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# Diffusion-based reverse membrane bioreactor for simultaneous bioconversion of high-inhibitor xylose-glucose media

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ABSTRACT

Two of the main hurdles in industrial production of second generation bioethanol are the high content of inhibitory compounds and presence of sequentially fermented hexose and pentose saccharides in the feedstock. In order to tackle these issues, the novel cell confinement approach in a reverse membrane bioreactor (rMBR), used in this study, proved to be promising for robust fermentation of high-inhibitory xylose-glucose media simulating a lignocellulosic hydrolysate. The high local cell concentration and concentration-driven diffusion-based mass transfer conditions in rMBR enhanced simultaneous utilization of sugars and boosted cell furfural tolerance/detoxification capacity. The diffusion rates of all compounds through the membrane were measured in a diffusion cell and in an rMBR. In the rMBR, yeast cells could readily convert high content of furfural (10 g/l) that is toxic to freely-suspended cells. Moreover, in the presence of 2.5 g/l of furfural, cells had the same performance as in medium with no inhibitor and could simultaneously convert glucose, xylose, and furfural with the latter two at the same rate with no lag phase. The performance of rMBR in remediating issues revolving around lignocellulosic bioethanol production covers the shortcomings of the conventional encapsulation technique and opens new areas of application for diffusion-based bioconversion systems.

## 1. Introduction

Although the application of MBRs in wastewater treatment dates back to late 1960s [1], their range of application has recently expanded to a great number of engineering processes from filtration to complex membrane bioreactors (MBR) [2,3]. Other than wastewater treatment, in recent decades, with the increasing demand for production of fuel from renewable sources to replace the depleting and environmentally polluting fossil-based fuels [4], there has been a great interest to use MBRs for biofuel production [5].

Bioethanol has been a biofuel of great interest to be produced and recovered feasibly using MBR technology [6]. In recent years, production of 2nd generation bioethanol from processing lignocellulosic materials (agricultural residues etc.) that are relatively cheap, abundant and from non-food or feed sources has gained great attention [7–9]. However, 2nd generation bioethanol fermentation has been limited by the process costs and production scale [10–12]. Lignocellulosic materials have a recalcitrant structure, mainly made up of cellulose, hemicellulose and lignin, that first needs to be opened up by intensive physical, thermal or thermochemical pretreatment, followed by enzymatic hydrolysis prior to fermentation [13]. During pretreatment, different hexose (glucose, mannose etc.) and pentose monosaccharides (xylose, arabinose etc.) and cell-inhibitory degradation by-products such as furan aldehydes (furfural and 5-hydroxymethyl furfural (HMF)) are produced [13–15]. On the other hand, wild-type yeasts cannot utilize pentose sugars and xylose-consuming recombinant yeasts consume sugars sequentially, *i.e.* utilize glucose first and then xylose only in glucose-deprivation conditions [16]. In addition, some strains of yeast are capable of converting some inhibitors such as furfural and HMF to the less inhibitory furfuryl alcohol and HMF alcohol, respectively [17,18]. However, presence of high content of furans during fermentation disturbs the cell's normal metabolic and physiologic condition by inhibiting cell growth and inactivating enzymes, changing cell membrane permeability and disturbing the cell redox balance [14,19].

In fermentation systems containing inhibitors, different sugars and freely suspended cells, all cells are exposed to the same level of medium constituents. This leads to a long lag phase (sometimes linked to medium detoxification) followed by priority-based substrate consumption. However, in recent years it has been reported that enhanced

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inhibitor tolerance and simultaneous utilization of different sugars can be achieved by providing high cell concentration microenvironments using cell encapsulation and/or flocculation techniques [20,21]. This cell-dense microenvironment controls the rate at which cells are exposed to different medium components attributing its success to the concentration gradient built over the membrane and cell aggregate [6]. However, several issues in the preparation and application of capsules have limited their scale and area of application. The process of cell encapsulation is time consuming and laborious [22]. Moreover, poor preparation can cause cell attachment to the outer surface of the capsules and also capsule breakage due to extensive gas formation in the capsule or high shear stress due to agitation can lead to cell escape [23,24]. These issues can be remediated using the new membrane cell confinement technique of rMBR [6].

The rMBR is a newly introduced type of immersed membrane bioreactor (iMBR) that has recently been applied in closed sachet [25] and multi-layer membrane column [25] configurations for biogas and in flat-sheet membrane panel configuration [24] for bioethanol production. While in the conventional iMBR, cells are suspended in the bulk medium and convective product separation happens through building a pressure gradient over the membrane surface, in rMBRs cells are confined between membrane layers and diffusional mass transfer happens in and out of the membrane bound area due to the presence of a concentration gradient [6]. As discussed in our previous review work [6], merging the benefits of conventional MBRs and cell encapsulation into the rMBR technique provides us with a promising approach for treatment of complex feed streams containing inhibitory compounds and mixtures of different sugars.

In this work, by benefiting from the membrane-assisted cell confinement technique of rMBR, we have tried to tackle some issues affiliated with 2nd generation bioethanol production by studying the possibility of simultaneous consumption of pentose and hexose saccharides along with detoxification of inhibitory compounds during fermentation. First the diffusion behavior of different chemical compounds was measured in semi-synthetic media representing lignocellulosic hydrolysate using a side-by-side diffusion cell. Then, rMBR fermentations were conducted at different concentrations of inhibitory compounds to observe the effect of environmental stress on cell metabolic activity by monitoring rates of consumption, production and detoxification of different compounds. The results of this study evaluate rMBRs capability of assisting the bioconversion of lignocellulosic material to bioethanol from an unconventional and interesting view point.

## 2. Materials and methods

### 2.1. Diffusion rate measurement

A diffusion cell (Side-Bi-Side, PermeGear Inc., Hellertown, PA, USA) was applied in order to have an understanding of the diffusion rate and flux of different compounds involved in fermentation through the membrane used for cell encasement. A simple scheme of the diffusion cell is presented in Fig. 1. The diffusion cell consists of a donor and a receptor chamber each of 60 ml volume connected through an opening (orifice) of 30 mm diameter (area 7.07 $cm^2$). The diffusion cell is water-jacketed and the temperature is maintained at 30 °C (chosen fermentation temperature) by a water-circulating water bath. In order to simulate the conditions used for actual rMBR fermentation cycles, single membrane layers were isolated from 2nd generation IPC (Integrated Permeate Channel) dual layer membranes. An IPC membrane typically contains two Polyethersulfone (PES) membrane layers, with an average pore size of 0.3 µm. Single membrane layers were obtained by slicing such IPC membrane into half. These were used to separate the donor (contains the diffusant(s)) and receptor (contains no or very low concentration of the diffusant(s)) compartments in the diffusion cell. In order to have homogeneous concentrations at all time, both receptor and donor cells were stirred at 500 rpm using a double-core H-series magnetic stirrer (PermeGear Inc., Hellertown, Pennsylvania).

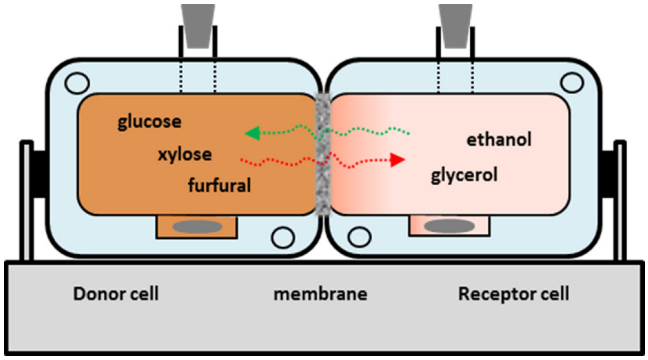


**Fig. 1.** The schematic of the side-by-side diffusion cell used in this study.

In order to simulate the rMBR fermentation conditions and to measure the counter-diffusion behavior of different compounds, the donor compartment mainly contained the substrates glucose, xylose and furfural while the receptor cell had only ethanol and glycerol. The used membrane layers were first soaked in NaOH 2% for 15 min and then rinsed with distilled water, before and after each diffusion cycle. The diffusion cycle was 12 h with 2 h sampling intervals. At every sampling 1 ml aliquot was withdrawn from the receptor cell and replaced with the same amount of fresh receptor medium. The changes in the concentrations of compounds in the receptor cell were measured using high performance liquid chromatography (HPLC) (Walters 2695, Walters Corporation, Milford, USA) (Section 2.4). To have steady state diffusion across the membrane, sink conditions should be provided in the diffusion cell *i.e.* the receptor cell is kept at zero concentration of diffusants [26]. However, this cannot be completely achieved in a static diffusion cell, therefore, the sink condition has been redefined as the condition at which the diffusant concentration in the receptor cell is less than 10% of its saturation solubility concentration [26]. The concentration of compounds in the donor compartment was chosen to be comparable to that of acid pretreated and enzymatically hydrolyzed wheat straw hydrolysate [27].

After each concentration measurement, the cumulative amount released per unit area of membrane (Q) was calculated for different compounds using Eq. (1) according to K.D. Thakker and W.H. Chern [28]:

$$Q = \left\{ C_n V + \sum_{i=1}^{n-1} C_i S \right\} / A \tag{1}$$

Where Q is the cumulative amount of a compound passed through the surface area of the membrane (g/$cm^2$), S is the sample aliquot volume (1 ml), V is the volume of each chamber (60 ml), A is the membrane surface area (7.07 $cm^2$), $C_n$ is the receptor cell concentration (g/ml) at the nth sampling and $\sum_{i=1}^{n-1} C_i S$ is the total amount of a compound released from the 1 st to the n-1th sampling intervals.

Graphs of the cumulative amount (Q) versus time were plotted and a regression line was estimated for the linear region of the graph. As the sink conditions exist, the slope of the adapted regression line represents flux (J) of a component per unit area of membrane surface [29].

The apparent permeability coefficient ($K_p$) of compounds through the membrane layer was estimated using Eq. (2) [30]:

$$K_p = \left(\frac{1}{AC_0}\right)\left(\frac{dM}{dt}\right) \tag{2}$$

Where A is the membrane surface area, $C_0$ is the initial concentration of the compound in the donor cell and (dM/dt) is the flux of the compound through the membrane.