Asian Journal of Natural Sciences (AJNS)
Vol. 3, No. 3, 2024 : 149-158

Lignosulfonate-Chitosan Based Hydrogel Production for Water Storage with Laccase by *Trametes versicolor*

Danu Risqi Yudiargo[1], Endry Nugroho Prasetyo[2]*
Institut Teknologi Sepuluh Nopember
Corresponding Author: Endry Nugroho Prasetyo enpracorp@gmail.com

A R T I C L E I N F O

Keywords: Laccase, Hydrogels, Trametes versicolor, Lignosulfonate, Chitosan

Received : 03, July
Revised : 25, July
Accepted: 08, August

A B S T R A C T

The combination of lignosulfonate and chitosan biopolymers will provide a highly air-soluble structure for a copolymer water storage hydrogel due to the large number of hydrophilic functional groups. Laccase plays a role in catalyzing the electron oxidation reaction of compounds that occur in lignosulfonate so that the opportunity to bind to chitosan is also greater. The aim of this study was to fabricate lignosulfonate-chitosan based hydrogel for water storage with laccase by Trametes versicolor. The hydrogel was fabricated by adding laccase to the lignosulfonate and chitosan solution, then added with acetic acid, PVA, and glutardialdehyde with vigorous stirring. The water absorption value of the water storage hydrogel that was successfully created was 7.18%

INTRODUCTION

Land with high levels of drought requires more water to irrigate agricultural land. Water storage hydrogel is known to be able to improve the physical properties of soil while increasing the efficiency of water use in dry and semi-dry areas (Albalasmeh et al., 2022). Water storage hydrogel is a group of polymer materials, the hydrophilic structure of which makes it able to hold large amounts of water in its three-dimensional network (Ahmed, 2015). Enzymatic cross-linking has the primary benefit of allowing the hydrogel to cross-link under mild conditions without requiring the use of low molecular weight chemicals (monomers, initiators, or cross-linking agents), irradiation, or previous polymer functionalization to promote cross-linking (Bustamante-Torres et al., 2021). Laccase catalyzes the oxidation of various substrates plus the reduction of molecular oxygen to water by a one-electron oxidation mechanism (Saoudi & Ghaouar, 2019). Chitosan is a hydrophilic polymer and has strong water solubility with numerous hydroxyl (−OH) and amino (−NH2) groups (Qiao et al., 2021). Laccase can catalyze the formation of phenolic-chitosan hydrogel (Huber et al., 2017). In the presence of small redox mediators, laccases provide a broad oxidation repertoire including non-phenolic substrates (Upadhyay, Shrivastava, & Agrawal, 2016). Utilization of lignin-based synthetic hydrogel is one prospective way to recycle lignosulfonate pulping waste (Wang et al., 2016). Hydrogels based on lignosulfonates or modified lignosulfonate raw materials are mainly prepared by crosslinking or crosslinking with other polymers (Teng et al., 2017). Oxidized phenolic laccase is a potential chitosan crosslinking agent as a new environmentally friendly approach to synthesize chitosan hydrogels (Huber et al., 2017).

Polyvinyl alcohol (PVA) is a common non-toxic water-soluble polymer with a polyhydroxy structure that rapidly dissolves in water and leaves no trace, making it useful in a variety of applications (Jiang et al., 2024). The growing interest in PVA-based hydrogels is evidenced by the increasing number of original research articles and reviews published in recent years (Bercea, 2024). The most popular technique for creating PVA hydrogels is to use dialdehydes as cross-linkers and it has been discovered that the hydroxyl group of PVA forms an acetal bond with aldehydes, and that the actual reaction of glutaraldehyde or glyoxal occurs under moderate circumstances (Aflatounian, Sharzehee, & Mashroteh, 2024). Glutaraldehyde has been widely employed in the chemical cross-linking of chitosan, and it is mostly used for cross-linking when a second polymer is introduced to chitosan to modify its characteristics (Ahmadi, Oveisi, Samani, & Amoozgar, 2015). The aim of this study was to fabricate lignosulfonate-chitosan based hydrogel for water storage with laccase by Trametes versicolor.

LITERATURE REVIEW

The process of crosslinking polymers to create hydrogels can be conducted in the presence of enzymes (Husain, Gupta, Alashwal, & Sharma, 2018). The oxidation of lignosulfonates with fungal oxidoreductases, particularly laccases, leads to increased molecular weight through cross-linking of lignin phenols. This process generates radicals in lignin, potentially leading to C–C bond cleavage and depolymerization, although repolymerization typically prevails (Rodríguez-Escribano, de Salas, Pardo, & Camarero, 2017). Laccase can oxidize lignin into radicals linked to water-soluble chitosan fragments through a process called radical coupling or Michael addition, which creates a stable gel made of chitosan linked to lignin (Beer et al., 2020). The preparation of hydrogel sponges from the gelatin/chitosan (GEL/CTS) matrix was successful with improved compressibility and thermal properties by adding the polyols like polyvinyl alcohol and polyethylene glycol- polypropylene glycol-polyethylene glycol (Vo, Vo, Tran, & Pham, 2022). In order to create a hydrogel that had physicochemical properties comparable to those of vitreous humor, polyvinyl alcohol and glutaraldehyde were used as cross-linkers (Morandim-Giannetti et al., 2018). The cross-linking of chitosan and PVA results in the formation of a porous hydrogel structure characterized by significant flexibility, durability, and the ability to absorb water effectively (Zhu et al., 2022).

METHODOLOGY

Lignosulfonate-Chitosan Hydrogel Fabrication

The hydrogel preparation was started by adding 1 mL of laccase to the lignosulfonate and chitosan solution. The lignosulfonate and chitosan solution was made by mixing 0.8 g of lignosulfonate and 0.8 g of chitosan into 17 mL of distilled water. Then the solution was stirred for 15 minutes and left for 10 minutes. The solution was then added with 2.2 g of acetic acid with vigorous stirring for 30 minutes at a temperature of 50°C. Then, 2.5 g of PVA was added to the chitosan solution with continuous stirring for 30 minutes at a temperature of 50°C. Then, the solution was added with 20 µL of Glutardialdehyde and stirred for 15 minutes. The solution was poured into a petri dish and dried in an oven. After 24 hours, the dry hydrogel was measured for dry weight. The dry hydrogel was added with 50 mL of distilled water, then the wet weight was measured after 1 hour.

RESEARCH RESULT

The results of water absorption capacity per gram of dry hydrogel 1 hour after adding 50 mL of distilled water can be seen in table 1.

Table 1. Water Absorption Capacity Results

Dry Weight (g)	Wet Weight (g)	Initial Volume (mL)	Final Volume (mL)	Water Absorption Capacity per gram of dry hydrogel 1 hour after adding 50 mL of distilled water (%)
3.9	23.4	50	22	7.18

Based on the analysis that has been carried out, the dry weight is 3.9 g, the wet weight is 23.4 g, the final volume is 22 mL, and the Water Absorption Capacity per gram of dry hydrogel 1 hour after adding 50 mL of distilled water is 7.18%. The results of lignosulfonate-chitosan-based hydrogel fabrication can be seen in Figure 1.

Figure 1. Lignosulfonate-Chitosan Hydrogel Fabrication

DISCUSSION

Laccase is a blue copper oxidoreductase that oxidizes a variety of substrates, including phenolic compounds, while also reducing molecular oxygen to air (Mayolo-Deloisa, González-González, & Rito-Palomares, 2020). Laccase is notable for its ability to oxidize a wide range of substrates, including o- and p-diphenols, polyphenols, aminophenols, aromatic amines, and aliphatic amines, as well as its high stability under harsh conditions such as extreme pH or high temperatures (Janusz et al., 2020). Laccase oxidizes phenolic residues in lignosulfonates create phenoxyl radicals and simultaneously reduces molecular oxygen to air (Mayr et al., 2023). Laccase oxidizes lignosulfonates, producing phenoxyl radicals that react with SucCTS's single bond (−NH2) group via radical coupling or Michaeĺs addition (Haske-Cornelius et al., 2019). Adding laccase to the lignosulfonate-chitosan caused oxidation and the formation of reactive phenoxyl radicals in lignin, which cross- linked with the reactive CTS-−NH2 groups (Beer et al., 2020). Enzymes often exhibit a high degree of substrate specificity, potentially avoiding side reactions during cross-linking (Bustamante-Torres et al., 2021). The hydrophilic functional groups adhere to the polymer's backbone, allowing the hydrogels to absorb water more easily (Shajahan Haima et al., 2021).

The blending of chitosan (CS) with hydrophilic polymers, such as Polyvinyl alcohol (PVA), has been shown to significantly enhance the water absorbency of hydrogels (Michalik & Wandzik, 2020). Polyvinyl alcohol (PVA) is a common water-soluble polymer compound (Zhang, Liu, Lin, & Wang, 2020). PVA-based hydrogels are gaining significant attention owing to their functional versatility, which arises from a distinctive three-dimensional network structure that is marked by high water content and remarkable water absorption capabilities (Liang et al., 2024). The water solubility of PVA requires cross-linking to be performed prior to the synthesis of hydrogels (Supriya Bhatt, Thakur, & Nune, 2023). Glacial acetic acid was the most effective in increasing the hydrogel's porosity (Nie et al., 2020). Acetic acid was added to the powdered carboxymethyl sago starch (CMSS) as a crosslinking agent to create smart hydrogel (Tuan Mohamood, Zainuddin, Ahmad Ayob, & Tan, 2018).

The chitosan molecule swells when the -OH bonds to the water molecule in the diluted acid solution, protonating the −NH2 on the molecule to −NH3+ and breaking the hydrogen connection between the molecules (Tang, Zhao, Yuan, Chen, & Leng, 2020). Glacial acetic acid was incorporated into the mixture to facilitate the complete dissolution of chitosan (CS) during the process of magnetic stirring (M. Wang, Hu, Li, Peng, & Zeng, 2022). It has been found that crosslinking chitosan with crosslinking agents like sodium tripolyphosphate or glutaraldehyde is a practical and efficient way to enhance its chemical and physical characteristics for use in various applications (OU & BO, 2017).

Glutaraldehyde is extensively utilized as a crosslinking agent owing to its ease of handling, affordability, high reactivity, excellent solubility in water, reduced toxicity, and adequate crosslinking ability (Zainal et al., 2021). Because it interacts effectively with the functional groups of polymers to form a stable and long-lasting hydrogel network, glutaraldehyde is an effective crosslinker for hydrogels (Gadaime, S. M. N. Mydin, Govindasamy, Bustami, & Sreekantan, 2025). Glutaraldehyde has been used as a crosslinking agent in the synthesis of gelatin/CMC hydrogels (Sahar et al., 2023). Glutaraldehyde has been widely employed in the chemical cross-linking of chitosan, and it is mostly used for cross-linking when a second polymer is introduced to chitosan to modify its characteristics (Ahmadi et al., 2015). Glutaraldehyde was used to crosslink the hydroxyl groups of bacterial cellulose with the gelatin's amine groups (Treesuppharat, Rojanapanthu, Siangsanoh, Manuspiya, & Ummartyotin, 2017).

CONCLUSIONS AND RECOMMENDATIONS

The results of making lignosulfonate-chitosan-based hydrogels for water storage using laccase of T. versicolor obtained a water absorption capacity/g of 7.18%.

ADVANCED RESEARCH

Suggestions for further research include the need to apply hydrogel water storage to agricultural land.

ACKNOWLEDGEMENTS

We would like to express my sincere appreciation to the Institut Teknologi Sepuluh Nopember and individuals whose contributions and support have greatly enhanced the quality and rigour of this research.

REFERENCES

Aflatounian, A., Sharzehee, M., C Mashroteh, H. A. (2024). Preparation of stable polyvinyl alcohol/polyurea hydrogel using oligomeric compounds containing urea-bonded. Results in Materials, 22, 100564. https://doi.org/10.1016/j.rinma.2024.100564

Ahmadi, F., Oveisi, Z., Samani, S. M., & Amoozgar, Z. (2015). Chitosan based hydrogels: characteristics and pharmaceutical applications. Research in Pharmaceutical Sciences, 10(1), 1–16.

Ahmed, E. M. (2015). Hydrogel: Preparation, characterization, and applications: A review. Journal of Advanced Research, 6(2), 105–121. https://doi.org/10.1016/j.jare.2013.07.006

Albalasmeh, A. A., Mohawesh, O., Gharaibeh, M. A., Alghamdi, A. G., Alajlouni, M. A., & Alqudah, A. M. (2022). Effect of hydrogel on corn growth, water use efficiency, and soil properties in a semi-arid region. Journal of the Saudi Society of Agricultural Sciences, 21(8), 518–524. https://doi.org/10.1016/j.jssas.2022.03.001

Beer, B., Bartolome, M. J., Berndorfer, L., Bochmann, G., Guebitz, G. M., & Nyanhongo, G. S. (2020). Controlled enzymatic hydrolysis and synthesis of lignin cross-linked chitosan functional hydrogels. International Journal of Biological Macromolecules, 161, 1440–1446. https://doi.org/10.1016/j.ijbiomac.2020.08.030

Bercea, M. (2024). Recent Advances in Poly(vinyl alcohol)-Based Hydrogels. Polymers, 16(14), 2021. https://doi.org/10.3390/polym16142021 Bustamante-Torres, M., Romero-Fierro, D., Arcentales-Vera, B., Palomino, K.,

Gadaime, N. K. R., S. M. N. Mydin, R. B., Govindasamy, G. A., Bustami, Y., & Sreekantan, S. (2025). Advanced Hydrogel Dressing with Zinc Oxide-Copper Oxide Nanocomposite for Effective Wound Management: Mechanochemistry, Antibacterial Efficacy, Cytocompatibility and Wound Healing Potentials. Journal of Polymers and the Environment, 33(3), 1601–1614. https://doi.org/10.1007/s10924-024-03468-2

Haske-Cornelius, O., Bischof, S., Beer, B., Jimenez Bartolome, M., Olatunde Olakanmi, E., Mokoba, M., … Nyanhongo, G. S. (2019). Enzymatic synthesis of highly flexible lignin cross-linked succinyl-chitosan hydrogels reinforced with reed cellulose fibres. European Polymer Journal, 120. https://doi.org/10.1016/j.eurpolymj.2019.08.028

Huber, D., Tegl, G., Baumann, M., Sommer, E., Gorji, E. G., Borth, N., … Guebitz, G. M. (2017). Chitosan hydrogel formation using laccase activated phenolics as cross-linkers. Carbohydrate Polymers, 157, 814–822. https://doi.org/10.1016/j.carbpol.2016.10.012

Husain, M. S. B., Gupta, A., Alashwal, B. Y., & Sharma, S. (2018). Synthesis of PVA/PVP based hydrogel for biomedical applications: a review. Energy

Sources, Part A: Recovery, Utilization, and Environmental Effects, 40(20), 2388– 2393. https://doi.org/10.1080/15567036.2018.1495786

Janusz, G., Pawlik, A., Świderska-Burek, U., Polak, J., Sulej, J., Jarosz-Wilkołazka, A., & Paszczyński, A. (2020, February 1). Laccase properties, physiological functions, and evolution. International Journal of Molecular Sciences, Vol. 21. MDPI AG. https://doi.org/10.3390/ijms21030966

Jiang, H., Yang, L., Tian, G., Huang, X., Xu, D., Li, L., … Li, Y. (2024). Water-soluble and environmentally friendly polyvinyl alcohol/straw aerogel for sustainable packaging. Industrial Crops and Products, 222, 119895. https://doi.org/10.1016/j.indcrop.2024.119895

Liang, X., Zhong, H.-J., Ding, H., Yu, B., Ma, X., Liu, X., … He, J. (2024). Polyvinyl Alcohol (PVA)-Based Hydrogels: Recent Progress in Fabrication, Properties, and Multifunctional Applications. Polymers, 16(19), 2755. https://doi.org/10.3390/polym16192755

Magaña, H., & Bucio, E. (2021). Hydrogels Classification According to the Physical or Chemical Interactions and as Stimuli-Sensitive Materials. Gels, 7(4), 182. https://doi.org/10.3390/gels7040182

Mayolo-Deloisa, K., González-González, M., & Rito-Palomares, M. (2020). Laccases in Food Industry: Bioprocessing, Potential Industrial and Biotechnological Applications. Frontiers in Bioengineering and Biotechnology,8. https://doi.org/10.3389/fbioe.2020.00222

Mayr, S. A., Wagner, S., Nagl, R., Pellis, A., Gigli, M., Crestini, C., … Schwaiger, N. (2023). Physicochemical Insights into Enzymatic Polymerization of Lignosulfonates. ACS Sustainable Chemistry and Engineering, 11(50),17739–17751. https://doi.org/10.1021/acssuschemeng.3c05521

Michalik, R., & Wandzik, I. (2020, October 1). A mini-review on chitosan-based hydrogels with potential for sustainable agricultural applications. Polymers, Vol. 12, pp. 1–16. MDPI AG. https://doi.org/10.3390/polym12102425

Morandim-Giannetti, A. de A., Rubio, S. R., Nogueira, R. F., Ortega, F. dos S., Magalhães Junior, O., Schor, P., & Bersanetti, P. A. (2018). Characterization of PVA/glutaraldehyde hydrogels obtained using Central Composite Rotatable Design (CCRD). Journal of Biomedical Materials Research Part B: Applied Biomaterials, 106(4),1558–1566. https://doi.org/10.1002/jbm.b.33958

Nie, G., Hong, K., Zhang, E., Liu, N., Wang, M., Wang, L., & Zang, Y. (2020). Fabrication of a porous chitosan/poly-(γ-glutamic acid) hydrogel with a high absorption capacity by electrostatic contacts. International Journal of Biological Macromolecules, 159, 986–994. https://doi.org/10.1016/j.ijbiomac.2020.05.112

OU, A., & BO, I. (2017). Chitosan Hydrogels and their Glutaraldehyde-Crosslinked Counterparts as Potential Drug Release and Tissue Engineering Systems - Synthesis, Characterization, Swelling Kinetics and Mechanism. Journal of Physical Chemistry & Biophysics, 07(03). https://doi.org/10.4172/2161-0398.1000256

Qiao, Z., Lv, X., He, S., Bai, S., Liu, X., Hou, L., … Yang, H. (2021). A mussel-inspired supramolecular hydrogel with robust tissue anchor for rapid hemostasis of arterial and visceral bleedings. Bioactive Materials, 6(9), 2829– 2840. https://doi.org/10.1016/j.bioactmat.2021.01.039

Rodríguez-Escribano, D., de Salas, F., Pardo, I., & Camarero, S. (2017). High-throughput screening assay for laccase engineering toward lignosulfonate valorization. International Journal of Molecular Sciences, 18(8). https://doi.org/10.3390/ijms18081793

Sahar, F., Riaz, A., Malik, N. S., Gohar, N., Rasheed, A., Tulain, U. R., … Shah,S. I. (2023). Design, characterization and evaluation of gelatin/carboxymethyl cellulose hydrogels for effective delivery of ciprofloxacin.Polymer Bulletin, 80(11),12271–12299. https://doi.org/10.1007/s00289-022-04657-6

Saoudi, O., & Ghaouar, N. (2019). Biocatalytic characterization of free and immobilized laccase from Trametes versicolor in its activation zone. International Journal of Biological Macromolecules, 128, 681–691. https://doi.org/10.1016/j.ijbiomac.2019.01.199

Shajahan Haima, J., Narayanan Nair, S., Juliet, S., Ravindran Nisha, A., Nair, B., & Nair Dhanushkrishna, B. (2021). Synthesis and characterisation of glutaraldehyde cross-linked κ-carrageenan-gelatin hydrogel. ~ 459 ~ Journal of Pharmacognosy and Phytochemistry, 10(1), 459–463. Retrieved from www.phytojournal.com

Supriya Bhatt, S., Thakur, G., & Nune, M. (2023). Preparation and characterization of PVA/Chitosan cross-linked 3D scaffolds for liver tissue engineering. Materials Today: Proceedings. https://doi.org/10.1016/j.matpr.2023.02.251

Tang, S., Zhao, L., Yuan, J., Chen, Y., & Leng, Y. (2020). Physical hydrogels based on natural polymers. In Hydrogels Based on Natural Polymers (pp. 51– 89). Elsevier. https://doi.org/10.1016/B978-0-12-816421-1.00003-3

Teng, X., Xu, H., Song, W., Shi, J., Xin, J., Hiscox, W. C., & Zhang, J. (2017). Preparation and Properties of Hydrogels Based on PEGylated Lignosulfonate Amine. ACS Omega, 2(1), 251–259. https://doi.org/10.1021/acsomega.6b00296

Treesuppharat, W., Rojanapanthu, P., Siangsanoh, C., Manuspiya, H., & Ummartyotin, S. (2017). Synthesis and characterization of bacterial cellulose and gelatin-based hydrogel composites for drug-delivery systems. Biotechnology Reports, 15, 84–91. https://doi.org/10.1016/j.btre.2017.07.002

Tuan Mohamood, N. F. A.-Z., Zainuddin, N., Ahmad@Ayob, M., & Tan, S. W. (2018). Preparation, optimization and swelling study of carboxymethyl sago starch (CMSS)–acid hydrogel. Chemistry Central Journal, 12(1), 133. https://doi.org/10.1186/s13065-018-0500-8

Upadhyay, P., Shrivastava, R., & Agrawal, P. K. (2016). Bioprospecting and biotechnological applications of fungal laccase. 3 Biotech, 6(1), 15. https://doi.org/10.1007/s13205-015-0316-3

Vo, T. S., Vo, T. T. B. C., Tran, T. T., & Pham, N. D. (2022). Enhancement of water absorption capacity and compressibility of hydrogel sponges prepared from gelatin/chitosan matrix with different polyols. Progress in Natural Science: Materials International,32(1), 54–62. https://doi.org/https://doi.org/10.1016/j.pnsc.2021.10.001

Wang, M., Hu, D.-D., Li, Y.-D., Peng, H.-Q., & Zeng, J.-B. (2022). Biobased mussel-inspired underwater superoleophobic chitosan derived complex hydrogel coated cotton fabric for oil/water separation. International Journal of BiologicalMacromolecules, 209, 279–289. https://doi.org/10.1016/j.ijbiomac.2022.04.007

Wang, X., Zhou, Z., Guo, X., He, Q., Hao, C., & Ge, C. (2016). Ultrasonic-assisted synthesis of sodium lignosulfonate-grafted poly(acrylic acid-co-poly(vinyl pyrrolidone)) hydrogel for drug delivery. RSC Advances, 6(42), 35550–35558. https://doi.org/10.1039/C6RA03398A

Zainal, S. H., Mohd, N. H., Suhaili, N., Anuar, F. H., Lazim, A. M., & Othaman,R. (2021). Preparation of cellulose-based hydrogel: a review. Journal of Materials Research and Technology, 10, 935–952. https://doi.org/10.1016/j.jmrt.2020.12.012

Zhang, Z., Liu, Y., Lin, S., & Wang, Q. (2020). Preparation and properties of glutaraldehyde crosslinked poly(vinyl alcohol) membrane with gradient structure. Journal of Polymer Research, 27(8), 228.https://doi.org/10.1007/s10965-020-02223-0

Zhu, M., Liu, X., Tian, Y., Caratenuto, A., Chen, F., & Zheng, Y. (2022). Dome-arrayed chitosan/PVA hydrogel-based solar evaporator for steam generation. Scientific Reports, 12(1), 4403. https://doi.org/10.1038/s41598- 022-08589-z