Sustainable Batch Production of Biosynthesized Nanoparticles

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Abstract

The production of biosynthesized nanoparticles is a slow process with complex isolation and collection procedures due to adhesion to the cell surface. Isolating the microorganism by immobilization in alginate beads will allow for easier recovery of nanoparticles. Immobilized *Clostridium pasteurianum* was able to reduce Pd ions efficiently through multiple batch production cycles with an average of 99.5% reduction in the metal ions. Microscopic imaging, diffraction and spectroscopic analyses of the Pd nanoparticles formed using immobilized cultures showed similar size distribution to particles collected from suspended cultures and confirmed the particles were metallic Pd. The Pd nanoparticles also retained their catalytic activity, as shown through the reduction of 450 μ M methyl orange in less time than the controls. These results demonstrate the effectiveness of the immobilization method for batch production of biosynthesized nanoparticles with little to no difference in particle size and properties.

Keywords

Nanoparticles, Immobilization, Biosynthesis, Alginate

1. Introduction

The biosynthesis of nanoparticles (bio-NPs) has accrued interest as a relatively simple, sustainable, and environmentally friendly method for the production of nanoparticles [1]. Biosynthesis processes can make use of plant extracts, bacteria, bacterial extracts, enzymes, and fungi in place of dangerous chemicals [1-5]. Bio-NPs have been shown to exhibit higher catalytic reactivity and greater specific surface area compared to nanoparticles synthesized through chemical methods [6, 7]. However, more research is needed in order to improve the production and application of biosynthesized nanoparticles. Production rates of bio-NPs can be impacted due to toxicity levels from the nanoparticles on the microorganisms at increased

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5 concentrations [8]. The low production rate can be exacerbated further due to the product lost during the
6 collection method. Through the use of immobilization, the microorganisms can be kept isolated from the
7 nanoparticles, making the separation of microorganisms and Bio-NPs nonessential. The use of
8 immobilization has been shown to eliminate inhibition caused by high concentrations of substrate and
9 product production [9] which, could reduce the toxicity of the nanoparticles. The use of 2 % alginate has the
10 most desirable characteristics and is one of the most widely used methods for whole cell entrapment [10].
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17 This work focuses on the production of nanoparticles through the use of immobilized *C.*
18 *pasteurianum* in 2% alginate beads. The use of alginate beads for the immobilization process should remove
19 the microorganism from the solution without inhibiting the formation of nanoparticles.
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23 **2. Experimental**

24 All media and solutions were prepared using deionized water (DiH₂O) that was boiled for 15 min
25 while being purged with nitrogen gas. All reagents were of analytical grade or better. *Clostridium*
26 *pasteurianum* BC1 (ATCC No. 53464) were used in all experiments. Samples of the isolated strain were
27 cultivated under sterile anaerobic batch conditions in a medium consisting of 5 g/L yeast extract, 10 g
28 ammonia chloride, 0.5 g calcium chloride, 0.3 g glycerol phosphate, 0.2 g magnesium sulfate, 0.1 g peptone,
29 0.1 g yeast extract, and 0.003 g iron sulfate at pH of 6.8. The optical density of bacterial solution used was
30 0.6 determined by measuring bacterial broth using a spectrophotometer at a wavelength of 600 nm. Cell
31 immobilization was performed in an anaerobic glove box with 2% (w/w) Na-alginate solution as discussed in
32 Nordmeier *et al.* [10]. Beads made from 1 ml of the bacteria/alginate mixture, were then placed into new
33 media vials and incubated for 48 h prior to use in order reach 20 psi. A Pd stock solution of 1000 mg/L was
34 made with Disodium tetrachloropalladate. The Pd(II) solution was added to cultures to achieve an initial
35 concentration of 20 mg/L. The media was centrifuged to collect the resulting particulates. The supernatant
36 was then removed for analysis and the particulates were collected for further characterization. New media
37 was then added to the beads, this marked the start of the next cycle. The reusability of the beads was
38 evaluated by 3 cycles of fresh media. The particulates were characterized with samples are characterized
39 transmission electron microscope with an Energy-dispersive X-ray spectroscopy (TEM-EDS, JEOL-JEM
40 21000F), and powder X-ray powder diffraction (XRD, Rigaku SmartLab diffractometer). The supernatant
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was analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer 8000) and a high performance liquid chromatograph (HPLC, Shimadzu LC-20AB) equipped with a refractive index detector (RID-10A) and an Aminex HPX-87H ion exclusion column from Bio-Rad. Cultures with Pd were inoculated with a 10 mM solution of methyl orange (MO) to reach a final concentration of 450 μ M of MO. The concentration of MO and its degradation product (N,N-Dimethyl-benzene-1,4-diamine) in the culture was monitored spectrophotometrically at 479 nm and 244 nm respectively, at 2 min time intervals as previously described in Johnson *et al.*[11].

3. Results and discussion

3.1. Bead Regeneration

The production of acetate, pressure and the glucose consumption was measured at the start and end of each cycle in order to monitor the viability of the immobilized culture. The pressure of the reactors reached on average 21 psi after 48 h of incubation throughout each cycle (Fig. 1A). The reactors were able to consume approximately 61-75% of the glucose while producing 0.72-0.81 g/L of acetate throughout the cycles, Fig. 1B. The first cycle of the reactors consume the least amount of glucose but was able to produce similar pressure and acetate concentrations as subsequent cycles in the same time frame.

Fig. 1: (A) The pressure of the immobilized batch reactors at time 48 h for each cycle. (B) The concentration of the acetate that was produced over the time course and the amount of glucose that was consumed by the immobilized bacteria.

3.2. Particle formation

C. pasteurianum is able to produce Pd NPs through a process described previously by one of the authors [12] and this process should not be inhibited by the immobilization technique and therefore the ability of immobilized *C. pasteurianum* to produce nanoparticles was tested using Pd(II) ions. Immobilized cultures supplemented with Pd(II) had a 99% Pd reduction which is similar to that of the controls which had 99.7% removal. The immobilized beads of *C. pasteurianum* were then transferred to fresh media and allowed to incubate for 48 h before supplementing with the metal ions. Subsequent cycles of the beads exhibited similar reduction of Pd in all 3 cycles. Three cycles were completed for the biosynthesis of Pd. There was no significant detected cell leakage from the alginate beads during any of the cycles as determined by UV-vis and TEM analysis. The precipitant from cultures supplemented with Pd produced by suspended and immobilized *C. pasteurianum* was collected for TEM analysis. TEM analysis of the collected samples demonstrated the presence of 2-7 nm size particulates in the precipitant (Fig. 2 A and B). EDS analysis confirmed the presents of Pd in both samples. The size distribution and morphology of the particles collected from the immobilized cultures were comparable to those of the suspended cultures. SEM analysis also corroborates the formation of nanoparticles in both the suspended and immobilized cultures; however, both samples exhibited agglomeration (see Supporting information). XRD analysis of the collected Pd nanoparticles exhibited the diffraction pattern of Pd with a FCC crystal structure, in accordance with JCPDS Card No. 01-088-2335, as shown in Fig. 2C. The particulates produced from the immobilized culture were compared to the particulates produced from the suspended culture. The broadening of the peaks are explained by Chidambaram *et al.*[12].

Fig. 2: TEM images of nanoparticles with EDS of the particles shown in panel (A) Pd nanoparticles produced through control culture (B) Pd produced through immobilized culture (C) XRD spectra of suspended bacteria formed Pd nanoparticles (---) and immobilized bacteria formed Pd nanoparticles (—).

3.3. Methyl Orange Degradation

Immobilized cultures were supplemented with 10 mg/L Pd to test the catalytic activity of the Pd nanoparticles. 10 mg/L Pd was chosen for this analysis because at higher Pd concentrations, the degradation occurred too rapidly to determine a rate. Once vials were supplemented with 10 mg/L Pd, the vials were allowed to sit for 5 min before 450 μ M of MO was introduced. Samples were collected every 2 min and analyzed by UV-Vis. The 450 μ M of MO was degraded below the level of detection within 6 min. As shown in Fig. 3, control experiments using heat-killed cells of *C. pasteurianum* BC1 and Bio-Pd-NPs did not

degrade MO to any significant extent, while a culture of *C. pasteurianum* without the addition of 10 ppm Pd was able to reach complete degradation of 450 μM MO in 140 min. These results are in agreement with earlier work from this laboratory on degradation of azo compounds by *C. pasteurianum* [11, 13] and the mechanism behind such degradation. Briefly, the $\text{N}=\text{N}$ (azo) bond is broken down and hydrogenated, leading to the formation of aminated daughter compounds, namely sulfanilic acid and N,N-Dimethylbenzene-1,4-diamine. Sulfanilic acid can be identified using UV-Visible spectroscopy at 244nm and was seen in all experiments as reported earlier [11, 13].

Fig. 3: MO degradation between controls and immobilized cultures supplemented with 10 mg/L Pd.

4. Conclusion

The results reported in this article demonstrate, for the first time, the bioreduction of Pd(II) ions by immobilized *C. pasteurianum*. Immobilized *C. pasteurianum* had comparable reduction of the Pd(II) ions to that of the control cultures and showed similar physical and catalytic characteristics to those formed in suspended cultures. This supports the use of immobilization for improving the collection of biosynthesized nanoparticles as well as for use in sustainable batch production of nanoparticles.

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Figure Captions:

Fig. 1: (A) the pressure of the immobilized batch reactors at time 48 h for each cycle. (B) The concentration of the acetate that was produced over the time course and the amount of glucose that was consumed by the immobilized bacteria.

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