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Research article

Synthesis and characterization of stabilized oxygen-releasing CaO_2 nanoparticles for bioremediation

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ABSTRACT

Bioremediation is one of the general methods to treat pollutants in soil, sediment, and groundwater. However, the low concentration and restricted dispersion of dissolved oxygen (DO) in these areas have limited the efficiency of remediation especially for microorganisms that require oxygen to grow. Calcium peroxide (CaO_2) is one of the oxygen-releasing compounds and has been applied to magnify the remediation efficacy of polluting areas. In this study, CaO_2 nanoparticles (NPs) were synthesized and evaluated by wet chemistry methods as well as dry and wet grinding processes. The characteristics of CaO_2 particles and NPs were analyzed and compared by dynamic light scattering, transmission electron microscopy, scanning electron microscopy, and X-ray powder diffraction. Our results showed that wet-grinded CaO_2 NPs had an average particle size of around 110 nm and were more stable compared to other particles from aggregation and sedimentation tests. In addition, we also observed that CaO_2 NPs had better DO characteristics and patterns; these NPs generated higher DO levels than their non-grinded form. Accordingly, our results suggested that wet-grinding CaO_2 particles to nanoscale could benefit their usage in bioremediation.

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1. Introduction

Due to industrial developments, the accidental spread of industrial pollutants is a major issue that is becoming increasingly concerning. Accordingly, the scientific community has investigated various methods to eliminate these pollutants from the environment. In situ chemical oxidation (ISCO) is especially promising and has gained significant traction over the last few decades: ISCO treatment is fast, inexpensive to implement, effective, and has a low maintenance-cost (Romero et al., 2009).

The ideal ISCO compound will not only oxidize pollutants, destroying the contaminants by converting them into innocuous compounds, but also encourage the growth of aerobic microorganisms, which, via bioremediation, will further promote the degradation of pollutants (Mosmeri et al., 2017; Wu et al., 2015).

CaO_2 nanoparticles (NPs) are one type of compound that is being researched for use as the active compound in ISCO: CaO_2 reacts with water by the Fenton reaction, producing oxygen-containing OH radicals that readily supply oxygen to the polluted site (Northup and Cassidy, 2008). The supplied oxygen simultaneously oxidizes pollutants and feeds aerobic microorganisms (Arienzo, 2000; Mosmeri et al., 2017). Other compounds can achieve similar effects, such as H_2O_2. The key difference, however, is the efficiency of oxygen delivery; Researchers have found that CaO_2 is more efficient than other potential compounds, such as the aforementioned H_2O_2, in terms of oxygen generation (Ndjou'ou and Cassidy, 2006). Further investigations into the Fenton reaction of CaO_2 have shown that CaO_2 is more efficient as an oxidant than traditional liquid H_2O_2 treatment at all pH levels: CaO_2 only releases H_2O_2 upon dissolution which reduces the loss of O_2 from volatilization (Northup and Cassidy, 2008). For all of these reasons CaO_2 has the potential to become a low-cost and relatively low maintenance remediation technology.

Different material combinations to obtain effective CaO_2 NPs for biodegradation have been tested. Ingredients including CaO_2, citrate, polyvinyl alcohol, and bamboo biochar with one specific ratio were tested for their optimal oxygen-releasing capacity in terms of biochar CaO_2 beads synthesis (Wu et al., 2015). The beads were then studied for the bioremediation of benzene, toluene, ethylbenzene, and xylene (BTEX) in relation to their oxygen releasing properties. The results showed that biochar CaO_2 beads could help microorganisms remove up to 99% of toluene in a polluted

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environment. In addition, another study concluded that the oxygen-releasing characteristic of CaO_2 effectively degraded organic matter in the sediment of a pond from 18% to 4%. Non-CaO_2 treatment groups showed no indication of organic matter degradation (Nykanen et al., 2012). Moreover, the oxygen release rate of CaO_2 beads could be controlled and regulated simply via linking them with alginate and aluminum materials to obtain a higher dissolved oxygen (DO) concentration for use in remediation (Lee et al., 2014).

Although the fate of NPs in the environment has been noticed and the particles have been inspected under various electron microscopes (Hsiung et al., 2016; Peng et al., 2015; Shih et al., 2012; Tso et al., 2010), the aggregation and sedimentation properties of CaO_2 has never been studied in previous references (Khodaveisi et al., 2011; Li et al., 2009; Mende et al., 2003). Therefore, the aggregation and sedimentation properties of CaO_2 particles were evaluated and discussed in this study.

Recently, researchers have used top-down and bottom-up methods to synthesize these particles. In the past, researchers have used precision milling to generate NPs in a cost-effective manner (Ding et al., 2012; Koch, 1997; Mende et al., 2003). For example, through milling, big particles could be grinded to solvent-free nanoscale zero-valent iron (ZVI). The results showed that after 8 h of grinding, microscale ZVI was reduced to sizes below 50 nm (Li et al., 2009). In addition, when polyethylene glycol (PEG) was used to stabilize the zinc peroxide particles, the particles' average diameter was further lowered to ~10 nm and the data from dynamic light scattering (DLS) measurements showed the aggregation size of these particles was about 500 nm (Rosenthal-Toib et al., 2008). In a similar study involving PEG-synthesized CaO_2 NPs, transmission electron microscopy (TEM) images showed the particle sizes were around 15–25 nm (Khodaveisi et al., 2011). More recently, CaO_2 NPs also had a good remediation efficiency of benzene in groundwater (Mosmeri et al., 2017). CaO_2 particles synthesized by the treatment of pre-grinded $Ca(OH)_2$ with H_2O_2 also presented nanoscale sized particles under the inspection of a scanning electron microscope (SEM) (Qian et al., 2013). Recently, CaO_2 NPs were synthesized from $Ca(NO)_3$ (Kaewdee et al., 2016). At around neutral pH, CaO_2 NPs had a good removal efficiency to treat arsenic (Olyaie et al., 2012).

Diesel is a mixture of long chain carbon compounds that can be accidentally released from the oil reserving tanks to the environment, causing a myriad of environmental problems. Although microbial diesel degradation is now a popular remediation method (Careghini et al., 2015; Lin et al., 2008; Mnif et al., 2015; Nwankwegu et al., 2016; Wang et al., 2016; Wu et al., 2017), its usage and interactions with CaO_2 are seldom studied. In addition, the study of aggregation and precipitation properties of CaO_2 and related characteristics were limited as these factors greatly affect oxygen delivery when applied to bioremediation.

In this study, we aim to optimize the synthesis of CaO_2 NPs that could help the microbial degradation of diesel in terms of improving CaO_2 particles' oxygen releasing pattern or particle dispersion in the presence of diesel-contaminated media.

2. Materials and methods

2.1. Chemical reagents and equipment

Hexadecyltrimethylammonium bromide (CTAB, ≥ 99% purity) and PEG-200 (200 g/mol) and were purchased from Sigma-Aldrich. Triton X-100 (TX-100) was purchased from Acros. Methanol was purchased from Honeywell (HPLC grade). Commercial A CaO_2 (CaO_2-A, ≥ 75% purity, He Cheng Chemical), commercial B CaO_2 (CaO_2-B, ≥ 75% purity, J&L Environmental Technology), and BYK 2000 as a dispersant (BYK Additives & Instruments) were purchased. The water used in this study was double-distilled and deionized with a Milli-Q water purification system (Millipore).

2.2. Preparation of nanoscale CaO_2 particles

CaO_2-A was used as the raw material in the wet and dry grinding procedures. First, 50 g of CaO_2-A was mixed with water containing equal amounts of dispersant to obtain 50 g/L of mixed solution. The CaO_2-A mixed solutions were infused into a NETZSCH Minicer's chamber containing ZrO_2 grinding beads (0.3–0.4 mm) and grinded at 17 °C and 3000 rpm for 2 h. The resulting product was dried in an oven set to 80 °C before performing further experiments.

The dry grinding process was similar to the wet grinding process, except mixing with water and dispersant. Some wet synthesis methods such as adding PEG, CTAB, TX-100 as the stabilizer were also evaluated. For example, the synthesis of CaO_2 NPs using PEG (PEG-CaO_2) was referred to a previous study without modifications (Khodaveisi et al., 2011).

2.3. Characterization of the commercial CaO_2 and CaO_2 NPs

The CaO_2 samples were analyzed by optical microscopy, TEM (JEM-1200EX), SEM (JEOL JSM-7600F), and by X-Ray diffraction (XRD, beamline BL 13A1, National synchrotron radiation research center (NSRRC), Taiwan). The wavelengths of X-ray were 1.02805 Å and 1.02703 Å, and the scanning range of 2θ was 10–70°. The scanning rate was set to 0.05°/min. DLS (Mastersizer 2000 particle size analyzer and Zetasizer, Nano ZS, Malvern) and Fourier-transform infrared spectroscopy (FTIR, NSRRC BL 14A1, Magna 860 Thermo-Nicolet Instruments) were used to investigate the morphology, the size distribution, the zeta-potential, and the composition of the CaO_2 NPs at room temperature.

2.4. Aggregation and sedimentation of CaO_2 in the solution

The measurements of the aggregation and the sedimentation properties of CaO_2 particles were conducted in water. After dissolution in water, the mixture was sonicated for 15 min. The aggregation and the sedimentation behaviors of the sonicated samples were monitored by DLS and UV–Vis (550 nm), respectively.

2.5. Effects of DO on cell growth during the biodegradation of diesel

The diesel-degrading microorganisms were cultivated in a medium containing diesel (1000 ppm), NH_4Cl (0.5 g/L), Na_2HPO_4 (1.3 g/L), KH_2PO_4 (0.8 g/L), $CaCl_2$ (0.02 g/L), $MgSO_4 \cdot 7H_2O$ (0.2 g/L), and Triton X-100 (0.05%). In each experiment, 0.4 g/L of microorganism was inoculated with the addition of different CaO_2 particles (0.1%). Cell growth was measured based on the dry weight at every sampling point, while the DO in the base medium was measured by a DO meter (HI 9142, Hanna Instruments). The concentrations of diesel were extracted from media following the procedure recommended in U.S.EPA test methods 3510C (US EPA, 1996) and then quantified by using a gas chromatograph (Agilent 6890) equipped with a DB-5 capillary column and a flame ionization detector.

3. Results and discussion

3.1. The morphology and size of CaO_2 particles

Optical microscopic images of commercial CaO_2-A and CaO_2-B particles revealed that their size were similar, both having an estimated average diameter of 20,000 nm (Fig. 1). The hydrodynamic particle size of CaO_2-A was larger than the detection limit of

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