

# Xylitol production by liquid emulsion membrane encapsulated yeast cells

Sarote Sirisansaneeyakul,<sup>a\*</sup> Rittikorn Chainoy,<sup>a</sup> Wirat Vanichsriratana,<sup>a</sup> Thongchai Srinophakun<sup>b</sup> and Yusuf Chisti<sup>c</sup>

## Abstract

**BACKGROUND:** Liquid emulsion membrane (LEM)-encapsulated live cells can be used to produce various products. This work reports on LEM-encapsulated cells for producing xylitol and models the production process.

**RESULTS:** Encapsulated cells of *Candida mogii* ATCC 18364 were used to produce xylitol from xylose. Soybean oil LEM consisting of 5% (w/v) lanolin and microwaxes was found most suitable for this process. The LEM-encapsulated cells were immobilized in a tubular biocatalytic loop. Xylitol was produced under oxygen-limited and aerobic conditions. Xylitol productivity and yield were 0.005 g L<sup>-1</sup> h<sup>-1</sup> and 0.52 g g<sup>-1</sup>, respectively, for oxygen-limited operation. Under aerobic conditions, xylitol productivity increased greatly to 0.022 g L<sup>-1</sup> h<sup>-1</sup>, but yield on xylose declined to 0.49 g g<sup>-1</sup>. A mathematical model successfully described substrate consumption and product formation in the LEM-immobilized cell system.

**CONCLUSION:** Potentially, immobilized cell LEM systems are useful for certain fermentations and they can be successfully modeled, as shown by the example of xylitol from xylose process.

© 2009 Society of Chemical Industry

**Keywords:** xylitol; xylose; liquid emulsion membrane; cell encapsulation; *Candida mogii*

## NOMENCLATURE

$a$	Interfacial area of the liquid emulsion per unit volume of outer phase (m <sup>-1</sup> )	$N_S$	Flux of xylose (g xylose m <sup>-2</sup> h <sup>-1</sup> )
$A_C$	Interfacial area of the liquid emulsion (m <sup>2</sup> )	$[\text{NaOH}]_{in}^0$	Sodium hydroxide concentration in inner phase (kmol m <sup>-3</sup> )
$C_X$	Biomass concentration (g DCW L <sup>-1</sup> )	$[\text{NaOH}]_{out}$	Sodium hydroxide concentration in outer phase (kmol m <sup>-3</sup> )
$C_{xyl}$	Xylose concentration (g xylose L <sup>-1</sup> )	$P$	Permeability coefficient (m s <sup>-1</sup> )
$(C_{xyl})_0$	Xylose concentration at $t = 0$ (g xylose L <sup>-1</sup> )	$q_{xyl}$	Specific uptake rate of xylose (g xylose g DCW <sup>-1</sup> h <sup>-1</sup> )
$(C_{xyl})_t$	Xylose concentration at $t = t$ (g xylose L <sup>-1</sup> )	$q_{xyl}^m$	Maximum specific uptake rate of xylose (g xylose g DCW <sup>-1</sup> h <sup>-1</sup> )
$C_{xyt}$	Xylitol concentration (g xylitol L <sup>-1</sup> )	$q_{xyt}$	Specific formation rate of xylitol (g xylitol g DCW <sup>-1</sup> h <sup>-1</sup> )
$C_{xyl}^E$	Xylose concentration in liquid emulsion (g xylose L <sup>-1</sup> )	$r_{xyl}$	Volumetric rate of xylose consumption in the absence of diffusion limitation (g xylose L <sup>-1</sup> h <sup>-1</sup> )
$C_{xyl}^{out}$	Xylose concentration in outer phase (g xylose L <sup>-1</sup> )	$(r_{xyl})^{diff}$	Volumetric rate of xylose consumption in the presence of diffusion limitation (g xylose L <sup>-1</sup> h <sup>-1</sup> )
$C_{xyt}^E$	Xylitol concentration in liquid emulsion (g xylitol L <sup>-1</sup> )		
$C_{xyt}^{out}$	Xylitol concentration in outer phase (g xylitol L <sup>-1</sup> )		
DCW	Dry cell weight (g L <sup>-1</sup> )		
$F$	Volumetric flow rate (L h <sup>-1</sup> )		
HLB	Hydrophile–lipophile balance		
HLB <sub>Mixture</sub>	HLB of mixture		
HLB <sub>Span</sub>	HLB of Span <sup>®</sup> 80		
HLB <sub>Tween</sub>	HLB of Tween <sup>®</sup> 40		
$J$	Transport flux of xylose (g xylose m <sup>-2</sup> h <sup>-1</sup> )		
$K_{xyl}$	Saturation constant based on xylose (g xylose L <sup>-1</sup> )		
$L$	Percent leakage (%)		
LEM	Liquid emulsion membrane		
$N_p$	Flux of xylitol (g xylitol m <sup>-2</sup> h <sup>-1</sup> )		

\* Correspondence to: Sarote Sirisansaneeyakul, Department of Biotechnology, Kasetsart University, Bangkok 10900, Thailand. E-mail: sarote.s@ku.ac.th

a Department of Biotechnology, Kasetsart University, Bangkok 10900, Thailand

b Department of Chemical Engineering, Kasetsart University, Bangkok 10900, Thailand

c Institute of Technology and Engineering, Massey University, Private Bag 11 222, Palmerston North, New Zealand

$(r_{xyt})_E$	Rate of xylitol formation in liquid emulsion (g xylitol $L^{-1} h^{-1}$ )
$r_{xyt}^m$	Maximum consumption rate of xylose (g xylose $L^{-1} h^{-1}$ )
$R$	Internal radius of tube (m)
SLM	Silicone tube supported liquid membrane
SLM- $n$	Immobilized cell fermentation run $n$ ( $n = 1-4$ )
SSE	Standard squared error
$t$	Time (h)
$V_E$	Liquid emulsion volume (L)
$V_{in}^0$	Initial volume of inner phase (mL)
$V_O$	Oil phase volume (mL)
$V_{out}$	Outer phase volume (L)
$V_W$	Water phase volume (mL)
$X_{Tween}$	Volume fraction of Tween <sup>®</sup> 40
$Y_{P/S}^{max}$	Maximum theoretical xylitol yield based on xylose (g xylitol g xylose <sup>-1</sup> )
$Z$	Length of silicone tube (m)
$\eta$	Effectiveness factor

## INTRODUCTION

Xylitol is a five-carbon sugar alcohol that is found in small quantities in many fruits and vegetables. Xylitol is nearly as sweet as table sugar sucrose. Unlike sucrose, xylitol is well tolerated by diabetics and does not cause tooth decay. As a consequence, xylitol is in demand in sugar-free confectionery.<sup>1</sup> Consumption of xylitol may provide other health benefits.<sup>2,3</sup>

Xylitol is produced commercially by chemical modification of wood xylose. This production method is expensive. An alternative production strategy is to use microorganisms that can ferment D-xylose to xylitol. Microbial production methods have been reviewed extensively.<sup>4-7</sup> At present microbial production is not competitive with the chemical modification route for making xylitol, but this is expected to change, particularly through the use of recombinant microorganisms.<sup>8</sup> Biochemistry of microbial production of xylitol has been reviewed by Granstrom *et al.*<sup>9</sup> This work is focused on development and demonstration of a novel liquid emulsion membrane (LEM)-immobilized live cell system for producing xylitol from D-xylose, as a possible alternative to existing microbial production technologies.

Preliminary screening suggests *Candida mogii* as a promising microorganism for producing xylitol;<sup>10</sup> however, production rate and yield are relatively low.<sup>11</sup> One strategy for enhancing productivity of microbial processes is to use a high concentration of immobilized cells in the bioconversion reactor. Immobilized cells can be recovered easily and reused in subsequent fermentations. Therefore, the use of immobilized *C. mogii* was investigated with a novel immobilization approach.

Although many methods have been developed to immobilize cells in matrices such as calcium alginate gels,<sup>12</sup> these methods are expensive and difficult to use in large-scale processing. An unconventional alternative immobilization method is the use of LEM. A LEM is a kind of double emulsion that is formed when a water-in-oil emulsion (w/o) is gently dispersed in a second aqueous phase, that is, the outer phase. The inner and outer phases are kept apart by a layer of water-immiscible liquid to form water-in-oil-in-water (w/o/w) emulsion. LEMs can provide a high surface area to volume ratio for mass transfer.<sup>13</sup> Transportation of solute through the LEM occurs either by passive transport or by carrier-facilitated transport that depends on the system used. LEMs are easily prepared and relatively inexpensive.<sup>14</sup>

Numerous applications of LEMs have emerged,<sup>15-18</sup> but they have been barely investigated for immobilizing cells<sup>19,20</sup> and enzymes.<sup>21</sup> A major reason for this is a limited long-term stability of LEMs. Furthermore, organic solvents such as xylene and octane that are widely used in LEMs<sup>20,21</sup> as the membrane phase are toxic to cells. These solvents are generally volatile and flammable.

The objectives of this work were to develop a stable w/o/w emulsion to encapsulate the whole live cells of *C. mogii* and to use the selected LEM system in xylitol fermentation. Instead of traditional solvents, this study made use of nontoxic and inexpensive liquid paraffin and soybean oil as solvent phases for making the LEMs. The oil-coated aqueous droplets of immobilized cells were themselves immobilized by adsorption on the inner wall of a silicone tube reactor. The impact of dissolved oxygen and cell density on production rate and yield of xylitol were investigated. Xylitol production in LEM was mathematically modeled for understanding the effects of nutrient transport on production in this novel immobilization system.

## MATHEMATICAL MODEL

The main reactions involved in metabolism of D-xylose to xylitol in *C. mogii* ATCC 18364 have been clearly described.<sup>10</sup> The extracellular xylose is first internalized by the yeast and reduced to xylitol with the enzyme xylose reductase in a step that consumes NADPH. Part of the xylitol produced is excreted and the rest is converted to xylulose through the action of NAD<sup>+</sup>-dependent xylitol dehydrogenase. When the yeast cells are encapsulated within the w/o/w-type LEM, the extracellular xylose must first diffuse through the liquid membrane from the outer phase before it can be transported into the cells (Fig. 1). Similarly, intracellular xylitol excreted by the cells must move through the LEM to the outer phase as the extracellular product.

Bioreaction performance of immobilized catalysts is generally assessed in terms of an effectiveness factor ( $\eta$ ), or the ratio of the rate of substrate consumption in the presence of diffusion limitation to the rate of substrate consumption in the absence of any diffusion limitation. For xylose fermentation, the effectiveness factor is as follows:

$$\eta = \frac{(r_{xyt})^{diff}}{r_{xyt}} \quad (1)$$

where  $(r_{xyt})^{diff}$  is the rate of xylose consumption in diffusion-limited immobilized cells and  $r_{xyt}$  is the rate of xylose consumption in the absence of diffusion limitation.

If the liquid film resistance is assumed to be negligible around the liquid membrane and the uptake rate of xylose by the cells in the absence of diffusion limitation (i.e.,  $r_{xyt}$ ) follows Michaelis-Menten type kinetics, then Eqn (1) can be rearranged to the following:

$$(r_{xyt})^{diff} = \eta \left( \frac{r_{xyt}^m C_{xyt}^{out}}{K_{xyt} + C_{xyt}^{out}} \right) \quad (2)$$

In Eqn (2),  $r_{xyt}^m$ ,  $C_{xyt}^{out}$  and  $K_{xyt}$  are the maximum consumption rate of xylose, xylose concentration in the outer phase and the substrate saturation constant, respectively. Equation (2) assumes a constant concentration of immobilized biomass in the liquid emulsion.

If the medium flows through a tubular loop of immobilized cells without backmixing, the typical case for tubular flow, the axial change in concentration of xylose and xylitol may be evaluated

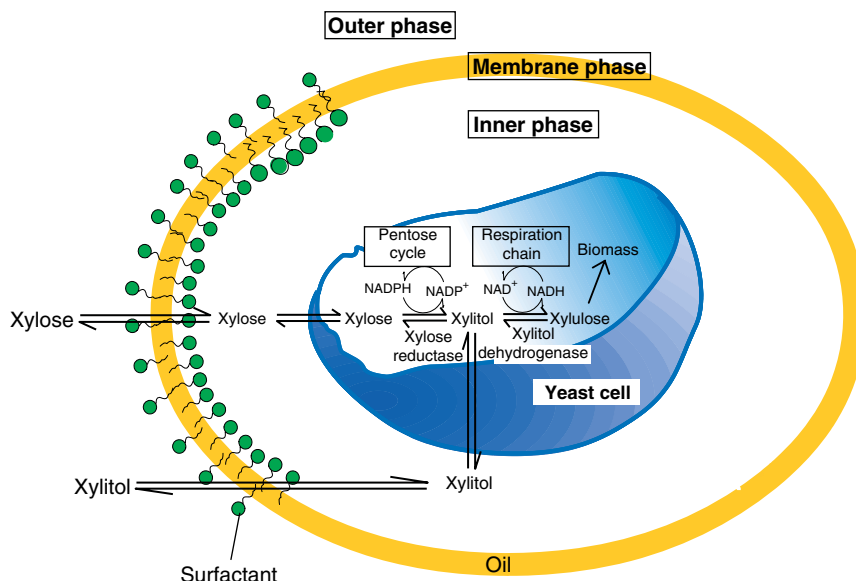


Figure 1. Simplified metabolism of xylose in LEM-encapsulated yeast cell.

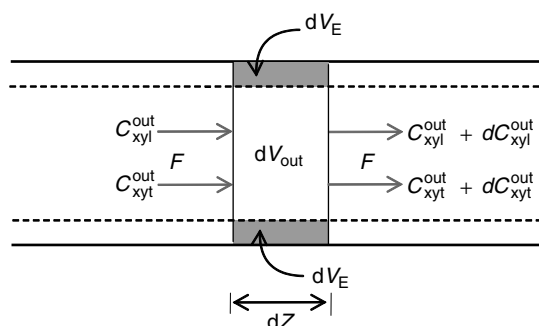


Figure 2. Axial concentration changes in silicone tube supported LEM.

by considering a differential volume of the loop as shown in Fig. 2. The medium enters and leaves the differential volume at a volumetric flow rate  $F$ . The medium, or the outer phase, has a xylose concentration of  $C_{\text{xylose}}^{\text{out}}$  and a xylitol concentration of  $C_{\text{xylitol}}^{\text{out}}$ , at the entrance to the differential volume. The fluid stream exiting the differential volume has undergone a small change in concentration of substrate (i.e.,  $dC_{\text{xylose}}^{\text{out}}$ ) and product (i.e.,  $dC_{\text{xylitol}}^{\text{out}}$ ). This change in concentration includes the transfer of xylose from the outer phase to the immobilized phase and transfer of xylitol in the opposite direction.

When xylose transfers to the internal LEM from the outer phase, the diffusion rate of xylose is related to the flux of xylose,  $N_S$ , and the interfacial area of the liquid emulsion,  $A_C$ , as follows:

$$V_{\text{out}} \left( \frac{dC_{\text{xylose}}^{\text{out}}}{dt} \right)_{\text{tran}} = -N_S A_C \quad (3)$$

where  $C_{\text{xylose}}^{\text{out}}$  is the concentration of xylose in the outer phase and  $V_{\text{out}}$  is the outer phase volume. In Eqn (3),  $(dC_{\text{xylose}}^{\text{out}}/dt)_{\text{tran}}$  is the rate of change of xylose concentration in the outer phase due to transport to the internal LEM phase. Because the interfacial area of the liquid emulsion per unit volume of the outer phase is as

follows:

$$a = \frac{A_C}{V_{\text{out}}} \quad (4)$$

Eqn (3) modifies to the following:

$$\left( \frac{dC_{\text{xylose}}^{\text{out}}}{dt} \right)_{\text{tran}} = -N_S a \quad (5)$$

Similarly, the change in concentration of xylose in the LEM because of substrate consumption under diffusion limitation is obtained from the following mass balance:

$$V_E \frac{dC_{\text{xylose}}^E}{dt} = N_S A_C - (r_{\text{xylose}})^{\text{diff}} V_E \quad (6)$$

In Eqn (6),  $C_{\text{xylose}}^E$  is the concentration of xylose in the liquid emulsion membrane and  $V_E$  is the volume of liquid emulsion membrane. At steady state, with substitution of Eqn (2) in Eqn (6), we obtain the following equation:

$$N_S A_C = (r_{\text{xylose}})^{\text{diff}} V_E = \eta \left( \frac{r_{\text{xylose}}^m C_{\text{xylose}}^{\text{out}}}{K_{\text{xylose}} + C_{\text{xylose}}^{\text{out}}} \right) V_E \quad (7)$$

or

$$N_S = \eta \left( \frac{r_{\text{xylose}}^m C_{\text{xylose}}^{\text{out}}}{K_{\text{xylose}} + C_{\text{xylose}}^{\text{out}}} \right) \frac{V_E}{A_C} \quad (8)$$

From Fig. 2, the material balance of xylose in the outer phase within the differential volume  $dV_{\text{out}}$  is as follows:

$$dV_{\text{out}} \frac{dC_{\text{xylose}}^{\text{out}}}{dt} = F(C_{\text{xylose}}^{\text{out}}) - F(C_{\text{xylose}}^{\text{out}} + dC_{\text{xylose}}^{\text{out}}) + dV_{\text{out}} \left( \frac{dC_{\text{xylose}}^{\text{out}}}{dt} \right)_{\text{tran}} \quad (9)$$

Substituting the xylose diffusion rate from Eqn (5) into Eqn (9) leads to the following:

$$dV_{\text{out}} \frac{dC_{\text{xylose}}^{\text{out}}}{dt} = -F dC_{\text{xylose}}^{\text{out}} - N_S a dV_{\text{out}} \quad (10)$$

At steady state, with substitution of Eqn (8) in Eqn (10), we obtain the following:

$$FdC_{\text{xyl}}^{\text{out}} = -N_S a dV_{\text{out}} = -\eta \left( \frac{r_{\text{xyl}}^m C_{\text{xyl}}^{\text{out}}}{K_{\text{xyl}} + C_{\text{xyl}}^{\text{out}}} \right) \frac{V_E a}{A_C} dV_{\text{out}} \quad (11)$$

In view of the definition in Eqn (4) and assuming a constant volumetric flow rate  $F$  ( $F = dV_{\text{out}}/dt$ ), the rate of xylose uptake by the supported LEM-encapsulated cells as given by Eqn (11), becomes

$$\frac{dC_{\text{xyl}}^{\text{out}}}{dt} = -\eta \left( \frac{r_{\text{xyl}}^m C_{\text{xyl}}^{\text{out}}}{K_{\text{xyl}} + C_{\text{xyl}}^{\text{out}}} \right) \frac{V_E}{V_{\text{out}}} \quad (12)$$

As with xylose, when xylitol transports to the outer phase from the internal LEM, the diffusion rate of xylitol is related to the flux of xylitol ( $N_P$ ) and the surface area of the liquid emulsion,  $A_C$ , as follows:

$$V_{\text{out}} \left( \frac{dC_{\text{xyl}}^{\text{out}}}{dt} \right)_{\text{tran}} = N_P A_C \quad (13)$$

In Eqn (13),  $C_{\text{xyl}}^{\text{out}}$  is the xylitol concentration in the outer phase and  $(dC_{\text{xyl}}^{\text{out}}/dt)_{\text{tran}}$  is the rate of change of xylitol concentration in the outer phase due to diffusion from the internal LEM phase. Substitution of Eqn (4) in Eqn (13) leads to the following equation:

$$\left( \frac{dC_{\text{xyl}}^{\text{out}}}{dt} \right)_{\text{tran}} = N_P a \quad (14)$$

Similar to Eqn (6), the change in quantity of xylitol in the emulsion membrane is related to the rate of formation of xylitol and its transport to the outer phase; thus:

$$V_E \frac{dC_{\text{xyl}}^E}{dt} = (r_{\text{xyl}})_{\text{E}} V_E - N_P A_C \quad (15)$$

In Eqn (15),  $C_{\text{xyl}}^E$  is the concentration of xylitol in the LEM. The rate of formation of xylitol is related to xylose consumption by the yield coefficient, as follows:

$$(r_{\text{xyl}})_{\text{E}} = (r_{\text{xyl}})^{\text{diff}} Y_{\text{P/S}}^{\text{max}} \quad (16)$$

In Eqn (16),  $Y_{\text{P/S}}^{\text{max}}$  is the maximum theoretical xylitol yield based on xylose. At steady state, with Eqn (16) substituted in Eqn (15), we obtain the following:

$$N_P A_C = (r_{\text{xyl}})_{\text{E}} V_E = (r_{\text{xyl}})^{\text{diff}} Y_{\text{P/S}}^{\text{max}} V_E = \eta \left( \frac{r_{\text{xyl}}^m C_{\text{xyl}}^{\text{out}}}{K_{\text{xyl}} + C_{\text{xyl}}^{\text{out}}} \right) Y_{\text{P/S}}^{\text{max}} V_E \quad (17)$$

or

$$N_P = \eta \left( \frac{r_{\text{xyl}}^m C_{\text{xyl}}^{\text{out}}}{K_{\text{xyl}} + C_{\text{xyl}}^{\text{out}}} \right) \frac{Y_{\text{P/S}}^{\text{max}}}{A_C} V_E \quad (18)$$

where  $(r_{\text{xyl}})_{\text{E}}$  is the rate of formation of xylitol in liquid emulsion membrane.

From Fig. 2, the material balance for xylitol over the differential volume,  $dV_{\text{out}}$ , is the following:

$$dV_{\text{out}} \frac{dC_{\text{xyl}}^{\text{out}}}{dt} = F(C_{\text{xyl}}^{\text{out}}) - F(C_{\text{xyl}}^{\text{out}} + dC_{\text{xyl}}^{\text{out}}) + dV_{\text{out}} \left( \frac{dC_{\text{xyl}}^{\text{out}}}{dt} \right)_{\text{tran}} \quad (19)$$

Substitution of the xylitol transport rate from Eqn (14) into Eqn (19) provides the following equation:

$$dV_{\text{out}} \frac{dC_{\text{xyl}}^{\text{out}}}{dt} = -FdC_{\text{xyl}}^{\text{out}} + N_P a dV_{\text{out}} \quad (20)$$

At steady state, with Eqn (18) substituted for  $N_P$ , Eqn (20) becomes

$$FdC_{\text{xyl}}^{\text{out}} = N_P a dV_{\text{out}} = \eta \left( \frac{r_{\text{xyl}}^m C_{\text{xyl}}^{\text{out}}}{K_{\text{xyl}} + C_{\text{xyl}}^{\text{out}}} \right) \frac{Y_{\text{P/S}}^{\text{max}}}{A_C} V_E a dV_{\text{out}} \quad (21)$$

In view of Eqn (4) and a constant  $F$ , the rate of xylitol production by the LEM-encapsulated cells in Eqn (21) becomes

$$\frac{dC_{\text{xyl}}^{\text{out}}}{dt} = \eta \left( \frac{r_{\text{xyl}}^m C_{\text{xyl}}^{\text{out}}}{K_{\text{xyl}} + C_{\text{xyl}}^{\text{out}}} \right) \frac{V_E}{V_{\text{out}}} Y_{\text{P/S}}^{\text{max}} \quad (22)$$

Equations (12) and (22) comprise the model of xylose consumption and xylitol production by *C. mogii* encapsulated in the supported LEM system.

## MATERIALS AND METHODS

### Liquid emulsion membrane (LEM) systems

#### Paraffin LEM system

Paraffin LEM was prepared and stability tested as described by Pal *et al.*<sup>21</sup> For preparing the membrane phase, liquid paraffin (15 mL; ASP Ajax Finechem, Taren Point, Australia) was vigorously mixed with different concentrations (1–8%, w/v; g per 100 mL) of the stabilizing surfactant Span<sup>®</sup> 80 (sorbitan monooleate; Fluka Chemika, Buchs, Switzerland) in different experiments. The inner phase was prepared as 10 mL of 0.1 mol L<sup>-1</sup> NaOH. For a specified membrane to inner phase ratio of 1:0.67, the inner phase was added dropwise to the membrane phase under vigorous agitation (1000 rpm magnetic stirrer; CAT-M6, Schott, Mainz, Germany) over a period of 15 min. This produced a water-in-oil (w/o) emulsion. Subsequently, a 20 mL portion of the emulsion was dispersed in the outer phase of 200 mL distilled water under mild agitation at 600 rpm. This produced a w/o/w type of LEM in which the inner phase was 0.1 mol L<sup>-1</sup> NaOH.

Some paraffin LEM preparations used a mixture of Span<sup>®</sup> 80 and Tween<sup>®</sup> 40 (polyoxyethylene sorbitan mono-palmitate; Fluka Chemika) in the outer phase, for stabilization. Use of mixed surfactants allowed the hydrophilic–lipophilic balance (HLB) to be adjusted to 11, a value that has been found suitable for stabilizing o/w emulsions of paraffin.<sup>22</sup> The desired HLB value was obtained by mixing Tween<sup>®</sup> 40 and Span<sup>®</sup> 80 in a volume ratio of 3:2. HLB of the mixed coemulsifier was calculated using the following equation:<sup>23</sup>

$$\text{HLB}_{\text{Mixture}} = X_{\text{Tween}} \text{HLB}_{\text{Tween}} + (1 - X_{\text{Tween}}) \text{HLB}_{\text{Span}} \quad (23)$$

where  $X_{\text{Tween}}$  is the volume fraction of Tween<sup>®</sup> 40 in the surfactant mixture,  $\text{HLB}_{\text{Tween}}$  (= 15.6) is the HLB for pure Tween<sup>®</sup> 40 and  $\text{HLB}_{\text{Span}}$  (= 4.3) is the HLB for pure Span<sup>®</sup> 80.

The coemulsifier mixture concentration was varied from 0.1% to 0.6% (w/v) of the outer phase in different experiments. In experiments that varied the concentration of the coemulsifier, the concentration of Span® 80 in the membrane phase was always 6% (w/v). The latter had been established to be optimal in earlier experiments.

LEM stability was quantified by monitoring the pH change in the outer phase due to leakage of the encapsulated alkali, thus:

$$[\text{NaOH}] = 10^{(\text{pH}-14)} \quad (24)$$

Alkali leakage to the outer phase was assumed to be solely due to rupture of the membrane. Percentage leakage ( $L$ ) of alkali was calculated as follows:

$$L = \frac{[\text{NaOH}]_{\text{out}} V_{\text{out}}}{[\text{NaOH}]_{\text{in}}^0 V_{\text{in}}^0} \times 100 \quad (25)$$

In Eqn (25),  $[\text{NaOH}]_{\text{out}}$  and  $[\text{NaOH}]_{\text{in}}^0$  are the concentrations of NaOH in the outer and inner phases, respectively.  $V_{\text{out}}$  and  $V_{\text{in}}^0$  are the outer and inner phase volumes, respectively. An initial rate of leakage ( $\% L \text{ min}^{-1}$ ) was determined as the slope of the initial part of a plot of  $L$  versus time.

#### Soybean oil LEM system

Stability of soybean oil LEM was assessed visually by observing the phase separation behavior after the emulsion had been prepared. Lanolin (Suksapanpanich, Bangkok, Thailand) and microwaxes (Symbols Service Co. Ltd, Bangkok, Thailand) were used as emulsifying and stabilizing agents. The inner phase consisted of distilled water (10 mL) containing varying amounts (0–30% (w/v); g 100 mL<sup>-1</sup> inner phase) of lanolin. The membrane phase material was prepared by melting microwaxes in 15 mL of refined soybean oil (Cook Brand, Thanakorn Vegetable Oil Products Co., Ltd, Samutprakarn, Thailand) to give varying concentrations (0–7% (w/v), g microwax 100 mL<sup>-1</sup> membrane phase) of microwaxes. The concentration of lanolin in the inner phase was either 5% or 10%, depending on experiment. The ratio of the membrane phase to inner phase in the LEM was 1 : 0.67. After each phase had been prepared, the inner phase was added dropwise to the membrane phase over a period of 15 min. During the addition, the membrane phase was vigorously agitated (1000 rpm; magnetic stirrer; CAT-M6, Schott). Samples (5 mL) of the resulting w/o emulsion were transferred to test tubes (16 × 100 mm) and allowed to stand at room temperature. The test tubes were photographed every 24 h to measure phase separation, i.e. stability.

A separate series of experiments were performed to assess the impact of the ratio of the membrane and inner phase volumes on stability. In these experiments, the ratio of inner to membrane phase volumes was 1 : 1, 1 : 1.5, 1 : 2, 1 : 2.5 and 1 : 3. The volume of the membrane phase was always 10 mL. Both the inner phase and the membrane phase contained 5% each of lanolin and microwaxes as this concentration had been found to be optimal from the earlier described series of experiments.

#### Permeability of the LEM systems

A series of experiments were performed to determine the permeability of xylose in paraffin and soybean oil LEMs. For this, LEMs were prepared in accordance with protocols that had been found (see earlier sections) to give most stable LEMs. Instead of 0.1 mol L<sup>-1</sup> NaOH, or distilled water, a 10 mL aqueous solution

of 10 g L<sup>-1</sup> xylose was encapsulated in the inner phase of the emulsion. These emulsions of 25 mL were coated onto the inner surface of silicone tubes (Dura® EZ-TG105, N.C.R. Rubber Industry Co. Ltd, Rayong, Thailand; 5 × 9 mm; 1.5 m long) by pumping (peristaltic pump; RP-1000, Eyela, Tokyo, Japan) the emulsion through the tube on a once through basis at a flow rate of 200 mL h<sup>-1</sup>. The total volume of the emulsion that flowed out of the fully drained tube was measured to determine the volume that had been coated (i.e.,  $V_E$ ). Subsequently, 50 mL of distilled water as the outer phase was circulated through the LEM-coated tube at a flow rate of 300 mL h<sup>-1</sup>. The water was sampled (0.4 mL) every 5–15 min from the recirculation tank, to determine the concentration of xylose that diffused from the inner phase of the supported LEM. Xylose was analyzed colorimetrically using the method of Deschatelets and Yu.<sup>24</sup>

Permeability coefficient was calculated according to the method of Kedari *et al.*<sup>25</sup> First, the transport flux of xylose ( $J$ ) was calculated as follows:

$$J = \frac{C_{\text{xy}} V_{\text{out}}}{A_C t} \quad (26)$$

where  $C_{\text{xy}}$ ,  $V_{\text{out}}$ ,  $A_C$  and  $t$  are xylose concentration, outer phase volume, membrane surface area, and time, respectively. The permeability coefficient ( $P$ ) of xylose was then estimated:

$$P = \frac{J}{C_{\text{xy}}} = \frac{dC_{\text{xy}}}{dt} \frac{V_{\text{out}}}{A_C} \frac{1}{C_{\text{xy}}} \quad (27)$$

Integration of Eqn (27) between the limits  $t = 0$ ,  $C_{\text{xy}} = (C_{\text{xy}})_0$  and  $t = t$ ,  $C_{\text{xy}} = (C_{\text{xy}})_t$ , led to the following linear equation:

$$\ln(C_{\text{xy}})_t = \ln(C_{\text{xy}})_0 + \frac{P A_C}{V_{\text{out}}} t \quad (28)$$

$\frac{P A_C}{V_{\text{out}}}$  was determined as the slope of a plot of  $\ln(C_{\text{xy}})_t$  versus time. Thus,  $P$  could be calculated. The surface area of LEM supported on the inner surface of the silicone tube was estimated using the following equation:

$$A_C = 2\pi rZ = 2\pi Z \sqrt{R^2 - \frac{V_E}{\pi Z}} \quad (29)$$

where  $r$ ,  $R$ ,  $Z$ , and  $V_E$  are the inner radius of the silicone tube with and without the emulsion coated, the tube length, and the volume of emulsion coated on the inner surface of the tube, respectively. The  $A_C$  value was 0.1651 m<sup>2</sup> for a tube that was 15 m long and 5 mm in internal diameter.

Furthermore, in the tube-supported soybean oil LEM system with an optimal lanolin concentration of 5%, the additional influence of concentrations of microwaxes (i.e., 5%, 7%, and 10%) and volume ratio of membrane phase to inner phase (i.e., 1 : 0.67 and 1 : 2) on permeability of xylose were examined. At the membrane to inner phase volume ratio of 1 : 2, the volumes of membrane and inner phases were 10 and 20 mL, respectively.

#### Fermentation

##### Microorganism, media, and inocula

The yeast *Candida mogii* ATCC 18364 was used for converting xylose to xylitol.<sup>10</sup> The yeast was maintained on potato dextrose agar (PDA) at 4 °C. The minimal medium used for growth contained (per liter of solution): 18.75 g KH<sub>2</sub>PO<sub>4</sub>, 6 g (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 1.13 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.1 g CaCl<sub>2</sub>, 36.5 mg myo-inositol, 18.2 mg

calcium pantothenate, 3.66 mg thiamine-HCl, 0.9 mg pyridoxal-HCl, 0.018 mg biotin, 9.1 mg FeCl<sub>3</sub>, 6.4 mg MnSO<sub>4</sub>·H<sub>2</sub>O, 5.46 mg ZnSO<sub>4</sub>·7H<sub>2</sub>O, 1.46 mg CuSO<sub>4</sub>·5H<sub>2</sub>O, and a specified carbon source.

A loopful of yeast from a slant culture was transferred to a test tube containing 2.5 mL of growth medium with an initial pH of 4.5. The growth medium contained 10 g L<sup>-1</sup> glucose and 5 g L<sup>-1</sup> xylose as a mixed carbon source. The tube was held on a rotary shaker at 30 °C and 250 rpm for 24 h. The broth was used to inoculate a 125 mL shake flask and a 500 mL shake flask. The inoculum size was 10% v/v in both cases. The small and large flasks contained 22.5 mL and 225 mL of minimal media (see above), respectively. The inoculated flasks were incubated at 30 °C, 250 rpm, for 24 h. Fermentation broth (1–2 L) from four to eight 500 mL flasks was pooled and centrifuged at 1753 × *g* for 15 min (Spectrafuge 16 M, Labnet, Edison, NJ, USA) at 4 °C to obtain the desired cell quantity of 4–10 g dry weight. This biomass was used in the cell immobilization procedure.

#### Cell immobilization

In view of the results of LEM stability and xylose permeability experiments, only the soybean oil LEM was used in immobilizing the yeast cells. A soybean oil LEM with 5% lanolin in the inner phase and 5% microwaxes in the membrane phase had been found (see earlier sections) to be optimal from the point of view of stability and xylose transport. Consequently, this LEM was used in cell immobilization. Either 100 mL or 150 mL identical batches of LEM were prepared. The ratio of the membrane phase to inner phase was 1 : 2 v/v. Microwaxes (5%) were melted and dissolved in an appropriate volume of soybean oil. The oil was then sterilized at 121 °C for 15 min. The inner phase was prepared by mixing yeast cells in sterile distilled water at various specified concentrations in different experiments. The inner phase contained 5% lanolin. The inner phase was now added dropwise over a period of 15 min to the soybean oil membrane phase with vigorous agitation (1000 rpm, turbine agitator RZR1; Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). This resulted in the formation of a w/o emulsion. A peristaltic pump was used to feed the emulsion

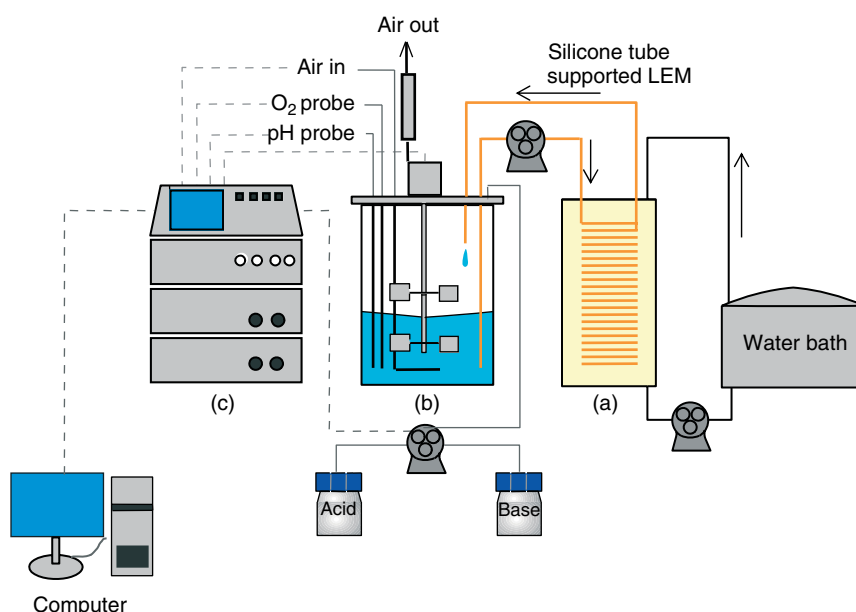
through a pre-sterilized (121 °C, 15 min) silicone tubing of 15 m length that had been rolled into a coil supported on a wire frame. The flow rate was 300 mL h<sup>-1</sup>. The coated tube was then washed by circulating 1 L of sterile distilled water through it at 300 mL h<sup>-1</sup>. This tube-supported LEM system was used in xylose fermentation.

#### Immobilized cell fermentation

Fermentations were carried out batchwise. A 2 L stirred-tank bioreactor (BioFlo 110, New Brunswick Scientific, Edison, NJ, USA) was used as the feed reservoir. The feed was pumped from the reservoir to the immobilized cell tubular loop and back to the reservoir (Fig. 3). The flow rate was constant at 300 mL h<sup>-1</sup>. A peristaltic pump was used. The stirred reservoir initially was filled with 1 L of the outer phase and 0.6 L of minimal medium supplemented with 10 g L<sup>-1</sup> xylose. The volume ratios of emulsion to outer phase were as specified in Table 1 for the different runs. The fermentation temperature was constant at 30 °C in both the stirred reservoir and the tubular loop. The latter was held in a thermostated water bath. The pH was controlled at pH 6 in the stirred reservoir by automatic addition of 3 mol L<sup>-1</sup> NaOH, as needed. The dissolved oxygen concentration in the stirred reservoir was maintained at either 30% of air saturation, or >70% of air saturation in different experiments. The dissolved oxygen level was controlled automatically by changes in both the aeration rate and agitation speed, as necessary. The fermentation conditions used in the various runs (SLM-*n*; *n* = 1–4) are summarized in Table 1.

#### Analytical methods

Fermentation broth was sampled periodically from the stirred tank. Suspended cells were recovered by initial centrifugation and subsequent filtration through a 0.45 μm cellulose acetate membrane filter (catalog no. 11 106-050 N; Sartorius AG, Goettingen, Germany). The cells were washed twice with distilled water and dried to a constant weight at 105 °C to obtain the dry weight of the freely suspended cells. The supernatant of the broth sample was



**Figure 3.** Combined stirred-tank–tubular-loop LEM bioreactor: (a) silicone tubular loop supporting the LEM; (b) well-mixed nutrient reservoir with environmental controls; and (c) monitoring and control unit.

**Table 1.** Fermentation conditions used with soybean oil LEM encapsulated cells

Operational parameter	Batch fermentation			
	SLM-1	SLM-2	SLM-3	SLM-4
1. Volume ratio of LEM to culture broth	1 : 10	1 : 4	1 : 4	1 : 4
2. Ratio of membrane to inner phase volumes	1 : 2	1 : 2	1 : 2	1 : 2
3. Culture broth volume (mL)	1000	600	600	600
4. Initial xylose concentration ( $\text{g L}^{-1}$ ) <sup>a</sup>	10	10	10	10
5. LEM volume (mL)	100	150	150	150
Inner phase (mL)	66.67	100	100	100
Lanolin concentration (% w/v)	5	5	5	5
Initial cell concentration ( $\text{g L}^{-1}$ ) <sup>a</sup>	4.2	3.89	6.2	15.31
Membrane phase volume (mL)	33.33	50	50	50
Microwaxes concentration ( $\text{g L}^{-1}$ )	5	5	5	5
6. Dissolved oxygen (%)	>70	>70	30	30

<sup>a</sup> Based on volume of broth.

used for spectrophotometric (UV 1210, Shimadzu Corporation, Kyoto, Japan) determinations of xylose<sup>24</sup> and xylitol.<sup>26</sup>

### Parameter estimation

The kinetic parameters (i.e.,  $r_{\text{xy}}^m$ ,  $K_{\text{xy}}$ ,  $Y_{\text{P/S}}^{\text{max}}$  and  $\eta$ ) of the model were estimated from experimental data. The estimation procedure searched the values of the parameters that minimized the difference between the model-predicted fermentation profiles and the experimentally observed data. The parameter values that best fitted the measured data were taken as the optimal ones. A minimum of the sum of squares of the function (FUN) used for data fitting was presented as  $\min \sum \{ \text{FUN}(X)^2 \}$ , where  $X$  is the parameter value obtained from the best fit of the nonlinear function. MATLAB® (The MathWorks, Inc., Natick, MA, USA) was used for estimating the model parameters.

## RESULTS AND DISCUSSION

### Liquid emulsion membrane development

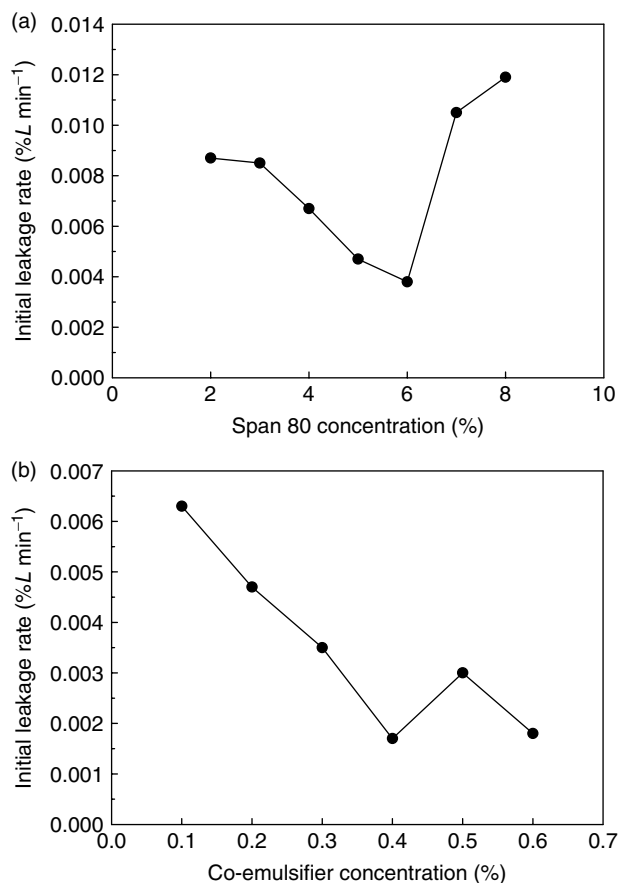
#### Liquid paraffin LEM system

Stability of the paraffin LEM was characterized in terms of the initial leakage rate of alkali, as explained in 'Paraffin LEM system', above. The initial leakage rate is shown in Fig. 4(a) for various concentrations of Span® 80 emulsifying surfactant in the membrane phase. The outer phase was distilled water. The initial leakage rate declined to a minimum value at Span® 80 concentration of around 6%. The improved stability, i.e., a declining initial leakage rate, observed with increasing concentration of Span® 80 over the concentration range of 2–6%, was due to increased coverage of the surface of microdroplets with the surfactant. At 6% Span® 80 concentration, the microdroplets became fully coated with a monolayer of the surfactant<sup>21</sup> and were, therefore, most stable. LEM stability decreased dramatically as the concentration of Span® 80 exceeded 6%. This was because

the excess added surfactant interacted with the surfactant that already coated the droplets, to form reversed micelles of the surfactant. Formation of reversed micelles can actually reduce the amount of surfactant available for coating the droplets and hence a reduced stability of LEM.

In subsequent work with liquid paraffin LEM, the Span® 80 concentration in the membrane phase was always held at the optimal 6%. At this fixed concentration of Span® 80, the effect of the presence of a coemulsifier mixture (Tween® 40 and Span® 80 at a volume ratio of 3 : 2) in the outer phase was evaluated on stability. The coemulsifier concentration varied from 0.1% to 0.6%. The LEM stability increased, i.e., the initial leakage rate declined, with increasing concentration of the coemulsifier up to a concentration value of 0.4% (Fig. 4(b)). The specific coemulsifier composition that was used had an HLB value of 11. This value has been observed to improve stability of paraffin-in-water emulsions in prior studies.<sup>27</sup> Presence of some Span® 80 in the outer phase probably minimized losses of this surfactant from the surface of the droplets to the outer phase by reducing the concentration gradient that drives mass transfer. Presence of Tween® 40 apparently stabilized the water film outside the microdroplets, as has been observed in the past.<sup>28</sup>

Although paraffin LEM with 6% Span® 80 in the membrane phase and 0.4% coemulsifier in the outer phase had excellent stability, this LEM was poor with respect to permitting mass transfer of xylose (see 'Soybean oil LEM system', above). Because of a poor mass transfer capability, the use of this LEM in xylose



**Figure 4.** Stability of the paraffin LEM systems prepared with different concentrations of (a) Span® 80 in the membrane phase and (b) Tween® 40/Span® 80 coemulsifier in the outer phase.

fermentation was impractical. Therefore, paraffin LEM was not examined further.

#### Soybean oil LEM system

To establish a stable LEM system of soybean oil, effects of lanolin and microwaxes, as emulsifiers in the inner and membrane phases, respectively, were investigated. As shown in Fig. 5(a), lanolin concentrations of 5–30% in combination with 0.5% microwaxes produced emulsions that separated into two layers within 24 h of standing at room temperature. The oil layer rose above the emulsion layer. To improve emulsion stability, different concentrations (1–7%) of microwaxes were tested in combination with 5% and 10% lanolin in different experiments. The results are shown in Fig. 5(b) and (c). Emulsions that were stable for more than 7 days under gravity at room temperature were obtained when microwax concentration was >4% and lanolin concentration was 5% (Fig. 5(b)). Equally stable emulsions were produced at microwax concentration of >3% in combination with a lanolin concentration of 10% (Fig. 5(c)). Microwaxes dissolved in soybean oil clearly increased emulsion stability by increasing the membrane viscosity. Presence of microwaxes greatly reduced the amount of lanolin that was needed to form stable emulsions.

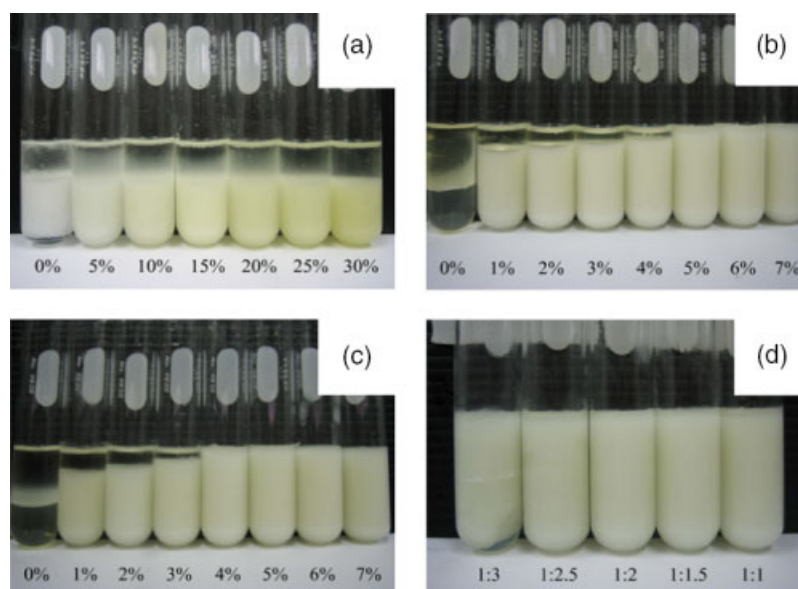
In view of the results in Fig. 5(b) and (c), the optimal emulsion composition for stability with the least amount of emulsifying agents used was a formulation that contained 5% each of lanolin and microwaxes. This composition was used in cell immobilization studies with the soybean oil LEM system. For this fixed composition, the effects on stability of varying the volume ratio of membrane phase to inner phase were examined. Potentially, this ratio can be used to make the membrane thinner and, therefore, improve the mass transfer of xylose. Furthermore, various volume ratios of the membrane phase to inner phase can be used to increase the inner droplet volume available for immobilizing the yeast. As shown in Fig. 5(d) for membrane to inner phase volume ratios ranging from 1 : 1 to 1 : 2.5, the emulsions were visibly stable for more than 7 days at room temperature. At a higher volume ratio of 1 : 3, some phase separation was observed within 24 h of standing at room temperature.

Interfacial area ( $A_c$ ) values and permeability coefficients ( $P$ ) of xylose for tube-supported soybean oil LEM (5% lanolin in the inner phase) with various concentrations of microwaxes in the membrane phase are shown in Table 2 for two different ratios of the membrane phase to inner phase volumes (i.e.,  $V_o/V_w$ ). At any given concentration of microwaxes, the xylose permeability was higher at the membrane to inner phase volume ratio of 1 : 2, presumably because the membrane was thinner compared to the case when the phase volume ratio was 1 : 0.67 (Table 2). For any given ratio of phase volumes, the  $P$ -value for xylose declined with increasing concentration of microwaxes in the membrane phase (Table 2). This was because the viscosity of the membrane phase increased with increasing amount of dissolved microwaxes. The interfacial area  $A_c$  of the supported LEM did not vary much with changes in microwax concentration and the ratio of phase volumes (Table 2). In view of the data in Table 2, the optimal formulation of soybean oil LEM that was not only highly stable but also permitted good transport of xylose was one with 5% microwaxes in the

**Table 2.** Permeability coefficient ( $P$ ) of xylose through the supported soybean oil LEM system

Microwaxes (% w/v)	Surface area, $A_c$ ( $\times 10^{-2} \text{ m}^2$ )		Permeability coefficient, $P$ ( $\times 10^{-7} \text{ m s}^{-1}$ )	
	$V_o/V_w = 1 : 0.67$	$V_o/V_w = 1 : 2$	$V_o/V_w = 1 : 0.67$	$V_o/V_w = 1 : 2$
5	2.01	1.96	4.72	6.37
7	1.96	2.10	3.31	4.76
10	1.86	1.96	1.34	4.33

$V_o/V_w$  is the volume ratio of membrane to inner phases. The surface area of the liquid emulsion supported on the inner surface of the silicone tube was calculated using Eqn (29). The volume of coated liquid emulsion on the silicone surface ( $V_E$ ) was varied by changing the amount of microwaxes from 6 to 11 mL in a 1.5 m long silicone tube.



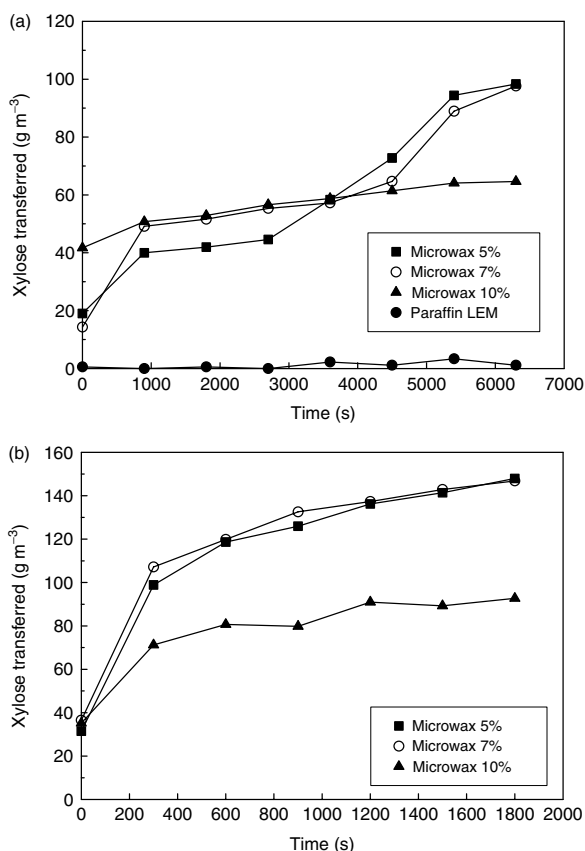
**Figure 5.** Stability of the soybean oil LEM systems prepared with: (a) different percentages of lanolin; (b) different amounts of microwaxes containing 5% lanolin; (c) different amounts of microwaxes containing 10% lanolin; and (d) different volume ratios of membrane and inner phases.

membrane phase, 5% lanolin in the inner phase, and a membrane to inner phase volume ratio of 1 : 2.

The total quantity of xylose transferred from the inner to the outer phase, per unit volume of the outer phase, is shown in Fig. 6 for various contact times and volume ratios of the membrane to inner phases. Clearly, the paraffin LEM did not allow significant transport of xylose. All soybean oil LEMs were far superior to the liquid paraffin LEM in terms of transport of xylose. This is because paraffin oil is a highly hydrophobic hydrocarbon compared with soybean oil, which is a triglyceride. For the soybean oil LEMs, for any given value of the phase volume ratio, the rate of xylose transport was comparable for microwax concentrations of 5% and 7%, but mass transfer became poorer as the concentration of microwaxes was raised to 10% (Fig. 6). As noted earlier, an increasing concentration of microwaxes in the membrane phase increased membrane viscosity and this explains the negative effect of increasing microwax concentration on xylose transport. As shown in Fig. 6, soybean oil LEM with a membrane to inner phase volume ratio of 1 : 2 gave superior mass transfer rates compared with the LEM at a phase volume ratio of 1 : 0.67. This was because of the effect of phase volume ratio on the thickness of the membrane, as pointed out previously.

### Production of xylitol by LEM-encapsulated yeast cells

The soybean oil LEM that had been optimally formulated (see 'Soybean oil LEM system', above) for high stability under quiescent



**Figure 6.** Total xylose transferred per unit volume of outer phase, for various contact times between the supported and outer phases. Only the soybean oil LEM contained microwaxes at the concentrations specified. The volume ratios of membrane to inner phases were: (a) 1 : 0.67 and (b) 1 : 2.

conditions and high transport rates of xylose, was used to immobilize the yeast cells (see 'Cell immobilization', above). The cell-containing LEM was supported by adsorption on inner walls of a silicone tubing (see 'Cell immobilization', above) and used for converting xylose to xylitol in a stirred-tank-tubular-loop combination bioreactor (Fig. 3). The unsupported LEM system could not be used in a freely suspended state because it was unstable in this state in the presence of mechanical agitation. Tests conducted with freely suspended LEM in the stirred tank showed extensive leakage of cells and coalescence of the LEM droplets.

In the supported state, the cell-containing LEM was quite stable in batch fermentation studies that lasted up to 120 h. Various fermentation conditions were assessed in attempts to optimize the immobilized cell bioreaction system. The focus was mainly on assessing the effects of dissolved oxygen and immobilized cell concentration on the fermentation. The conditions used in the various fermentations are summarized in Table 1. The fermentation profiles are shown in Fig. 7 for four batch fermentations.

Concentration of leaked cells did not exceed 260 mg L<sup>-1</sup> in any of the fermentations (Fig. 7(a)–(d)), indicating a stable system of immobilized cells. In the aerobic batch fermentation SLM-1 (Fig. 7(a)), the ratio of immobilized emulsion volume to the total broth volume was 1 : 10. As a consequence of a relatively small volume of the active immobilized emulsion, the rate of conversion of xylose was low (Fig. 7(a)). The yield and productivity of xylitol were 0.31 g g<sup>-1</sup> and 0.0078 g L<sup>-1</sup> h<sup>-1</sup>, respectively.

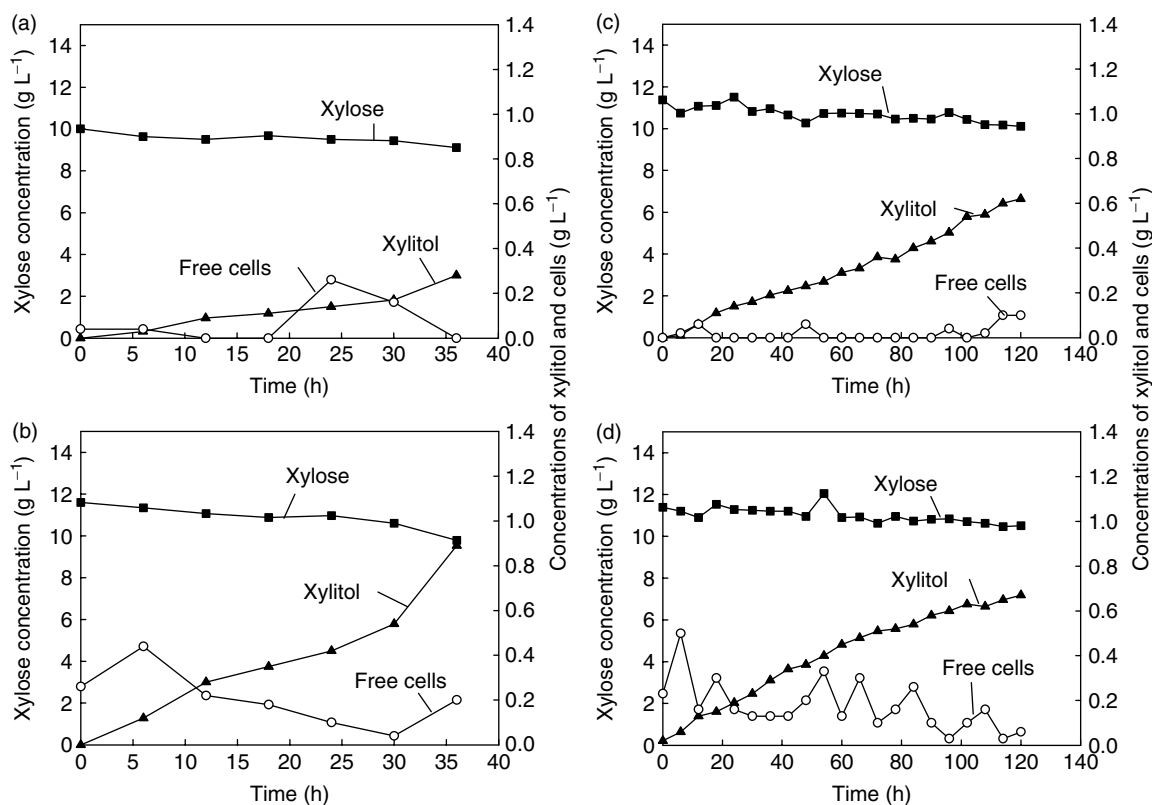
In fermentation SLM-2 (Fig. 7(b)), the bioactive proportion of the total broth volume was increased so that the emulsion-to-broth volume ratio was 1 : 4. Other conditions were the same as in fermentation SLM-1 (Fig. 7(a)). This increase in the relative volume of the bioactive emulsion increased the volumetric rate of xylose consumption from 0.0187 to 0.0416 g L<sup>-1</sup> h<sup>-1</sup> (Table 3). Similarly, the volumetric rate of xylitol production increased from 0.0078 g L<sup>-1</sup> h<sup>-1</sup> to 0.0216 g L<sup>-1</sup> h<sup>-1</sup> (Table 3), a 2.8-fold increase. Simultaneously, the yield of xylitol increased 1.6-fold, to 0.49 g g<sup>-1</sup>.

Fermentations SLM-3 (Fig. 7(c)) and SLM-4 (Fig. 7(d)) were carried out under oxygen-limited conditions with dissolved oxygen levels of less than 30% of air saturation. Doing this increased the xylitol yield in comparison with the highly aerated fermentations SLM-1 and SLM-2 (Table 3), but the uptake rate of xylose was significantly reduced (Table 3). Under conditions of oxygen limitation, an increase in the amount of coenzyme

**Table 3.** Kinetic parameters of fermentation using soybean oil LEM-encapsulated cells

Fermentation run	Xylitol yield (g g <sup>-1</sup> )	Volumetric rates (g L <sup>-1</sup> h <sup>-1</sup> )		Specific rates (g g <sup>-1</sup> h <sup>-1</sup> )	
	$Y_{P/S}$	$\frac{dC_{\text{xyt}}^{\text{out}}}{dt}$	$\frac{dC_{\text{xyt}}^{\text{out}}}{dt}$	$q_{\text{xyt}}$	$q_{\text{xyt}}$
SLM-1	0.31	0.0187	0.0078	0.0044	0.0017
SLM-2	0.49	0.0416	0.0216	0.0107	0.0056
SLM-3	0.52	0.0090	0.0050	0.0014	0.0008
SLM-4	0.52	0.0093	0.0055	0.0006	0.0004

xyt, xylt and out represent xylose, xylitol and the outer phase, respectively.



**Figure 7.** Xylose and xylitol concentration profiles during fermentation by the tube supported LEM-encapsulated yeast cells: (a) aerobic conditions under a  $V_{LEM}/V_{Broth}$  ratio of 1:10; (b) aerobic conditions under a  $V_{LEM}/V_{Broth}$  ratio of 1:4; (c) oxygen-limited conditions under a  $V_{LEM}/V_{Broth}$  ratio of 1:4 and  $C_X = 6.2$  g L<sup>-1</sup>; and (d) oxygen-limited conditions under a  $V_{LEM}/V_{Broth}$  ratio of 1:4 with  $C_X = 15.3$  g L<sup>-1</sup>.

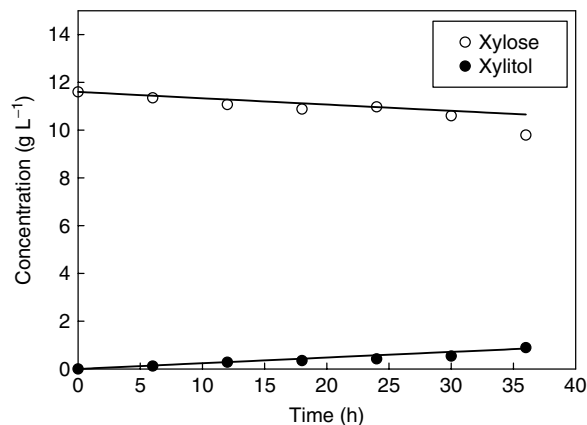
NADH reduces considerably the NAD<sup>+</sup>-dependent activity of xylitol dehydrogenase. This reduces the conversion of xylitol to D-xylulose that is known to occur under aerobic conditions.<sup>10</sup> This explains the increased yield of xylitol in a low-oxygen environment.

Attempts were made to counteract a reduced uptake rate of xylose by the cells under low oxygen conditions, by increasing the concentration of the immobilized cells. Thus in fermentation SLM-4 the immobilized biomass concentration was 2.5-fold greater than in fermentation SLM-3, but this did not increase the xylose uptake rate. Evidently, a high concentration of immobilized cells in the LEM reduced mass transport of xylose by reducing the free space available for diffusion.

### Xylitol fermentation model

The model parameters in Eqns (12) and (22) were estimated from experimental data obtained during batch production of xylitol under aerobic conditions with the volume ratios of LEM/broth and membrane/inner phases of 1:4 and 1:2, respectively. The estimated values of parameters were obtained by minimizing the mean weighted square error between the experimental data and model-simulated values. As shown in Fig. 8 for fermentation SLM-2, the model-predicted profiles of xylose consumption and xylitol production agreed closely with the measured data. The estimated model parameter values are given in Table 4.

The model estimated values for the maximum volumetric rate of xylose uptake ( $r_{xy}^m$ ) and the saturation constant based on xylose ( $K_{xy}$ ) are comparable to the values reported previously<sup>11</sup> for these parameters in batch fermentation with freely suspended cells. However, at 0.257 g xylose g DCW<sup>-1</sup> h<sup>-1</sup>, the maximum specific



**Figure 8.** Comparison of experimentally observed (symbols) and model predicted (solid lines) concentration profiles for batch fermentation with broth recycle (i.e., run SLM-2).

rate of xylose consumption ( $q_{xy}^m$ ) calculated using an immobilized biomass concentration of 3.89 g DCW<sup>-1</sup> L<sup>-1</sup> in this work, was lower than the value that has been reported<sup>11</sup> for fermentation with freely suspended cells. For the latter,  $q_{xy}^m$  is 0.342 g xylose g DCW<sup>-1</sup> h<sup>-1</sup>.

At 0.90, the maximum conversion yield of xylose to xylitol ( $Y_{P/S}^{\max}$ ) in Table 4 is identical to the theoretical yield for aerobic free suspension fermentation when only the NADPH-dependent xylose reductase functions.<sup>29</sup> The estimated effectiveness factor ( $\eta$ ) value of 0.20 confirmed that diffusion of xylose limited this immobilized

**Table 4.** Estimated values of model parameters

Parameter	Value	Units
$r_{\text{xyt}}^m$	1.00	g xylose L <sup>-1</sup> h <sup>-1</sup>
$K_{\text{xyt}}$	10.00	g xylose L <sup>-1</sup>
$Y_{P/S}^{m\max}$	0.90	g xylitol g xylose <sup>-1</sup>
$\eta$	0.20	–

The mean weighted square error of parameter estimation, SSE, was 0.958.

cell fermentation. The observed rate of xylose consumption was only about 20% of that expected in a fermentation with freely suspended cells, a system without diffusion limitation. Clearly, it is necessary to improve the xylose mass transport in LEMs to achieve an immobilized cell fermentation performance that is comparable to that of freely suspended cells.

## CONCLUSIONS

Liquid emulsion membrane (LEM) systems of soybean oil and paraffin were investigated for use in immobilized cell fermentations of xylose to xylitol. Stable formulations could be developed both for soybean oil LEM and liquid paraffin LEM. The poor xylose transport characteristics of liquid paraffin LEM made its use for fermentation impractical. Soybean oil LEM permitted sufficiently good transport of xylose, for successful use in immobilized cell fermentation. Yeast cells were immobilized in the inner phase of the LEM. The LEM containing the immobilized cells was supported on the inside wall of a tubular loop bioreactor, as unsupported LEMs were unstable in a mechanically agitated bioreactor. The optimal soybean oil LEM formulation had 5% microwaxes in the membrane oil phase and 5% lanolin in the inner aqueous phase. The optimal volume ratio of membrane to inner phases was 1 : 2.

A mathematical model invoking diffusion limitation of xylose in the immobilized cell system satisfactorily fitted the measured concentration profiles of xylose and xylitol during the fermentation. The model substantiated the existence of a significant diffusion limitation. Aerobic fermentation afforded greater xylitol productivity in comparison with oxygen limited fermentation, but the yield of xylitol on the substrate was lower in aerated fermentations.

The optimally formulated soybean oil LEM system stably encapsulated the cells; however, diffusive transport of the substrate and product in the LEM membrane phase need to be improved substantially for this system to be as effective as a free-suspension cell fermentation.

## ACKNOWLEDGEMENTS

This work was supported by Thailand Research Fund (TRF) under the research grant MRG485S020 awarded in 2005. The authors are grateful to Assistant Professor Dr Pakamon Chitprasert, Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, for valuable guidance particularly on emulsion systems.

## REFERENCES

- Pepper T and Olinger PM, Xylitol in sugar free confection. *Food Technol* **42**:98–104 (1988).
- Uhari M, Tapiainen T and Kontiokari T, Xylitol in preventing acute otitis media. *Vaccine* **19**:144–147 (2000).

- Mattila PT, Svanberg MJ, Jamsa T and Knuutila MLE, Improved bone biochemical properties in xylitol-fed aged rats. *Metabolism* **51**:92–96 (2002).
- Nigam P and Singh D, Processes for fermentative production of xylitol – a sugar substitute. *Process Biochem* **30**:117–124 (1995).
- Saha BC and Bothast RJ, Microbial production of xylitol. *ACS Symp Ser* **666**:307–319 (1997).
- Winkelhausen E and Kuzmanova S, Microbial conversion of D-xylose to xylitol. *J Ferment Bioeng* **86**:1–14 (1998).
- Granstrom TB, Izumori K and Leisola M, A rare sugar xylitol. Part II. Biotechnological production and future applications of xylitol. *Appl Microbiol Biotechnol* **74**:273–276 (2007).
- Povelainen M and Miasnikov AN, Production of xylitol by metabolically engineered strains of *Bacillus subtilis*. *J Biotechnol* **128**:24–31 (2007).
- Granstrom TB, Izumori K and Leisola M, A rare sugar xylitol. Part I. The biochemistry and biosynthesis of xylitol. *Appl Microbiol Biotechnol* **74**:277–281 (2007).
- Sirisansaneeyakul S, Staniszewski M and Rizzi M, Screening of yeast for production of xylitol from D-xylose. *J Ferment Bioeng* **80**:565–570 (1995).
- Tochampa W, Sirisansaneeyakul S, Vanichsriratanana W, Srinophakun P, Bakker HHC and Chisti Y, A model of xylitol production by the yeast *Candida mogii*. *Bioprocess Biosyst Eng* **28**:175–183 (2005).
- Willaert RG, Baron GV and De Backer L (eds), *Immobilised Living Cell Systems: Modelling and Experimental Methods*. Wiley, Chichester (1996).
- Patnaik PR, Liquid emulsion membranes: principles, problems and applications in fermentation processes. *Biotechnol Adv* **13**:175–208 (1995).
- Simmons DK, Sheldon WM and Pradeep KA, Enzymes in liquid membrane: reaction and bioseparation. *ACS Symp Ser* **419**:108–129 (1990).
- Melzner D, Tilkowski J, Mohrmann A, Poppe W, Halwach W and Scholler K, Selective extraction of metal by the liquid membrane technique. *Hydrometallurgy* **13**:105–123 (1984).
- Scholler C, Chaudhuri JB and Pyle DL, Emulsion liquid membrane extraction of lactic acid from aqueous solution and fermentation broth. *Biotechnol Bioeng* **42**:50–58 (1993).
- Kakoi T, Horinouchi N, Goto M and Nakashio F, Selective recovery of palladium from a simulated wastewater by liquid surfactant membrane process. *J Membr Sci* **118**:63–71 (1996).
- Lee SC, Lee KH, Hyun GH and Lee WK, Continuous extraction of penicillin G by an emulsion liquid membrane in a counter current extraction column. *J Membr Sci* **24**:43–51 (1997).
- Mohan RR and Li NN, Nitrate and nitrite reduction by liquid membrane encapsulated whole cells. *Biotechnol Bioeng* **17**:1137–1156 (1975).
- Prichanont S, Leak DJ and Stuckey DC, The solubilization of mycobacterium in water in oil microemulsion for biotransformation: system selection and characterization. *Colloids Surfaces A* **166**:177–186 (2000).
- Pal P, Datta S and Bhattacharya P, Multi-enzyme immobilization in eco-friendly emulsion liquid membrane reactor a new approach to membrane formulation. *Sep Purif Technol* **27**:145–154 (2002).
- Fox C, Cosmetic emulsions, in *Emulsions and Emulsion Technology*, Part 2, ed. by Lissant KJ. Dekker, New York, pp. 701–933 (1974).
- Griffin WC and Lynch MJ, Surface active agents, in *Handbook of Food Additives* (2nd edn), ed. by Furia TE. CRC Press, Boca Raton, FL, pp. 397–429 (1972).
- Deschatelets L and Yu EKC, A simple pentose assay for biomass conversion study. *Appl Microbiol Biotechnol* **24**:379–397 (1986).
- Kedari CS, Pandit SS, Misra SK and Ramanujam A, Mass transfer mechanism of the carrier-facilitated transport of uranium (VI) across 2-ethylhexyl phosphonic acid mono-2-ethylhexyl ester immobilized liquid membranes. *Hydrometallurgy* **62**:47–45 (2001).
- Alder L and Gustafsson L, Polyhydric alcohol production and intracellular amino acid pool in relation to halotolerance of the yeast *Debaryomyces hansenii*. *Arch Microbiol* **124**:123–130 (1980).
- McClements DJ, *Food Emulsions: Principles, Practice and Techniques*. CRC Press, Boca Raton, FL (1999).
- Dickinson E and Stainsby G, *Colloids in Food*. Applied Science, London (1982).
- Barbosa MFS, de Medeiros MB, Mancilha IM, Schneider H and Lee H, Screening of yeasts for production of xylitol from D-xylose and some factors which affect xylitol yield in *Candida guilliermondii*. *J Ind Microbiol* **3**:241–251 (1988).