, E. Gil, D. Cianciosi, J. Zhang, M. Elexpuru-Zabaleta, J. Simal-Gandara, F. Giampieri, M. Battino, Antioxidants. 9 (2020) 619.
[16] G. El-Saber Batiha, A. Magdy Beshbishy, L.G. Wasef, Y.H.A. Elewa, A.A. Al-Sagan, A. El-Hack, E. Mohamed, A.E. Taha, Y.M. Abd-Elhakim, H. Prasad Devkota, Nutrients 12 (2020) 872.
[17] A. Nakamura, M. Arimoto, K. Takeuchi, T. Fujii, Biol. Pharm. Bull. 25 (2002) 569–572.
[18] M. Johnson, O.N. Olaleye, O.S. Kolawole, Microbiol. Res. J. Int. 14 (2016) 1–11.
[19] S.K. Jensen, Lipid Technol. 20 (2008) 280–281.
[20] T. Qian, Y. Wang, T. Fan, G. Fang, D. Zhou, Sci. Rep. 6 (2016) 33630.
[21] G. Chu, J. Zhao, Y. Huang, D. Zhou, Y. Liu, M. Wu, H. Peng, Q. Zhao, B. Pan, C.E.W. Steinberg, Environ. Pollut. 240 (2018) 1–9.
[22] H. Peng, P. Gao, G. Chu, B. Pan, J. Peng, B. Xing, Environ. Pollut. 229 (2017) 846–853.
[23] S.M. Villota, H. Lei, E. Villota, M. Qian, J. Lavarias, V. Taylan, I. Agulto, W. Mateo, M. Valentin, M. Denson, ACS Omega 4 (2019) 7088–7095.
[24] L. Liu, Y. Li, S. Fan, Processes 7 (2019) 891.
[25] F. Ateş, Ö. Özcan, Eur. J. Eng. Technol. Res. 3 (2018) 6–11.
[26] M. Hassan, Y. Liu, R. Naidu, S.J. Parikh, J. Du, F. Qi, I.R. Willett, Sci. Total Environ. 744 (2020) 140714.
[27] S. Durairaj, S. Srinivasan, P. Lakshmanaperumalsamy, Electron. J. Biol. 5 (2009) 5–10.
[28] B. Chand, J. Microbiol. Biotechnol. Food Sci. 2 (4) (2021) 2481–2491.