Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 693-699 | ISSN:2251-6727

The Effect of Boric Acid Composition as Additional Cross-Linking Agent in SA-PVA Matrix Immobilized *Pseudomonas aeruginosa* for Methylene Blue Decolorization

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(Received: 07 October 2023 Revised: 12 November Accepted: 06 December)

KEYWORDS

biodecolorization, boric acid, methylene blue, Pseudomonas aeruginosa

ABSTRACT:

Methylene blue (MB) dye is commonly used in the textile industry because of its low cost and abundance properties. Nevertheless, the disposal of MB wastewater can cause environmental pollution and disrupt the ecosystem life. To overcome this problem, biodecolorization by using microorganisms such as Pseudomonas aeruginosa bacteria is one of the best methods for dye removal. P. aeruginosa can be immobilized on sodium alginate-polyvinyl alcohol (SA-PVA) matrix to enhance its capability to decolorize dye. The SA and PVA are well-known as good materials for immobilization. The beads of SA-PVA@P. aeruginosa used in this study were prepared through a cross-linking process with $CaCl_2$ and boric acid as the cross-linking agents. This study aimed to investigate the effect of boric acid composition as additional cross-linking agent in SA-PVA matrix on Pseudomonas aeruginosa's ability to degrade MB through immobilization. The peaks of many constituents of the beads existing in FTIR spectra indicated that the cross-linking process in the bead fabrication was successfully done. The result showed that the most optimum percentage of MB decolorization by P. aeruginosa immobilized in SA-PVA matrix was 86.252% which was obtained in the beads cross-linked by using $CaCl_2$ and boric acid composition of 4:5, followed by 4:7 (83.619%), 4:3 (81.022%), 4:1 (79.212%), and 4:0 (77.140%). In addition, the temperature and time incubation study obtained that the most optimum MB decolorization percentage was reached at 40 °C and 24 hours incubation time.

1. Introduction

Environmental pollution in water bodies due to many pollutants such as synthetic dye effluents, pesticides, petroleum hydrocarbons, and heavy metals is a global problem faced by many countries [1-4]. In textile industry, methylene blue (MB) dye is commonly used because of its low-cost and abundance properties. MB is used as a dye for wool, silk, and cotton [5]. In the dyeing process, 10-15% of dyes are discharged into the environment in the form of wastewater [1]. Unfortunately, acute MB exposure can cause several health risks, such as high blood pressure, increased heart rate, gastrointestinal pain, dizziness, diarrhea, fever, and nausea [6]. Meanwhile, MB might block incoming light and reduce oxygen concentrations in the water so that it causes disruption to aquatic ecosystem life [7].

There are some methods that have been used for treating synthetic dye waste, including physical, chemical, and biological methods [8]. Bioremediation is one of the biological methods for decreasing dye pollutants in water by using microorganisms, such as bacteria. Moreover, this method is an efficient, sustainable, and relatively inexpensive dye waste treatment [9, 10]. In bioremediation, bacteria act as remediation or degradation agents to change pollutant structures into harmless metabolites [11].

Pseudomonas aeruginosa is one of microorganisms that is capable to degrade aromatic compounds, including dye [12]. A previous study reported that *P. aeruginosa* could degrade methyl red dye at 40 °C with 64.69% of decolorization percentage [13]. Due to the influence of the decolorization performance of *P. aeruginosa*, immobilization techniques on microorganisms can be used. Compared with free cells, immobilization technique has many advantages in terms of protecting

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JCHR (2023) 13(6), 693-699 | ISSN:2251-6727

microorganisms from toxic environment, also enhancing the metabolic activity and reusability [14].

Sodium alginate (SA) is a natural polysaccharide that is commonly used as cell immobilization matrix due to its natural abundance, non-toxic, and biodegradable properties [15]. Nevertheless, SA has low mechanical stability which causes it to be easily crushed. SA can be combined with polyvinyl alcohol (PVA) which is non-toxic and has high mechanical strength. In this way, PVA contributes to the durability and stability of the beads, while SA improves the surface properties of the beads and reduces the tendency to agglomerate [9].

The SA-PVA immobilized *P. aeruginosa* beads in this study were prepared via the cross-linking method by using $CaCl_2$ and boric acid as cross-linking agents. Previous studies reported that the optimum composition of cross-linking agents that used in the matrix preparation could increase the mechanical stability and the decolorization performance of biocomposite materials [16]. Therefore, in this study, the different compositions of boric acid as the additional cross-linking agent with $CaCl_2$ in SA-PVA matrix for *P. aeruginosa* immobilization on MB decolorization were studied. We also investigate the factors influencing the optimal conditions for the best cross-linking agent composition used in the matrix.

2. Material And Methods

Chemicals

Chemicals used in the study were analytical grade purchased from different companies. MB (MB, C16H18N3SCl) dye was purchased from Merck, USA. Nutrient agar (NA) and Luria Bertani (LB) broth were bought from Merck, Germany. Immobilization materials were using Sodium alginate (SA) from Himedia, polyvinyl alcohol (PVA), $CaCl_2$, and boric acid bought from Merck, Germany. Alcohol 70%, methanol, and demineralized water were purchased from PT. Sumber Ilmiah Persada, Indonesia.

Culture Preparation

P. aeruginosa cultures were obtained from the collection of the Microbial Chemistry Laboratory, Department of Chemistry, Institut Teknologi Sepuluh Nopember, Indonesia. The stock culture of *P. aeruginossa* was regenerated on NA by streaking technique and incubated at 37 °C for 24 hours. The colony was inoculated into 10 mL of LB broth media and pre-incubated at 37 °C for 24 hours with 140 rpm shaking condition. The culture was then moved and incubated in 180 mL of LB broth media until its stationary phase [17]. The prepared culture was used for immobilization in SA-PVA matrix.

Immobilization of P. aeruginosa in SA-PVA Matrix

SA 2% (*w/v*) and PVA 4% (*w/v*) were mixed in demineralized water until 100 mL and heated to 100 °C in stirring condition with a magnetic stirrer. Afterwards, the mixture was sterilized at 120 °C for 15 minutes in the autoclave. The SA/PVA hydrogel was mixed by using a homogenizer with 5 mL of the prepared bacterial aqueous culture of *P. aeruginosa*. In the next steps, beads were formed by dripping the hydrogel solution by using a syringe into crosslinking agents ($CaCl_2$: boric acid) with compositions of 4:0, 4:1, 4:3, 4:5, and 4:7. The beads were washed with sterile demineralized water.

Materials Characterization

SA-PVA with immobilized *P. aeruginosa* were characterized with a Fourier Transform Infrared (FTIR, Shimadzu 8400S) to determine their structural properties. The

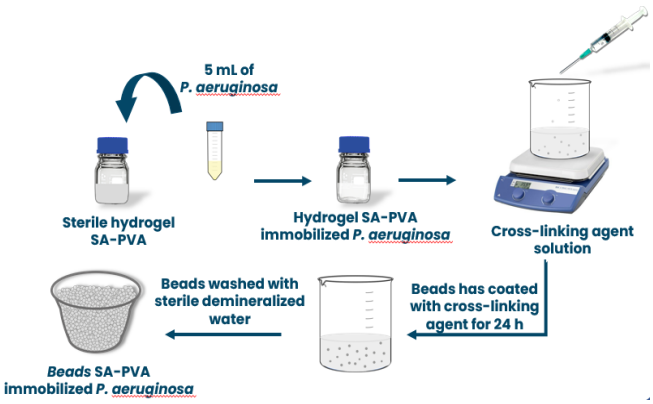

Fig 1. Schematic of cross-linking process in the bead's preparation

The functional groups that play a role in the biodecolorization of MB in the beads used in this study were analyzed for their adsorption at 400-4000 cm^{-1}. Prior to analysis, the beads were crushed and formed into tablet plates with KBr pellets.

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JCHR (2023) 13(6), 693-699 | ISSN:2251-6727

MB Decolorization

MB decolorization was experimented in 100 mL of Erlenmeyer by using 3 g of beads and 10 mL of MB 50 mg/L. The treated solution was incubated at 37 °C for 24 hours with 140 rpm stirring condition. Afterwards, the MB filtrate was separated through decantation and centrifuged for 10 minutes at 2000 rpm. The supernatant was decanted for analysis of decolorization percentage by using UV-Vis's spectrophotometer at 664 nm wavelength. This experiment was performed in triplicate (n=3). The percentage of decolorization was calculated by using Equation 1,

$$\% \text{ decolorization} = \frac{C_0 - C_t}{C_0} \times 100\% \quad (1)$$

where C_0 is the concentration of control (MB 50 mg/L), and C_t is the concentration of MB after treated using beads [18]. The treatment with the highest decolorization percentage was used for further experiment.

Effect of Various Temperature and Time Incubation

The effect of temperature in MB decolorization was studied at 25, 30, 35, 40, and 45 °C with initial MB concentration of 50 mg/L and incubation time of 24 hours. In the other hand, the variation of incubation time that used in this study was 6, 12, 18, 24, and 30 hours. All the results were analyzed by UV-Vis's spectrophotometer. These experiments were performed in triplicate (n=3).

3. Result And Discussion

Preparation of SA-PVA@P. aeruginosa Beads

In the fabrication process, SA-PVA@*P. aeruginosa* beads are formed through a cross-linking method by using various cross-linking agents. The illustration scheme of the cross-linking process was depicted in Fig. 1. SA could be cross-linked by $CaCl_2$, while PVA could be cross-linked by boric acid. $CaCl_2$ as the cross-linking agent exchanged Na^+ ions in SA with Ca^{2+} ions so that a coordination structure with the polyguluronate segment of SA is formed [19, 20]. According to Zain et al. [21], the polyguluronate residues in alginate bind more effectively with Ca^{2+} ions than the polymannuronate residues. SA acted as a ligand by binding calcium ions to itself. On the other hand, tetrahydroxyborate ions in the boric acid cross-linked the alcohol groups of PVA. These linkages appear almost identical to chemical bonds, except they are labile polar covalent interactions. They are constantly and rapidly break and reform, resulting in unusual intermediate solid and liquid properties [22]. Jóźwiak et al. [16] reported that the use of the effective cross-linking agent composition enhanced the efficiency of dye decolorization and strength of the beads prepared. Therefore, the composition of $CaCl_2$ and boric acid as the cross-linking agent was studied.

The resulting beads of SA-PVA@*P. aeruginosa* from the cross-linking process using $CaCl_2$ and boric acid have a diameter of around 4 mm, white colour, smooth surface, and no tails. Physically, the beads become sturdier with the increasing concentration of boric acid added to the cross-linking agent solution. The beads of SA-PVA@*P. aeruginosa* is shown in Fig 2.

Fig 2. Beads of SA-PVA immobilized *P. aeruginosa*

MB Biodecolorization Test and Beads Characterization

MB concentration and the ability of SA-PVA@*P. aeruginosa* beads to decolorize MB in this study were analyzed by using a UV-Vis's spectrophotometer. SA and PVA could absorb pollutants due to the presence of -COOH and -OH in the structure of SA and PVA, respectively [14]. SA and PVA can also be used as carbon and energy sources by immobilized bacteria [23]. Meanwhile, in the immobilized beads, *P. aeruginosa* played a role not only in absorbing MB but also in degrading it. It was because *P. aeruginosa* produces several enzymes which can biotransform the structure of pollutants to be more harmless [24].

The efficiency of MB decolorization by using SA-PVA@*P. aeruginosa* beads in this study were summarized in Table 1. SA-PVA@*P. aeruginosa aeruginosa* beads prepared by the cross-linking process using $CaCl_2$ and boric acid with the composition of 4:5

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JCHR (2023) 13(6), 693-699 | ISSN:2251-6727

have the highest MB decolorization percentage, approximately 86.252%. It was followed by the $CaCl_2$ and boric acid compositions of 4:7 (83.619%), 4:3 (81.022%), 4:1 (79.212%), and 4:0 (79.212%), and 4:0 (77.140%). The gap percentage of MB decolorization by SA-PVA-*P. aeruginosa* beads with boric acid as the additional cross-linking agent and without boric acid was approximately 10.356%.

Beads, as an adsorbent, were designed to have a porous structure with a negatively charged surface so that it could absorb more cationic dye, such as MB. Meanwhile, boric acid as an additional cross-linking agent acted to enhance the mechanical stability of the beads due to the decolorization process and contributed more active sites in the beads to bind MB. SA-PVA matrix are also used as a protector for *P. aeruginosa* bacteria so that its enzymatic activity in eliminating MB becomes more optimal [25]. *Pseudomonas* is a ligninolytic enzyme-producing bacteria whose role is to break down the lignin structure. This enzyme can break down dyes that have a compound structure like lignin, such as MB [23]. Like other dye-degrading bacteria, *P. aeruginosa* is known to be able to produce manganese peroxidase (MnP), laccase, oxygenase, *N*-demethylase, lipase, and lignin peroxidase (LiP), which are the main factors of the lignin-degrading enzyme system [11, 12].

FTIR spectra characterization of SA-PVA@*P. aeruginosa* beads by using boric acid as the additional cross-linking agent and $CaCl_2$ in this study was depicted in Fig 1. The beads have broad peaks around 3210 cm^{-1}, which were ascribed from the O-H stretching vibrations of SA and PVA as matrix constituents [27]. The PVA band of aliphatic stretching C-H vibration appeared at 2950 cm^{-1} [9]. The bands at 1624 and 1420 cm^{-1} might be attributed to the asymmetrical and symmetrical stretching of C=O vibrations, respectively [28]. The bands at 1193 and 1112 cm^{-1} indicated the stretching vibration of asymmetrical and symmetrical aliphatic C-O. Meanwhile, O-H bending was obtained at 1331 cm^{-1} [29].

Regarding boric acid which was used as additional cross-linking agent in beads preparation, the boric acid peak appeared at 1420 and 637 cm^{-1} due to B-O stretching and O-B-O bending vibrations, indicating that the cross-linking process was successfully done. In addition, a peak at 1288 cm^{-1} has been attributed to the asymmetrical stretching of B-O-C vibrations [20, 21]. These three peaks confirmed the reaction of boric acid with the hydroxyl group of PVA in SA-PVA@*P. aeruginosa* beads.

Table 1. MB decolorization by using SA-PVA@*P. aeruginosa* beads with different cross-linking agents

$CaCl_2$: boric acid (%*w/v*)	Final Concentration of MB (mg/L)	Decolorization (%)
4:0	11.472 ± 0.236	77.140 ± 0.689
4:1	10.433 ± 0.215	79.212 ± 0.539
4:3	9.526 ± 0.118	81.022 ± 0.128
4:5	6.276 ± 0.150	86.252 ± 0.442
4:7	8.222 ± 0.087	83.619 ± 0.065

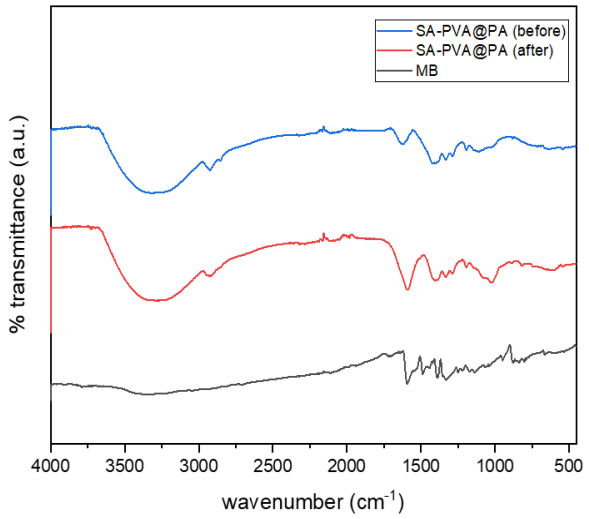

Fig 3. FTIR spectra of SA-PVA@*P. aeruginosa*

The SA-PVA@*P. aeruginosa* beads after MB decolorization showed no significant differences in FTIR spectra with the beads before decolorization (Fig. 3). The peaks at 3290 cm^{-1} indicated the stretching vibration of hydroxyl group which overlapped with the stretching vibration of the secondary amine group [28]. The strong peak at 1600 cm^{-1} indicated the asymmetrical C-O stretching vibration which overlapped with the C=C stretching vibration of MB structure [9, 30]. Furthermore, the new peak at 814 cm^{-1} was attributed as the bending vibration of C-N [31].

Effect of Temperature and Incubation Time on MB Decolorization

Since SA-PVA@*P. aeruginosa* beads prepared with $CaCl_2$ and boric acid compositions of 4:5 as cross-linking agents had the most optimum percentage of MB

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decolorization, thus that beads have been chosen for further analysis of the temperature and incubation time effect. The temperature variations used in this study were 25, 30, 35, 40, and 45 °C referring to the optimum temperature range used for *P. aeruginosa*. The MB decolorization results which were influenced by various incubation temperatures were summarized in Table 2.

Table 2. The effect of temperature on MB decolorization by using SA-PVA@*P. aeruginosa* beads cross-linked by $CaCl_2$: boric acid (4:5)

Temperature (°C)	Final Concentration of MB (mg/L)	Decolorization (%)
25	13.834 ± 0.229	72.434 ± 0.660
30	11.680 ± 0.215	76.726 ± 0.642
35	9.526 ± 0.033	81.020 ± 0.260
40	6.275 ± 0.059	87.449 ± 0.114
45	16.121 ± 0.229	67.883 ± 0.180

The highest MB decolorization percentage by SA-PVA@*P. aeruginosa* beads in this study was obtained at 40 °C followed by 35, 30, 25, and 45 °C. The metabolic activity of microorganisms such as *P. aeruginosa* bacteria in degrading dye pollutants was affected by temperature. Each enzyme produced by bacteria has varying characters depending on the type of microbes [31]. At a too low temperature, the enzymes become inactive so that the biodecolorization efficiency will be inhibited, whereas at a too high temperature the enzyme is denatured so that the decolorization capacity will be decreased [33].

In addition, the incubation time effect on MB decolorization by using SA-PVA@*P. aeuginosa* was study at 6, 12, 18, 24, and 30 hours. Initial MB concentration was 50 mg/L with 40 °C incubation temperature at 120 rpm stirring condition. Table 3 showed that at 6 hours until 24 hours incubation times, MB decolorization process was increased along with the decreasing of the concentration. At 30 hours incubation time, the final concentration was a little bit lower than 24 hours incubation. It could be suggested that 24 hours was the optimum incubation time for this experiment.

Table 3. The effect of incubation time on MB decolorization by using SA-PVA@*P. aeruginosa* beads with $CaCl_2$: boric acid (4:5)

Incubation Time (h)	Final Concentration of MB (mg/L)	Decolorization (%)
6	23.509 ± 0.260	53.162 ± 0.328
12	17.009 ± 0.182	66.112 ± 0.408
18	10.886 ± 0.164	78.308 ± 0.516
24	6.993 ± 0.075	86.067 ± 0.209
30	6.904 ± 0.204	86.268 ± 0.439

4. Conclusion

The different composition of $CaCl_2$ and boric acid as cross-linking agents in SA-PVA immobilized *P. aeruginosa* beads for MB decolorization was investigated. The $CaCl_2$ and boric acid of 4:5 had the highest MB decolorization among others, which was approximately 86.252%. The optimum incubation temperature was obtained at 40 °C for 24 hours incubation time.

Acknowledgments

The authors are grateful to Lembaga Pengelola Dana Pendidikan (LPDP), The Ministry of Finance of Indonesia, for giving full funding to the author to continue the master's study. This research was also supported by an international seminar and thesis research fund by LPDP.

Reference:

1. K. Selvam, K. Swaminathan, and Keon-Sang Chae, "Microbial decolourization of azo dyes and dye industry effluent by *Fomes lividus*", World Journal of Microbiology and Biotechnology, vol. 19, pp. 591-593, 2003. https://doi.org/10.1023/A:1025128327853.
2. A. Sariwati, A. S. Purnomo, "The Effect of *Pseudomonas aeruginosa* Addition on 1,1,1-Trichloro-2,2-bis(4-chlorophenyl) ethane (DDT) Biodegradation by Brown-rot Fungus *Fomitopsis pinicola*", Indonesian Journal of Chemistry, vol. 18, no. 1, 2018. https://doi.org/10.22146/ijc.25158.
3. R. M. Al-zahrani, F. Al-otibi, N. Marraiki, R. I. Alharbi, H. A. Aldehaish, "Biodegradation of Petroleum Hydrocarbons by *Drechsleraspicifera* Isolated from Contaminated Soil in Riyadh, Saudi Arabia", Molecules, vol. 27, no. 19, 2022. https://doi.org/10.3390/molecules27196450.
4. A. R. P. Hidayat, A. R. Widyanto, Asranudin, R. Ediati, D. O. Sulistiono, H. S. Putro, D. Sugiarso,

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 693-699 | ISSN:2251-6727

D. Prasetyoko, A. S. Purnomo, H. Bahruji, B. T. I. Ali, I. S. Caralin, “Recent Development of Double Chamber Microbial Fuel Cell for Hexavalent Chromium Waste Removal”, Journal of Environmental Chemical Engineering, vol. 10, 107505, 2022. https://doi.org/10.1016/j.jece.2022.107505

5. P. O. Oladoye, T. O. Ajiboye, E. O. Omotola, and O. J. Oyewola, "Methylene Blue Dye: Toxicity and Potential Elimination Technology from Wastewater", Results in Engineering, vol. 16, 100678, 2022. https://doi.org/10.1016/j.rineng.2022.100678.
6. U. Yunusa, B. Usman, and M. B. Ibrahim, "Cationic Dyes Removal from Wastewater by Adsorptive Method: A Systematic In-Depth Review". Algerian Journal of Chemical Engineering, vol. 2, pp. 6-40, 2021. https://doi.org/10.5281/ZENODO.5101197.
7. R. Jayalakshmi, K. Soundaranayaki, and M. S. Kannan, "Removal of Methylene Blue Dye from Textile Wastewater Using Vertical Flow Constructed Wetland", Materials Today: Proceedings, vol. 77, pp. 365–370, 2023. https://doi.org/10.1016/j.matpr. 2022.12.030.
8. A. Malik, “Metal Bioremediation Through Growing Cells”, Environment Interfaces, vol. 30, pp. 261–78, 2004, https://doi.org/10.1016/j.envint.2003.08.001
9. B. Nabilah, A. S. Purnomo, D. Prasetyoko, and A. A. Rohmah, "Methylene Blue Biodecolorization and Biodegradation by Immobilized Mixed Cultures of *Trichoderma viride* and *Ralstonia pickettii* into AA-PVA-Bentonite Matrix", Arabian Journal of Chemistry, vol. 16, no. 8, 104940, 2023. https://doi.org/10.1016/j.arabjc.2023.104940.
10. H. D. Rizqi and A. S. Purnomo, "The ability of Brown-Rot Fungus *Daedalea dickinsii* to Decolorize and Transform Methylene Blue Dye", World Journal of Microbiology and Biotechnology, vol. 33, no. 5, 92, 2017. https://doi.org/10.1007/s11274-017-2256-z.
11. A. S. Purnomo, D. Maulianawati, I. Kamei, “*Ralstonia pickettii* Enhance the DDT Biodegradation by *Pleurotus eryngii*”, Journal of Microbiology and Biotechnology, vol. 29, no. 9, pp. 1424-1433. https://doi.org/10.4014/jmb.1906.06030.
12. R. Kishor, D. Purchase, G. D. Saratale, F. L. F. Romanholo, C. M. Hussain, S. I. Mulla, and R. N. Bharagava, "Degradation Mechanism and Toxicity Reduction of Methyl Orange Dye by A Newly Isolated Bacterium *Pseudomonas aeruginosa* MZ520730, Journal of Water Process Engineering, vol. 43. 102300, 2021. https://doi.org/10. 1016/j.jwpe.2021.102300.
13. O. Anjaneya, S. Y. Souche, M. Santoshkumar, and T. B. Karegoudar, "Decolorization of Sulfonated Azo Dye Metanil Yellow by Newly Isolated Bacterial Strains: *Bacillus* sp. strain AK1 and *Lysinibacillus* sp. strain AK2", Journal of Hazardous Materials, vol. 190, no. 3, pp. 351–358, 2011. https://doi.org/10.1016/j.jhazmat.2011.03. 44.
14. E. Eroglu, V. Agarwal, M. Bradshaw, X. Chen, S. M. Smith, C. L. Raston, and I. K. Swaminathan, “Nitrate removal from liquid effluents using microalgae immobilized on chitosan nanofiber mats”, Green Chemistry, vol. 14, pp. 2682-2685, 2022. https://doi.org/10.1039/ C2GC35970G.
15. F. Zahakifar, A. R. Keshtkar, and M. Talebi. “Synthesis of Sodium Alginate (SA)/ Polyvinyl Alcohol (PVA)/ Polyethylene Oxide (PEO)/ ZSM-5 Zeolite Hybrid Nanostructure Absorbent by Casting Method for Uranium (VI) Adsorption from Aqueous Solutions”, Progress in Nuclear Energy, vol. 134, 103642, 2021. https://doi.org/10.1016/j.pnucene.2021103642.
16. T. Jóźwiak, U. Filipkowska, P. Szymczyk, J. Rodziewicz, and A. Mielcarek, "Effect of Ionic and Covalent Crosslinking Agents on Properties of Chitosan Beads and Sorption Effectiveness of Reactive Black 5 Dye", Reactive and Functional Polymers, vol. 114, pp. 58–74, 2017. https://doi.org/10.1016/j.reactfunctpolym.2017.03. 007.
17. A. S. Purnomo, K. Ashari, and F. T. Hermansyah, "Evaluation of The Synergistic Effect of Mixed Cultures of White-Rot Fungus *Pleurotus ostreatus* and Biosurfactant-Producing Bacteria on DDT Biodegradation", Journal of Microbiology and Biotechnology, vol. 27, no. 7, pp. 1306–1315, 2017. https://doi.org/10.4014/jmb.1701.01 073.
18. A. A. Rohmah, A. S. Purnomo, and W. N. Safitri, "Biodecolorization of Methylene Blue by Using *Bacillus subtilis* Immobilized into SA-PVA-Bentonite Matrix in Mineral Salt Medium and Non-nutritious Medium", Indonesian Journal of Chemistry, vol. 22, no. 6, 1637, 2022. https://doi.org/10.22146/ijc.76080.
19. H. Lu, J. A. Butler, N. S. Britten, P. D. Venkatraman, and S. S. Rahatekar, “Natural Antimicrobial Nano Composite Fibres Manufactured from A Combination of Alginate and Oregano Essential Oil”, Nanomaterials, vol. 11, no. 8, 2062, 2021. https://doi.org/10.3390/nano11082062.
20. A. Merakchi, S. Bettayeb, N. Drouiche, L. Adour, and H. Lounici, “Cross-linking and Modification of

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 693-699 | ISSN:2251-6727

Sodium Alginate Biopolymer for Dye Removal in Aqueous Solution", Polymer Bulletin, vol. 76, no. 7, pp. 3535–3554, 2019. https://doi.org/10.1007/s00289- 018-2557-x.

21. N. A. M. Zain, M. S. Suhaimi, A. dan Idris, "Development and Modification of PVA–Alginate as A Suitable Immobilization Matrix", Process Biochemistry, vol. 46, no. 11. pp. 2122–2129, 2011. https://doi.org/10.1016/j.procbio. 2011.08.010.
22. P. Pattanapipitpaisal, N. L. Brown, and L. E. Macaskie, "Chromate Reduction by Microbacterium *Liquefaciens* Immobilised in Polyvinyl Alcohol", Biotechnology Letter, vol. 23, pp.61-65, 2001. https://doi.org/10.1023/A:102675 0810580.
23. B. Liu, J. Zhang, and H. Guo, "Research Progress of Polyvinyl Alcohol Water-resistant Film Materials", Membranes, vo. 12, no. 3, 347, 2022. https:doi.org/10.390/ membranes12030347.
24. S. Bhandari, D. K. Poudel, R. Marahatha, S. Dawadi, K. Khadayat, S. Phuyal, S. Shrestha, S. Gaire, K. Basnet, U. Khadka, and N. Parajuli, "Microbial Enzymes Used in Bioremediation", Journal of Chemistry, vol. 2021, 8894512, 2021. https://doi.org/1 0.1155/2021/8894512.
25. H. M. Lee, H. R. Kim, E. Jeon, H. C. Yu, S. Lee, J. Li, D. Kim, "Evaluation of the Biodegradation Efficiency of Four Various Types of Plastics by *Pseudomonas aeruginosa* Isolated from the Gut Extract of Superworms", Microorganisms, vol 8, no. 9, 1341, 2020. https://doi.org/ 10.3390/microorganisms8091341.
26. L. Bandounas, N. J. Wierckx, J. H. Winde, and H. J. Ruijssenaars, "Isolation and Characterization of Novel Bacterial Strains Exhibiting Ligninolytic Potential", BMC Biotechnology, vol. 11, no.1, 94, 2011. https://doi.org/ 10.1186/1472-6750-11-94
27. E. Baigorria, L. A. Cano, L. M. Sanchez, V. A. Alvarez, and R. P. Ollier, "Bentonite-composite Polyvinyl Alcohol/alginate Hydrogel Beads: Preparation, Characterization and Their Use as Arsenic Removal Devices", Environment Nanotechnology Monitoring Management, vol. 14, 100364, 2020. https://doi.org /10.1016/j.enmm.2020.100364.
28. A. Yumin, D. Liguo, Y. Yi, and J. Yongna, "Mechanical Properties of An Interpenetrating Network Poly(vinyl Alcohol)/alginate Hydrogel with Hierarchial Fibrous Structures", RSC Advances, vol. 11632, 2022. https://doi.org/10.1039/d1ra07368k.
29. T. R. Alkas, R. Ediati, T. Ersam, R. Nawfa, and A. S. Purnomo, "Fabrication of Metal-Organic Framework Universitetet I Oslo-66 (Uio-66) and Brown-Rot Fungus *Gloeophyllum trabeum* Biocomposite (UiO-66@GT) and Its Application for Reactive Black 5 Decolorization", Arabian Journal of Chemistry, vol. 15, no. 10, 104129, 2022. https://doi.org/10.1016/ j.arabjc. 2022.104129.
30. L. Sun, J. Wang, J. Liang, and G. Li, "Boric Acid Cross-linked 3D Polyvinyl Alcohol Gel Beads by NaOH-Titration Method as A Suitable Biomass Immobilization Matrix", Journal of Polymer and The Environment, vol. 28, pp. 532-541, 2020. https://doi.org/ 10.1007/s10924-019-01610-z.
31. A. F. Isik, N. O. S. Keskin, and Y. Ulcay. "Synthesis and In Vitro Antimicrobial Characterization of Boron-PVA Electrosspun Nanofibers", The Journal of Textile Institute, 2018. https://doi.org/10.10180/00405 000.2018.1496989.
32. S. Wahyuni, A. Khaeruni, A. S. Purnomo**,** Asranudin, Holilah, Fatahu, "Characterization of Mannanase Isolated from Corncob Waste Bacteria", Asian Journal of Chemistry, vol. 29, no. 5, pp. 1119-1120, 2017. https://doi.org/10. 14233/ajchem.2017.20437.
33. M. P. Alves, R. L. Salgado, M. R. Eller, R. S. Dias, S. O. de Paula, and A. F. de Carvalho, "Temperatue Modulates the Production and Activity of a Metalloprotease from *Pseudomonas fluorescens* 07A in Milk", Journal of Diary Science, vol. 101, no. 2, pp. 992-999, 2016. https://doi.org/ 103168/jds.2017-13238.