



A Review Study of Immobilized Microbial-Nanoparticles: Techniques and Biotechnology Applications

Rana Abbas Azeez^{1*}, Muayad M. Hasan¹, Nabil Majd Alawi², and Hoang M. Nguyen³

¹ Petroleum Technology Department, University of Technology-Iraq, Baghdad-Iraq

² Chemical Engineering Department, University of Technology-Iraq, Baghdad-Iraq

³ University of Science and Technology, University of Dannang, Da Nang, Vietnam

Article information

Article history:

Received: August, 14, 2022

Accepted: October, 01, 2022

Available online: October, 08, 2022

Keywords:

Wastewater treatment;
Bioremediation; Biosorption;
Immobilization microbial cells;
Nanoparticles; Nano-biocatalyst.

*Corresponding Author:

Rana Abbas Azeez

Email: rana.a.azeez@uotechnology.edu.iq

Abstract

Immobilization of microbial cells on nanomaterials opens up broad prospects in biotechnology applications including environmental pollution control and enhancement of biofuel production. Cell-immobilization contributes to improved microbial performance, protection of microbes, and further biomass degradation. Moreover, nanoparticles as biomass support are an advanced strategy in bioremediation. The nanoparticles are interesting for immobilizing microbes due to their unique physicochemical properties. This review throws some light on the recent advances in the immobilization of microbial cell techniques onto nanoparticles as a carrier where they are subsequently used as a nano-bio-stimulator in bioprocessing. The benefits and drawbacks of immobilization techniques are included. The biotechnology applications of nano-biocatalyst in bioprocessing are covered including biosorption of pollutants from wastewater and stimulants in biofuel production processes for green energy generation. The use of nano-bio-catalysts in bioprocesses is an effective strategy for achieving economic feasibility in many processes. Furthermore, future research directions for microbial cell immobilization techniques are discussed.

DOI: <http://doi.org/10.55699/ijogr.2022.0202.1030>, Department of Petroleum Technology, University of Technology-iraq

This is an open access article under the CC BY 4.0 license <http://creativecommons.org/licenses/by/4.0>

1. Introduction

Environmental pollution in soil, air, and aquatic systems results in serious problems for human health. Thus, the degradation of the natural environment in many places around the world is of growing concern [1]. Some of these pollutants are non-biodegradable chemical compounds frequently introduced into ecosystems from industrial discharge, petroleum production activities, agricultural, fungicides, and mining activities [2]. Traditional physicochemical processes are frequently utilized to decline the toxicity from effluents among several techniques

such as ion exchange [3], adsorption [4], ozonization treatment [5], membrane filtration [6], and photocatalysis [7]. The traditional processes result in some drawbacks such as high cost of operation and maintenance and the production of toxic sludge. Bioremediation is preferred for removing environmental pollutants such as chemicals or organic and inorganic compounds due to their ability to degrade organic compounds under extreme conditions and being cost-effective and environmentally friendly.

Bioremediation improves naturally present remedial activities that rely on microorganisms to break down, transform, or remove dangerous organic pollutants with several mechanisms like biosorption, bioaccumulation, and biodegradation [8]. Bioaccumulation is the gradual accumulation of pollutants into the living tissue of microbial [9]. Biodegradation is the breakdown of organic materials by microorganisms [10]. Biosorption is an eco-friendly and economical mechanism for removing the pollutants from effluents, especially the non-biodegradable, which utilize various types of dead and living cells as well as agriculture waste as an efficient biosorbent [11-14]. Several studies have been reported with different waste types such as agriculture and industrial waste as efficient biosorbent [11,12,15]. The benefits of the biosorption over other mechanisms applied are the availability of biosorbent, fast, and high removal efficiency of contaminants via high binding sites under different conditions of pH and temperature [11]. In the last years, the biosorption technique has been extensively investigated using microbial cells as a biosorbent to remove organic and chemical compounds in addition to toxic metals. Compared to other adsorbents used, microbial cells like microalgae, fungi, bacteria, and yeasts outperform due to the presence of different functional groups on the wall tissue of microorganisms. Both dead and living cells have been utilized to remove pollutants [13,16].

Immobilization of microbial cells is a promising technique utilized in various scientific and industrial sectors. These techniques restrain the microbial cells in a particulate form which is considered a bio-catalyst to remove pollutants from wastewater [17]. Immobilization of microbial cells on the solid surface gives mechanical strength, and rigidity to the microbial cells as well as gives the utmost potential for its regeneration and reuse. Various techniques have been applied to immobilize microbes, which are preferred over free cells mainly used in biosorption [18]. Moreover, microbial cell immobilization has been utilized in various support materials like alumina, calcium alginate, and nanoparticle. Appropriate support material selectivity plays an important role in the immobilization of biomass [17,18]

Recently, nanotechnology has demonstrated a new generation of environmental treatment techniques that can be applied to remove toxic pollutants, especially heavy metals and dyes [19, 20]. Nanoparticles are flexible and versatile support for living cells, due to their unique characteristics [18]. In this review, the techniques applied in microbial cell immobilization are covered as well as the benefits and drawbacks of each technique. Moreover, important biotechnology applications of these techniques are also addressed.

2. Carriers materials of microbial cells

The selection of the appropriate support materials is a crucial carrier in obtaining an effective microbial cell immobilization technique. The almost utilized materials are known to be divided into the organic such as (Ca-alginate, and chitin), inorganic such as (activated carbon, zeolite, and ceramics) and composite carriers like (polyvinyl alcohol), as well as biomass materials [21,22]. Preferred materials for wastewater treatment from environmental pollutants to support materials should have such merits as non-biodegradable, insoluble, porous, non-toxic, light in weight, high in biomass retention, chemically stable, and allowing simple immobilization process at low cost [11,12]. The main features of inorganic carriers are thermostable and resistance to microbial degradation. On the other hand, biomass materials are derived from animals, plants, and microbes, and when compared with inorganic materials, they reveal better performance such as perfect biocompatibility and unique physical characteristics, as well as a good mass transfer performance, economical, non-toxicity, and easy immobilization techniques [23,24]

Nanotechnology, like nanomaterials technology, is a promising technology that is leading to an industrial revolution where industrial applications of nanomaterials are constantly increasing. Nanotechnologies provide high characteristics materials with unique activities and support scientific investigations in the development of specific processes and equipment for various applications such as energy, health, environmental treatment, and agriculture [25]. Recently, nanoparticles have been utilized as a support for microorganisms immobilization [19,20,24]. Nanoparticles form an innovative and interesting matrix for the immobilization of microbial cells due to their unique physicochemical properties including a high surface area, mass transfer resistance, high mechanical strength, and efficient diffusion. The immobilization of microbial cells on the surface of magnetic nanoparticles is an emerging technique with benefits such as biocompatibility and chemical activity [26,27]. In wastewater

treatment, magnetic nanoparticles are remarkable as support materials due to their high surface area, and super-paramagnetic properties. Some characteristics of magnetic nanoparticles iron (II, III) oxide widely applied in various areas of applications are given below [28].

- 1- Black or brownish-black with a metallic gloss.
- 2- High magnetic susceptibility.
- 3- Higher chemical stability.
- 4- Narrow size distribution.
- 5- High surface reactivity.
- 6- The magnetic motion can be adjusted by the size of the nanoparticle cluster.
- 7- Small grains of magnetite are very common in igneous and metamorphic rocks.

Magnetic nanoparticles have an additional benefit that they can be simply separated from the continuous phase by utilizing an external magnetic field. Therefore, the immobilization of biomass in magnetic nanoparticles makes biomass more applicable in industrial sectors [29]. The main challenges of immobilization of microbial cells on the magnetic nano-based are the fabrication costs for a certain time. Recently, developed nanomaterials, such as Fe_3O_4 -NPs, have attracted great interest from researchers due to their important properties related to their size, shape, and distribution, which reflect their uses in various fields of applications [30]. The green synthesis of Fe_3O_4 -NPs was presented as a simple, fast and environmentally friendly process to synthesize magnetite nanoparticles more easily and safely of application. Several researchers have successfully synthesized Fe_3O_4 -NPs using plant extracts [27,29].

3. Immobilization techniques of microbial cells

The basic classification of cell immobilizing mechanisms depends on the interaction patterns or bonds involved between cells and their support carriers. Mechanisms for binding microbial cells to carriers utilizing artificial ways are adsorption and covalent binding. On the other hand, entrapment and encapsulation are physically immobilized cells figure (1). The following subsections describe immobilizing techniques [31].

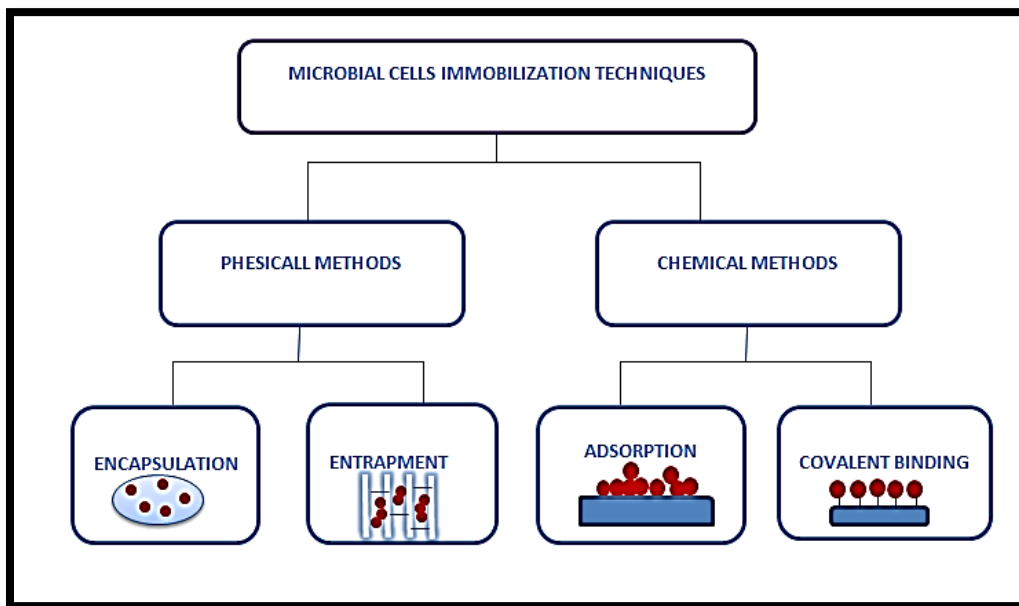


Figure 1: Scheme showing techniques of microbial cells immobilization [source: Authors]

3.1. Adsorption: Adsorption represents the simplest method of immobilizing microbial cells, cells are initially attracted to the surface of carriers, and then are adsorbed onto the matrix. This technique is essentially a physical binding between the surface of a water-insoluble carrier and the immobilized microbes, including physical interactions such as van der Waals interactions, ionic forces, and hydration [31,32]. Therefore, due to the taxonomic and metabolic status of the cells that limit the affinity between cell and carrier, this status is important

for cell sorption via its influence on the immobilization efficiency. Obviously, different types of microbes demonstrate various adsorption behaviors [33]. Adsorption is more appropriate for the immobilization of microbes when compared to the entrapment technique [34]. The selection of suitable sorbents can maintain microbes' metabolic activity and catalyze microbial metabolism, as well as preserve the microbes against toxic agents. Support materials with a high surface area, void volume, permeability, and durability are advised for adsorption studies [33,34]. O. Długosz et al. (2020) succeeded in using α -amylase immobilized on the surface of metal oxide NPs as a biosorbent [35].

3.2. Covalent binding: Covalent binding immobilization is represented as one of the commonly used techniques of cell immobilization. It is an economical technique based on forming the covalent bond between functional groups in microbes' walls and on the carrier material in the presence of a binding agent [36]. The functional groups present in microbial cell walls are such as carboxyl, phosphoryl, and amide groups [37]. Glutaraldehyde (GA) and carbodiimide hydrochloride (EDC) are used as binding agents in immobilization. Covalent binding agents are usually toxic and as cells inhibitor. Therefore, the covalent binding technique is essentially utilized for enzyme immobilization instead of whole-cell immobilization [38]. S.Ding et al. (2015) showed that the immobilization enzyme onto nanoparticles schemes has preserved or even boosted immobilized enzyme performance due to the size of nanoparticle and surface chemistry in addition to the density of immobilized enzymes [39]. A. Rasheed et al. (2020) [40] immobilized *Pseudomonas aeruginosa* on the surface of economical carriers eggshell powder and used glutaraldehyde as a binding agent for removal of Cr (VI) from aqueous solution. The reaction chemistry of immobilization is described in Figure (2).

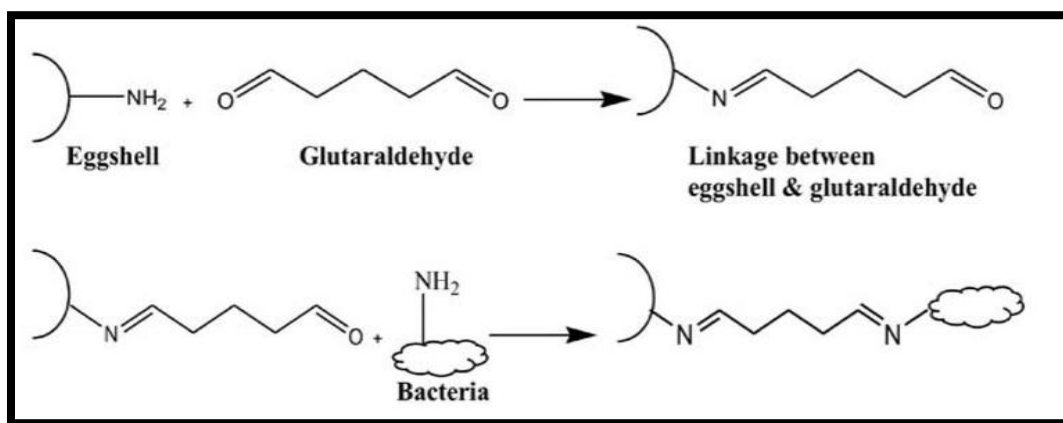


Figure 2: Reaction chemistry of immobilization technique [40].

3.3. Encapsulation: Encapsulation, or the so-called coating, represents as a special microbial immobilizing of the trap. It is a physicochemical or mechanical mechanism to trap the encapsulated material [41]. Encapsulation of biomass is surrounded by a semi-permeable membrane. The permeability of the membrane allows the influx of free substrates and nutrients while the biomass is restricted by the membrane walls. This technique limits entry to the inside of the capsule, which in turn protects the biomass from external conditions, and also prevents the biomass from increasing its metabolic activity and generation of metabolites. It is an effective technique in many studies on laboratory and commercial scales, and it is one of the most widely applied techniques through small-scale immobilization. Encapsulation in beads or granules with a suitable size, mechanical strength, and porosity is one of the most common [42]. Coating magnetic nanoparticles MNPs with organic materials such as alginate has been commonly used to preserve agglomerated microorganisms and increase their stability. Moreover, the coating can also provide functional groups for effective biosorption [43] M. Yao et al. (2018) [44] showed perfect viability when encapsulating the probiotics (*Pediococcus pentosaceus* Li05) in alginate-gelatin microbeads and MgO NPs. On the other hand, N. Suvarli1 et al. (2022) [45] successfully conjugated β -


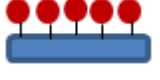

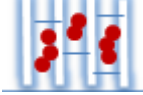
Galactosidase onto the surface of polymer NPs and encapsulated these enzyme-conjugated nanoparticles (ENPs).

3.4. Entrapment: Entrapment is the most extensively applied mechanism for immobilizing microbes. It depends on the enclosure of microbes inside a rigid network [46]. The trap itself is airtight enough to prevent cells from being released while still allowing substrates and products to diffuse. Therefore, this technique is one of the least disruptive mechanisms for inhibiting the movement of microbes where microbial entrapment uses naturally occurring gel polymers [22,47]. The porous polymer is utilized to enclose microbes. Microbial entrapment in alginate gel is widely used due to its mild conditions and simplicity of methodology [48,49]. It has been routinely utilized in biotransformation and fermentation operations to manufacture antibiotics, enzymes, alcohols, and organic acids [50,51]. W. Tian et al. (2007) [52] were able to use a biosorbent prepared by immobilizing the *Sphingomonas sp.* on Fe₃O₄ NPs and gellan gum as a modified entrapment technique for bioremediation of wastewater.

4. Benefits and drawbacks of microbial immobilization techniques.

All techniques used for microbial immobilization including encapsulation, covalent binding, entrapment, and adsorption utilized to achieve biocatalyst have benefits and drawbacks as shown in Table 1.

Table 1: Benefits and drawbacks of microbial immobilization techniques

Adsorption 		Covalent binding 	
Benefits	Drawbacks	Benefits	Drawbacks
It is mild, rapid, simple, cheap, and efficient. It does not need chemical additives, as well as the potential of carrier reloading.	The binding force is weak between microbes and carriers due to the high leakage rate of the adsorbed cell from the carrier. To restrain microbes' desorption from the matrix, powerful adsorption between cells and carriers must be established depending on the type of appropriate adsorbents.	It can improve the catalytic activity by stabilizing the active conformation.	Difficult to prevent cell damage during immobilization. The complicated immobilization conditions result in a remarkable loss of enzyme activity through altering protein structure.
Z. B. Bouabidi et al. (2018) [31], Z. Bayat et al. (2015) [32],		K. Abdollahi et al. (2017) [53], M. Garmroodi et al. (2016) [54]	
Encapsulation 		Entrapment 	
Benefits	Drawbacks	Benefits	Drawbacks
No chemical modification of the raw material is required which results in the proper activity of the inactivated microbes.	Declined mass transfer, creation of fragile immobilization capsules, and instability at specific acidity.	The formation of a barrier protective of the biomass prolongs catalyst viability, store, and augments catalyst mechanical strength.	Cell leakage, diffusion limitations, expensive, least loading capacity, and deactivation through immobilization.
M. E. Mahmoud et al. (2016) [55], J. K. Park and H. N. Chang (2000) [56], and C. S. M. Suzana et al. (2013) [57]		S. Gao et al. (2010) [58], I. Stolarzewicz et al. (2011)[59]	

5. Influential factors of immobilized microbial nanoparticles

The immobilization of microorganisms and adhesion to carrier surfaces are affected by several factors that affect its degrading capacity and its contaminant removal efficiency [60-62]. These factors are summarized below:

- 1- Surface characteristics of sorbents and charge surface.
- 2- Physiological state and the surface structure of microbes.
- 3- The composition and acidity of the medium can change the electrokinetic possibility of cells and the temperature, which affects the sorption of cells.

- 4- Nature of sorbents: Inorganic sorbents can be easily regenerated, and resist decomposition because they are soluble in alkaline solutions, while organic sorbents are chemically stable.

The metabolic activity of microorganisms on the surfaces is influenced by various factors including variation in acidity, existence and composition inhibitors, as well as the dosage of substrates and ions. Therefore, it is worthwhile to select suitable surface support for microbes and the type of microorganisms as well as the technique applied to provide excellent fabrication of the biocatalyst [60].

6. Biotechnology Application of Nano-cells immobilization

The application of cell-immobilization approaches to nanoparticles has attracted the attention of many researchers because it is necessary to apply these strategies on an industrial scale. Therefore, the main applications in the biosorption of pollutants from wastewater and other useful products will be summarized below.

6.1. Nano-cells immobilization in biosorption technique

Biosorption is an excellent technique in the bioremediation of wastewater and is characterized by wide availability, easy-to-use sorbents, and cost-effective and environmentally friendly operation [63]. It is a physicochemical technique that is similar to traditional mechanisms such as sorption, desorption, precipitation, and ion exchange. Moreover, it is described as a promising biotechnique for removing pollutants from wastewater to control environmental pollution. The characteristics that distinguish biosorption from the technique applied are that the essential sorbents are bio-materials or living and dead microorganisms [63,64].

Bioremediation of industrial wastewater containing heavy metals is receiving serious attention. Therefore, several studies have been conducted to remove heavy metals from wastewater using different sorbent materials [64,12]. It has been reported that the removal efficiency increases with the increase in the surface area of the sorbent material [12,63,64]. Moreover, the immobilization of microbial cells increases their stability and resistance to environmental conditions [31]. Q. Peng et al. (2010)[65] found that 96.8% removal efficiency Cu(II) at optimum conditions by immobilized *Saccharomyces Cerevisiae* on chitosan-coated magnetic NPs.

B. Ren et al. (2017) [66] found that the immobilization of spores of *Aspergillus niger* in Ca-alginate may enable the conservation of spores from environmental influences, thus maintaining high biosorption efficiency of Cr(VI). Table 2 reviewed the biosorption of heavy metals using various techniques of immobilization of microbial cells. It is clear from the table that the relevant data indicated a high efficiency of the adsorption capacity.

Table 2 : Previous studies related to heavy metals removal using nano-cells immobilization techniques.

Type of Metals	Kind of Microorganisms	Fabrication of Biosorbent	Optimum Operation Condition	Ref.
Sr(II)	<i>Kluyveromyces fragilis</i> yeast	Magnetic Fe ₃ O ₄ NPs	140.8mg adsorption capacity at 20 °C, and 20 min	[67]
Pb(II)	<i>Penicillium funiculosum</i> (enzymes)	Silicon dioxide NPs	1266.7 mmol /g adsorption capacity pH=5 after 20 min	[68]
Pb(II)	<i>Phanerochaete Chrysosporium</i> (fungus)	Ca-alginate-Fe ₃ O ₄ NPs	96.03% efficiency at pH 5.0 and 35 °C, and an initial concentration of 500 mg L ⁻¹ Pb(II)	[69]
Cu(II)	<i>Saccharomyces cerevisiae</i> (yeast)	water-based magnetic fluid stabilized perchloric acid	1.2 mmol/g adsorption capacity at 25 mg/L initial concentration of ions and pH above 5	[70]
Ni(II)	<i>Saccharomyces cerevisiae</i> (yeast)	nZVI	77% efficiency at pH 5, 3 g/L of biosorbent dosage, and 3 hrs	[71]
Sr(II)	<i>Saccharomyces cerevisiae</i> (yeast)	Chitosan-Fe ₃ O ₄ NP	81.96 mg adsorption capacity Sr(II)/g pH= 8, 5hs, and 30 mg adsorbent dosage.	[72]
Co(II)and Hg(II)	<i>Pleurotus eryngii</i> (Fungus)	Maghemite (γ-Fe ₂ O ₃).	More than 95% of Co(II) and Hg(II) at 150 mg /l of solid phase, and pH(4-5}	[73]
Cd ²⁺ and Pb ²⁺	<i>lactic acid bacteria cells</i>	magnetic NPs s	98.04 % pb ²⁺ and 99.06% Cd ²⁺ efficiency at 1 gm/L biosorbent dosage,60 min, and 28°C	[74]

Dyes are vastly utilized in industries and their release into the environment caused an environmental disturbance and increased water pollution [75]. Bioprocessing of dyes by microorganisms is carried out either by the

biosorption of dye molecules on biomass or by biodegradation of dyes. Biodegradation of Azo dyes is carried out using microbial culture, in which the metabolic activities of the microbial community in consortia lead to increased biodegradation and mineralization [76]. The immobilization of nano-cells as a nano-biosorbent is carried out to improve biosorption efficiency due to enhanced stability and protection of microbial cells [75]. Table 3 shows some studies developing new eco-friendly water treatment techniques, decolorizing effluents by fabrication nano-absorbent in the biosorption process.

Table 3: Previous studies related to decolorization by immobilization of microbial onto nanoparticles.

Type of dyes	Kind of microorganisms	Fabrication of biosorbent	Optimum Operation Condition	Ref.
Five types of organic dyes	<i>Sargassum muticum</i> (Macroalgae)	Iron oxide (NPs)	A higher adsorption capacity is 193.8 mg/ gm for acridine orange and (110.4 mg/g) is the lowest one for malachite green	[77]
Methylene blue (MB)	<i>Agrobacterium fabrum</i> (bacteria)	Iron oxide (NPs)	91 mg g ⁻¹ adsorption capacity at 200 ppm MB, pH=11, 160 rpm, and 25 °C.	[78]
Azo dyes (DO 34, CR, Orange II, and Red MX-5B)	<i>Bacillus subtilis</i> (bacteria)	Super paramagnetic Fe ₃ O ₄ NPs	~95% decolorization at pH = 7, 24 h, and 100 mg/L	[79]
Basic Red 46 azo dye (BR 46)	<i>Enterobacter cloacae</i> sp. (bacteria)	Coated cells with magnetic Fe ₃ O ₄ NPs	91.60 % at 200 ppm initial concentration of dye, 37 °C, and pH 7 after 24 hrs	[80]
Methyl orange (M.O)	<i>Saccharomyces Cerevisiae</i> (yeast)	Fe ₃ O ₄ NPs	96.52 % was at the pH 6.5, 50 mg/l, 1.5 g/l biosorbent dosages, 110 rpm, and 35 °C	[75]
Basic Blue 41 azo dyes	<i>Raoultella Ornithinolytica</i> sp. (bacteria)	Magnetic Fe ₃ O ₄ NPs	95.20% decolorization at 1600 ppm dyes, 5 ml/L biosorbent dosage, pH=8, 24 hrs, and 37 °C.	[81]
RO16 and Methylene Blue	<i>Manganese peroxidase</i> (MnP) enzyme	Iron oxide-Chitosan	98% RO16 and 96% MB decolorization at 50 ppm, pH=7, and 27 °C	[82]

6.2. Nano-cells immobilization in the hydrolysis of lignocellulosic material

The possibility of using nanocarriers of cells in biotechnology applications to obtain nano-biocatalysts could be an important strategy for achieving the economic feasibility of many processes [83]. Green energy can be obtained from lignocellulosic materials as feedstock by biotransformation to bioethanol [84]. Hydrolysis of lignocellulose materials by immobilized enzymes is preferred over MNPs as these nano-bio-catalysts can be easily recovered and reused which increases the life of the bio-catalyst, and reduces the cost of bio-catalytic synthesis [85]. Figure (3) shows the steps of biotransformation of lignocellulose into monosaccharides by immobilized enzymes on magnetic NPs [86].

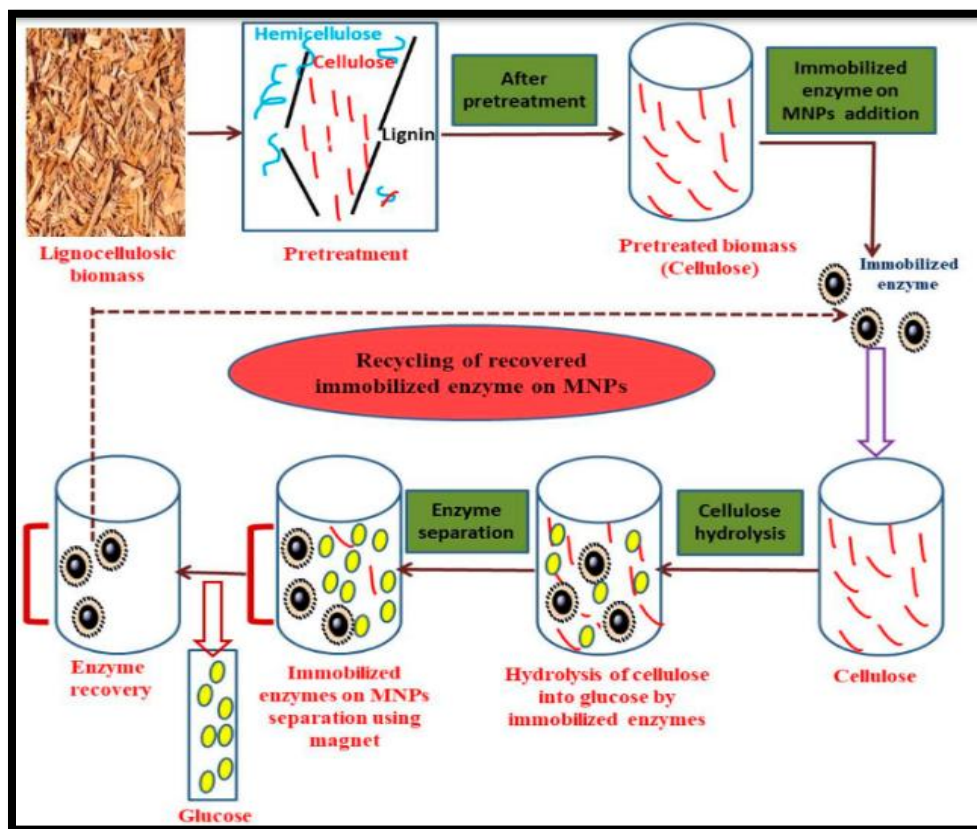


Figure 3: Schematic diagram of the hydrolysis of lignocellulosic material by enzymes immobilized on magnetic NPs to a monosaccharide [86].

6.3. Nano-cells immobilization as stimulants for biofuel production

In recent decades, biofuel production is rapidly increasing and expanding its extent from traditional to advanced biomass (2nd and 3rd generation biofuels). Biofuel production such as bioethanol and biodiesel are alternatives to fossil fuels to reduce the negative impacts on the environment [84].

Nanoparticles incorporated into biofuel production are a promising approach to obtaining efficient biomass degradation and accelerating biofuel production efficiency. V. Ivanova et al. (2011) [87] found an improvement in bioethanol production from corn starch by yeast cells trapped with alginate/magnetic nanoparticles that reached 264.0 g/L per hour while reducing the sugar concentration by 200 g/L. S. Sarwari et al. (2022)[88] obtained that the improvement of bioethanol production from wheat straw by immobilized *S. cerevisiae* SS-4 in calcium alginate/ magnetic NPs gave an optimum yield of (49.71g/L), while 45.66g/L of bioethanol was obtained by immobilized yeast cells in calcium alginate and (36.52g/L) by free cells at optimum conditions of pH 4.5, 72 hrs at 28 °C.

Biodiesel production with important properties has led to the expansion of the biofuel market as the most energy efficient while reducing environmental impacts [84]. X. Wang et al. (2011) [89] where they converted more than 88% to biodiesel in 192 hrs using a four-packed-bed reactor by immobilized lipase on Fe₃O₄ nanoparticles. On the other hand, M. Raita et al. (2015) [90] obtained 97.2% biodiesel production from palm oil by immobilized *Thermomyces lanuginosus* lipase on magnetic NPs under optimum conditions of 23.2 wt % enzyme loading, 4.7:1 methanol to oil, 50 °C and 24 hrs. Moreover, M. Karimi et al. (2016) [91] obtained 91% of biodiesel production from waste cooking by immobilized lipase on superparamagnetic iron-oxide NPs within 35 hrs.

5. Future Research Directions

The immobilization of microbial cells onto nanomaterials has been utilized in different biotechnological applications. Nano-based support materials have been increasingly used due to their important features such as large surface area, the ability to be used in various sectors, and efficient surface chemistry. The immobilized microbial nanoparticle technique is considered a green approach for environmental pollution management, due to

its high efficiency. The green synthesis of nanomaterials from plant extracts contributes to reducing costs [92]. Therefore, these environmentally friendly techniques will be a starting point for their use in various industrial, medical and biological wastewater treatment sectors. At present, many studies are still underrated to solve practical problems of the immobilization of microbial cells [93]. Simultaneously, the studies conducted on the use of immobilized cells on nanoparticles technique is increasing due to the enhanced microorganisms' effective [18]. Recent trends in the utilization of immobilized microbial nanoparticles enhance biogas production in the biotreatment of wastewater at an anaerobic condition [84, 94]. A. Ahmad, and S. S. Reddy (2020) found that the enhancement in the average biogas production reached 4.5 liters and reduction in the chemical oxygen demand is about 95.7% via immobilized consortium onto zinc oxide NPs in upflow anaerobic sludge blanket reactor [95]. Therefore, this strategy will contribute to the production of green energy and thus reduce gaseous emissions that cause environmental pollution. Finally, scientists should support and develop microbial immobilization mechanisms to obtain a novel nano-bio- catalyst with high properties.

6. Conclusion

Immobilization of cells or enzymes on nanoparticles is highly effective in biotechnology applications. Nanoparticles improve microbial performance due to their unique physicochemical properties such as high surface area, effective diffusion, and biocompatibility. Biotechnology applications include the application of nanobiocatalyst in the biosorption of contaminants from industrial effluents using various techniques including the fabrication of nano-biosorbents. The biodegradation of lignocellulosic materials into monosaccharides is obtained by hydrolysis. Moreover, bio-stimulates in biofuel production processes represent a renewable energy alternative to fossil fuels. Therefore, microbial nanoparticle immobilization techniques, as an emerging bioremediation approach with great potential, have been used in various biotechnology applications.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

- [1] I. Manisalidis, E. Stavropoulou, A. Stavropoulos, and E. Bezirtzoglou, "Environmental and Health Impacts of Air Pollution: A Review," *Front Public Health*, vol. 8, p. 1-13, 2020. doi: 10.3389/fpubh.2020.00014
- [2] H. Ali, E. Khan, and I. Ilahi, "Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation", *Journal of Chemistry*, vol. 2019, p. 1-14, 2019. <https://doi.org/10.1155/2019/6730305>
- [3] F. J. Alguacil, and E. Escudero. The removal of toxic metals from liquid effluents by ion exchange resins. Part XVII: Arsenic(V)/H+/Dowex 1x8," *Revista De Metalurgia*, vol. 58, p. e221. 2022. <https://doi.org/10.3989/revmetalm.221>.
- [4] E. H. Khader, Th. J. Mohammed, N. Mirghaffari, A. D. Salman, T. Juzsakova and Th. A. Abdullah," Removal of organic pollutants from produced water by batch adsorption treatment," *Clean Technologies and Environmental Policy*, vol. 24, p. 713–720, 2022. <https://doi.org/10.1007/s10098-021-02159-z>.
- [5] N.A. Al-Rubaiey," A trends in Ozone Treatment of Wastewater: a Review, vol. 2 p. 55-64, 2022.
- [6] B. Y. Sherhan, A. D. Abbas, Q. F. Alsalhy, T. K. Abbas, Y. M. Mahdi, N. A. Abdul Kareem, A. A. Rashad, Z. W. Rashad, and A. A. Shawkat," Produced Water Treatment Using Ultrafiltration and Nanofiltration Membranes," *Al-Khawarizmi engineering Journal*, vol. 12, p. 10-18, 2016.
- [7] B. Liu, B. Chen, and B. Zhang, Oily Wastewater Treatment by Nano-TiO₂-Induced Photocatalysis Seeking more efficient and feasible solutions," *IEEE Nanotechnology. Magazine*, Vol. 11, p.4-15, 2017. doi:10.1109/MNANO.2017.2708818.
- [8] Ü.D. Gül," Bioremediation of dyes in textile wastewater," *Türk Bilimsel Derlemeler Dergisi*. Vol. 11, p. 24-28, 2018. <https://dergipark.org.tr/en/pub/derleme/issue/41716/428216>.

- [9] A. Curtean-Ba ȃna ȃduc, A. Burcea, C.-M. Mihut, V. Berg, J.L. Lyche, and D. Ba ȃna ȃduc, "Bioaccumulation of persistent organic pollutants in the gonads of *Barbus barbus* (Linnaeus, 1758)," *Ecotoxicology Environmental Safety*, vol. 201, p. 110852, 2020. <https://doi.org/10.1016/j.ecoenv.2020.110852>.
- [10] R. A. Azeez, "A Study on The Effect Of Temperature on The Treatment of Industrial Wastewater Using *Chlorella Vulgaris* Alga," *Journal. Engineering Technology*, vol. 28, p. 785-792, 2010.
- [11] Z. Karm, A. D. Subhi, and R. S. Hamied, "Synthesis, characterization, and application of gamma-alumina as adsorbent material to enhance iron removal from produced water," *UPB Scientific Bulletin. B: Chemical Material Science*, vol. 82, p. 237-246. 2020. ISSN 1454-2331.
- [12] T .J. Mohammed, R. A. Azeez, and T. Lutffe, "Biosorption of copper from synthesized wastewater using agriculture waste (Roasted Date Pits)," *International Journal of Recent Scientific Research*, vol. 6, p.3063-3068, 2015.
- [13] E. Torres, "Biosorption: A Review of the Latest Advances," *Process*, vol. 8, p.1584, 2020. <https://doi.org/10.3390/pr8121584>.
- [14] G. Qadir, "Yeast a magical microorganism in the wastewater treatment," *Journal of Pharmacognosy and Phytochemistry*, vol. 8, p. 1498- 1500, 2019.
- [15] A. Adewuyi, "Chemically Modified Biosorbents and Their Role in the Removal of Emerging Pharmaceutical Waste in the Water System: Review," *Water*, vol. 12p. 1551, 2020. doi:10.3390/w12061551
- [16] R. O. Cañizares-Villanueva, "Biosorption of heavy metals by the use of microbial biomass," *Revista Latinoamericana de Microbiologia*, vol. 42, p. 131-143, 2000.
- [17] S. C. S. Martins, C. M. Martins, L. M. C.G. Fiúza and S. T. Santaella, "Immobilization of microbial cells: A promising tool for treatment of toxic pollutants in industrial wastewater." *African Journal of Biotechnology*, vol.12, p. 4412-4418, 2013.
- [18] E. C. Giese, D. D. V. Silva, A. F. M. Costa, S. G. C. Almeida, and K. J. Dussán, "Immobilized microbial nanoparticles for biosorption," *Critical Reviews in Biotechnology*, vol. 40, p. 653-666, 2020. DOI: [10.1080/07388551.2020.1751583](https://doi.org/10.1080/07388551.2020.1751583)
- [19] A. G. Zaki, Y. A. Hasanien, and A. S. Abdel-Razek, "Biosorption optimization of lead(II) and cadmium(II) ions by two novel nanosilica-immobilized fungal mutants," *Journal of Applied Microbiology*, vol. 133, p. 987-1000, 2022.
- [20] A. Nadi, D. Boyer, N. Charbonnel, A. Boukhriss, C. Forestier, and S. Gmouh, "Immobilisation of bacteria onto magnetic nanoparticles for the decolorisation and degradation of azo dyes," *IET Nanobiotechnology*, vol. 13, p. 144-149, 2019. doi: [10.1049/iet-nbt.2018.5026](https://doi.org/10.1049/iet-nbt.2018.5026) www.ietdl.org
- [21] L. Rusu, C-G. Grigoraş, A-L. Simion, E. M. Suceveanu, D. Şuteu, M. Harja, M, "Application of *Saccharomyces cerevisiae*/Calcium Alginate Composite Beads for Cephalexin Antibiotic Biosorption from Aqueous Solutions," *Materials*, vol. 14, p.4728, 2021 <https://doi.org/10.3390/ma14164728>
- [22] H. Merzendorfer, E. Cohen, "Chitin/Chitosan: Versatile Ecological, Industrial, and Biomedical Applications," *Extracellular Sugar-Based Biopolymers Matrices, Biologically-Inspired Systems*, vol 12. P. 541-624, 2019 https://doi.org/10.1007/978-3-030-12919-4_14
- [23] C. Cottet, Y. A. Ramirez-Tapias, J. F. Delgado, O. d. I. Osa, A. G. Salvay and M. A. Peltzer, "Biobased Materials from Microbial Biomass and Its Derivatives: Review" *Materials*, vol 13, p1263, 2020. doi:10.3390/ma13061263
- [24] H. M. Yusof, R. Mohamad, U. H. Zaidan, and N. A. Abdul Rahman, "Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: a review," *Journal of Animal Science Biotechnology*, vol 10, p. 1-22, 2019.
- [25] X. He, H. Deng, and H-M. Hwang, "The current application of nanotechnology in food and agriculture Review," *Journal of Food and Drug Analysis*, vol. 27,p. 1-21, 2019.
- [26] S. A. Ansarz, Q. Husain, "Potential applications of enzymes immobilized on/in nano materials: a review," *Biotechnology Advances*, vol. 30, p. 512-523, 2012.

- [27] M. Ince, O. K. Ince, B. Aydogdu, H. Alp, "Green-synthesis of superparamagnetic Fe₃O₄ /alginate bio-nanocomposites for heavy metal contamination removal from industrial wastewater," *Research Square*, vol. 2022. DOI: <https://doi.org/10.21203/rs.3.rs-1336564/v1>.
- [28] M. I. A. Abdel Maksoud, A. M. Elgarahy, C Farrell, A. Al-Muhtaseb, D. Rooney A. I. Osman," Insight on water remediation application using magnetic nanomaterials and biosorbents," *Coordination Chemistry Reviews*, vol. 403, p.213096, 2020.
- [29] S. Shukla, R. Khan, and A. Daverey" Synthesis and characterization of magnetic nanoparticles, and their applications in wastewater treatment: A review" *Environmental Technology & Innovation*, vol. 24, p. 101924, 2021.
- [30] I. Ali, C. Peng, I. Naz, Z. M. Khan, M. Sultan, T. Islama and Irfan A. Abbasi," Phytogenic magnetic nanoparticles for wastewater treatment: A review," *RSC Advances*,vol. 7, p. 40158-40178, 2017. DOI: [10.1039/c7ra04738j](https://doi.org/10.1039/c7ra04738j)
- [31] Z. B. Bouabidi, M. H. El-Naas, and Z. Zhang," Immobilization of microbial cells for the biotreatment of wastewater:A review," *Environmental Chemistry Letters*, vol.17, p. 241-257, 2018. <https://doi.org/10.1007/s10311-018-0795-7>
- [32] Z. Bayat, M. Hassanshahian, and S. Cappello," Immobilization of microbes for bioremediation of crude oil polluted environments: a mini review," *Microbiology Journal* , vol. 9, p.48–54, 2015.
- [33] M. Kubota, T. Nakabayashi, Y. Matsumoto, T. Shiomi, Y. Yamada, KeitaIno, H. Yamanokuchi, M. Matsui, T. Tsunoda, F. Mizukam, and K. Sakaguchi," Selective adsorption of bacterial cells onto zeolites," *Colloids and Surfaces B: Biointerfaces*, vol. 64, p. 88-97. 2008.
- [34] P. Monsan, G. Durand, and J. M. Navarro," Immobilization of microbial cells by adsorption to solid supports," *Methods Enzymology*, vol.135, p.307–318, 1987.
- [35] O. Długosz, J. Matysik, W. Matyjasik, and M. Banach," Catalytic and antimicrobial properties of α -amylase immobilised on the surface of metal oxide nanoparticles," *Journal of Cluster Science* , vol.32,p. 1609–1622, 2021. <https://doi.org/10.1007/s10876-020-01921-5>
- [36] D. A. Cowan, R. Fernandez-Lafuente," Enhancing the functional properties of thermophilic enzymes by chemical modificationand immobilization," *Enzyme Microbial Technology* 49:326–346, 2011. <https://doi.org/10.1016/j.enzmi.2011.06.023>
- [37] W. Jiang, A. Saxena, B. Song, B. B. Ward, T. J. Beveridge, and S. C. B. Myneni," Elucidation of Functional Groups on Gram-Positive and Gram-Negative Bacterial Surfaces Using Infrared Spectroscopy," *Langmuir*, vol. 20, p.11433–11442,2004. <https://doi.org/10.1021/la049043+>
- [38] H. H. Nguyen and M. Kim," An Overview of Techniques in Enzyme Immobilization," *Applied Science and Convergence Technology*, vol. 26, p.157-163,2017. <http://dx.doi.org/10.5757/ASCT.2017.26.6.157>
- [39] S. Ding, A. A. Cargill, I. L. Medintz, and J. C Claussen," Increasing the activity of immobilized enzymes with nanoparticle conjugation," *Curren Opinion in Biotechnology*, vol. 34, p.242-250, 2015. <https://doi.org/10.1016/j.copbio.2015.04.005>
- [40] A. Rasheed, T. Ghous, S. Mumtaz, M. N. Zafar, K. Akhter, R. Shabir, Zain-ul-Abdin, and S. S. Shafqa," Immobilization of *Pseudomonas aeruginosa* static biomass on eggshell powder for on-line preconcentration and determination of Cr (VI)," *Open Chemistry*, vol. 18, p. 303-313,2020.
- [41] S. Krishnamoorthi, A. Banerjee, and A. Roychoudhury," Immobilized enzyme technology: potentiality and prospects," *Journal of Enzymology and Metabolism*, vol. 1, p.104, 2015.
- [42] J. Burgain, C. Gaiani, M. Linder, and J. Scher," Encapsulation of probiotic living cells: from laboratory scale to industrial applications," *Journal of Food Engineering*, vol. 104, p.467–483, 2011. <https://doi.org/10.1016/j.jfoodeng.2010.12.031>
- [43] D.A. Alromi, S.Y. Madani, and A. Seifalian," Emerging Application of Magnetic Nanoparticles for Diagnosis and Treatment of Cancer," *Polymers (Basel)*, vol.13,p. 4146, 2021. doi: 10.3390/polym13234146. PMID: 34883649; PMCID: PMC8659429.

- [44] M. Yao, B. Li, H. Ye, W. Huang, Q. Luo, H. Xiao, D. J. Mc Clements and L. Li, "Enhanced viability of probiotics (*Pediococcus pentosaceus* Li05) by encapsulation in microgels doped with inorganic nanoparticles," *Food Hydrocolloids*, vol. 83, p. 246–252, 2018.
- [45] N. Suvarli, L. Wenger, C. Serra, I. Perner-Nochta, J. Hubbuch, and M. Wörner, "Immobilization of β -Galactosidase by Encapsulation of Enzyme-Conjugated Polymer Nanoparticles Inside Hydrogel Microparticles," *Frontiers Bioengineering Biotechnology*, vol. 9, p.818053, 2022. doi: 10.3389/fbioe.2021.818053.
- [46] J. A. Trelles, and C.W. Rivero, "Whole Cell Entrapment Techniques. In: Guisan, J., Bolivar, J., López-Gallego, F., Rocha-Martín, J. (eds) Immobilization of Enzymes and Cells. *Methods in Molecular Biology*, vol. 2100 p. 385–394, 2020. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-0215-7_25
- [47] D. Berillo, A. Al-Jwaid, and J. Caplin, "Polymeric Materials Used for Immobilisation of Bacteria for the Bioremediation of Contaminants in Water," *Polymers (Basel)*, vol. 13, p. 1073, 2021doi: 10.3390/polym13071073.
- [48] M. H. El-Naas, M.A. Alhaija, and S. Al-Zuhair, "Evaluation of an activated carbon packed bed for the adsorption of phenols from petroleum refinery wastewater," *Environmental Science Pollution Research*, vol. 24, p.1–10, 2017.
- [49] X. Bai, B. Yang, H. Gu, Z. Hua, L. Yu, and H. Shi, "Design of multi- *N*-functional magnetic PVA microspheres for the rapid removal of heavy metal ions with different valence," *Desalination Water Treatment*, vol. 56, p.1809–1819, 2015.
- [50] J. Al-Hilo, T. J. Mohammed, and R. A. Azeez, "The Production of Ethanol from Sugar Beet Waste by Immobilized *Saccharomyces Cerevisiae*," *Iraqi Journal of Chemical and Petroleum Engineering*, vol. 8, p. 25-30, 2007.
- [51] T. Seyedeh-Masoumeh, A. Ebrahiminezhad, M. B. Ghoshoon, A. Dehshahri, A. Berenjian, and Yo. Ghasemi, "2020. "Magnetic Immobilization of *Pichia pastoris* Cells for the Production of Recombinant Human Serum Albumin," *Nanomaterials*, vol. 10, p. 111, 2020. <https://doi.org/10.3390/nano1001011>
- [52] Y. Wang, Y. Tian, B. Han, H-b. Zhao, J-nan Bi, and B-l Cai, "Biodegradation of phenol by free and immobilized *Acinetobacter* sp. strain PD12," *Journal of Environmental Sciences(China)*, vol. 19, p.222–225, 2007.
- [53] K. Abdollahi, F. Yazdani, and R. Panahi, "Covalent immobilization of tyrosinase onto cyanuric chloride crosslinked amine-functionalized superparamagnetic nanoparticles: synthesis and characterization of the recyclable nanobiocatalyst," *International Journal of Biological Macromolecules*, vol. Part A 94, p. 396–405, 2017. <https://doi.org/10.1016/j.ijbiomac.2016.10.058>
- [54] M. Garmroodi, M. Mohammadi, A. Ramazani, M. Ashjari, J. Mohammadi, B. Sabour, and M. Yousefi, "Covalent binding of hyperactivated *Rhizomucor miehei* lipase (RML) on hetero-functionalized siliceous supports," *International Journal of Biological Macromolecules*, vol. 86, p. 208–215, 2016. <https://doi.org/10.1016/j.ijbio mac.2016.01.076>
- [55] M. E. Mahmoud, A. E. H. Abdou, S. M. S. Mohamed, M. M. Osman, "Engineered *Staphylococcus aureus* via immobilization on magnetic Fe₃O₄-phthalate nanoparticles for biosorption of divalent ions from aqueous solutions," *Journal of Environmental Chemical Engineering*, vol. 4, p.4:3810–3824, 2016.
- [56] J. K. Park, and H. N. Chang, "Microencapsulation of microbial cells. *Biotechnology Advances*, vol.18, p. 303-319, 2000.
- [57] S. C. S. Martins, C. M. Martins, L. M. C. G. Fiúza and S. T. Santaella, "Immobilization of microbial cells: A promising tool for treatment of toxic pollutants in industrial wastewater" *African Journal of Biotechnology*, vol. 12, p. 4412-4418, 2013. DOI: 10.5897/AJB12.2677
- [58] S. Gao, Y. Wang, X. Diao, G. Luo, and Y. Dai, "Effect of pore diameter and cross-linking method on the immobilization efficiency of *Candida rugosa* lipase in SBA-15," *Bioresource Technology*, vol. 101, p. 3830-3837, 2010.

- [59] I. Stolarzewicz, K. E. Bialecka-Florjańczy, E. Majewska, and J. Krzyczkowski, "Immobilization of yeast on polymeric supports." *Chemical and Biochemical Engineering Quarterly*, vol. 25, p.135-144, 2011.
- [60] J. Zdarta, A. S. Meyer, T. Jesionowski and M. Pinelo " A General Overview of Support Materials for Enzyme Immobilization: Characteristics, Properties, Practical Utility" *Catalysts*, vol. 8, p. 92, 2018. doi:10.3390/catal8020092
- [61] S. Cesarini, B. Infanzón, F. J. Pastor, and P. Diaz, "Fast and economic immobilization methods described for non-commercial *Pseudomonas* lipases," *BMC Biotechnology*, vol.14, p. 27 , 2014. <https://doi.org/10.1186/1472-6750-14-27>
- [62] M. L. Verma, M. Puri, and C. J. Barrow, " Recent trends in nanomaterials immobilised enzymes for biofuel production," *Critical reviews in biotechnology*, vol. 36, p.108-119, 2016. <https://doi.org/10.3109/07388551.2014.928811>
- [63] F. K. Al-Zuhairi, R. A. Azeez, S. A. Mahdi, W. A. Kadhim, and M. Kh. Al-Naamee, Removal oil from produced water by using adsorption method with adsorbent a Papyrus reeds. . *Journal Engineering Technology*, vol. 37, p. 157-165 doi: <http://dx.doi.org/10.30684/etj.37.5A.2>.
- [64] R. Li, B. Wang, A. Niu, N. Cheng, M. Chen, X. Zhang, Z. Yu, S. Wang, " Application of biochar immobilized microorganisms for pollutants removal from wastewater: A review," *Science of The Total Environment*, vol. 837, p. 0048-9697, 2022, <https://doi.org/10.1016/j.scitotenv.2022.155563>.
- [65] Q. Peng, Y. Liu, G. Zeng, W. Xu, C. Yang, and J. Zhang, " Biosorption of copper(II) by immobilizing *Saccharomyces cerevisiae* on the surface of chitosan-coated magnetic nanoparticles from aqueous solution," *Journal of Hazardous Materials*, vol. 177, p. 676-682, 2010. <https://doi.org/10.1016/j.jhazmat.2009.12.084>.
- [66] B. Ren, J. Wang, W. Zhang, K. Kan, L. Zhao, X. Song, C. Cui, "Removal of Cr(VI) from solution by free and immobilised spores of *Aspergillus niger*," *Micro & Nano Letters*, vol. 12, p. 615–617. 2017. doi:10.1049/mnl.2017.0043
- [67] Y-Q.Ji, Y-T. Huu, Q. Tian, X-Z. Shao, J. Li, M. Safarikova, and . Safarik, " Biosorption of strontium ions by magnetically modified yeast cells," *Seperation science and technology*, vol. 45, p. 45, p.499–1504, 2010. DOI: [10.1016/j.biortech.2011.11.081](https://doi.org/10.1016/j.biortech.2011.11.081)
- [68] M. E. Mahmoud, A. A. Yakout, H. Abdel-Aal and M. M. Osman, "High Performance SiO₂-Nanoparticles-Immobilized-Penicillium Funiculosum for Bioaccumulation and Solid Phase Extraction of Lead," *Bioresource Technology*, vol. 106, p. 125-132, 2012. [.http://dx.doi.org/10.1016/j.biortech.2011.11.081](http://dx.doi.org/10.1016/j.biortech.2011.11.081)
- [69] P. Xu, G. Zeng, D. Huang, S. Hu, C. Feng, C. Lai, M. Zhao, C. Huang, N. Li, Z. Wei, and G. Xie, Synthesis of iron oxide nanoparticles and their application in *Phanerochaete chrysosporium* immobilization for Pb(II) removal," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 419, p. 147-155, 2013. <https://doi.org/10.1016/j.colsurfa.2012.10.061>.
- [70] L. Uzun, N. Sağlam, M. Safarikova, I. Safarik, A. Denizli, " Copper Biosorption on Magnetically Modified Yeast Cells Under Magnetic Field," *Separation Science and Technology*, vol. 46, p. 1045–1051, 2011. doi:10.1080/01496395.2010.541400
- [71] U. A. Guler, and M. Ersan, "S. cerevisiae cells modified with nZVI: a novel magnetic biosorbent for nickel removal from aqueous solutions," *Desalination and Water Treatment*, vol. 57, p. 7196-7208, 2015. doi:10.1080/19443994.2015.101399
- [72] Y. Yin, J. Wang, X. Yang, J. Hu, and W. Li, " Removal of Strontium Ions by Immobilized *Saccharomyces Cerevisiae* in Magnetic Chitosan Microspheres," *Nuclear Engineering and Technology*, vol. 47, p. 172-177, 2017. doi:10.1016/j.net.2016.09.00.
- [73] S. Ozdemir, S. Mohamedsaid, E. Kilinc, A. Yıldırım, and M. Soylak, " Application of magnetized fungal solid phase extractor with Fe₂O₃ nanoparticle for determination and preconcentration of Co(II) and Hg(II) from natural water samples. *Microchemical Journal*, vol. 143, p. 198-204, 2018. Doi:[10.1016/j.microc.2018.07.032](https://doi.org/10.1016/j.microc.2018.07.032)
- [74] X. Li, Q. Ming, R. Cai, T. Yue, Y. Yuan, Z. Gao, and Z. Wang, " Biosorption of Cd²⁺ and Pb²⁺ from apple juice by the magnetic nanoparticles functionalized lactic acid bacteria cells, *Food Control*, vol. 109, p.106916, 2020. <https://doi.org/10.1016/j.foodcont.2019.106916>

- [75] R. A. Azeez, and F. K. I. Al-Zuhairi, "Biosorption of dye by immobilized yeast cells on the surface of magnetic nanoparticles," *Alexandria Engineering Journal*, vol. 61, p.5213–5222, 2022. <https://doi.org/10.1016/j.aej.2021.10.044>, 2021.
- [76] A. Rane, and S. J. Joshi, "Biodecolorization and Biodegradation of Dyes: A Review," *The Open Biotechnology journal*, vol. 15, p. 97-108, 2021. DOI: [10.2174/1874070702115010097](https://doi.org/10.2174/1874070702115010097)
- [77] R. Angelova, E. Baldikova, K. Pospiskova, Z. Maderova, M. Safarikova, and I. Safarik, "Magnetically Modified *Sargassum Horneri* Biomass as an Adsorbent for Organic Dye Removal," *Journal of Cleaner Production*, vol. 137, p. 189–194, 2016. doi:10.1016/j.jclepro.2016.07.068
- [78] S. Sharma, A. Hasan, N. Kumar, and L. M. Pandey, "Removal of methylene blue dye from aqueous solution using immobilized *Agrobacterium fabrum* biomass along with iron oxide nanoparticles as biosorbent," *Environmental science and pollution research international*, vol. 25, p. 21605–21615, 2018. <https://doi.org/10.1007/s11356-018-2280-z>
- [79] A. Nadi, D. Boyer, N. Charbonnel, A. Boukhriss, C. Forestier, and S. Gmouh, "Immobilisation of bacteria onto magnetic nanoparticles for the decolorisation and degradation of azo dyes. IET Nanobiotechnology," vol. 13, p.144-149, 2019. doi: 10.1049/iet-nbt.2018.5026.
- [80] F. Bekhit, S. Farag, and A. M. Attia, "Decolorization and degradation of the Azo dye by bacterial cells coated with magnetic iron oxide nanoparticles," *Environmental Nanotechnology, Monitoring & Management*, vol. 14, p.100376,2020. <https://doi.org/10.1016/j.enmm.2020.100376>.
- [81] F. Bekhit, S. Farag, and A. M. Attia, "Characterization of Immobilized Magnetic Fe₃O₄ Nanoparticles on *Raoultella Ornithinolytica* sp. and Its Application for Azo Dye Removal," *Applied Biochemistry Biotechnology*, 2022. <https://doi.org/10.1007/s12010-022-04076-3>
- [82] S. M. Siddeeg, M. A. Tphoon, W. Mnif, F. B. Rebah, "Iron oxide/chitosan magnetic nanocomposite immobilized manganese peroxidase for decolorization of textile wastewater," *Processes*, vol.8, p. 5, 2020. [10.3390/pr8010005](https://doi.org/10.3390/pr8010005)
- [83] A. Shahbaz, N. Hussain, M. Z. Saleem, M. U. Saeed, M. Bilal, and H. M.N. Iqbal, "Nanoparticles as stimulants for efficient generation of biofuels and renewables," *Fuel*, vol. 319, p.123724, 2022. <https://doi.org/10.1016/j.fuel.2022.123724>
- [84] R. A. Azeez, and F. K. Al-Zuhairi, "Biofuels (Bioethanol, Biodiesel, and Biogas) from Lignocellulosic Biomass : A Review," *Journal of University of Babylon engineering science*, vol. 28, p. 202-215, 2020. <https://www.journalofbabylon.com/index.php/JUBES/article/view/2948>
- [85] J. Zdarta, A. S. Meyer, T. Jesionowski, and M. Pinelo, "A general overview of support materials for enzyme immobilization: Characteristics, properties, practical utility. *Catalysts*, vol. 8, p. 92, 2018.
- [86] M. Singhvi, and B. S. Kim, "Current Developments in Lignocellulosic Biomass Conversion into Biofuels Using Nanobiotechnology Approach" *Energies*, vol.13, p. 5300, 2020. <https://doi.org/10.3390/en13205300>
- [87] V. Ivanova, P. Petrova, and J. Hristov, "Application in the Ethanol Fermentation of Immobilized Yeast Cells in Matrix of Alginate/Magnetic Nanoparticles, on Chitosan-Magnetite Microparticles and Cellulose-coated Magnetic Nanoparticles," *International Review of Chemical Engineering*, vol. 3, p. 289-299, 2011.
- [88] S. Sarwari, A. Tahiri, I. U. Haq, and F. Anum, "Alkaline pre-treatment of wheat straw for production of ethanol using *Saccharomyces Cerevisiae SS-4* immobilized in nanoparticles," *Pakistan Journal Botany*, vol.54, p. 309-315, 2022. DOI: [http://dx.doi.org/10.30848/PJB2022-1\(45\)](http://dx.doi.org/10.30848/PJB2022-1(45))
- [89] X. Wang, X. Liu, C. Zhao, Y. Ding, and P. Xu, "Biodiesel production in packed-bed reactors using lipase-nanoparticle biocomposite. *Bioresource Technology*, vol. 102, p. 6352–6355, 2011.
- [90] M. Raita, J. Arnthong, V. Champreda, and N. Laosiripojana, "Modification of magnetic nanoparticle lipase designs for biodiesel production from palm oil," *Fuel Processing Technology*, vol. 134, p. 189–197, 2015.
- [91] M. Karimi, "Immobilization of lipase onto mesoporous magnetic nanoparticles for enzymatic synthesis of biodiesel," *Biocatalysis and Agricultural Biotechnology*, vol. 8, p. 182- 188, 2016.
- [92] M. Yusefi, K. Shameli, o. S. Yee, S.Y. Teow, Z. Hedayatnasab, H. Jahangirian, T. J. Webster, and K. Kuča, "Green Synthesis of Fe₃O₄ Nanoparticles Stabilized by a Garcinia mangostana Fruit Peel Extract for Hyperthermia and Anticancer Activities," *International Journal of Nanomedicine*, vol. 16, p. 2515-2532, 2021. <https://doi.org/10.2147/IJN.S284134>
- [93] C. Chen, J. Hu, and J. Wang, "Biosorption of uranium by immobilized *Saccharomyces cerevisiae*," *Journal of Environmental Radioactivity*, vol. 213, p. 106158. 2020
- [94] R. A. Azeez, F. K. Al-Zuhairi, "Bio-treatment Technologies of produced water: a review," *Engineering and*

technology journal, vol. 40,2022. <http://doi.org/10.30684/etj.2022.131480.1040>

- [95] A. Ahmad, and S. S. Reddy, "Performance evaluation of upflow anaerobic sludge blanket reactor using immobilized ZnO nanoparticle enhanced continuous biogas production," *Energies; Basel*, vol. 31, p. 330-347, 2019 . <https://doi.org/10.1177/0958305X19865967>