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ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 45 (2007) 1–9
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Cartridge filter systems containing immobilized enzymes Part I. Concept and features

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Received 10 February 2006; received in revised form 22 September 2006; accepted 29 September 2006
Available online 13 November 2006

Abstract

Immobilized enzyme particulates positioned on the upstream surface of a cartridge filter were shown to provide effective catalytic conversion of a recirculating solution of reactant to product. This arrangement provided for low-pressure drop operation and minimal pH change across the catalyst bed, and no measurable loss in catalytic function was observed over the course of tens of reaction cycles in the laboratory. Although 60 larger scale (100 L) reaction cycles were insufficient to conclusively differentiate the cartridge filter reaction system arrangement from slurry batch operation, isolation of the enzyme from mechanical and chemical stresses normally associated with slurry batch processing operations is expected to lead to enhanced catalyst lifetimes when conducting the 500–600 reaction cycles that are typically desired for industrial biocatalytic systems.

Keywords: Pig Liver Esterase; Penicillin G acylase; Cartridge filter reaction system; Immobilized enzyme; Biocatalysis

1. Introduction

Immobilized enzymes have been extensively utilized to conduct industrial scale synthetic reactions, often being employed in batch processing operations in which the immobilized enzyme particles are simply added to a stirred reactor [1]. There are at least two major problems associated with the slurry or batchwise utilization of an immobilized enzyme to conduct repetitive industrial syntheses. First, mechanical agitation required to provide adequate mass transfer of reactant to the heterogeneous catalytic site generally causes significant physical deterioration of the support particles. Initial catalyst separation operations that can be as simple as allowing the particles to settle and providing a means to essentially decant the supernatant become substantially more complex and costly as the catalyst particles fracture, eventually requiring centrifugation to separate catalyst from product solution. A second and equally problematic issue with many enzyme-catalyzed industrial reactions is pH control. Often an acidic or basic byproduct is created requiring addition of a pH correcting complementary basic or acidic solution to maintain a stable environment for the enzyme. Relatively high product solution concentrations are desired to facilitate product isolation, so typically very concentrated pH correcting solutions are utilized. When added to a heterogeneous reaction mixture and before homogenization can occur, extreme pH conditions can momentarily exist in the vicinity of a subset of the immobilized enzyme particles, causing irreversible damage and loss of catalytic function and activity.

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1381-1177/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2006.09.009

One of the most time-honored and effective methods of removing a particulate from a liquid stream is to utilize cartridge filters that exhibit significant particle removing capacities and the ability to rapidly process the stream. In one common arrangement, a filtering element of defined porosity is configured perpendicularly to the flow of the liquid stream in an arrangement known as direct flow filtration (also known as dead end filtration). This provides for deposition of the particles on the upstream surface of the filter element and a "clean" downstream filtrate.

It occurred to us that an arrangement of a shallow bed of immobilized enzyme particles positioned on the upstream surface of a cartridge filter could be very useful for conducting industrial scale synthetic operations. In contrast to conventional column or plug flow reactor arrangements of immobilized enzyme particles, a reactant solute flowing through a cartridge filter would be challenged by a catalytic column of particles having a very wide frontal surface area and shallow bed depth, and high conversion of reactant to product could conceivably be accomplished by multiple recirculation through the device. Configured in this fashion with the sensitive catalyst contained in a separate module and isolated from the mechanical and chemical stresses occurring in the reactor, catalyst separation issues and destructive changes in pH in the vicinity of the biocatalyst would be completely eliminated. A very thin column of particles would also allow for low pressure drop operation and cause small changes in pH to occur across the bed per pass which has been a source of instability and poor conversion with certain immobilized enzymes employed using reactor arrangements involving deeper catalyst beds [2]. A further advantage of the shallow bed depth is that low pressure drop operation could be maintained even with very small immobilized enzyme particles and their attending advantages in terms of high diffusion rates compared to larger macroporous particles. This proposed catalytic system should also scale fairly easily, as several multi-cartridge assemblies are presently available that offer a parallel arrangement of cartridge filters.

A highlight of our work describing 2-alkenyl azlactone technology has appeared [3] that contains brief mention of cartridge filters modified by inclusion of immobilized pig liver esterase (PLE). This report more fully examines the cartridge filter reaction system (CFRS) and discloses investigations that establish important process parameters for the system for conducting preparative organic reactions with both PLE and Penicillin G acylase (PGA). The synthetic potential of CFRS was shown to be essentially equivalent to slurry batch operation in terms of catalytic efficiency and conversion of reactant to product in a time efficient manner, while providing complete elimination of catalyst removal operations from product solutions as an inherent feature. The issue of greater longevity of an immobilized enzyme in CFRS due to its isolation from the chemical and mechanical rigors normally associated with stirred tank reactors was supported, but conclusive support for this latter performance feature awaits results from several hundred reaction cycles.

2. Experimental

2.1. Materials, equipment and methods

Synthesis conditions for Emphaze™ AB 1 Biosupport Medium, which is a macroporous, azlactone-functional polymer support has been reported [4]. As normally supplied by 3 M, the reactive support typically has an average particle diameter of about 60 μm. The Emphaze™ supports utilized in the present study consisted of fines from that manufacturing process which passed through a 38 μm sieve and had particle diameter sizes between 6 and 40 μm (mean = 21 μm). This size range was ideal for penetrating between the pleat tips of the cartridge filters and providing a more uniform layer of particles over the entire filter element surface area. The dispersion copolymer HEMA-30 was a terpolymer of trimethylolpropane trimethacrylate (50 pbw), 2-vinyl-4,4-dimethylazlactone (20 pbw) and 2-hydroxyethyl methacrylate (HEMA, 30 pbw) and was prepared according to a published procedure [5]. Eupergit™ C, pig liver esterase (PLE, EC 3.1.1.1), Penicillin G, potassium salt and 4-(2-hydroxyethyl)piperazine-1-propanesulfonic acid (EPPS) were purchased from Sigma. Phosphate buffered saline (PBS) was either purchased from Sigma or made directly from the reagents specified. Penicillin G acylase (PGA, EC 3.5.1.11) was obtained from Pharma Biotechnologie Hannover (Hannover, Germany). 1-Methoxy-2-propanol acetate (MPA), dimethyl 3-methylglutarate (DMMG), and triacetin were purchased from Aldrich. Cartridge filters were obtained from 3 M (Filtration Products, St. Paul, MN), and two models were utilized in the present study: (1) 3 M High Capacity Liquid Filter Cartridge Model 313B having an all polypropylene filter element with a 2 μm pore size rating, dimensions of 7.6 cm (diameter) × 25.4 cm (height), and 8361 cm^2 of filter element area; and (2) 3 M High Capacity Liquid Filter Cartridge Model 743B having an all polypropylene filter element with a 2 μm pore size rating, dimensions of 17.8 cm (diameter) × 101.6 cm (height), and 139,350 cm^2 of filter element area. The filter housing for the 25.4 cm units (Model PSCL) was purchased from Ametek Inc. (Sheboygan, WI), and a 3 M 740 Series Filter Housing was employed for the 101.6 cm cartridges. For 25.4 cm unit operation, a Millipore Peristaltic Pump (Model 802G230) and Norprene™ tubing were utilized for all particulate loading and synthetic operations conducted with flow rates of 1400 mL/min and higher. A smaller MasterFlex Model 7520-00 peristaltic pump was utilized for synthetic operations conducted with flow rates of 550 mL/min and lower. The pH stat arrangement consisted of a New Brunswick Scientific titrant pump Model pH 4000 (Edison, NJ), an Ingold pH Electrode (Model 9100232, Wilmington, MA), and a 100 mL burette containing the standard NaOH titrant. Homogenization of dispersion copolymer supports was conducted using a Willems Polytron Model PT 10-35 Tissue Homogenizer (available from Brinkman Instruments). GC Analyses were performed using a Hewlett-Packard Model 5880A Gas Chromatograph equipped with a DB-1 Column. Particle sizes were determined using a Coulter LS-100 Particle Size Analyzer.