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Characteristics of a novel low density cell-immobilized magnetic supports in liquid magnetically stabilized beds

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ABSTRACT

The adsorptive and hydrodynamic characteristics of low density non-porous magnetic supports used for biocatalyst immobilization have been investigated. The magnetic particles consist of a sand core have been covered with a magnetite layer followed by a layer of nano size activated carbon or fly ash with the aid of epoxy resin. The resulted particles showed good adsorbing behavior toward resting cells of *Escherichia coli* from batch culture of different cell concentrations. The maximum adsorption capacity for both particles were calculated using Langmuir isotherm model as 18 (1.22 million cells) and 14 (0.95 million cells) mg/g, respectively. The hydrodynamic study in a liquid magnetically stabilized beds confirms that these particles have lower fluidizing velocity and attain a higher expanded stabilized bed than particles of magnetic dense core. These results confirm the applicability of these particles to immobilize microorganisms for different applications. It was found that the conversion using these particles was 30% higher than that using traditional dense magnetic particles due to the longer residence times in the bed.

1. Introduction

Cell Immobilization has generated considerable interest in the field of bioreactor design and lead to the design of new contactors to accommodate the resulting relatively large and spherical immobilized biocatalyst particles. Among these new contactors, fluidized beds and packed beds are being considered as promising reactors suitable for carrying out bioprocesses with immobilized biocatalysts [1,2]. Subsequently, magnetically stabilized fluidized beds (MSFBs) have been applied to conduct bioprocesses with immobilized biocatalyst on magnetic particles as a solid phase, thereby presenting further options in continuous reactor systems [3–5]. This technology is expected to show potential applications in various areas such as bioseparations and immobilized biocatalyst systems [6–12].

Accordingly, many types magnetic carriers or supports for immobilized biocatalysts have been previously prepared and tested [13,14]. Non-porous magnetic supports as carrier for immobilization have many advantages as they seem to be more resistant to diffusional limitations, attrition, and fouling than porous supports [15,16]. In addition, these carriers usually have reasonable chemical inertness.

A variety of non-porous magnetic materials have been used, including iron, cobalt and their oxides methods of their preparation have been classified into four groups: direct use of a coupling agent, adsorption, encapsulation, and formation of a thin film of polymer. Goetz et al. [17,18] developed a versatile magnetic silica support which can be derivatized readily for both adsorption chromatography and enzyme immobilization by well-known techniques. This support was prepared by electrostatically depositing alternating layers of colloidal silica and cationic polymer onto macroscopic nickel core particles. The polymer is then burned out and the silica partially sintered to yield a porous shell with 5–80 m^2/g of surface area. Horak et al. [19] presented a comprehensive review for all

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Nomenclature

ACMC	Activated carbon magnetic core
ACSC	Activated carbon sand core
FAMC	Fly ash magnetic core
Bs	Saturation flux density (mT)
C	Aqueous cell concentration in equilibrium (mg/l)
CC	Cell count
d_p	Particle diameter (mm)
H_b	Bed height after applying the magnetic field (cm)
H_{bo}	The initial bed (cm)
MSC	Sand particles covered with magnetite
MSFBs	Magnetically stabilized fluidized beds
OD	Optical density (–)
Q_e	Adsorbed cell density (mg/g)
Q_m	Adsorption capacity (mg/g)
K_d	Dissociation constant (mg/l)
U_{mf}	Minimum fluidization velocity (m/s)
U_e	Expansion velocity (m/s)
SC	Sand core
ϵ_b	Bed porosity (–)
ϵ_{so}	The initial porosity of the magnetic particles (–)
ρ	Bed density(kg/m^3)
ρ_s	Particle density (kg/m^3)

published methods used in the preparation of magnetic nanoparticles and microspheres used as separation media in different fields of chemistry, biochemistry, biology, and environment protection. However, most of the previous methods led to the production of relatively high density magnetic particles since the core of the particles is usually made of dense magnetic material such as iron, cobalt, or magnetite. The values of the minimum fluidization velocity in MSFBs could vary from 3 to 6 times that in the absence of magnetic field depending on the particles properties and on the magnetic field intensity [20]. However, these values of high medium velocities are not applicable in bioreactors for two main reasons. First, bio reaction rates are generally low and in continuous processes the substrate should have enough residence time in the reactor to attain sufficient conversion. Second, liquid recirculation is not possible in processes accompanied by product inhibition. Furthermore, the use of high density magnetic support at high magnetic fields could lead to a state of aggregated frozen bed rather than stabilized fluid-like bed. For these reasons, a possible way to overtake the above limitations of using MSFBs as a bioreactor is to reduce the magnetic particles densities. Several reports indicated that MSFBs of low density magnetic particles can be operated at acceptable liquid flow rates with the aid of the stabilization action of the magnetic field [21,22]. However, most of these low density carriers are fragile and unable to be repeatedly cleaned with harsh reagents or to withstand sterilization conditions for repeated use [23]. For this reason, the main objective of the present work was to conduct a new, simple method for the preparation of non-porous low density magnetic supports for biocatalyst immobilization. The applicability of the prepared magnetic particles with different types and sizes was tested as a support for cell immobilization using resting cells of *Escherichia coli*. Indeed, the study assessed the hydrodynamics and catalytic properties of these particles in liquid magnetically stabilized bed.

2. Materials and methods

Sea sand of different sizes was obtained from the red sea, Yanboa beach. Magnetite (Fe_3O_4) of particles diameter (d_p) less 5 μm and activated carbon of nano size were obtained from Sigma–Aldrich, Germany. Acetophenone was obtained from Fulka, Chemie GmbH (Buchs, Switzerland), (*S*)-1-phenylethanol from Sigma–Aldrich, (Taufkirchen Germany) and 2-propanol from Carl Roth GmbH (Karlsruhe, Germany). β-NADH was obtained by Jülich Fine Chemicals (Jülich, Germany) and used as delivered. All other chemicals and reagents with high purity: buffer, salts and solvents were obtained from commercial sources. HPLC deionized water was used to prepare the required solutions.

Fly ash of nano-size was obtained from the activated sludge combustion pilot plant of Prof. Dr.-Ing. J. Werther (Institute of Solids Process Engineering & Particle Technology, Hamburg University of Technology, Hamburg–Harburg, Germany). Hot curing epoxy system, based on Araldite LY 564 which is a low-viscosity epoxy resin and the hardener Aradur 2954 which is a cycloaliphatic polyamine needed to prepare the epoxy resin were provided by Prof. Dr.-Ing. K. Schulte (Institute of Polymer Composites, Hamburg University of Technology, Hamburg–Harburg, Germany). Lyophilized *E. coli* tuner (DE3) cells containing alcohol dehdrogenase ADH-'A' were used for immobilization on the magnetic support. These cells were kindly provided by Prof. Wolfgang Kroutil, Institute of Chemistry, University of Graz (Graz, Austria) [24]. The cells were resting and not able to grow which facilitates an accurate measurement of enzyme activity during the adsorption process.

2.1. Preparation of magnetic supports

Sea sand was cleaned from impurities by rinsing with 0.1 M HCl solution followed by washing by distilled water and then by 0.1 M NaOH and finally washed with abundant amount of distilled water. The clean sand particles were left to dry at 105 °C for overnight then cooled, sieved into different fractions and kept in closed plastic bags. Araldite LY 564 (5 ml) and the hardener Aradur 2954 (2.1 ml) were mixed in a suitable glass dish under the fume hood for 5 min to ensure homogeneity. The mixture started to polymerize causing a modification of the chain structure to a cyclic solid of a high melting point. After this period, the viscosity of the mixture started to increase and the homogenous mixture of the epoxy resin which was formed by condensation was mixed with 10 g of 500 μm diameter sand particles. After 5 min of intensive manual mixing with suitable glass rod, the epoxy resin was equally distributed as a thin film which was coating the particles surface and starts to form strong bonds with it. At that point, 20 g of magnetite powder was added and thoroughly mixed until the magnetite powder covered the sand particles which disintegrated into individuals and left for 16 h under the hood to complete the solidification process. In the next day, the particles were sieved to remove the excess magnetite, washed several times with distilled water and dried at 105 °C for 3 h and cooled. These particles were then mixed again, for 5 min in the same procedure as before, with a mixture of 5 ml of Araldite LY 564 and 2.1 ml of the hardener Aradur 2954. 20 g of activated carbon powder or fly ash were added and thoroughly mixed until the carbon covered the whole magnetite layer. The resulting covered particles were left for 48 h to complete the solidification process at room temperature. Then, the cleaning procedure was repeated to get rid of the excess and unbounded activated carbon.

2.2. Characterization of magnetic supports

The magnetic particles were then sieved and subjected to several characterization experiments in order to measure the bulk density (ρ_b), molded density (ρ_s), bed porosity (ϵ_b), minimum fluidization velocity (U_{mf}) in the absence of magnetic field, and the saturation flux density (B_s). After that, the magnetic particles were kept in glass bottles for further immobilization experiments. Scaling up of this production method will be the subject of further experiments.