Biochemical Engineering Journal 107 (2016) 52–60

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Short communication

A novel bioreactor to study the dynamics of co-culture systems

M.H. Kim[a], M. Liang[a], Q.P. He[b,**], J. Wang[a,*]

[a] Department of Chemical Engineering, Auburn University, Auburn, AL 36849, USA
[b] Department of Chemical Engineering, Tuskegee University, Tuskegee, AL 36088, USA

ARTICLE INFO

Article history:
Received 16 June 2015
Received in revised form
20 November 2015
Accepted 26 November 2015
Available online 30 November 2015

Keywords:
Co-culture
Bioreactors
Control
Bioprocess monitoring
Fermentation
Ethanol

ABSTRACT

Co-culture strategy has drawn increasing interest in the last a few years to ferment the mixture of glucose and xylose or lignocellulosic hydrolysate. However, existing research has been mostly qualitative which mainly examined the ethanol production performance of the co-culture system such as final ethanol yield and productivity, with little or no attempt made to understand the dynamic interactions between the two microbes. This is partially due to the difficulties associated with monitoring and control of co-culture systems. In this work, we developed a bioreactor and associated protocols and control strategies to facilitate quantitative and systematic study of co-culture systems. In particular, the reported equipment, operation protocols and control strategies can deliver chemostat operation under controlled stable operation conditions by achieving stable oxygen utilization rates at various levels. In addition, the developed membrane-separated co-culture bioreactor enables independent control of the dissolved oxygen (DO) levels in each chamber, and easy tracking of individual biomass of each strain. The new protocol is a dual continuous/pseudo-continuous operation mode, which allows the control of constant biomass by adjusting the operation time of each mode, dilution rate and feed concentration. Experimental results on the co-culture system of *Saccharomyces cerevisiae* and *Scheffersomyces stipitis* are provided to demonstrate the capabilities of the developed co-culture bioreactor. With the developed equipment and protocol, simultaneous and complete utilization of both glucose and xylose was achieved around 70 h into the experiments we conducted, and was maintained as long as 800 h. Such complete utilization can be maintained even longer if desired. In addition, different OUR conditions (ranging 0.0036–0.0045 mmol O_2/gDCW/h) were tested under controlled chemostat. Under the different operation conditions tested, ethanol yields and conversion rates varied in the range of 0.12–0.44 g/g and 0.22–1.95 g EtOH/L/h, respectively, which are in line with results reported in the literature.

1. Introduction

Lignocellulosic biomass is an attractive, sustainable, and abundant carbon source for ethanolic fermentation process. However, the lack of microorganisms which can efficiently ferment all sugars derived from lignocellulosic biomass is one of the key barriers in industrializing lignocellulosic ethanol process. To overcome this barrier, numerous microorganisms have been constructed in the past few decades to ferment the mixture of glucose and xylose, the dominant hexose and pentose from lignocellulosic hydrolysate [1–3]. As an alternative, co-culture strategy has drawn increased attention for simultaneous conversion of glucose and xylose. Table 1 summarizes recent attempts in using co-culture systems for glucose/xylose or lignocellulosic hydrolysate fermentation with two older continuous co-culture studies. Existing results show that co-culture is a promising way to ferment mixed glucose and xylose for ethanol production, especially in reducing fermentation time and improving ethanol productivity. However, existing research mainly examined the ethanol production performance of the co-culture system such as final ethanol yield and productivity, with little or no attempt made to understand the dynamic interactions between the two microbes. In addition, major challenges associated with the co-culture strategy have not been adequately addressed, which include the incompatible oxygen conditions required by two microbes for optimal ethanol production, catabolite repression of xylose utilization when glucose is present, and the low ethanol tolerance of xylose-fermenting microorganisms [4–7].

In this work, we developed a new bioreactor and a new protocol to address some of the above-mentioned challenges, which

* Corresponding author at: Auburn University, 212 Ross Hall, Auburn, AL 36849, USA. Fax: +1 334 844 2063.
** Corresponding author at: Tuskegee University, 522B LHF Hall, Tuskegee, AL 36088, USA. Fax: +1 334 724 4188.
E-mail addresses: qhe@mytu.tuskegee.edu (Q.P. He), wang@auburn.edu (J. Wang).

http://dx.doi.org/10.1016/j.bej.2015.11.019
1369-703X/

Table 1
Recent work in ethanol production using co-culture systems (with two older continuous studies).

Reference	Co-culture strains	Operation mode	Best theoretical yield (%)	Best yield (g/g)	Best productivity (g/L/h)
[21,22]	*S. cerevisiae–S. stipitis*	Batch	78% at 200 h	0.40	1.26
			88% at 42 h	0.45	1.07
[23]	*Z. mobilis–S. stipitis* (reactors in series)	Continuous	92% for *Z. mobilis* with dilution rate 0.07 h^{-1} at 168 h	0.47	2.20
			88% for *S. stipitis* with dilution rate 0.048 h^{-1} at 168 h	0.45	0.54
[24,25]	*S. cerevisiae–S. arborariae*	Batch	94% at 250 h	0.48	0.11
	S. cerevisiae–C. shehatae		94% at 250 h	0.48	0.15
[26]	*S. cerevisiae–S. stipitis*	Batch	78% at 36 h	0.40	0.49
[27]	*S. cerevisiae–S. stipitis*	Batch	94% at 84 h	0.48	0.22
[28]	*S. cerevisiae–S. arborariae*	Batch	75% at 150 h	0.38	0.25
[29]	*S. cerevisiae–S. stipitis* (inactivate *S. cerevisiae*)	Batch	85% at 125 h	0.43	0.12
[30]	*S. cerevisiae–S. stipitis*	Batch	78% at 36 h	0.40	0.33
[31]	*(RD) S. cerevisiae–S. stipitis*	Continuous	82% at 240 h	0.42	0.075
[32]	*S. cerevisiae–S. stipitis* (reactors in series)	Continuous	64% at 816 h	0.33	0.43

allow the systematic investigation of the dynamic properties of a co-culture system: *Saccharomyces cerevisiae* and *Scheffersomyces stipitis*. The new equipment is a membrane-separated co-culture bioreactor which enables the independent control of the dissolved oxygen (DO) levels in each chamber, and the easy tracking of the biomass of each strain. The new protocol is a dual continuous/pseudo-continuous operation mode, which allows the control of constant biomass by adjusting the operation time of each mode, dilution rate and feed concentration. It is worth noting that membrane-separated bioreactors have been reported in different applications and they have many benefits, including obtaining high cell density culture, easy control or separation of cells, retention of cellulase, etc. [8–12]. However, one of the major issues with these systems is the membrane fouling due to the adhesion of cells on the filtration surface. In addition, some systems were found to be difficult to control the specific OUR [9]. In comparison, the system developed in this work could keep running for several months under controlled OUR and chemostat condition.

2. Materials and methods

2.1. Microorganisms and culture media

The strains used in this study were *S. cerevisiae* D5A and *S. stipitis* CBS 5773 which were obtained from Y.Y. Lee, Auburn University and ATCC, respectively. The frozen stock and culture media were the same as in Liang et al. [13]. The sugar concentrations of feed medium for pseudo-continuous fermentation were varied based on the operational conditions.

2.2. Reactor design and development

The schematic diagram and actual set-up of the developed membrane-separated bioreactor are shown in Fig. 1. The body of the bioreactor was constructed using polycarbonate, which consists of two chambers. By placing a microporous filter membrane (Biodyne® Plus, Pall Corporation, NY) between the two chambers, the two strains are separated by the membrane while various metabolites can exchange between the two chambers. The Biodyne® plus membrane is a nylon membrane which consists of Nylon 6,6 cast as a symmetric microporous membrane (pore size 0.45 μm) on non-woven polyester support and is sterilized together with the bioreactor using autoclave. The propellers of the agitators are driven by external magnetic fields; pH and temperature are under automatic feedback control (not shown in Fig. 1a). This two-chamber configuration enables us to easily monitor and control the biomass development of individual strains during fermentation. It also allows the independent control of different oxygen conditions for each strain by adjusting nitrogen and air gas flow rates to each chamber. In addition, by feeding the substrates into *S. cerevisiae* chamber, glucose can be completely consumed by *S. cerevisiae*, leaving only xylose reaching *S. stipitis* chamber. Therefore, the catabolite repression of xylose utilization can be alleviated.

2.3. Dual-mode operation

Pseudo-continuous operation is characterized by continuous nutrient feeding and continuous cell-free broth withdrawal, which can be achieved through a cell retention module to keep cells from effluent. The biggest challenge in pseudo-continuous operation is maintaining the stability and effectiveness of cell filtration module. Due to the continuous buildup of cells on the filtration surface, commercial filtration modules usually have limited operation time even with back-flash implemented periodically [9]. To address this difficulty, we have developed a cell retention module in-house, with the filtration surface located right against the propeller blades as shown in Fig. 1a. In this way the membrane (Supor800, pore size 0.8 μm, Pall Corporation, NY) surface is constantly cleaned through the shear stress generated by agitation, which allowed prolonged pseudo-continuous fermentation for months [13]. Because *S. stipitis* grows very slowly under oxygen limited condition, "wash-out" is often resulted under continuous operation. With pseudo-continuous mode as an option, the biomass of *S. stipitis* can be controlled by the dual-mode operation, which combines pseudo-continuous operation (for biomass accumulation) and continuous fermentation (for biomass maintaining or reduction). The operation time of each mode depends on the cell growth rate and dilution rate, and the detailed calculation of the operation time for each mode can be found in Appendix A.

2.4. Independent control of oxygen supply and monitoring of oxygen utilization rate

The conversion efficiency of xylose to ethanol by *S. stipitis* is highly influenced by oxygen utilization rate (OUR) [14–16]. In order to quantitatively study the effect of OUR on the fermentation performance and by-product secretion, maintaining stable OUR at different levels to achieve chemostat is necessary. It should be noted that OUR cannot be directly controlled as one cannot control how much oxygen cells will pick up, it can only be manipulated through controlling the composition of the feeding gas into the system. To achieve various stable OUR conditions, feed gas with different oxygen compositions but constant total gas flow rate has

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