Enhancement of Oxygen Transfer in Fermentation by Use of Functionalized Magnetic Nanoparticles

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ABSTRACT

Enhancement of oxygen mass transfer has been observed in the presence of colloidal dispersions of magnetite (Fe_3O_4) nanoparticles coated with oleic acid and a polymerizable surfactant. These fluids improve gas-liquid oxygen mass transfer up to 6 fold in an agitated, sparged, cell-free reactor and show remarkable stability in high-ionic strength media over a wide pH range. A combination of experiments using physical and chemical methods has been used to show that the mass transfer coefficient k_L and the volumetric mass transfer coefficient k_La are enhanced in the presence of nanoparticles. An increase of 40% in the oxygen uptake rate has been achieved in *Escherichia Coli* fermentation at a 5.5-L scale by using 0.6% w/w particles dispersed in fermentation media. The enhancement in mass transfer is directly translatable into increased fermenter productivity.

Keywords: magnetic nanoparticles, nanofluids, oxygen mass transfer enhancement, fermentation, mass transfer coefficient

1. INTRODUCTION

Maintaining an adequate oxygen supply to aerobic cell cultures has been a long-standing problem in fermentation technology. This problem is particularly amplified in high cell density cultures and in large scale operations, in which insufficient oxygen transfer rates limit cell growth and ultimately process productivity.

Our approach to reducing the oxygen transport limitation consists of adding functionalized magnetic nanoparticles to the fermentation medium; these materials consist of particles that have a magnetic core and two coatings. The magnetic core facilitates recovery of the fluid after the fermentation by passing it through a magnetic field; the first coating, made of oleic acid, confers high-oxygen storing capacity and the outer layer coating, made of surfactant (Hitenol-BC Polyoxyethylene alkylphenyl ether, Montello, Inc., Tulsa, OK), confers colloidal stability in water to the particle. Using magnetic nanoparticles presents several advantages compared to previous approaches [1, 2], including large interfacial areas and the possibility to readily recover the particles by High-Gradient Magnetic Separation [3, 4]. In addition, the nanoparticles are non-volatile and are synthesized with benign, low-cost chemicals, which makes their use attractive from environmental and economic standpoints [5].

2. EXPERIMENTAL METHODS

2.1 Nanoparticle Synthesis and Purification

A mixture of 94 g of ferric chloride hexahydrate (97% $FeCl_3 \cdot 6H_2O$) and 34.4 g ferrous chloride tetrahydrate (99% $FeCl_2 \cdot 4H_2O$) in 100 g of water was stirred at 80 ^oC under nitrogen sparging for 30 min in a round bottom flask. Subsequently, 100 g of potassium oleate (40 wt% paste in water, $CH_3(CH_2)_7CH=CH(CH_2)_7COOK$) was added, and the mixture was stirred for an additional 30 min. A 100 mL of an aqueous solution containing 28% ammonium hydroxide (NH_4OH) was added to the mixture, after which the solution immediately turned black because of the formation of magnetite. The reaction continued at 80 ^oC under stirring and nitrogen sparging for 30 min, after which it is assumed that oleic acid had completely coated the magnetite aggregates. Following the coating of the magnetite aggregates, 100 g of Hitenol–BC and 5 g of ammonium persulfate (>98% $(NH_4)_2S_2O_8$) were added to the reaction mixture. The reaction continued at 80 ^oC, under nitrogen sparging and vigorous stirring for 30 min. The solution was then cooled to room temperature and remained in the oven at 80 ^oC overnight, after which most of the residual ammonium hydroxide evaporated. The dispersion was dialyzed against distilled water (14,000 kDa MWCO dialysis membrane) in a 20 L container under mild stirring for 2 days to remove unreacted potassium oleate, Hitenol–BC, ammonium persulfate, and other salts and metal ions. Finally, the dialyzed solution was kept in the oven overnight at 80 ^oC, after which its solids contents were measured. The final solid contents were typically

between 15 to 25% in weight. This synthetic procedure yielded magnetic nanoparticles with an average number diameter between 20 and 25 nanometers (nm).

2.2 Nanoparticle Characterization

Nanoparticles were characterized by Dynamic Light Scattering (DLS) using a Brookhaven BI-200SM instrument at a measurement angle of 90°. The particle sizes cited here correspond to number averages. All measurements were recorded in quadruplicate and reported as average values. DLS measurements showed that particles have a hydrodynamic diameter of 20 nm over the pH range relevant to fermentation (around 7). Zeta potential measurements were recorded using a Brookhaven ZetaPals Zeta Potential Analyzer. Measurements were recorded in quadruplicate and reported as average values. Particles showed a strongly negative zeta potential of approximately -30 mV around pH 7.

3. RESULTS

3.1 Mass Transfer in Cell-Free Systems

3.1.1 Physical Method: Mass Transfer in a Stirred Beaker System

Mass transfer was characterized by a physical method in an agitated beaker. Experiments were conducted in a system that had a fixed and known gas-liquid contact area. The results thus obtained yielded information on the value of the mass transfer coefficient, k_L. This experimental setup was used to avoid problems associated with interfacial area and gas holdup. The experimental system was a cylindrical, 10.3 cm diameter beaker, containing 500 mL. The liquid was agitated by a 4.5 cm diameter, 4-bladed, axial-flow impeller (pitched-blade with each blade measuring 3 cm wide and 1.5 cm long). Dissolved oxygen was measured using a dissolved oxygen sensor (YSI 5010). The temperature was regulated at 37±0.5 °C with a water bath. Oxygen response curves were obtained by first sparging nitrogen until the dissolved oxygen concentration fell to zero and then monitoring the increase in dissolved oxygen concentration due to exposure of the liquid free surface to the room air. To ensure a constant gas-liquid interfacial area no air sparging was used during the second step.

Oxygen mass transfer into the aqueous liquid phase is enhanced in the presence of nanoparticles, as shown in Figure 1. The time required to reach saturation is reduced by approximately 25% in the presence of a nanoparticle mass fraction of ϕ = 0.005 (0.5% w/w). Further reductions are attained at larger particle holdups, but the effect is less pronounced above ϕ = 0.01. Values of the mass transfer coefficient k_L can be extracted from the data in Figure 1 by performing a mass balance on oxygen in the liquid phase

$$\frac{dC_{O_2,\text{bulk}}}{dt} = k_L a\left(C^*_{O_2} - C_{O_2,\text{bulk}}\right) \quad (1)$$

where a is the specific interfacial area, and $C_{O2,bulk}$ and C^*_{O2} are the liquid phase concentrations of oxygen in the well-mixed bulk and at saturation respectively.

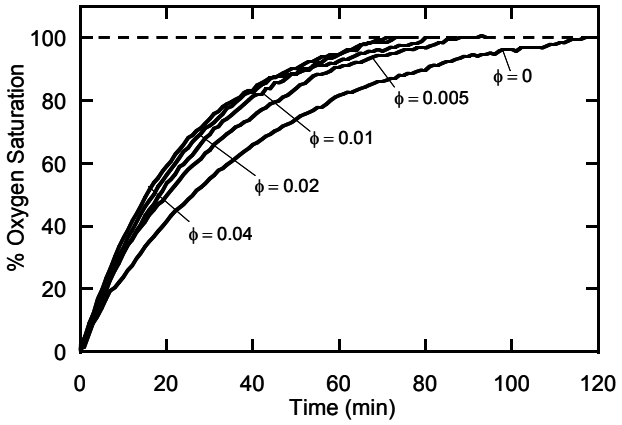

Figure 1: Response curves of dissolved oxygen in a stirred beaker at increasing nanoparticle holdup

The absolute enhancement in k_L , defined as

$$E = k_{L,\text{nanoparticles}} / k_{L,\text{control}} \quad (2)$$

is presented in Figure 2 as a function of the nanoparticle holdup at different agitation rates. Enhancement increases rapidly at low particle holdups and slowly at larger holdups (above ϕ = 0.01 approximately). It can also be observed that enhancement is greater at a lower agitation rate.

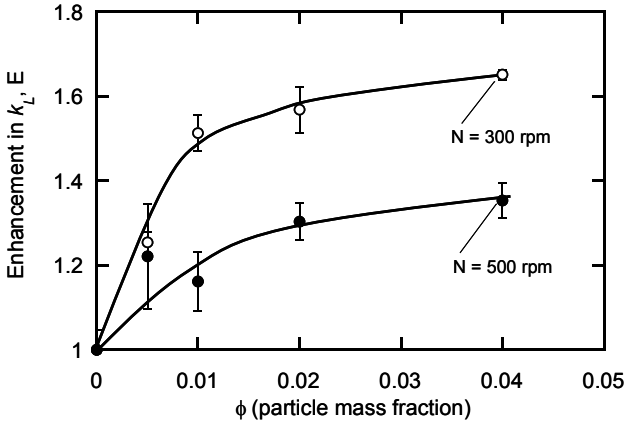

Figure 2: Enhancement in k_L as a function of nanoparticle holdup in at different agitation rates

3.1.2 Chemical Method: Sodium Sulfite Oxidation

A laboratory scale aerated and agitated fermentor was used for the determination of volumetric mass transfer coefficients (k_La) of oxygen. The sodium sulfite oxidation method was used. In the presence of a Cu^{2+} or Co^{2+} catalyst, sodium sulfite is oxidized according to the following reaction

$$Na_2SO_3 + \frac{1}{2}O_2 \xrightarrow{Cu^{2+}\text{or } Co^{2+}} Na_2SO_4 \quad (3)$$

The reaction rate can be adjusted by changing the catalyst concentration so that oxygen transport from the gas to the liquid becomes the limiting step. The oxygen uptake rate (OUR) is calculated by measuring the effluent gas composition with a mass spectrometer and performing a mass balance on oxygen of the gas phase through the reactor as follows

$$OUR = \frac{F_{N_2\,in}\left[\left(\frac{C_{O_2}}{C_{N_2}}\right)_{in} - \left(\frac{C_{O_2}}{C_{N_2}}\right)_{out}\right]}{V} \quad (4)$$

where OUR is the oxygen uptake rate, $F_{N2,in}$ is the flowrate of nitrogen entering the reactor, C_{O2} and C_{N2} are the concentrations of oxygen and nitrogen entering or exiting the reactor, and V is the working volume. The volumetric mass transfer coefficient can then be determined as

$$k_La = \frac{OUR}{C^*_{O_2} - C_{O_2,bulk}} \quad (5)$$

where C^*_{O2} is the average liquid phase saturation concentration in equilibrium with the inlet and outlet gas. Experiments were performed in a 5.5 L (working volume) stirred tank reactor (Biolafitte fermentor system, model BL 20.2), with an agitator with 2 Rushton 4-bladed turbine impellers. A 0.67 M sodium sulfite solution was loaded into the reactor, and then a $1 \cdot 10^{-3}$M solution of copper sulfate catalyst was added. The pH was initially adjusted to 8.0 with sulfuric acid. Temperature for the experimental runs was maintained at 37±0.5 ^oC. Results in Figures 3a and 3b show that a 4 fold enhancement of k_La can be obtained at a ϕ = 0.0025. At higher particle concentrations k_La is still further enhanced, up to 6 fold, but diminishing returns are obtained as the particle fraction approaches ϕ = 0.01

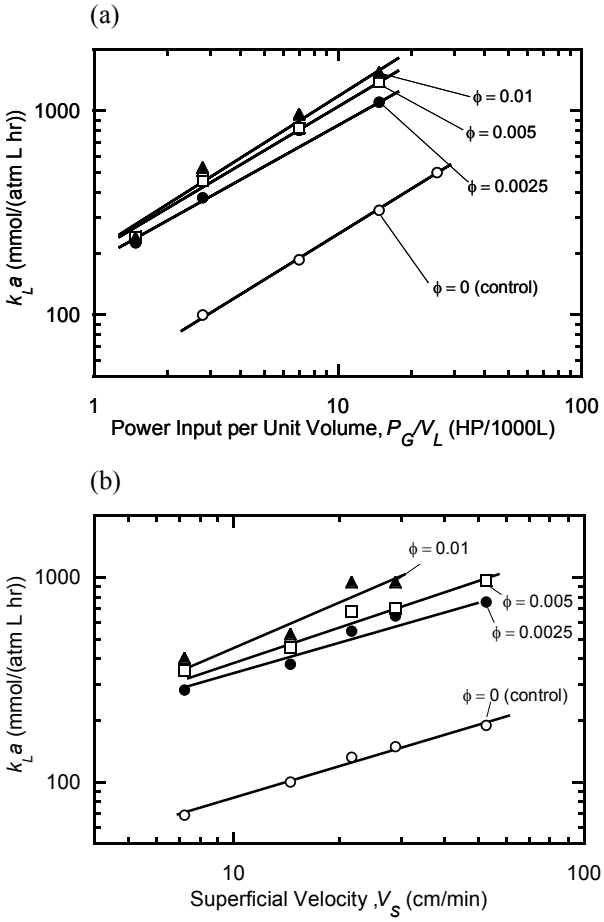

Figure 3: (a) k_La as a function of power input per unit volume at several nanoparticle mass fractions and at a superficial gas velocity of Vs= 14.5 cm/min (b) k_La as a function of superficial velocity at several nanoparticle mass fractions and at a power input per unit volume P_G/V_L of 2.8 HP/1000 L.

3.2 Mass Transfer in Fermentation

Fermentations at a 5.5 L scale in the presence of nanoparticles were conducted to characterize the enhancement of oxygen mass transfer in biological media and to quantify the enhancement of cell growth. The results were compared to a control run conducted in the absence of nanoparticles. The experiments were conducted without controlling dissolved oxygen levels. In this fashion, dissolved oxygen levels fell down to approximately zero (at around 6 hours for the control run) as cells proliferated and thereafter the cultures grew under oxygen mass transfer limitations. During the oxygen limited growth phase it was apparent that oxygen uptake rates were higher in the presence of nanoparticles, as shown in Figure 4. An increase of 40% in the oxygen uptake rate was achieved in *Escherichia Coli* fermentation by using 0.6% w/w

particles dispersed in fermentation media. Higher cell growth rates were maintained in the presence of nanoparticles when the cultures grew under an oxygen transfer–limited regime, *e.g.,* after 6 hours in the control run, or after 8 hours for $\phi = 0.0057$. This was supported by dry cell weight profiles, as shown in Figure 5. It can be observed in both Figures 4 and 5 that a nanoparticle concentration of $\phi = 0.02$ does not yield oxygen uptake rates or cell concentrations significantly higher than $\phi = 0.0057$.

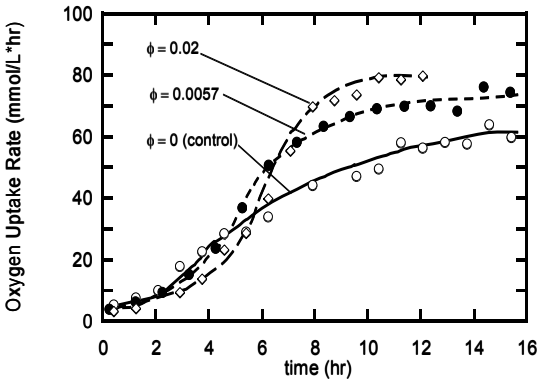

Figure 4: Oxygen uptake rate profiles during fed–batch fermentations of *Escherichia Coli* conducted at mass fractions of nanoparticles of $\phi = 0$, $\phi = 0.0057$, and $\phi = 0.02$.

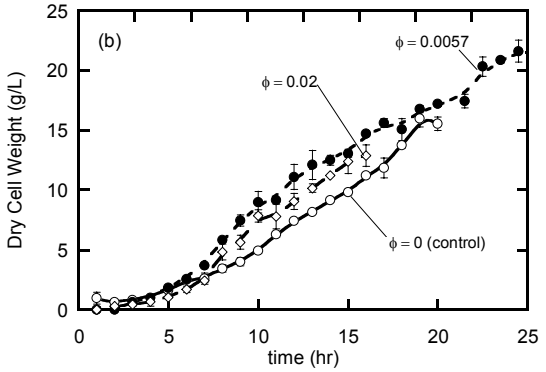

Figure 5: Dry cell weight during fed-batch fermentations of *Escherichia Coli* conducted at mass fractions $\phi = 0$, $\phi = 0.0057$, and $\phi = 0.02$ of oleic acid–coated nanoparticles

CONCLUSIONS

Colloidal dispersions of magnetite (Fe_3O_4) nanoparticles coated with oleic acid and a polymerizable surfactant enhance gas-liquid oxygen mass transfer up to 6-fold in a cell-free system. The dispersions show remarkable stability in high-ionic strength solutions and over a wide range of pH and can therefore be used in fermentation media. An increase of 40% in the oxygen uptake rate has been achieved in *Escherichia Coli* fermentation by using 0.6% w/w of particles. The enhancement in mass transfer is directly translatable into increased cell concentrations.

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