Biotechnology Advances 32 (2014) 514–525

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Multi-stage continuous high cell density culture systems: A review

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ARTICLE INFO

Article history:
Received 3 July 2013
Received in revised form 31 December 2013
Accepted 15 January 2014
Available online 21 January 2014

Keywords:
Multi-stage continuous bioreactors
High cell density culture
Product titer improvement
Productivity improvement

ABSTRACT

A multi-stage continuous high cell density culture (MSC-HCDC) system makes it possible to achieve high productivity together with high product titer of many bioproducts. For long-term continuous operation of MSC-HCDC systems, the cell retention time and hydraulic retention time must be decoupled and strains (bacteria, yeast, plant, and animal cells) must be stable. MSC-HCDC systems are suitable for low-value high-volume extracellular products such as fuel ethanol, lactic acid or volatile fatty acids, and high-value products such as monoclonal antibodies as well as intracellular products such as polyhydroxybutyric acid (PHB), microbial lipids or a number of therapeutics. Better understanding of the fermentation kinetics of a specific product and reliable high-density culture methods for the product-generating microorganisms will facilitate timely industrialization of MSC-HCDC systems for products that are currently obtained in fed-batch bioreactors.

Contents

1. Introduction . . . 515
   1.1. Product yield . . . 515
   1.2. Product titer . . . 515
   1.3. Bioreactor productivity . . . 515
2. High cell density culture systems . . . 515
   2.1. Immobilized cells . . . 515
      2.1.1. Cell entrapment in polymer matrix . . . 516
      2.1.2. Cell retention by membranes . . . 516
      2.1.3. Self-immobilized cells by flocculation or aggregation . . . 516
   2.2. Suspended cells . . . 517
      2.2.1. Membrane bioreactors in wastewater treatment . . . 517
      2.2.2. Monoclonal antibody production . . . 517
      2.2.3. Ectoine production using *Halobacteria elongata* . . . 517
      2.2.4. Cell recycling with upflow packed-bed . . . 517
3. Single stage continuous HCDC . . . 517
   3.1. History . . . 517
   3.2. Cell growth and product formation kinetics . . . 517
      3.2.1. HCDC with continuous cell mass production (CMP) reactor . . . 518
      3.2.2. Membrane fouling and long-term operation of HCDC . . . 518
      3.2.3. High purity oxygen-based cell culture . . . 518
4. Multistage continuous high cell density culture (MSC-HCDC) systems . . . 519
   4.1. History . . . 519
   4.2. Kinetics . . . 519
   4.3. High productivity and high product titer . . . 520

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0734-9750/$ – see front matter © 2014 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.biotechadv.2014.01.004

5. Applications of MSC-HCDC . . . 520
5.1. Volatile fatty acids production . . . 520
5.2. Lactic acid . . . 521
5.3. Ethanol production . . . 521
5.4. Intracellular products . . . 522
5.5. Simulation of MSC-HCDC bioreactors for high titer and productivity . . . 522
6. Concluding remarks . . . 523
Acknowledgments . . . 523
References . . . 524

1. Introduction

The term bioreactor refers to a device, or system that contains substrates and enzymes or cells as biocatalysts and provides an environment in which the biocatalysts can perform their functions.

The characteristics of enzyme biocatalysts resemble more or less those of chemical catalysts in that their activities degrade with time, whereas cells are self-multiplying living systems. Both types of biocatalysts have undergone successful developments in producing various products. However, this review will not include discussion on enzymes but instead focus on microbial cells including bacteria, yeasts and fungi, and on plant and animal cells, grown in a reactor or a system of reactors where bioreactions occur efficiently (Asenjo and Marchuk, 1995; Brauer, 1985; Cooney et al., 1985; Nielsen and Villadsen, 1994; Shuler and Kargi, 2002).

Bioreactions can be conducted in bioreactor systems with many diverse characteristics. Once a product is selected, we must consider various aspects of production using microbial cells, plant or animal cells, including the sterilization processes, bioreactor operation modes, product location (intracellular or extracellular) and separation methods or may even compare bioreactor production with competing manufacturing methods by biological or chemical means or a combination of both.

The manufacturing cost of a bioproduct consists of (1) raw materials, (2) utilities (e.g., steam and electricity), (3) labor, and (4) depreciation of the capital investment per unit quantity of a product. In addition, ~15% profit may be added. Depreciation is evaluated by dividing the total capital cost by the sum of the total manufactured quantities over the depreciation-years. The parameters that most affect the manufacturing costs are product yield and titer. In the case of high-value low-quantity products, the purification costs can exceed the culture costs.

1.1. Product yield

The cost of a bioproduct from feedstock consumption depends mainly on the cost of the raw materials, the conversion rate and the *product yield*:

$$\$/\text{kg}-\text{product} = \frac{\$/\text{kg-raw material}}{\text{Conv.}(\%) \times \text{Yield (kg-product/kg-raw material)}}. \quad (1)$$

The contribution of feedstock consumption to the final manufacturing cost declines when developing processes for less costly raw materials, higher conversion efficiency and product yield. For example, low-cost lignocelluloses have an advantage over sugarcane or grains (e.g., corn or wheat) for lowering fuel ethanol production cost. However, lignocelluloses have a lower substrate conversion and product yield than grains. Substituting grains with low-cost raw materials is not a simple solution.

1.2. Product titer

We need a *high-titer product* (kg/m^3 or g/L) because we must remove water or separate a product from broth, which has a water content of nearly 90%. Distillation or extraction is frequently used to concentrate water-soluble products from broth with low product titers. For example, we could obtain pure acetic acid from 1 m^3 of fermentation broth containing 3.5% (w/w) acetic acid by distilling or removing ~965 kg of water. In practice, it is very difficult to obtain pure acetic acid by distillation because of the water-acetic acid azeotrope. Having a higher product titer greatly reduces the cost of water removal because a 1% (w/w) solution of the product requires the removal of 990 L of water, while a 10% (w/w) solution needs the removal of 900 L of water. Currently, fermentation products are enriched by distillation or extraction, requiring expensive heat energy or solvents. However, if we could remove water from the fermentation broth using a non-phase-changing membrane technology, such as reverse osmosis or forward osmosis, the enrichment costs would be greatly reduced (McCutcheon et al., 2005; Mulder, 1996).

1.3. Bioreactor productivity

Productivity measures bioreactor efficiency in terms of kg-product/(m^3 bioreactor volume per unit time), which depends on biocatalysts such as bacterial cells, yeast, fungi, plant or animal cells and the mode of the bioreactor operation being batch, fed-batch, continuous or high cell density cultures. The volumetric productivity of a bioreactor (Q_p) can be expressed as the product of the specific productivity of cells ($q_{p/x}$) and the cell mass per unit volume, X, within the bioreactor (Cooney, 1983). However, various factors such as supplementation of carbon source and other nutritional components, C/N ratio, dissolved oxygen, and formation of products and byproducts that are inhibitory to cells also affect Q_p through their impact on cell physiology and metabolism, which consequently affect either $q_{p/x}$ or X or both.

Q_p can be increased by using a high performance strain ($q_{p/x}$) and a larger cell mass (X). In addition, sufficient oxygen supply is very important in aerobic high cell density culture to meet its requirement that significantly affects cellular physiology and metabolism. In the batch and fed-batch culture systems such as ethanol, lactic acid, or penicillin, the substrate for cell mass formation is usually minimized through optimization. A smaller cell mass reduces substrate consumption for biomass accumulation to improve product yield, although the less cell biomass lowers the productivity. Multistage continuous high cell density culture (MSC-HCDC) systems consist of n-serially connected continuous stirred tank reactors with either hollow fiber cell recycling or cell immobilization for high cell density culture.

The objective of this review is to introduce a MSC-HCDC bioreactor system that increases the productivities by maintaining high product titers for batch and fed-batch fermentations (Chang, 2011; Chang et al., 2011a). In short, we seek a method that can replace the current conventional fed-batch method with high productivity (Fig. 1). Bioprocessing for fuels and chemicals from biomass can be quite different from sugar-based fermentation products in terms of bioreactor productivity and titer (Lee et al., 2012).

2. High cell density culture systems

2.1. Immobilized cells

HCDC refers to approximately 10 times the normal cell density of a simple batch culture-. If 5–10 g/L of *Escherichia coli* cells is considered a normal cell density, 50–100 g/L would be called high cell density