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Green stabilization of nanoscale zero valent iron (nZVI) with rhamnolipids produced by agro-industrial waste: application on nitrate reduction

Cinthia Cristine de Moura

Tese apresentada ao Instituto de Pesquisa em Bioenergia de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências.

Orientador(a): Jonas Contiero

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TÍTULO DA TESE: Green stabilization of nZVI with rhamnolipids produced by agro-industrial waste: application on nitrate reduction

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"A lei da mente é implacável.

O que você pensa, você cria;

O que você sente, você atrai;

O que você acredita

Torna-se realidade".

Buda

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Resumo

A contaminação ambiental causada por compostos orgânicos é um importante problema que afeta solos e água superficiais. Para reduzir ou remover esses poluentes, os locais contaminados são geralmente tratados com métodos físicos e químicos. No entanto, a maioria dessas técnicas de remediação é custosa e geralmente leva à remoção incompleta e à produção de resíduos secundários. A nanotecnologia consiste na produção e aplicação de estruturas extremamente pequenas, cujas dimensões estão na faixa de 1 a 100 nm, neste cenário a nanopartícula de ferro zero valente representa uma nova geração de tecnologias de remediação ambiental. É não tóxica, abundante, barata, fácil de produzir, e seu processo de produção é simples. No entanto, a fim de diminuir a tendência de agregação, a nanopartícula de ferro zero é frequentemente revestida com surfactantes. A maioria dos surfactantes é quimicamente sintetizado a partir de fontes petroquímicas, eles são persistentes ou parcialmente biodegradáveis, enquanto oferecem baixos riscos à saúde humana, esses compostos podem prejudicar plantas e animais. Para diminuir o uso de métodos químicos, a síntese e estabilização verde de nanomateriais metálicos apresentam-se como uma opção menos perigosa ao meio ambiente. Os biossurfactantes podem potencialmente substituir qualquer surfactante sintético, eles são compostos extracelulares produzidos por microrganismos, como bactérias, e cultivados em diferentes fontes de carbono, podendo ser substratoshidrofílicos. Os biossurfactantes possuem uma grande variedade de estruturas químicas e propriedadesde superfície e entre eles estão os ramnolipídios que já foram intensamente investigados e estudados. Os ramnolipídios podem ser produzidos pela *Pseudomonas aeruginosa* em diferentes substratos, incluindo o glicerol. Uma produção bem-sucedida de biossurfactantes depende do uso de materiais renováveis e de baixo custo. O glicerol bruto, principal subproduto do processo de transesterificação em uma usina de biodiesel, é um substrato amplamente utilizado para a produção de ramnolipídios. A fim de obter a melhor temperatura e concentração inicial de glicerol, o design rotacional composto central e o método de superfície de resposta foram empregados para delimitar as melhores condições para aumentar a produção de ramnolipídeos e diminuir o glicerol remanescente no meio. Com o auxílio do Método de Superfície e Resposta foi possível verificar a viabilidade do uso do glicerol bruto livre de sal, atingindo uma produção de 2,63 g/L de ramnolipidios e depleção total da fonte de carbono, no meio otimizado contendo 25 g/L de fonte inicial de carbono a 32 ° C. Em seguida, as nanopartículas de ferro zero foram sintetizadas utilizando a redução química com borohidreto de sódio. Foram testadas duas metodologias: (i) adição de ramnolipídios durante a síntese química e (ii) adição após a síntese. As nanopartículas foram subsequentemente testadas quanto à eficiência na redução de nitrato em água subterrânea simulada sob condições anaeróbias em pH 4. A nanopartícula de ferro zero sintetizada adicionando ramnolipídios após a síntese, mostrou a melhor eficiência com uma taxa de remoção de cerca de 78% de remoção de nitrato e concentração inicial de nitrato de 25. mg/L. O método para preparar nanopartícula de ferro zero, usando ramnolipídios como agente estabilizador, mostrou-se uma alternativa promissora para a funcionalização de superfície da nanopartícula, em substituição a surfactantes sintéticos e tóxicos

Palavras chave: Biossurfactantes. Química verde. Design experimental. Glicerol. Nanopartícula de ferro zero valente. Águas subterrâneas.

Abstract

Environmental contamination caused by organic compounds is the most important challenge that affects a huge number of soils and water surfaces. To reduce or remove these pollutants, contaminated sites are usually treated using physical and chemical methods. However, most of these remediation techniques are expensive and commonly lead to incomplete removal and to the production of secondary wastes. Nanotechnology is the production and application of extremely small structures, whose dimensions are in the range of 1 to 100 nm and Nanoscale zero-valent iron represents a new generation of environmental remediation technologies, is non-toxic, abundant, cheap, easy to produce, and its reduction process requires little maintenance. Nonetheless, in order to diminish the tendency of aggregation, nanoscale zero-valent iron is often coated with surfactants. Most surfactants are chemically synthesized from petrochemical sources, they are slowly or partially biodegradable, while offer low harm to humans, such compounds can influence plants and animals. To decrease the use of chemical methods green synthesis and stabilization of metallic nanomaterials viable option. Biosurfactants can potentially replace virtually any synthetic they are extracellular compounds produced by microbes such as by bacteria and grown on different carbon sources containing hydrophobic/hydrophilic substrates. The biosurfactants have a wide variety of chemical structures and surface properties and among them is the rhamnolipids which have been intensively investigated and extensively reviewed, they can be produced by *Pseudomonas aeruginosa* from different substrates, including glycerol. Successful production of biosurfactants depends on the use of renewable materials and low cost. Crude glycerol is the primary byproduct of the transesterification process in a biodiesel plant and it is a widely used substrate for rhamnolipid production. To provide the best temperature and initial concentration of glycerol the central composite rotational design and response surface method were employed to increase rhamnolipids yield and lower the glycerol remaining in the medium. The response surface method methodology indicated the viability of the use of crude glycerol, reaching a production of 2.63 g/L of biosurfactant and total depletion of carbon source, at the optimized medium containing 25 g/L of initial carbon source at 32 °C. Then, the iron nanoparticles were synthesized using the chemical reduction of ferric ions with sodium borohydride. Were tested two methodologies: (i) adding rhamnolipids during the chemical synthesis and (ii) adding after the synthesis. The nanoparticles were subsequently tested for their efficiency in nitrate reduction in simulated groundwater under anaerobic conditions at pH 4. The nanoscale zero-valent iron synthetized adding rhamnolipids after the synthesis showed the best efficiency with a removal rate about 78% and initial nitrate concentration of 25 mg/L. The method for preparing nanoscale zero-valent iron using rhamnolipids biosurfactants as stabilizer was found as a promising alternative for the synthesis and surface functionalization of iron nanoparticles, in replacement to toxic synthetic surfactants

Key words: Biosurfactants. Green chemistry. Experimental design. Glycerol. Nanoscale zero-valent iron. Groundwater.

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Sumário

1. Introduction

Nanotechnology is the production and application of extremely small structures, at the level of atoms, molecules, and supramolecular structures, whose dimensions are in the range of 1 to 100 nm (ISO, 2009; NSET, 2003). Is an innovative alternative that can be used for the remediation of contaminated sites, it has the potential to significantly affect environmental protection, because it has the property to remove the finest contaminants from water supplies and air and mitigate pollutants in the environment (NSET, 2003). Environmental pollution by organic contaminants is a major problem today. Recently, there have been many reports of soil and surface water locations contaminated with organic pollutants (CETESB, 2016; EPA, 2002; EUROPEAN COMMISSION DG; ALERT; SERVICE, 2013), with a great impact on soil and groundwater.

Nanoscale zero-valent iron (nZVI) represents a new generation of environmental remediation technologies, because nZVI is non-toxic, abundant, cheap, easy to produce, and its reduction process requires little maintenance (FU; DIONYSIOU; LIU, 2014). To reduce problems with aggregation, nZVI is often coated with surfactants (CRANE; SCOTT, 2012; NSET, 2003). Surfactants play major roles improving the particle mobility (DUTRA, 2015) and lowering the interfacial tension, they also prevent coalescence of newly formed drops (MORSY, 2014).

Most of the commercially available surfactants are chemically synthetized , produced based on petrochemical sources (BANAT; MAKKAR; CAMEOTRA, 2000; GAUTAM; TYAGI, 2006; VAN BOGAERT et al., 2007). Furthermore, some of the surfactants are only slowly or partially biodegradable (KUMAR, 2019) contributing to environmental impact (HAUSMANN; SYLDATK, 2015). Thus, the rapid advances in biotechnology and increased environmental awareness, the chemically surfactants have increasingly been replaced by biologically synthetized surfactants (BANAT; MAKKAR; CAMEOTRA, 2000; GAUTAM; TYAGI, 2006). Surfactants present low toxicity to humans but can affect plants and animals, i.e. ethoxylated alcohols, found in laundry detergents are toxic to fish (MULLIGAN; YONG; GIBBS, 2001a).

Biosurfactants are an alternative, they can potentially replace the synthetic surfactant (REIS et al., 2013; SÁENZ-MARTA et al., 2015). Biosurfactants can be applied in bioremediation field, to clean the contaminated soil and water (THAVASI, 2011), they present advantages in microbial enhance oil recovery (MEOR) , when compared to the synthetic surfactants (BANAT et al., 2010). They have low toxicity and high biodegradability and biocompatibility, additionally, present functionality under extreme conditions of temperature, pH, salinity; with possibility of production from renewable sources (BANAT et al., 2010; COOPER, 1986; DESAI; BANAT, 1997a) and the in situ application (WANG et al., 2016)

Pseudomonas aeruginosa has the ability to synthesize a glycolipid-type biosurfactant, rhamnolipids this was first reported in 1949 by Jarvis et al., appud (MAIER; SOBERÓN-CHÁVEZ, 2000). It can produce rhamnolipids from substrates including C11 and C12 alkanes, succinate, pyruvate, citrate, fructose, glycerol, olive oil, glucose and mannitol (ROBERT et al., 1989). They have been intensively investigated and extensively reviewed (MAIER; SOBERÓN-CHÁVEZ, 2000; NITSCHKE; COSTA; CONTIERO, 2005; OCHSNER; HEMBACH; FIECHTER, 1996). The application of biosurfactants in the bioremediation has become one of the methods used in the remediation of contaminated sites. They can be used to clean the contaminated soil and water (THAVASI, 2011). The use of a raw material of agro-based wastes, low-cost renewable substrates and the new research about rhamnolipids applications on biodegradation and toxicity are worth further investigation and may make biosurfactants a versatile sustainable molecule. The industrial conversion of renewable resources into useful compounds has been receiving much attention; the use of crude glycerol is becoming very important from the environmental point of view (MORITA et al.; 2007; EASTERLING et al., 2009). It is a widely used substrate for rhamnolipid production (SYLDATK et al. 1985) since a wide variety of microorganisms, as *Pseudomonas aeruginosa*, can utilize glycerol as a source of carbon and it is often formed as an intermediate in both the aerobic and anaerobic catabolism of lipids and glucose. (JOHNSON, TACONI, 2007; HAUSMANN; SYLDATK, 2015).

The hypothesis of this work lies in the fact that the rhamnolipids produced by *Pseudomonas aeruginosa* strain, using glycerol as a substrate may significantly increase the stabilization of nanoparticles of zero valent iron. It can become a novel application for biosurfactants allied to a consequently reduction on the costs of the process due to the use of a renewable substrate.

3. Objectives

General

Stabilization of de nanoscale zero-valent iron (nZVI) aiming the biosurfactant produced by *Pseudomonas aeruginosa* using glycerol as a carbon source.

Specific

1. A bibliographic survey about the state of the art of nZVI and biosurfactants;

2. Optimization of temperature and initial carbon source for the production of rhamnolipids and glycerol consumption using *Pseudomonas aeruginosa* LBI 2A1;

3. Production, stabilization and characterization of nZVI using rhamnolipids as capping agent;

4. Study the performance of bare nZVI and stabilized nZVI in nitrate removal.

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General Conclusions

This work has shown how it is possible to use *P. aeruginosa LBI 2A1* rhamnolipids as carbon source crude glycerol for stabilization nanoscale zero valent iron (nZVI). Chapter 1 presents a general introduction, the importance of biosurfactants, the use of renewable sources for its production, such as crude glycerol and also the importance of nZVI for groundwater remediation. The bibliographic survey from Chapter 2 shows the gaps that still exist about the green stabilization of nZVI using biosurfactants and how little attention has been given to this technique and the need for further researches. In Chapter 3 the Central Composite Rotatable Design (CCRD) and Response Surface Method (RSM) using pro-analysis glycerol and crude glycerol proved to be efficient for optimizing the best culture conditions using *P. aeruginosa LBI 2A1*. The best results were obtained using 25g / L salt free glycerol (GFS) at 32 ° C and pH 7, with a 2.68 g/L production of ramnolipids. In chapter 4 we observed the production and stabilization of nZVI using two different methodologies, according to the Zeta Potential analysis, the best functionalization happened with nZVI-S, when the nanoparticle was stored in rhamnolipids solution. XRD analyzes indicate that nanoparticles remain stable for at least 1 month. Efficiency on nitrate removal also indicated that nZVI-S is better, removing up to 78.62% of nitrate. This work provides unpublished evidences on the use of biosurfactant rhamnolipids as nZVI green stabilizers and their subsequent use for nitrate removal on simulated groundwater.