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NATURAL CARRIERS FOR BACTERIAL IMMOBILIZATION USED IN BIOREMEDIATION

BY

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Abstract. In the last few decades, the environment has been drastically polluted due to the fast industrialization and other anthropic activities. As a boomerang effect, different areas around the world started to shift their original state to one where life itself is continuously diminished, mostly resulting from hazardous circumstances. Microorganisms, in particular bacterial strains have a key role in the bioremediation process due to their efficiency, friendly-environment effect and cheap cost of use. Bioremediation started traditionally using just free cells as a decontaminating tool, however, in couple of years, immobilized microorganisms gained a lot of attention due to their higher stability, retention in time and biotransformation efficiency. Aforementioned immobilization can be realized either on natural or synthetic carriers. The main goal of this paper is to summarize and bring in the spotlight some relevant data regarding the microbial immobilization on specific natural carriers (derived from biomass conversion) and its importance for environmental remediation.

Keywords: feedstock, porous materials, persistent organic pollutants, microbial biofilm, bioremediation.

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1. Introduction

Environmental issues have risen significant concerns in the last decades on account of intensive agriculture, industrialization and anthropic activities (Verma, 2021). Among the most critical pollutants that affect both the environment and human health, are: chemical fertilizer, pesticides, herbicides, insecticides, hydrocarbons, heavy metals and nuclear wastes (Sharma, 2020).

Of all the technologies and methods for cleaning up the pollutants, bioremediation has come out as a desirable approach due to its low cost and ability to hinder the formation and accumulation of contaminants (Ojewumi *et al.*, 2018). Although, the recent advances in microbial immobilization technology (MIT) have been rapidly applied, due to the fact that it provides high activity, rapid reaction rate and high stability for microorganisms involved in bioremediation as well as good efficiency of the process (Rui *et al.*, 2022).

MIT aims to fix microorganisms on specific carriers by physic and chemical methods, in order to prolong their lifespan and keep cells activity (Wang *et al.*, 2020). MIT overcomes the shortcomings of difficult microorganisms' separation or secondary pollution. Moreover, in many cases, the carriers are also conducive to recycling (Wang *et al.*, 2019). And furthermore, through immobilization process the microorganisms have the advantages of high and stable biomass, not easy to lose, fast reaction speed, strong resistance to toxicity (Ting *et al.*, 2013). However, occasionally, the matrix may produce inhibitors resulting in microorganisms' activity loss. Since the microbial activity could be affected by the performance of the carrier materials, it is essential to select suitable carriers for immobilized microorganisms.

A diversity of natural and synthetic carriers has been studied in the last years: porous ceramics, polyvinyl alcohol (PVA), silica and carbon materials, etc. (Rui *et al.*, 2022). Some of them have drawbacks such as high cost, secondary reactions, poor fixation effect and weak applicability. The research progress of microbial immobilization on natural carbon materials has been recently reviewed (Wu *et al.*, 2021) and pointed out some aspects that need to be further studied, especially in terms of carrier's selection (Murshid and Dhakshinamoorthy, 2020). Ideal immobilized carrier materials should be non-toxic, easily available, highly stable, as well as possess large specific surface area (SSA) and good mass transfer. Therefore, the selection of an ideal carrier is the target and difficulty in microbial immobilization technology (Rui *et al.*, 2022).

This work aims to enhance our current knowledge on application of microorganism immobilization technology in environmental bioremediation highlighting the carriers. The specific objectives are: (1) to outline the available sources of natural materials used as carriers; (2) to summarize the immobilized microorganisms used in this process; (3) to discuss the major groups of pollutants subjected to the immobilized microorganism degradation; (4) to provide perspectives on promising applications of immobilized bacterial strains on carbonaceous materials used as carriers.

2. Materials used as carriers

Microbial immobilization requires specific materials for a better efficiency and survivability. Porous material which will be used as carriers need to respect a series of standards such as: easily accessible, inexpensive, environmentally friendly and be able for regeneration. An ideal immobilization process needs to take into account that carrier properties are highly important. Such an example would be carriers that are used in adsorption or binding, they need high porosity level and a good surface area for microbial aggregates (Bayat *et al*., 2015). Porous material starts to get more and more attention due to their benefic impact on long term in bioremediation process (Martins *et al*., 2013). The nature of this materials is shifting in the same time with the type of the bioremediation process will be applied. For example, in bioaugmentation highly biodegradable and nutrient rich carriers are more suitable (Bayat *et al*., 2015; Martins *et al*., 2013).

Carriers, as other materials, are categorized as inorganic and organic or synthetic and natural (Dzionek *et al*., 2016). Due to their chemical and physical resistance, inorganic carriers either natural or synthetic are of interest for bioremediation researches. Such kind of materials can be found abundantly in the environment (magnetite, volcanic rocks, vermiculite) or produced industrially (ceramics, silica-based materials, porous glass and nanoparticles) (Daumann *et al*., 2014; Salis *et al*., 2009). Despite advantages, especially in development of hybrid carriers based on synthetic nanoparticles and a natural polymer matrix, the reduced number of functional groups could be an important drawback (Kourkoutas *et al*., 2004).

Synthetic carriers (such polystyrene, polypropylene, polyvinyl chloride and polyvinyl alcohol) could be of interest considering the higher number of functional groups and the possibility to tailor the macromolecular structure and the suitable molecular weight (Banerjee *et al*., 2015; Lin *et al*., 2015). Although, some features (porosity, polarity, pore diameter, etc.) can be controlled during synthesis till to a certain point, resulting new synthetic carriers that could have different shapes (tubes, membranes, coatings). These materials are also quite cheap and easily available on market (Bayat *et al*., 2015; Siripattanakul and Fürhacker, 2014; Yujian *et al*., 2006).

Natural organic carriers have many functional groups, which stabilize microorganisms and offer them the necessary support for biodegradation process, this class includes: bagasse, rice, diatomite alginate, κ-carrageenan, chitosan, straw, charcoal, husks of sunflower seeds, plant fibers, corncob, mycelium, sawdust, etc. (Table 1) (Marchlewicz *et al*., 2017 ; Gentili, 2006 ; Obuekwe and Al-Muttawa, 2001; Pattanasupong *et al*., 2004). Most of the wastes produced by industries or agriculture ends up by being unused and dumped. These wastes possess good characteristics for a suitable carrier being hydrophilic and cost effective while the low resistance to biodegradation limit their use (Bayat *et al*., 2015; Cubitto and Gentili, 2015).

Nearly all materials used as carriers show specific properties when they are treated thermically. In the following part will discuss some of the feedstock to obtain such kind of carriers (Daumann *et al*., 2014).

Plant fibers constitute some of the most applied material in immobilization processes due to their availability and cheap costs to process. Loofah sponge obtained from either *Luffa cylindrica* or *Luffa aegyptiaca* (Table 1) possess a high porosity (up to 95%) a very low density (approx. 0.05 g/cm^3). The fiber matrix provides a good shelter for microorganism to form a healthy and stable biofilm and the nutrient exchange is high as well. (Akhtar *et al*., 2008; Iqbal *et al*., 2005). Also, the loofah powder could be an important source of carbon for the white rot fungi (Mazmanci and Ünyayar, 2005).

Sugarcane bagasse is obtained from *Saccharum officinarum* (Table 1). Using this fiber can be beneficial for the immobilization process due to the pore size approx. 0.5–5 μm and fiber parallel structures ideal for bacterial and fungal hyphae attachment (Basak *et al*., 2014). The immobilization of *Phanerochaete chrysosporium* on this carrier increase the manganese peroxidase activity for anthracene microbial degradation (Mohammadi and Nasernejad, 2009). By immobilizing a strain of *Acinetobacter venetianus* on bagasse, the degradation rate of tetradecane was more efficient (Daumann *et al*., 2014). The use of a *Candida tropicalis* strain immobilized on bagasse improved the PHB5 remove (Basak *et al.*, 2014; Lin *et al*., 2014).

Sawdust is one of the major wastes resulted from wood industry and agriculture, which has been utilized as carrier for bacterial cells. For degradation of crude petroleum, a strain of *Arthrobacter* sp. was immobilized on sawdust and the enzymatic activity has increased for up to one month and a half of stockpiling between 25°C - 45°C (Obuekwe and Al-Muttawa, 2001). Sawdust has an overly complex design giving exceptionally high surface areas to cell connection, good porosity and hydrophobicity. The bacterial immobilization can be realized with minimum costs and with immediate results (Podorozhko *et al*., 2008).

Corncob is a material that offer many benefits being vigorous, permeable and punctured and having a high-water holding limit, 4 cycles of bioremediation (Rivelli *et al*., 2013; Labana *et al.*, 2005). Mishra *et al.* (2001) have investigated corncobs much closely and find out that are great carriers for bioremediation. Plangklang *et al*. 2012 observed that a strain of *Burkholderia cepacia* formed a biofilm on the outer layer of corncob, due to the limited space to attach the new cells which formed were unattached from the biofilm and lost they capacity to survive. Using corncob powder Rivelli *et al*. (2013) showed that bacterial capacity including retention time and degrading rates of the immobilized microorganisms can be highly increased.

Tezontle is a volcanic stone used in the last decades in bioremediation processes (Table 1). This rock is ordinarily utilized in Mexico as a structure material, and has a trademark ruddy tone (because of the presence of iron particles). The surface is profoundly permeable and punctured, which gives a decent spot to biofilm development (Yáñez-Ocampo *et al*., 2009). Santacruz et al. 2005 showed that immobilization of *a Pseudomonas fluorescens* strain on a tezontle support led to a DDT and 2,4-dichlorophenoxyacetic high degradation. Yáñez-Ocampo *et al*. (2009) observed that biodegradation of methyl-parathion and tetrachlorvinphos (TCPV), both insecticides, was more efficient using a bacterial consortium immobilized on tezontle powder rather than using only microorganisms.

Table 1
Natural carries and microorganisms used in bioremediation processes

Natural Carrier	Thermo-chemical conversion process	Immobilized bacterial strain	Removed pollutant	References
Rice husk	-	*Rhodococcus sp.*	4-chlorobenzoate	Verma, 2021
Wheat bran	-	*Eschirichia coli AtzA*	Atrazine	Sahoo *et al*. (2019)
Hair mats	-	*Pseudomonas putida BCRc14349*	Phenol, trichloroethane	Rui et al. (2022)
Alginate	-	*Pseudomonas sp.* *P. fluorescens G7*	Cadmium and Zinc	Ting *et al*. (2013)
Alginate, agar, polyacrylamide	-	*Pseudomonas fluorescens-CS2*	Ethylbenzene	Parameswarappa *et al*. (2008)
Puffed foxed tail mitted	-	*Rhodococcus sp. F92*	Various petroleum products	Sharma *et al*. (2020)
Biochars				
Sawdust	Pyrolysis	*Arthrobacter sp.*	Petroleum oil	Podorozhko *et al*. (2008)
Plant fibers (Loofah sp.)	Pyrolysis	*Bacillus cereus*	Aromatic hydrocarbons	Maliji *et al*. (2013)
Corncob powder	Pyrolysis	*Pseudomonas sp.* *Rhodococcus sp.*	Hexadecane	Rivelli *et al*. (2013)

Coco-peat	Pyrolysis	*P. fluorescens*	DDT (pesticide)	Nunal *et al.* (2014)
Cotton fibers	Pyrolysis	*Acinetobacter sp.*	n-Heptadecane	Lin *et al.* (2015)
Papaya stem	Pyrolysis	*Mycoplana sp. MVMB2*	Phenanthrene	Lakshmi *et al.* (2012)
Bagasse	Pyrolysis	*Pseudomonas fluorescens*	2,4-dinitrotoluene	Murshid *and* Dhakshina moorthy, (2020)
Cotton fibers	Pyrolysis	*Vibrio sp. HC-3B*	Crude oil	Lin *et al.* (2018)
Wood chip wax	Pyrolysis	*Pseudomonas fluorescens*	2,4-dinitrotoluene	Martins *et al.* (2013)
Bamboo	Fast pyrolysis	*Burkholderia xenovorans LB400*	Arochlor 1221, 1232, and 1242	Wasi *et al.* (2013)
Chitosan	Slow pyrolysis	*Pseudomonas putida BCRc14349*	Phenol, trichloroethane	Lukasz *et al.* (2013)
Husks of sunflower seeds	Slow pyrolysis	*Rhodococcus sp.*	Crude oil	Cubitto and Gentili, (2015)
Hydrochars				
Plant residues or Biochar	HTC (220°C)	*Pseudomonas putida*	Phenanthrene and pyrene	Wang *et al.*, 2019
Peanut shell powder	HTC (220°C)	*Mycobacterium gilvum*	Pyrene	Bayat *et al.* (2015)
Corncob-sodium alginate	HTC (225°C)	*Burkholderia sp.*	di-n-octyl phthalate	Massalha *et al.* (2017)
Tezontle	HTC (230°C)	*P. aeruginosa*	Styrene	Mooney *et al.* (2006)
Corn cobs	HTC (235°C)	*Pseudomonas sp.*	Biphenyl	Jiang *et al.* (2006)

Coco-peat, husks of sunflower seeds and cotton strands are feedstocks which can be great carriers for bioremediation processes (Cubitto and Gentili, 2015; Lin *et al.*, 2015; Nunal *et al.*, 2014). Crude petroleum contamination was remediated by a strain of *Rhodococcus spp.* immobilized on sunflower seed husks, or by *Acinetobacter sp.* biofilm.

This data can give a hint on the future perspectives of using immobilized microorganisms in environmental bioremediation.

3. Types of pollutants that are degraded using immobilized microorganisms

Petroleum hydrocarbons (PHCs) are the primary constituents in crude oil, gasoline and a variety of solvents and penetrating oils. Crude oil is a mixture, mainly formed by hydrocarbon molecules which are found in the underground reservoirs in a liquid phase. After the extraction this liquid can be processed into petroleum products such as heating oil, gasoline, diesel fuel, liquid petroleum gas, etc.) (Lin *et al*., 2018). Once spilling into soil, PHCs might impact the physicochemical properties of soil and the dispersions of soil microbial networks (Sheng *et al*., 2021). Likewise, soil PHCs pollution have triggered serious natural and human wellbeing concerns (Miri *et al*., 2019). Bioremediation of diesel-contaminated groundwater and seawater by diesel-degrading consortia immobilized on biochar was an effective, economic, and environmentally friendly method in simulated groundwater and seawater environments (Rivelli *et al*., 2013). All immobilized cells show high mechanical strength in long-term degradation processes and high reusability for diesel degradation (Labana *et al*., 2005). The immobilized petroleum hydrocarbons-degrading bacteria on carriers also had great potentials in the bioremediation of petroleum hydrocarbon contaminated soils (Galitskaya *et al*., 2016; Liang *et al*., 2009). The biodegradation efficiency of petroleum hydrocarbons, bacterial population and activity were accelerated after immobilization, this can be due to the high level of oxygen content, nutrients, petroleum hydrocarbons mass transfer and water holding capacity of the soil amended with the carrier (Liang *et al*., 2009).

Polycyclic aromatic hydrocarbons (PAHs) are another class of chemicals that causing cancer and mutagenic disorders. Most part of them are produced from burning or pyrolysis of natural substances and are pervasively present in the climate (Nunal *et al*., 2014). Past investigations revealed that PAH-degraders (*Pseudomonas aeruginosa and Cupriavidus sp*.) immobilized on a carrier essentially improved degradation of acenaphthene and benzo[a]pyrene from soil/wastewater (Basak *et al*., 2014). In another review, two PAHs-degrading microorganisms, *Pseudomonas putida* (Table1) and an unidentified native bacteria captured in biochar-sodium alginate dots increased the degradation of PAHs and moderated soil contamination, contrasted with free cells (Akhtar *et al*., 2008). The bioremediation efficiencies of PAHs with 4-and 5-rings by immobilized cells were higher than that of PAHs with 3-and 6-rings (Wang *et al*., 2019). Comparative discoveries were accounted for in the biodegradation of PAHs in polluted soils by the composites of PAH-degrading *Mycobacterium gilvum* and carrier, it was observed a higher resistance limit and degrading rate to phenanthrene, fluoranthene and pyrene (Banerjee *et al*., 2015).

Benzenes like phenol, nitrobenzene, and toluene are generally utilized in paintings, glues, pesticides, and plastics (Salis *et al*., 2009). They are a threat to the environment and all forms of life due to the high toxicity and mutagenic capacities (Daumann *et al*., 2014). Nonylphenol (NP)-degrading microorganisms immobilized on biochar showed higher resistance and degradation rates levels than biochar alone or free cells (Wang *et al*., 2020). Toluene-degrading organisms (*Pseudomonas sp*.) trapped in biochar pores with polyvinyl liquor (PVA) act as a cross-connecting bond in the bio-streaming channel and could keep up with high cell movement, thus enhancing the resistance against high levels of toluene (Lin *et al*., 2018). The cell-biochar biodegradation of toluene included two phases. Toluene was first adsorbed on the outer layer of biochar in the main phase, and afterward the run off toluene was totally degrades by toluene-degrading microorganisms in the second phase (Lin *et al*., 2015; Lin *et al*., 2018). The benefits of immobilized degrading microbes attached on a carrier offer a higher stability and resilience than free cells also efficiency is progressive (Zhuang *et al*., 2015). Some phenol-degrading microorganisms were effectively when were immobilized on biochar and the immobilized cells showed a great potential for the adsorption and biodegradation of phenols (Ehrhardt and Rehm, 1989; Kwon *et al*., 2009; Massalha *et al*., 2017). Another study shows that, dibutyl phthalate (DBP)-degrading bacteria (*Bacillus siamensis strain T7)* immobilized on biochar helped the degradation of DBP in soil by taking up high levels of the pollutant because of the biofilm formation stability and division rates of bacteria. Spills of DBP to soil affect biota directly and with severe consequences but microbial immobilization can restable the equilibrium (Wang *et al*., 2019).

Heavy metals came as a significant issue due to the great industrialization form 18th century and urbanization which has brought huge quantities of metals into the environment (Yang *et al*., 2019). Heavy metals can perturbate the environment and have a serious impact on the general wellbeing, as a result of their high harmfulness and cancer-causing nature even at low levels of exposure (Nagajyoti *et al*., 2010). Microorganisms immobilized on biochar have been applied in order to degrade some of the most trouble causing metals like Cd (II), Cu (II), Pb (II), Mn (II) and Hg (II). The primary components, responsible for higher Cd (II) levels in soil/wastewater, include electrostatic forces, particle exchange, and surface complexation with the other compounds from environment (e.g., carboxyl, hydroxyl, carbonyl, and phosphodiester) on the outer layer of biochar-microalgae immobilized edifices (Sheng *et al*., 2021). As for the reduction of Mn (II), Mn (II)- oxidizing bacterium (*Streptomyces violarus* strain SBP1) was immobilized on biochar. The data highlighted that Mn (II) reduction was synergistic with the biofilm formation on the biochar and adsorption by biochar, and this led to a natural oxidation (Mn (II) > Mn (III) > Mn (IV) (stable particulate) by SBP1 (Youngwilai *et al*., 2020). Microorganisms immobilization with biochar as a carrier are intensely researched in remediating wastewater contaminated with numerous heavy metals. Wang *et al*. (2019) showed that the mineral parts of biochar and the bounds of the co-sorbents worked as sorption points for Pb (II), while the solid phase affected the link between Hg (II) and natural sulphur formation (thiols) in cells. The co-sorbents with various sorption destinations for Pb (II) and Hg (II) had an easier path just with biochar alone due to the synchronous degradation of Pb (II) and Hg (II) (Wang *et al*., 2019).

4. Future perspectives of using carbonaceous materials as carriers

Altogether, bacterial strains could improve the tolerance and degradation efficiency against a high concentration of pollutants being immobilized on appropriate carrier. However, the practical application of MIT is restricted due to the complexity of the operation and demands a better investigation.

Thus, the selection of microorganisms (*Pseudomonas sp., Bacillus sp.* or *Vibrio sp.*) and carriers plays a vital role in the practical bioremediation processes. The main function of microorganisms is to adsorb and break down the pollutant molecules, while the main function of carrier materials is to protect and supply with nutrients the microorganisms.

Therefore, future research should be focused on the development of a cost-effective, physic and chemically stable, with sufficient adsorption sites and good biocompatibility carrier for immobilization of microorganisms. In this perspective, carbonaceous materials like biochar/hydrochar obtained from sustainable feedstock could represent a good alternative as natural carriers being capable to transfer nutrients and electrons, thereby improving the ability of microorganisms towards biodegradation.

The data related to the interactions between bacterial strains, carriers and pollutants needs environmental optimization. Also, the formed biofilm can enhance the capacity of biodegradation, in fact more strains can secure a better rate of degradation and resistance towards pollutants. Therefore, a deeply explore of the interaction mechanism between materials and microorganisms to achieve low operating costs and high pollutant removal efficiencies have to be conducted.

5. Conclusions

The literature has point out that the immobilized microorganisms on various carriers have a decisive result in the bioremediation process, due to their capacity to degrade different contaminates.

This paper gives an outline on more used natural carriers as well as in the carrier's effectiveness against toxic pollutants and their application either in soil or wastewater remediation.

The literature reveals that natural organic carriers have many functional groups, which stabilize microorganisms. Most of them also poses good characteristics for a suitable carrier being hydrophilic, biodegradable, biocompatible, and inexpensive.

Nearly all materials used as natural carriers show better specific properties when they are treated thermically (biochar, hydrochar).

Studies have shown that adsorption and entrapment are the most well-known mechanism for the remove or degradation of toxic compounds.

The immobilized cells have been successfully applied in the presence of heavy metals, natural toxins, exhaust gases, etc. The immobilization of metal-tolerant microorganisms on natural carriers lead to a higher removal capacity of toxic metals, particularly As (III) and Cr (VI) than alone carrier or free cells.

Various bacteria immobilized contribute to the complete degradation of organic pollutants (anthracene, PHB5, DDT and 2,4-dichlorophenoxyacetic, petroleum et al) within a short time, which was unrealizable for carriers alone or free bacterial cells.

Overall, microbial immobilization technology is considered to be an effective and appealing biological approach for soil, wastewater and off-gases bioremediation and carriers are of critical interest for the smooth running and process effectiveness.

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MATERIALE SUPORT DE ORIGINE NATURALĂ PENTRU IMOBILIZAREA BACTERIILOR UTILIZATE ÎN BIOREMEDIERE

(Rezumat)

În ultimele decenii mediul înconjurător a fost puternic poluat din cauza industrializării rapide și a altor activități antropice. Ca efect de bumerang, diferite zone din lume au început să se transforme în areale în care viața este în mod continuu diminuată, în mare parte ca urmare a degradării calităţii factorilor de mediu. Microorganismele, în special bacteriile au un rol important în bioremedierea mediului datorită eficienței, impactului redus asupra mediului și costurilor mici de utilizare. Bioremedierea a fost aplicată la început folosind doar culturi de microorganisme ca instrument pentru decontaminare. Insă, în câțiva ani microorganismele imobilizate au devenit de interes datorită stabilității mai bune, rezistenţei mai bune în timp și eficienței biotransformării. Imobilizarea poate fi realizată fie pe materiale suport naturale, fie pe suporți sintetici. Scopul principal al acestei lucrări este de a evidenția date privind imobilizarea bacteriană pe suporți naturali precum și utilizarea acestora în îndepărtarea unor poluanți prioritari pentru remedierea mediului.