

Influence of Different Solvents on the Structure of ZIF-67 for Enzyme Immobilization Matrices

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ABSTRACT

Metal-organic framework (MOF) is a crystalline structure which has high porosity and surface area. MOFs' design and functionality are versatile and stem from limitless combination of metals and organic linkers, which has proven stability in processes operated at high pH and temperature and resistant to various chemicals. These features are effective in biocatalysis as enzymes need protection from harsh environments. Nevertheless, during the synthesis of Enzyme-Metal organic complexes (E-MOF), the process must be designed in a delicate way such as avoiding harsh solvent (methanol) and room temperature synthesis, to preserve protein microenvironment. The study is aimed at analyzing the effect of different solvents; ultrapure water (which consists only H⁺ and OH⁻ ions) and methanol towards ZIF-67 morphology. ZIF-67(W) shows leaf-like morphology with crystal size in the range of 10-16µm while ZIF-67(M) shows rhombic dodecahedron morphology with crystal size in the range of 200-400nm. It was found that ZIF-67(M) has better thermal stability compared to ZIF-67(W). The relationship between the different solvents used to synthesize ZIF-67 and in-situ enzyme encapsulation is discussed by considering the feasibility of ZIF-67 synthesis with water as an eco-friendly solvent with good characteristics. Preliminary result showed that more than 90% of methylene blue can be degraded by laccase@ZIF-67(W).

Keywords: ZIF-67; biocatalysis; laccase; metal organic framework; enzyme immobilization

INTRODUCTION

Metal-organic frameworks (MOF) are nanoparticles that consist of metal ions and organic ligands that are constructed together forming a crystalline structure, like a cage (W. Zhang & Pinna, 2024). There are thousand types of MOFs have been reported up to these days which grouped into Isoreticular MOFs, Zeolitic Imidazolate Frameworks (ZIFs), Porous Coordination Networks (PCNs), Materials Institute Lavoisier (MIL), Porous Coordination Polymers (PCPs) and University of Oslo (UiO) MOFs among others (Yusuf et al. 2022). The major characteristics of MOFs are high surface area, tunable pore sizes, and versatile structural and chemical properties which provide wide applications in gas storage, separation, and catalysis. ZIF-67 is one type of MOF from the Zeolitic

Imidazolate Framework (ZIF) family, constructed from cobalt ion and 2-methylimidazole organic ligand, producing purple color powder. ZIF-67 are highly versatile materials with extensive applications in enzyme technology, catalysis, environmental remediation, drug delivery, biosensing, and organic synthesis (G. Zhong et al. 2018). It was reported that ZIF, in general, can enhance enzyme stability, activity, and reusability by providing a protective and conducive microenvironment and hence enable efficient biocatalytic reactions and improve the durability of enzymes under extreme conditions (Xu et al. 2021).

Integration of enzymes with MOF is governed according to the different functionalities in processes and typically involves encapsulation (Chai et al. 2021), (Nadar et al. 2020), adsorption (Jia et al. 2019), (L. H. Liu et al. 2021) or covalent bonding (Patra et al. 2016).

Adsorption involves physically adsorbing the enzyme onto the MOF surface, leveraging the high surface area and pore volume of MOFs to increase enzyme loading. Covalent bonding, on the other hand, chemically links the enzyme to the MOF, ensuring strong attachment and reducing leaching. Encapsulation or in-situ synthesis involves synthesizing the MOF in the presence of the enzymes, trapping the enzyme within the MOF structure. This integration can be applied in various areas such as biosensors, bioremediation, drug synthesis, and CO₂ reduction (Bilal et al. 2019).

Tuning the MOF structure is important in achieving the target application as the different parameters can bring different results for the MOF (Han et al. 2024). MOF can be altered in its structure by using different concentration ratios of metal ion to organic ligand, different solvents during synthesis, MOF post modification, and different synthesis duration (Bergaoui et al. 2021; Thakare & Ramteke, 2018). In terms of solvent as a parameter, Ahmad, S.Z.N., and a co-worker (2023) (Ahmad et al. 2023) conducted the synthesis of ZIF-8 by using different solvents; water, methanol, and ammonia for heavy metal removal. It was found that the morphology of ZIF-8 (water) resembled the mixture of a cubic and rhombic dodecahedron with truncated corners, whereas ZIF-8(methanol) occurred as the most stable rhombic dodecahedron and ZIF-8 (ammonia) appeared as cubes with truncated corners. In a study by Jian and team (2015) (Jian et al. 2015), even though using only water as the solvent in the synthesis, the morphology of the MOF transitions from a multilayered structure to characteristic ZIF-8 crystals as the water amount is reduced. Traditionally, dimethylformamide (DMF) and methanol are among the most common solvents used to synthesize MOF (Kumar et al. 2020). Gao J. and team (2020) (Gao et al. 2020) used DMF in synthesizing ZIF-7 before immobilizing it to the membrane. Ta D. et al. (2018) (Ta et al. 2018) used methanol in constructing nano-ZIF-8 with high yield. However, using this solvent can degrade the enzyme due to its harsh nature during enzyme immobilization, especially in-situ encapsulation hence reducing the biocatalytic efficiency. Cyrene or dihydrolevoglucosenone is the alternative green solvent but Cyrene requires a high-temperature and long-duration synthesis process due to the high viscosity (J. Zhang et al. 2016). Zhang J and co-workers (2016) (J. Zhang et al. 2016) used Cyrene at 110°C for 10 hours for ZIF-8 synthesis in a 2-dram vial. This condition will also affect the enzyme if the in-situ encapsulation method is to be applied. Other methods such as adsorption, covalent bonding, and infiltration encapsulation may be used as MOF has already been constructed (Guajardo & Domínguez de María, 2020). Using water as the solvent for synthesis is widely used as water is not expensive, can be synthesized at ambient temperature and

atmospheric pressure, and is readily available (Shi et al. 2017).

In this study, ZIF-67 is synthesized by using ultrapure water and methanol, denoted as ZIF-67(W) and ZIF-67(M) respectively. The solvent effect upon morphology, size, and characteristics of the ZIF-67 with control concentration and temperature is discussed in detail in terms of solvent polarity. The preliminary result of laccase@ZIF-67 through the in-situ encapsulation synthesis method towards the degradation of methylene blue is included to assess the feasibility of enzyme-MOF hybrid in bioremediation of phenolic compound.

METHODOLOGY

MATERIALS

Cobalt nitrate (R&M), 2-methylimidazole 99% (Merck), methanol 99% (Merck) used as a solvent in ZIF-67(M) synthesis and ultrapure water used in synthesis ZIF-67(W). These solvents are used to dissolve cobalt nitrate and 2-methylimidazole in respective synthesis. Methylene blue is supplied by R&M (Selangor, Malaysia) and Laccase from *Trametes versicolor* is supplied by Merck (Darmstadt, Germany).

SYNTHESIS ZIF-67(W), ZIF-67(M) AND LACCASE@ZIF-67(W)

ZIF-67(W) and ZIF-67(M) are synthesized by following the report from Wang *et al.* (2020) with some modifications. ZIF-67(W) is prepared by adding cobalt nitrate solution (0.045M, 5mL) into 2-methylimidazole solution (0.36M, 5mL) by using ultrapure water as a solvent for both solutions. For laccase@ZIF-67(W), 2 mg of laccase is added into the mixture. It is then stirred for 2 hours at room temperature and stays undisturbed overnight at room temperature. This mixture of solution is then centrifuged at 5000 rpm for 30 minutes. The precipitate is collected by removing the supernatant and rinsing it with ultrapure water. The precipitate is then dried at 60°C. ZIF-67(M) is prepared by the same procedure but with methanol as solvent. Both powders are characterized for their morphology.

CHARACTERIZATION

ZIF-67(W) and ZIF-67(M) are characterized by using Field Emission Scanning Electron Microscopy (FESEM), X-ray diffraction (XRD), Fourier Transform Infrared (FTIR), Thermogravimetric Analysis (TGA), Brunauer-Emmett-

Teller (BET) analyses. The morphology of the ZIF-67 is studied by using the FESEM model Zeiss Supra VP FESEM operated at 10 kV. XRD model Rigaku Ultima IV is used for the study of ZIF-67 crystallinity. The XRD analysis uses Cu K α radiation with a range of 5° to 50°, a scanning rate of 2°/min, width step of 0.02° at 40kV and 40mA. FTIR analysis used the Perkin-Elmer model to scan the wavenumber from 515 cm⁻¹ to 4000 cm⁻¹. TGA measurement is conducted on the Mettler Toledo TGA/DSCI STAR System model with N₂ atmosphere gas at temperatures from 25°C to 700°C with a heating rate of 10°C/min. BET analysis is performed by using model Micromeritic 3Flex 3500 with the degassed temperature of 150°C for 3 hours.

BIOCATALYTIC DEGRADATION OF METHYLENE BLUE

10 mg of laccase@ZIF-67(W) is added into 100 mL of 19.2 ppm of methylene blue solution and let sit for 4 weeks at room temperature in a covered beaker. The remaining concentration of methylene blue in the beaker is analyzed using UV-VIS spectrophotometer UV-1280 (Shimadzu, Japan) at a wavelength of 665nm every 7 days. The percentage degradation of methylene blue (MB) is calculated as follows:

$$\text{MB degradation (\%)} = \frac{([\text{MB}]_{\text{initial}} - [\text{MB}]_{\text{final}})}{[\text{MB}]_{\text{initial}}} \times 100 \quad (1)$$

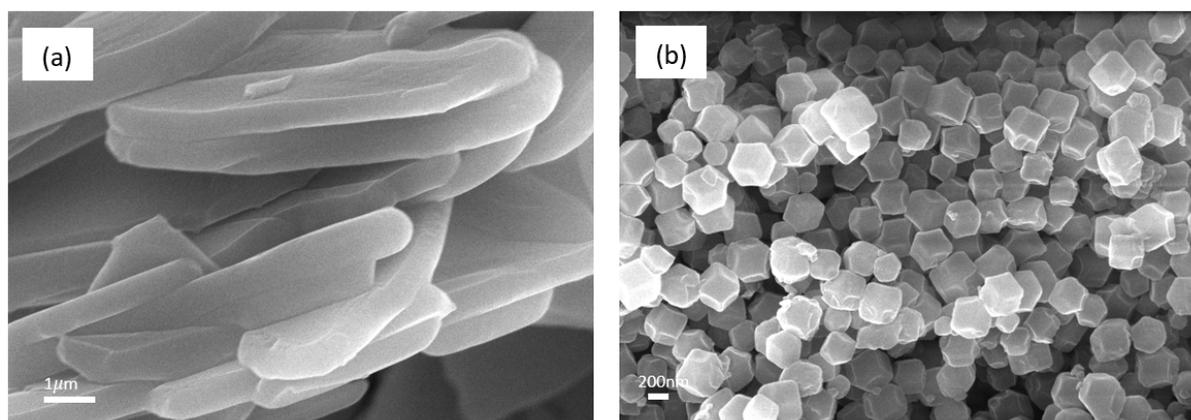


FIGURE 1. FE-SEM of ZIF-67 synthesis in different solvents (a) water, ZIF-67(W) (b) methanol, ZIF-67(M)

FTIR analysis of different solvents during ZIF-67 synthesis is shown in Figure 2(b). FTIR analysis is used to identify the presence of functional groups in ZIF-67. Both ZIF-67(W) and ZIF-67(M) have similar FTIR patterns that were reported by Rafiei S. *et al.* (2018) (Rafiei *et al.* 2018) and Hu Y. *et al.* (2019) (Hu *et al.* 2019). The band

RESULTS AND DISCUSSION

CHARACTERIZATION OF ZIF-67

The morphology of ZIF-67 structures by using different solvents during synthesis is shown in Figure 1. ZIF-67(W) shows non-uniform leaf-like structure while ZIF-67(M) shows a definite rhombic dodecahedron morphology. Using the same magnification, the FESEM image reveals that ZIF-67(W), with a crystal size range of 10–16 μm , has larger crystals compared to ZIF-67(M), which has a size range of 200–400 nm. This shows that different solvents can tune the crystal size and shape.

The XRD patterns of ZIF-67 crystallinity in different solvents during synthesis are shown in Figure 2(a). ZIF-67(M) has a similar degree of the peak with the JCPDS card of ZIF-8 (00-062-1030) and as reported by Yiwen Hu *et al.* (2019) (Hu *et al.* 2019). Characteristic peaks of ZIF67(M) are found at 2Theta (°) = 7.34, 10.48, 12.78, 14.62, 16.36, 19.7, 22.24, 24.6, 25.8, 26.76. ZIF-67(W) shows a similarity with the degree of the peak of simulated ZIF-L crystallinity (Yu *et al.* 2022). The characteristic peaks of ZIF-67(W) at 2Theta (°) = 7.44, 7.84, 8.96, 10.44, 11.08, 11.6, 13.02, 13.6, 14.78, 15.2, 15.74, 16.78, 17.3, 18.1, 19.2, 21.26, 21.84, 23.48, 25.0, 27.9, 29.14, 29.68, is similar to those reported by Yu C. *et al.* (2022).

at 3137 and 3000 cm⁻¹ from ZIF-67(W) and at 3164 and 2990 cm⁻¹ from ZIF-67(M) shows the stretch mode of C-H from imidazole ring and aliphatic chain from imidazole residue, and the band at 1561 cm⁻¹ from ZIF-67(W) and at 1570 cm⁻¹ from ZIF-67(M) show the stretch mode of C=N of imidazole ring (Rafiei *et al.* 2018). The peak below 800 cm⁻¹ shows the

characteristic of the out-of-plane bend mode of the imidazole rings (Hu et al. 2019). This shows that both ZIF-67(W) and ZIF-67(M) have the same bonding

interaction during nucleation and crystallization even though they have different morphology and crystallinity based on FESEM and XRD patterns.

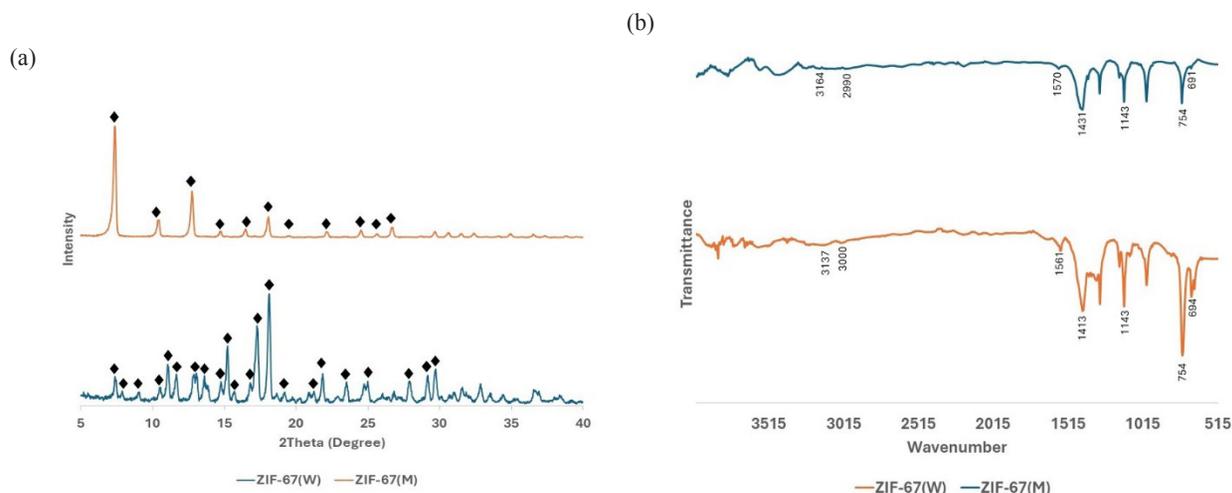


FIGURE 2. (a) XRD patterns (b) FTIR pattern for ZIF-67(W) and ZIF-67(M)

Thermogravimetric analysis shows the thermal stability and degradation behavior of ZIF-67 as a function of increasing temperature by analysing the remaining weight percentage (Fig. 3). The synthesized ZIF-67 in methanol or water are similar to the former reports (Sun et al. 2016). ZIF-67(M) is more thermally stable up to 500°C compared to ZIF-67(W) which only stable up to 300°C. This is indicated by the plateau of the curves at the temperature range. The initial weight loss at 100°C and below for both curves corresponds to the removal of adsorbed water. ZIF-67(W) shows weight percent reduction from 86% to 76% at a range of 288°C to 316°C due to degradation of 2-methylimidazole. Right after that, ZIF-67(W) shows a slight plateau at a range of 300°C to 500°C before further mass loss. The second weight percent reduction graph shows that the crystal structure is starting to collapse at 500°C for both ZIF-67(W) and ZIF-67(M) (Shi et al. 2017). The weight of ZIF-67(W) drops to 26% while ZIF-67(M) drops to 48% at 700°C. This shows that ZIF-67(M) has a better structure and does not easily collapse compared to ZIF-67(W). This is because the rhombic dodecahedron morphology is more stable compared to the leafy-like morphology (Khan et al. 2018).

Table 1 shows the BET surface area and the pore size of ZIF-67(W) and ZIF(M). From the Table, the ZIF-67(M) has a higher surface area compared to ZIF-67(W). This result is consistent with the result in Figure 1 of FESEM of both ZIF-67(W) and ZIF-67(M) where ZIF-67(M) has a smaller crystal size compared to ZIF-67(W). The pore size of the ZIF-67(W) is larger

than ZIF-67(M) because ZIF-67(W) is a leaf-like crystal that has a surface that has a larger face based on Figure 1 of FESEM. Furthermore, the BET surface area for ZIF-67(M) has similar to the reported by Duan C. et al. (2022) (Duan et al. 2022). They stated that the surface area of standard morphology of ZIF-67 (rhombic dodecahedron), which is more than 1700 m²/g. Meanwhile, the surface area of ZIF-67(W) with morphology of leafy structure, like ZIF-L, have is different with what reported of ZIF-L (67.02 m²/g) (Nasir et al. 2018) and the lowest surface area by Li J et al. (2023) with 38.49 m²/g (Li et al. 2023). This result may be attributed to the incomplete removal of the supernatant from the precipitate during sample preparation, as a portion of the supernatant was likely retained within the precipitate. Consequently, residual unreacted precursor could have obstructed the pores of the ZIF-67(W) structure.

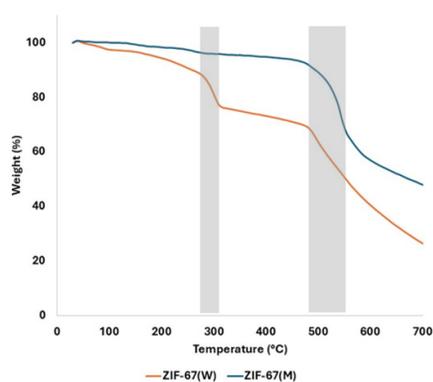


FIGURE 3. Thermogravimetric curve pattern on ZIF-67(W) (orange) and ZIF-67(M) (blue)

TABLE 1. The BET surface area and pore size of ZIF-67(W) and ZIF-67(M)

Sample	BET surface area (m ² /g)	Pore size (nm)
ZIF-67(W)	3.67	6.33
ZIF-67(M)	1749.66	1.69

These differences in morphological and characteristic are the result of the solvent polarity in deprotonating the ligand (in this case 2-methylimidazole) (Akhundzadeh Tezerjani et al. 2021). Methanol is better in the deprotonation of ligands compared to water. Deprotonation of ligand gives effect to the nucleation and crystallization (W. Zhang & Pinna, 2024). During the dissolution of the ZIF-67 precursor, both precursor cobalt ion and 2-methylimidazole ligand dissolve better in methanol, making it a homogenous solution. Methanol helps in coordinating the precursor solution by arranging the nitrogen atom of the imidazole ring to cobalt, forming cobalt (II)-imidazolate complexes and maintaining a homogenous solution that leads to better ZIF-67 nucleation. From that, methanol solvent produces a standard rhombic dodecahedron. It shows that methanol is involved and played a role in the crystallization of ZIF from the precursor dissolution until the final crystal nucleation (Lewis et al. 2023). Plus, ZIF-67 crystallization in methanol produces porous crystals with standard morphology and stronger structure as resulting from this study. Methanol is a volatile chemical, which gives the advantage in the purification of ZIF-67(M) by reducing residue left.

Meanwhile, water is also good in ligand deprotonation, but the hydroxide ion easily deprotonates the cobalt ion, thus ZIF-67(W) nucleation is slow due to less cobalt ion interaction with ligand anions (Akhundzadeh Tezerjani et al. 2021). From that, water introduces different coordination structures due to its high polarity and hydrogen bonding which lead to various crystal nucleation and crystal growth. This will also result in various crystal sizes and morphologies. According to Davoodi et al. (2021) (Davoodi et al. 2021), the leaf-like two-dimensional structure of MOF is constructed when specifically using water as a solvent. This report corroborates the result of this study. Most MOFs are sensitive to water, making them unstable, experiencing phase changes, and crystal structures easily collapse (B. Zhang et al. 2015). In making standard morphology of ZIF by using water as the solvent, some changes are needed on the condition during synthesis such as temperature, pH, and additives usage. This adjustment still gives various crystal morphology and size during the adjustment (Shi et al. 2017). As reported by Cui J. et al (2017) (Cui et al. 2017), standard rhombic dodecahedrons of ZIF-8 crystal can be achieved with a ratio concentration of metal ion to ligand 1:20.

RELATION BETWEEN DIFFERENT SOLVENT IN SYNTHESIS OF ZIF-67 AND ENZYME IMMOBILIZATION

When selecting a solvent for synthesizing ZIF-67 for enzyme immobilization purpose, several factors should be considered. These include the feasibility of synthesizing ZIF-67 in the chosen solvent, the use of an environmentally friendly solvent, the solvent's ability to promote efficient nucleation and crystallization, and its role in enhancing the desirable characteristics of the resulting ZIF. These solvent criteria are important especially for in-situ enzyme encapsulation into MOF since the enzyme and MOF precursor will be synthesized together in one pot and the enzyme is sensitive to harsh environments.

Based on synthesis criteria and discussion, both water and methanol can be utilized in the synthesis of ZIFs or MOFs. Previous research has demonstrated that these solvents effectively crystallize ZIFs even under ambient temperature and atmospheric pressure conditions (Lei et al. 2023; Qian et al. 2012). During the synthesis, nucleation and crystallization occurred for both solvents; water and methanol. The difference is the polarity of the solvent and the interaction of the solvent and precursor ions toward nucleation. Methanol which is used as a solvent will produce a standard crystal of MOF. Water still constructs the ZIF crystal even though the crystal is not standard and uniform compared to methanol. Based on the morphology, crystallinity, and characteristics specifically from this paper, ZIF-67(M) shows the uniform-shaped crystals compared to ZIF-67(W).

This non-standard crystal synthesized by using water as a solvent can be used in other applications suitable for such morphology such as enzyme immobilization (S. Zhang et al. 2024; L. Zhong et al. 2021). According to Ren S. *et al.* (2018) (Ren et al. 2018), enzymes immobilized on flower-like morphology of ZIF-8 have higher enzyme activity compared to enzymes immobilized on standard polyhedron morphology due to wide pore size for the enzyme to catalysis. ZIF-67(M) also shows a withstanding structure toward high temperatures with more than 40% weight left at temperature 700°C compared to ZIF-67(W). ZIF-67(W) still can be used as an enzyme protector for temperatures less than 500°C.

Based on environmentally friendly criteria, water is the best solvent to use in the synthesis of MOF. The synthesis of the MOFs with water as a solvent has been extensively studied (Kumar et al. 2020). Water is considered as safest, cheapest, and easiest in the final treatment method meanwhile methanol is very volatile and flammable. Methanol must be handled with caution and all procedures involving it should be performed

within a fume hood to prevent exposure to harmful vapors. The comparison of solvent based on the criteria is shown in Table 2.

TABLE 2. Comparison of water and methanol as solvent based on the criteria

Criteria	Water as solvent	Methanol as solvent
Easy to synthesize	✓	✓
Environmentally friendly	✓	
Good nucleation and crystallization		✓
Good characteristic	✓	✓

The synthesis of ZIF-67 using water and methanol as solvents involves distinct processes that influence the resulting material's properties. When using water as the solvent, the synthesis typically benefits from being more environmentally friendly and cost-effective, as water is non-toxic and readily available. Water-based synthesis often leads to the formation of ZIF-67 crystals with high purity and well-defined morphology, albeit sometimes with longer crystallization times due to water's higher polarity and hydrogen bonding. In contrast, methanol, a polar aprotic solvent, facilitates faster crystallization of ZIF-67, often resulting in smaller, more uniform crystals with potentially higher surface area and porosity. Methanol's lower polarity compared to water can lead to differences in nucleation and growth kinetics, producing materials with varying degrees of crystallinity and particle size distribution. While both solvents are effective, the choice between water and methanol depends on the desired properties of the ZIF-67 and the specific application requirements.

Ecologically friendly solvents play a major role in the manufacture of ZIF and the immobilization of the enzyme in all conditions. Because enzymes are more environmentally benign than chemical catalysts when utilized for catalysis (Chapman et al. 2018). Under the environmental considerations, it is appropriate to use ZIF which undergoes an eco-friendly synthesis process as a shield to the enzyme (K. G. Liu et al. 2021). Using water as a solvent is an example of this context of MOF synthesis for enzyme immobilization, regardless of its drawbacks; forming of non-standard crystal structures. Even with these non-standard crystals, these MOFs have larger pore sizes giving the upper hand in enzyme immobilization and biocatalysis.

BIOCATALYTIC DEGRADATION OF METHYLENE BLUE

The study hypothesized that Laccase@ZIF-67 complexes, which was prior synthesized with water can adsorb and degrade methylene blue. To test the hypothesis, a significantly high concentration of methylene blue (19.2 ppm) is mixed with laccase@ZIF-67(W) in a covered beaker and let react at room temperature for 28 days. The enzyme loading of the laccase@ZIF-67(W) is 100% even after centrifuge. Figure 4(a) shows the trend of methylene blue remaining concentration in the beaker after each week. The degradation is the most rapid in the first 7 days with an average of 47% reduction (Figure 4b). The final concentration of methylene blue after 28 days is analyzed to be 1.2 ppm and this contributes to 93.5% degradation. A visual image of significant color reduction of methylene blue from Day 1 to Day 28 can be seen in Figure 4(c). This preliminary result shows that using water as solvent in the synthesis of the enzyme-metal organic framework (E-MOF) can be used to encapsulate enzyme (laccase). The laccase encapsulated within the ZIF-67(W) stays active for at least 28 days and is able to continuously degrade methylene blue for the period. At day 28, a sample is analyzed for the presence of enzyme which is hypothesized could be leaking out from the MOF. The results show no enzyme leakage. The leafy-like morphology of ZIF-67(W), even though it is not as definite structure as ZIF-67(M) (Fig. 1b), is able to encapsulate laccase within the framework, and the porous surface of ZIF-67(W) allows for substrate-enzyme contact, enabling adsorption and biodegradation of methylene blue. The pristine of ZIF-67(W) shows high adsorption at first 7 days of 66.1% but gradually slow to 83.8% at 28 days. This show that the ZIF-67(W) nearly saturated with the methylene blue. Compared to classical adsorption process of dyes which can cause secondary waste of toxic saturated adsorbers, Laccase@ZIF-67(W) is an excellent option to be applied because it can adsorb and simultaneously degrade the dyes to its nontoxic features.

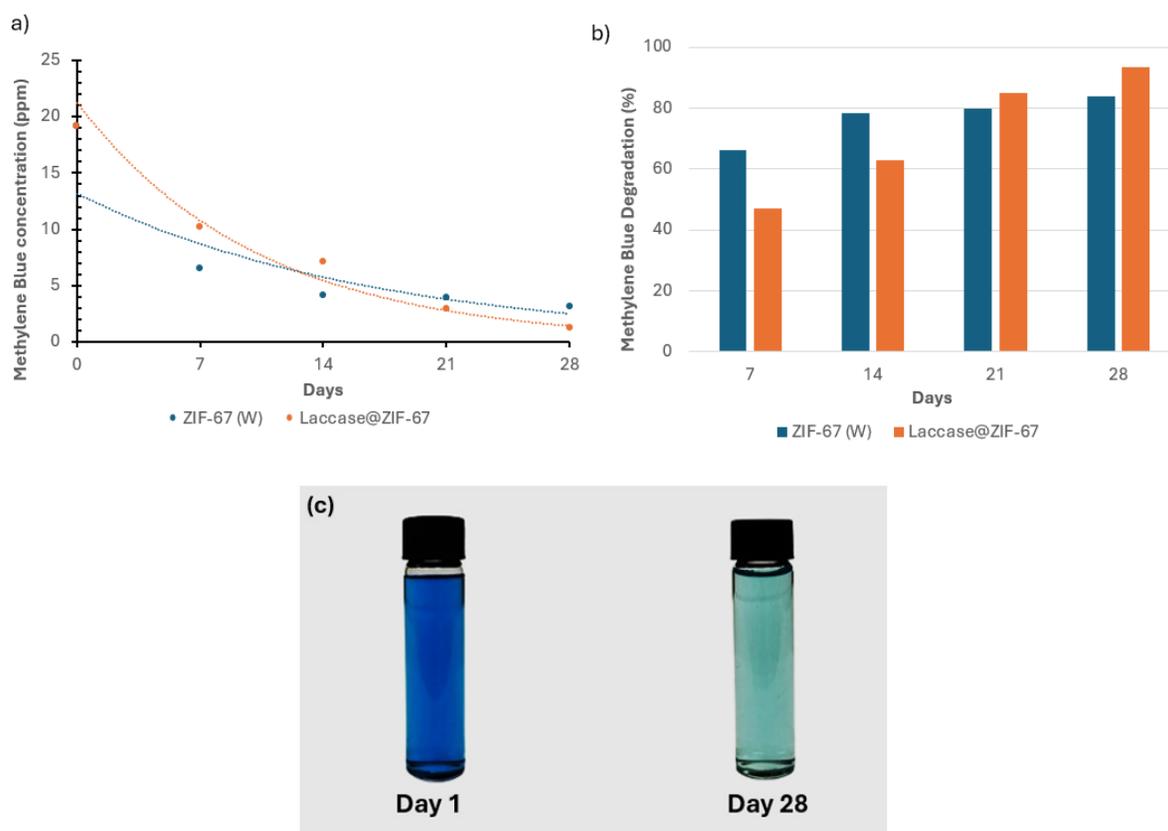


FIGURE 4. Feasibility Result of Biocatalytic Reduction of Methylene Blue by laccase@ZIF-67(W); (a) Methylene Blue concentration analyzed each week for a month; (b) percentage degradation of Methylene Blue; (c) comparison colour of methylene blue of Day 1 and Day 28

CONCLUSION

In summary, this study successfully synthesized ZIF-67 by using different solvents; water (ZIF-67(W)) and methanol (ZIF-67(M)). From this preparation, both ZIF-67 constructed different morphology, crystallinity, and sizes. ZIF-67(W) formed leaf-like morphology with a crystal size range of 10-16 μ m while ZIF-67(M) formed rhombic dodecahedron morphology with a crystal size of 200-400nm. The percent weight of ZIF-67(M) is 48% at temperature 700°C while ZIF-67(W) is 26%, showing ZIF-67(M) has a more thermally stable structure compared to ZIF-67(W). Methanol aids in organizing the precursor solution by aligning the nitrogen atom of the imidazole ring with cobalt, resulting in the formation of cobalt(II)-imidazolate complexes and hence sustaining a uniform solution, which enhances ZIF-67 nucleation. Whereas water, while being eco-friendly solvent and can function the same way, results in longer crystallization time and introduces different coordination structures which leads to different morphology. Even though water produced non-standard crystals, these crystals can help enhance the

enzyme activity by their mesopore size giving a wide opening to the enzyme active site to reach the substrate. It can be concluded that water is a good solvent to use in the synthesis of laccase@ZIF-67. The preliminary result in methylene blue degradation by using laccase@ZIF-67(W) shows a 93.5% degradation from the initial 19.2 ppm after 4 weeks.

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DECLARATION OF COMPETING INTEREST

None.

REFERENCES

- Ahmad, S. Z. N., Salleh, W. N. W., Yusof, N., Yusop, M. Z. M., Hamdan, R., & Ismail, A. F. 2023. Synthesis of zeolitic imidazolate framework-8 (ZIF-8) using different solvents for lead and cadmium adsorption. *Applied Nanoscience* 13(6): 4005–4019. <https://doi.org/10.1007/s13204-022-02680-7>
- Akhundzadeh Tezerjani, A., Halladj, R., & Askari, S. 2021. Different view of solvent effect on the synthesis methods of zeolitic imidazolate framework-8 to tuning the crystal structure and properties. *RSC Advances* 11(32): 19914–19923. <https://doi.org/10.1039/d1ra02856a>
- Bergaoui, M., Khalfouli, M., Awadallah-F, A., & Al-Muhtaseb, S. 2021. A review of the features and applications of ZIF-8 and its derivatives for separating CO₂ and isomers of C₃- and C₄- hydrocarbons. *Journal of Natural Gas Science and Engineering* 96(June): 104289. <https://doi.org/10.1016/j.jngse.2021.104289>
- Bilal, M., Adeel, M., Rasheed, T., & Iqbal, H. M. N. 2019. Multifunctional metal-organic frameworks-based biocatalytic platforms: Recent developments and future prospects. *Journal of Materials Research and Technology* 8(2): 2359–2371. <https://doi.org/10.1016/j.jmrt.2018.12.001>
- Chai, M., Razmjou, A., & Chen, V. 2021. Metal-organic-framework protected multi-enzyme thin-film for the cascade reduction of CO₂ in a gas-liquid membrane contactor. *Journal of Membrane Science* 623(January): 118986. <https://doi.org/10.1016/j.memsci.2020.118986>
- Chapman, J., Ismail, A. E., & Dinu, C. Z. 2018. Industrial applications of enzymes: Recent advances, techniques, and outlooks. *Catalysts* 8(6). <https://doi.org/10.3390/catal8060238>
- Cui, J., Feng, Y., Lin, T., Tan, Z., Zhong, C., & Jia, S. 2017. Mesoporous Metal-Organic Framework with Well-Defined Cruciate Flower-Like Morphology for Enzyme Immobilization. *ACS Applied Materials and Interfaces* 9(12): 10587–10594. <https://doi.org/10.1021/acsami.7b00512>
- Davoodi, M., Davar, F., Rezayat, M. R., Jafari, M. T., & Shalan, A. E. 2021. Cobalt metal-organic framework-based ZIF-67 for the trace determination of herbicide molinate by ion mobility spectrometry: investigation of different morphologies. *RSC Advances* 11(5): 2643–2655. <https://doi.org/10.1039/d0ra09298c>
- Duan, C., Yu, Y., & Hu, H. 2022. Recent progress on synthesis of ZIF-67-based materials and their application to heterogeneous catalysis. *Green Energy and Environment* 7(1): 3–15. <https://doi.org/10.1016/j.gee.2020.12.023>
- Gao, J., Mao, H., Jin, H., Chen, C., Feldhoff, A., & Li, Y. 2020. Functionalized ZIF-7/Pebax® 2533 mixed matrix membranes for CO₂/N₂ separation. *Microporous and Mesoporous Materials* 297. <https://doi.org/10.1016/j.micromeso.2020.110030>
- Guajardo, N., & Domínguez de María, P. 2020. Assessing biocatalysis using dihydrolevoglucosenone (Cyrene™) as versatile bio-based (co)solvent. *Molecular Catalysis* 485. <https://doi.org/10.1016/j.mcat.2020.110813>
- Han, Z., Yang, Y., Rushlow, J., Huo, J., Liu, Z., Hsu, Y. C., Yin, R., Wang, M., Liang, R., Wang, K. Y., & Zhou, H. C. 2024. Development of the design and synthesis of metal-organic frameworks (MOFs) - from large scale attempts, functional oriented modifications, to artificial intelligence (AI) predictions. *Chemical Society Reviews*. <https://doi.org/10.1039/d4cs00432a>
- Hu, Y., Song, X., Zheng, Q., Wang, J., & Pei, J. 2019. Zeolitic imidazolate framework-67 for shape stabilization and enhanced thermal stability of paraffin-based phase change materials. *RSC Advances* 9(18): 9962–9967. <https://doi.org/10.1039/c9ra00874h>
- Jia, Y., Chen, Y., Luo, J., & Hu, Y. 2019. Immobilization of laccase onto meso-MIL-53(Al) via physical adsorption for the catalytic conversion of triclosan. *Ecotoxicology and Environmental Safety* 184(September): 109670. <https://doi.org/10.1016/j.ecoenv.2019.109670>
- Jian, M., Liu, B., Liu, R., Qu, J., Wang, H., & Zhang, X. 2015. Water-based synthesis of zeolitic imidazolate framework-8 with high morphology level at room temperature. *RSC Advances* 5(60): 48433–48441. <https://doi.org/10.1039/c5ra04033g>
- Khan, I. U., Othman, M. H. D., Ismail, A. F., Ismail, N., Jaafar, J., Hashim, H., Rahman, M. A., & Jilani, A. 2018. Structural transition from two-dimensional ZIF-L to three-dimensional ZIF-8 nanoparticles in aqueous room temperature synthesis with improved CO₂ adsorption. *Materials Characterization* 136: 407–416. <https://doi.org/10.1016/j.matchar.2018.01.003>
- Kumar, S., Jain, S., Nehra, M., Dilbaghi, N., Marrazza, G., & Kim, K. H. 2020. Green synthesis of metal-organic frameworks: A state-of-the-art review of potential environmental and medical applications. *Coordination Chemistry Reviews* 420: 213407. <https://doi.org/10.1016/j.ccr.2020.213407>
- Lei, Y., Yang, H., Xie, J., Chen, Q., Quan, W., & Wang, A. 2023. Synthesis of strong magnetic response ZIF-67 for rapid adsorption of Cu²⁺. *Frontiers in Chemistry* 11. <https://doi.org/10.3389/fchem.2023.1135193>
- Lewis, A., Butt, F. S., Wei, X., Mazlan, N. A., Chen, Z., Yang, Y., Yang, S., Radacsi, N., Chen, X., & Huang, Y. 2023. Crystallization and phase selection of zeolitic imidazolate frameworks in aqueous cosolvent systems: The role and impacts of organic

- solvents. *Results in Engineering* 17: 100751. <https://doi.org/10.1016/j.rineng.2022.100751>
- Li, J., Zhang, Y., Zhao, H., & Sui, G. 2023. Preparation of 2D ZIF-L and its antibacterial and antifouling properties. *Nanomaterials* 13(1). <https://doi.org/10.3390/nano13010202>
- Liu, K. G., Sharifzadeh, Z., Rouhani, F., Ghorbanloo, M., & Morsali, A. 2021. Metal-organic framework composites as green/sustainable catalysts. *Coordination Chemistry Reviews* 436: 213827. <https://doi.org/10.1016/j.ccr.2021.213827>
- Liu, L. H., Chiu, R. Y., So, P. B., Lirio, S., Huang, H. Y., Liu, W. L., & Lin, C. H. 2021. Fragmented α -amylase into microporous metal-organic frameworks as bioreactors. *Materials* 14(4): 1–8. <https://doi.org/10.3390/ma14040870>
- Nadar, S. S., Vaidya, L., & Rathod, V. K. 2020. Enzyme embedded metal organic framework (enzyme-MOF): De novo approaches for immobilization. *International Journal of Biological Macromolecules* 149: 861–876. <https://doi.org/10.1016/j.ijbiomac.2020.01.240>
- Nasir, A. M., Md Nordin, N. A. H., Goh, P. S., & Ismail, A. F. 2018. Application of two-dimensional leaf-shaped zeolitic imidazolate framework (2D ZIF-L) as arsenite adsorbent: Kinetic, isotherm and mechanism. *Journal of Molecular Liquids* 250: 269–277. <https://doi.org/10.1016/j.molliq.2017.12.005>
- Patra, S., Sene, S., Mousty, C., Serre, C., Chaussé, A., Legrand, L., & Steunou, N. 2016. Design of laccase-metal organic framework-based bioelectrodes for biocatalytic oxygen reduction reaction. *ACS Applied Materials and Interfaces* 8(31): 20012–20022. <https://doi.org/10.1021/acsami.6b05289>
- Qian, J., Sun, F., & Qin, L. 2012. Hydrothermal synthesis of zeolitic imidazolate framework-67 (ZIF-67) nanocrystals. *Materials Letters* 82(2012): 220–223. <https://doi.org/10.1016/j.matlet.2012.05.077>
- Rafei, S., Tangestaninejad, S., Horcajada, P., Moghadam, M., Mirkhani, V., Mohammadpoor-Baltork, I., Kardanpour, R., & Zadehahmadi, F. 2018. Efficient biodiesel production using a lipase@ZIF-67 nanobioreactor. *Chemical Engineering Journal* 334: 1233–1241. <https://doi.org/10.1016/j.cej.2017.10.094>
- Ren, S., Feng, Y., Wen, H., Li, C., Sun, B., Cui, J., & Jia, S. 2018. Immobilized carbonic anhydrase on mesoporous cruciate flower-like metal organic framework for promoting CO₂ sequestration. *International Journal of Biological Macromolecules* 117: 189–198. <https://doi.org/10.1016/j.ijbiomac.2018.05.173>
- Shi, Z., Yu, Y., Fu, C., Wang, L., & Li, X. 2017. Water-based synthesis of zeolitic imidazolate framework-8 for CO₂ capture. *RSC Advances* 7(46): 29227–29232. <https://doi.org/10.1039/c7ra04875k>
- Sun, W., Zhai, X., & Zhao, L. 2016. Synthesis of ZIF-8 and ZIF-67 nanocrystals with well-controllable size distribution through reverse microemulsions. *Chemical Engineering Journal* 289: 59–64. <https://doi.org/10.1016/j.cej.2015.12.076>
- Ta, D. N., Nguyen, H. K. D., Trinh, B. X., Le, Q. T. N., Ta, H. N., & Nguyen, H. T. 2018. Preparation of nano-ZIF-8 in methanol with high yield. *Canadian Journal of Chemical Engineering* 96(7): 1518–1531. <https://doi.org/10.1002/cjce.23155>
- Thakare, S. R., & Ramteke, S. M. 2018. Postmodification of MOF-5 using secondary complex formation using 8-hydroxyquinoline (HOQ) for the development of visible light active photocatalysts. *Journal of Physics and Chemistry of Solids* 116: 264–272. <https://doi.org/10.1016/j.jpics.2018.01.032>
- Wang, H., Jiao, F., Gao, F., Zhao, X., Zhao, Y., Shen, Y., Zhang, Y., & Qian, X. 2017. Covalent organic framework-coated magnetic graphene as a novel support for trypsin immobilization. *Analytical and Bioanalytical Chemistry* 409(8): 2179–2187. <https://doi.org/10.1007/s00216-016-0163-z>
- Wang, Z., Ren, D., Yu, H., Jiang, S., Zhang, S., & Zhang, X. 2020. Study on improving the stability of adsorption-encapsulation immobilized Laccase@ZIF-67. *Biotechnology Reports* 28: e00553. <https://doi.org/10.1016/j.btre.2020.e00553>
- Xu, S., Liang, J., Mohammad, M. I. B., Lv, D., Cao, Y., Qi, J., Liang, K., & Ma, J. 2021. Biocatalytic metal-organic framework membrane towards efficient aquatic micropollutants removal. *Chemical Engineering Journal* 426. <https://doi.org/10.1016/j.cej.2021.131861>
- Yu, C., Kim, Y. J., Kim, J., & Eum, K. 2022. ZIF-L to ZIF-8 Transformation: Morphology and Structure Controls. *Nanomaterials* 12(23). <https://doi.org/10.3390/nano12234224>
- Yusuf, V. F., Malek, N. I., & Kailasa, S. K. 2022. Review on metal-organic framework classification, synthetic approaches, and influencing factors: Applications in energy, drug delivery, and wastewater treatment. *ACS Omega* 17(49): 44507–44531. <https://doi.org/10.1021/acsomega.2c05310>
- Zhang, B., Zhang, J., Liu, C., Sang, X., Peng, L., Ma, X., Wu, T., Han, B., & Yang, G. 2015. Solvent determines the formation and properties of metal-organic frameworks. *RSC Advances* 5(47): 37691–37696. <https://doi.org/10.1039/c5ra02440d>
- Zhang, J., White, G. B., Ryan, M. D., Hunt, A. J., & Katz, M. J. 2016. Dihydrolevoglucosenone (Cyrene) as a green alternative to n,n-dimethylformamide (DMF) in MOF synthesis. *ACS Sustainable Chemistry and Engineering*, 4(12): 7186–7192. <https://doi.org/10.1021/acssuschemeng.6b02115>

- Zhang, S., Gan, Y., Wang, H., Qi, X., Su, P., Song, J., & Yang, Y. 2024. Enhancing chymotrypsin activity and stability of capillary immobilized enzyme microreactors using zeolitic imidazolate frameworks as encapsulation materials. *Analytical Chemistry*. <https://doi.org/10.1021/acs.analchem.4c01425>
- Zhang, W., & Pinna, N. 2024. Metal organic frameworks synthesis: The versatility of triethylamine. *Chemistry - A European Journal*. <https://doi.org/10.1002/chem.202304256>
- Zhong, G., Liu, D., & Zhang, J. 2018. The application of ZIF-67 and its derivatives: Adsorption, separation, electrochemistry and catalysts. *Journal of Materials Chemistry A* 6(5): 1887–1899. <https://doi.org/10.1039/c7ta08268a>
- Zhong, L., Feng, Y., Hu, H., Xu, J., Wang, Z., Du, Y., Cui, J., & Jia, S. 2021. Enhanced enzymatic performance of immobilized lipase on metal organic frameworks with superhydrophobic coating for biodiesel production. *Journal of Colloid and Interface Science* 602: 426–436. <https://doi.org/10.1016/j.jcis.2021.06.01>