Student Collaboration in a Series of Integrated Experiments to Study Enzyme Reactor Modeling with Immobilized Cell-Based Invertase

Experiment 2: Modeling of enzymatic reactors

Supporting Information

Instructors Notes

The experimental work described in the main manuscript as Experiment 2 is lectured as the second part of an integrative study in enzyme catalysis and application, and involves two laboratory sessions of three hours each (preparation and operation).

The main goal of this activity is to evaluate the performances of a CSTR (Continuous Stirred Tank Reactor), a PFR (Plug Flow Reactor) and a FBR (Fluidized Bed Reactor) containing invertase from *Saccharomyces bayanus* cells immobilized in calcium alginate, operating in continuous mode at optimal conditions (45°C and pH 4.5). For each type of reactor, an experimental conversion profile as a function of the feed flow rate imposed is established and this profile is compared with predicted theoretical profiles (according to equations 2 and 3 in the manuscript), based upon kinetic parameters (for free and immobilized enzyme) obtained by the students in two previous laboratory classes.

The two laboratory sessions (preparation and operation) are scheduled for 3 groups of students (each studying one type of reactor) with 3-4 students/group. At the end, the groups share the results for overall analysis/discussion in the written report.

Materials

Lyophilized *Saccharomyces bayanus* yeast cells for champagne production were purchased from Lallemand Inc. (Montreal, Canada); D-Sucrose, sodium alginate and sodium-potassium tartrate were obtained from BDH-Prolabo (VWR International, Radnar, PA/USA). D-Glucose, 3,5-dinitrosalicylic acid and sodium hydroxide were purchased from Merck (Darmstadt, Germany) and acetic acid, sodium acetate and calcium chloride were from Panreac (Castellar del Vallès, Spain). All reagents needed are commercially available products of analytical grade, from the above specified or alternative suppliers.

The following solutions are prepared beforehand (by a Lab technician) and supplied to the students:

- 20 mM acetate buffer pH 4.5 with 1% (w/v) $CaCl_2$
- 2% (w/v) $CaCl_2$
- Sucrose solution 10 $g.L^{-1}$ in 20 mM acetate buffer pH 4.5 with 1% (w/v) $CaCl_2$

• DNS reagent.

Table A1 in Appendix 1 summarizes the CAS-numbers for all the reagents used as well as links to safety data information.

Equipment

The equipment necessary includes:

- One jacketed glass vessel reactor ($V_T \approx$ 30 mL) with a rubber lid with double perforation, one jacketed glass column reactor ($d_{int} \approx$ 1 cm; L≈ 28.5 cm) and one jacketed glass column reactor ($d_{int} \approx$ 2.3 cm; L≈ 18 cm). The reactors used in this lab activity were made at the faculty glass workshop. However, similar glass equipment can be commercially acquired from laboratory glassware suppliers.
- One peristaltic pump (*e.g.*, Watson Marlow 181, for flow rates of 1 to 5 mL min^{-1})
- One gear pump (*e.g.*, ISMATEC Reglo-Z 121, for flow rates of 5-100 $mL.min^{-1}$)
- Silicon tubing with varied cross-sectional areas, plastic connectors/reductors and Hoffman tweezers.
- One magnetic stirrer
- One thermo circulator (*e.g.,* BBraun® Thermomix M)
- One dry bath or a boiling water bath and a pan.
- Vortex mixers
- One (or more) UV/Vis spectrophotometer (*e.g.,*T80 from PG Instruments Ltd) for absorbance readings
- Automatic pipettes
- Glass tubes and supports
- Chronometers
- Measuring cylinders (10, 25, 50 and 100 mL)

The experimental setups for all reactors are prepared before the classes by a Laboratory technician and are shown in Figures 1-3.

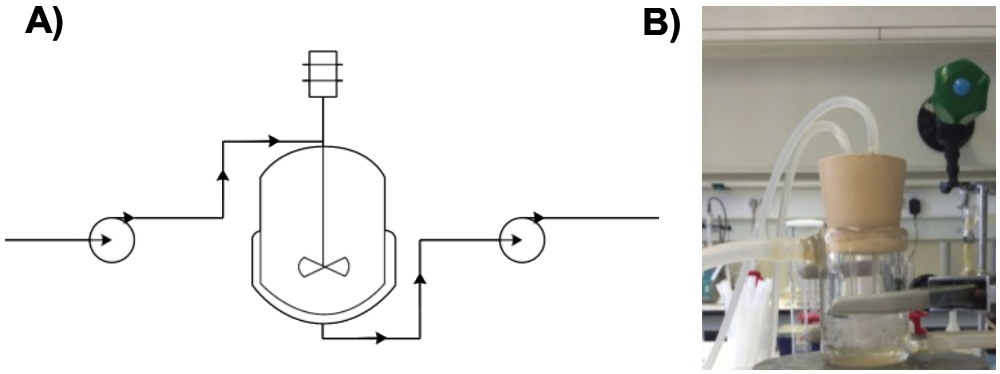

Figure 1- **A)** Schematic representation of CSTR; **B)** Experimental setup used in the laboratory sessions: a jacketed glass vessel reactor ($V_T \approx$ 30 mL) connected to a water bath, and a perforated rubber lid, with inlet and outlet silicone tubes (of appropriate internal and external diameter) connected to a peristaltic pump (flow rate 1-5 mL.min^{-1}). Inside: alginate beads containing *S. bayanus* cells.

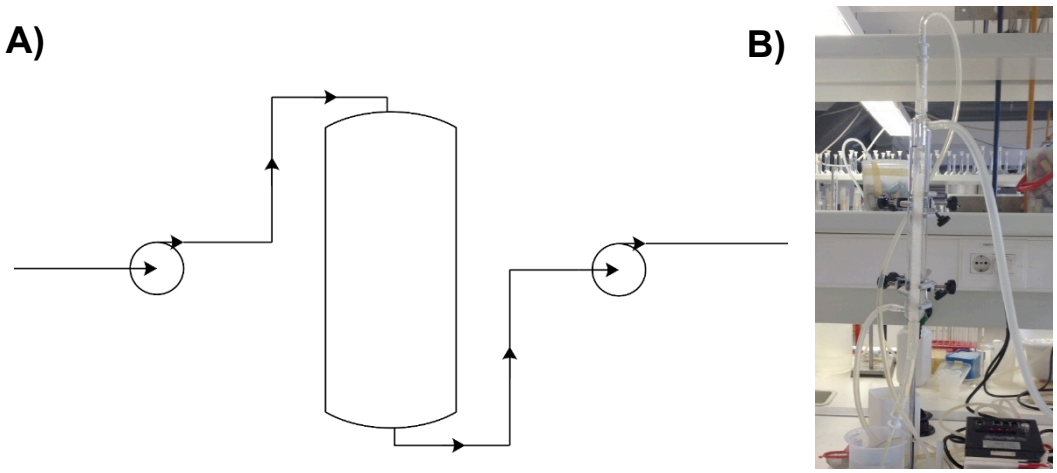

Figure 2- **A)** Schematic representation of PFR; **B)** Experimental setup used in the laboratory sessions: a jacketed glass column reactor ($d_{int} \approx$ 1 cm; L≈ 28.5 cm) connected to a water bath. The inlet (top) and outlet (bottom) are connected to a peristaltic pump (flow rate 1-5 mL.min^{-1}). Inside: alginate beads containing *S. bayanus* cells.

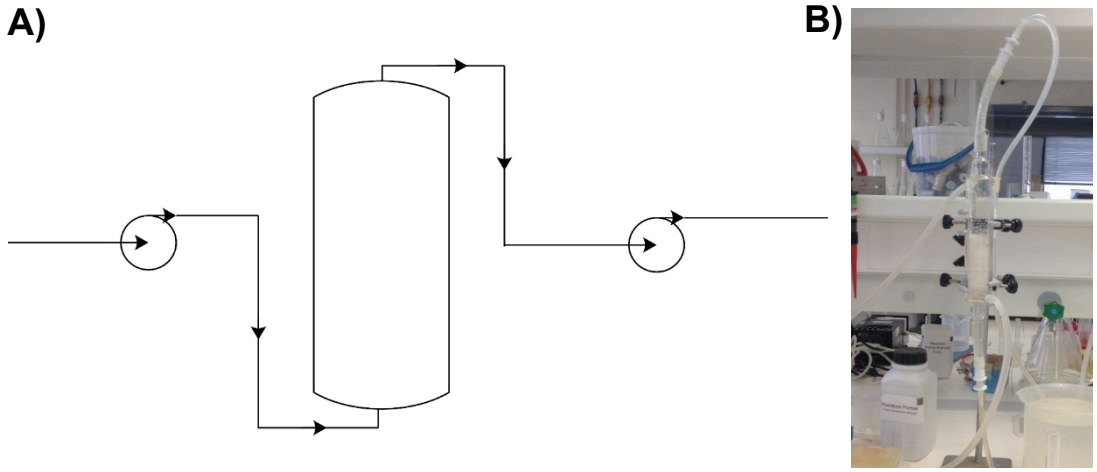

Figure 3 - **A)** Schematic representation of FBR; **B)** Experimental setup used in the laboratory sessions: a glass column reactor ($d_{int} \approx$ 2.3 cm; L≈ 18 cm) with a jacket connected to a water bath with the inlet (bottom) connected to a gear pump (flow rate 10-100 mL.min^{-1}). Outlet is at the top, discharging the liquid into a container. Inside: alginate beads containing *S. bayanus* cells.

Methods

Determination of total reducing sugars (TRS)

The dinitrosalicylic acid (DNS) reagent method for determination of reducing sugars is used for the detection of fructose and glucose (products of reaction, designated as total reducing sugars or TRS). This method is based on the formation of a colorimetric complex resulting from the reduction of DNS, which absorbs at 540 nm (*see* [1,2]).

Protocol: To 0.5 mL of sample, 0.5 mL of DNS reagent is added and the mixture is heated for 5 min in boiling water or a dry bath at 100ºC. After cooling the sample under running tap water at room temperature, 5 mL of distilled water is added and the absorbance at 540 nm is read against a blank of the sucrose solution subjected to the same treatment. The absorbance is converted into total reducing sugars (TRS) concentration through a calibration curve obtained with solutions of glucose with known concentrations (0.2, 0.4, 0.6, 0.8, 1.0 $mg.mL^{-1}$) in 20 mM acetate buffer pH 4.5 (supplied by the lab technician or obtained by the students in a previous session).

Preparation of the DNS reagent

Dissolve 5 g of 3,5-dinitrosalicilic acid in 100 mL of 2N sodium hydroxide, at room temperature; Then add 500 mL of distilled water and 300 g of sodium-potassium tartrate. Fill the solution with distilled water up to 1 L and let it dissolve to clear orange solution.

Calibration curve

(The calibration curve for DNS is either obtained by the students is a previous lab session or supplied by the lab technician)

Table S1. Absorbance at 540 nm of different glucose standard solutions.

Glucose (g/L)	Abs 540 nm
0.0	0.000
0.2	0.112
0.4	0.241
0.6	0.352
0.8	0.491
1.0	0.592

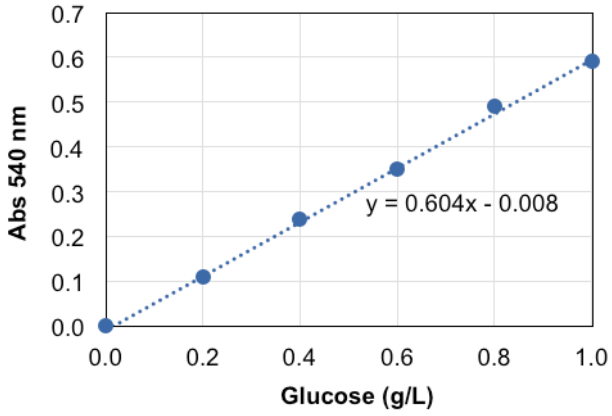

Figure S1. Calibration curve for the DNS method.

First laboratory session for reactor modeling (preparation)

(All the data presented were gathered from students Excel files/reports)

1. Measure of volumetric flow rates: pump calibration

The first thing students do in the lab is to regulate the pumps (associated with each reactor) at different rotation speeds in order to operate at the desired flow rates for the study in continuous operation. Two to three measures are made for each flow rate and the speed (usually given as a percentage) to impose a given flow rate is registered.

Material necessary: a measuring cylinder (10-15 mL) and a chronometer

Table S.1 – Examples of flow rate measures obtained with a Watson Marlow 181 peristaltic pump, used for the operation of CSTR and PFR, at different rotation speeds displayed as a % of maximum speed.

Pump % V (mL)	t (min)	Q ($mL.min^{-1}$)
15.7		
2	2.078	0.9625
2	2.055	0.973
2	2.008	0.996
32.6		
2	0.985	2.030
2	0.894	2.237
49.4		
2	0.653	3.063
2	0.595	3.361
66.2		
2	0.469	4.264
2	0.4608	4.340
85.1		
2	0.361	5.540
2	0.372	5.376

Table S.2 – Examples of flow rate measures obtained with an ISMATEC Reglo-Z 121 gear pump, used for operation of the FBR, at different rotation speeds displayed as a % of maximum speed.

Pump V (mL)	t (min)	Q (mL.min^{-1})
5%		
5	1.38	3.614
5	1.37	3.658
5	1.37	3.658
8%		
5	0.70	7.100
5	0.74	6.775
5	0.74	6.732
10%		
5	0.41	12.165
5	0.42	12.029
5	0.41	12.245
14%		
5	0.28	17.910
5	0.27	18.856
5	0.29	17.113
18%		
10	0.33	30.000
10	0.34	29.455
22%		
10	0.26	38.314
10	0.27	37.612
26%		
10	0.22	45.283
10	0.22	46.261
10	0.21	47.059
30%		
10	0.17	58.366
10	0.17	60.000
10	0.17	60.000
35%		
10	0.13	74.719
10	0.13	75.282
10	0.13	75.949
40%		
20	0.26	77.121
20	0.26	76.775
20	0,26	78.023

Note: The numeric digits in the tables do not follow the rules of significant digits as students utilize these data for intermediate calculations (in Excel spreadsheets).

With the data in Tables S1-S3 a linear variation between the rotation speed (displayed as a '%' of maximum speed in the pump) and the volumetric flow rate – commonly referred as the pump calibration – is established (Figure S1). From the linear regression equation, the % of rotation to obtain a given flow rate is obtained.

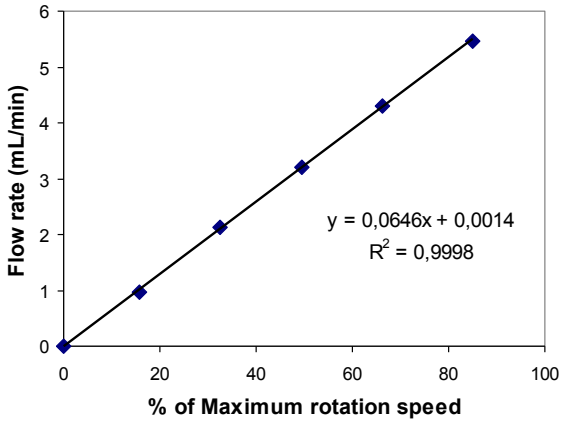

Figure S1 – Example of a calibration curve for a Watson Marlow peristaltic pump, used for operation of CSTR and PFR.

The pump calibration curves are used in the second section to operate the different types of reactors at desired flow rates (1-5 $mL.min^{-1}$ for CSTR and PFR; 5-100 $mL.min^{-1}$ for FBR).

2. Estimation of Cross-Sectional Area for FBR

The cross-sectional area (A) of FBR is estimated by flowing off a volume of buffer corresponding to a known column height (Ex. Δh=3-5 cm marked on the wall of the column reactor) into a measuring cylinder. Two to three measures should be made and the average of $\Delta V/\Delta h$ calculated.

The total volume of the reactor is given by the product $A \times h$, where h is the total bed height (liquid + alginate beads).

The value of A is necessary for estimation of the minimum fluidization velocity for the FBR (an additional exercise explained ahead).

3. Immobilization of *S. bayanus* cells

Follow the information in the *Students Handout* 2 (A study with invertase from *Saccharomyces bayanus* cells immobilized in calcium alginate. 2. Modeling of enzymatic reactors). Yeast cells (containing invertase) are immobilized in calcium alginate in the "Preparation session" and stored at 4ºC in 2%(w/v) calcium chloride solution until the next session (operation).

Second laboratory session for reactor modeling (operation)

(All the data presented were gathered from students Excel files/reports)

In this laboratory session, students fill up the reactors with the immobilized enzyme and study the operation in continuous for the flow rates established in the preparatory session. The experimental procedures are described in detail in the Students Handout 2.

CSTR

For operation studies, the alginate beads are suspended in 10 mL of the sucrose feed solution, and the reactor is operated with a peristaltic pump connected to both the inlet and outlet streams at flow rates of 1-5 $mL.min^{-1}$. The experimental setup for the CSTR is depicted in Figure 1.

PFR

The reactor is filled with the alginate beads in substrate solution or 20 mM acetate buffer pH 4.5 (see Students Handout 2). After packing, the volume of liquid is adjusted to 0.5-1.0 cm above the bed height (Figure 2) and the reactor operated as referred above for CSTR. The experimental setup for the PFR is depicted in Figure 2.

FBR

The calcium alginate beads are packed in the reactor, and the column is then filled up with feed solution at a low flow rate (≤ 10 mL min^{-1}) to prevent bed fluidization. The reactor is operated for flow rates in the range of 5-100 $mL.min^{-1}$. The experimental setup for the FBR is represented in Figure 3.

Sampling

For all reactors, the samples removed from the outlet stream at steady state for each flow rate are analysed by the DNS method to determine the Total Reducing Sugar (TRS) concentration. Generally, samples from lower flow rates have to be diluted in order to fit in the calibration curve (typical dilutions for each type of reactor are given bellow in Tables S.5, S.8 and S.11).

The results obtained from DNS analysis of the samples allow the calculation of an experimental conversion degree (X) for each flow rate:.

$$x = \text{Moles of sucrose converted} / \text{Moles of initial sucrose}$$

Table S.3 – Parameters necessary to calculate experimental conversion

Parameters		
[Sucrose] feed ($g.L^{-1}$)	10	
Sucrose Molecular Weight ($g.mol^{-1}$)	342	
Glucose/Fructose Molecular Weight ($g.mol^{-1}$)	180	
TRS Trendline		
Abs (540 nm)= 0.604 x TRS ($g.L^{-1}$) – 0.008 **		

** This trendline is either obtained by the students in a previous laboratory session or supplied by the Lab technician (*See* Notes for Instructors 1: "A study with invertase from *Saccharomyces bayanus* cells free and immobilized in calcium alginate. 1. Determination of kinetic parameters").

Example of calculation of one experimental conversion:

(*See* Table S.5)

The moles of sucrose converted are equal to moles of total reduced sugars obtained (glucose + fructose) that are indistinctly detected by the DNS method. Therefore, for an initial absorbance at 540 nm of 0.214 (with a tenfold dilution):

$$(0.214 + 0.008)/0.604) \times 10 = 3.675\ gTRS.L^{-1}$$ (in the outlet stream at stationary state)

Considering the example of a residual TRS concentration of 0.040 $g.L^{-1}$ measured in the feed solution (10 $g.L^{-1}$ in sucrose):

$$x = \{(3.675-0.04) \times 342/360\} /10 = 0.345$$

where 360 Da is the combined molecular weight of both sugars (glucose + fructose) and 342 Da is the sucrose molecular weight.

For all reactors

The working volume (which is ≈10 mL in all cases) should be measured and confirmed at the end of operation (by draining the volume of liquid in the reactor into a measuring cylinder).

Reactor Modeling

Students obtain the data required for reactor modeling in two previous laboratory sessions (*See* Students Handout 1 and Instructors Notes 1). The kinetic parameters obtained for free enzyme (intrinsic enzyme parameters) are used to model ideal reactors whereas the

corresponding parameters for the immobilized enzyme (apparent enzyme parameters) allow the modeling of real reactors.

Table S.4 – Kinetic data necessary for reactor modeling.

Kinetic Modeling Data*		
	Ideal	**Real**
Vmax.specific = v_w (g Suc.g cell^{-}min^{-1})	0.650	0.425
KM (g Suc/L)	11.7	19.0

*Data of Table 1 in the main manuscript, obtained by the students in two previous sessions

Note: The data for reactor modeling presented bellow are represented in Figure 3 of the main manuscript.

CSTR

1. Calculation of experimental conversion for each flow rate

Table S.5 - Experimental conversion as a function of feed flow rate for the CSTR.

Experimental data				
Q_{mean} (mL.min^{-1})*	**A540**	**Dilution factor**	**C (g TRS.L^{-1})**	**Experimental TRS Conversion**
Blank	0.000	-	0	-
Sucrose Solution 10 g/L	0.016	-	0.040	-
0.977	0.214	10	3.675	0.345
2.134	0.137	10	2.401	0.224
3.212	0.089	10	1.606	0.149
4.302	0.178	5	1.540	0.143
5.458	0.156	5	1.358	0.125

* **Note: Q enters in the modeling equation in L.min^{-1}**

2. Modeling of an ideal CSTR

Using the model equation of CSTR (eq. 2 in the main manuscript), with the kinetic parameters for the free enzyme and the Solver Tool of the Excel find the theoretical values of conversion that solve the equation.

Table S.6 – Modeling of an ideal CSTR.

Ideal Reactor Model		
v_w.W/Q (gSuc.L^{-1})	**S_0.x+Km.(x/1-x) (gSuc.L^{-1})**	**TRS Conversion to equal the two expressions**
-	-	-
-	-	-
33.257	33.257	0.692
15.231	15.231	0.473
10.118	10.118	0.358
7.554	7.554	0.286
5.954	5.954	0.235

W (mass of immobilized cells; see Immobilization section in Students Protocol)

3. Modeling of a real CSTR

Using the model equation of CSTR (eq. 2 in the main manuscript), with the kinetic parameters for the immobilized enzyme and the Solver Tool of the Excel find the theoretical values of conversion that solve the equation.

Table S.7 – Modeling of a real CSTR.

Real Reactor Model		
v_w.W/Q (gSuc.L^{-1})	S_0.X+Km.(X/1-X) (gSuc.L^{-1})	TRS Conversion to equal the two expressions
-	-	-
-	-	-
21.745	21.745	0.473
9.959	9.959	0.275
6.616	6.616	0.197
4.939	4.939	0.152
3.893	3.893	0.123

W (mass of immobilized cells; see Immobilization section in Students Protocol)

PFR

1. Calculation of experimental conversion for each flow rate

Table S.8 - Experimental conversion as a function of feed flow rate for the PFR

Q_{mean} (L.min^{-1})	A540	Dilution factor	C (g TRS.L^{-1})	Experimental TRS Conversion
Blank	0.000	--	0	-
Sucrose Solution 10 g.L^{-1}	0.016	---	0.040	-
0.001	0.401	10	6.772	0.640
0.002	0.272	10	4.636	0.437
0.003	0.208	10	3.576	0.336
0.004	0.317	5	2.690	0.252
0.005	0.275	5	2.343	0.219

2. Modeling of an ideal PFR

Using the model equation of PFR (eq. 3 in the main manuscript), with the kinetic parameters for the free enzyme and the Solver Tool of the Excel find the theoretical values of conversion that solve the equation.

Table S.9 – Modeling of an ideal PFR.

Ideal Reactor Model		
v_w.W/Q (gSuc.L^{-1})	S_0.x-Km.ln(1-x) (gSuc.L^{-1})	TRS Conversion to equal the two expressions
-	-	-
-	-	-
97.5000	97.5000	≈0.999
48.7500	48.7500	0.965
32.5000	32.5000	0.869
23.5625	23.5625	0.747
19.5000	19.5000	0.666

W (mass of immobilized cells; see Immobilization section in Students Protocol

3. Modeling of a real PFR

Using the model equation of PFR (eq. 3 in the main manuscript), with the kinetic parameters for the immobilized enzyme and the Solver Tool of the Excel find the theoretical values of conversion that solve the equation.

Table S.10 – Modeling of a real PFR

Real Reactor Model		
v_w.W/Q (gSuc.L^{-1})	S_0.x-Km.ln(1-x) (gSuc.L^{-1})	TRS Conversion to equal the two expressions
-	-	-
-	-	-
63.7500	63.7500	0.943
31.8750	31.8750	0.726
21.2500	21.2500	0.561
15.4063	15.4063	0.440
12.7500	12.7500	0.377

W (mass of immobilized cells; see Immobilization section in Students Protocol)

FBR

A similar approach is used for the FBR using equation 3 in the main manuscript as the model equation.

1. Calculation of experimental conversion for each flow rate

Table S.11 - Experimental conversion as a function of feed flow rate for the FBR

Experimental data				
Q_{mean} (mL.min^{-1})*	**A540**	**Dilution factor**	**C (g TRS.L^{-1})**	**Experimental TRS Conversion**
Blank	--	--	0	-
Sucrose Solution 10 g.L^{-1}	0.014	--	0.036	-
3.644	1.104	2	3.682	0.346
6.869	0.72	2	2.411	0.226
12.146	0.387	2	1.308	0.121
17.960	0.304	2	1.033	0.095
29.728	0.287	1	0.488	0.043
37.978	0.234	1	0.401	0.035
46.201	0.199	1	0.343	0.029
59.455	0.179	1	0.310	0.026
75.317	0.149	1	0.260	0.021
77.307	0.124	1	0.219	0.017
89.022	0.104	1	0.185	0.014
99.728	0.091	1	0.164	0.012

*** Note: Q enters in the modeling equation in L.min^{-1}**

2. Modeling of an ideal FBR

Using the model equation of FBR (eq. 3 in the main manuscript), with the kinetic parameters for the free enzyme and the Solver Tool of the Excel find the theoretical values of conversion that solve the equation.

Table S.12 – Modeling of an ideal FBR.

Ideal Reactor Model		
v_w.W/Q (g Suc.L^{-1})	**S_0.x-Km.ln(1-x) (g Suc.L^{-1})**	**TRS Conversion to equal the two expressions**
-	-	-
-	-	-
35.677	35.677	0.898
18.925	18.925	0.653
10.703	10.703	0.424
7.238	7.238	0.302
4.373	4.373	0.190
3.423	3.423	0.151
2.814	2.814	0.125
2.187	2.187	0.098
1.726	1.726	0.078
1.682	1.682	0.076
1.460	1.460	0.066
1.304	1.304	0.059

W (mass of immobilized cells; see Immobilization section in Students Protocol)

3. Modeling of a real FBR

Using the model equation of FBR (eq. 3 in the main manuscript), with the kinetic parameters for the immobilized enzyme and the Solver Tool of the Excel find the theoretical values of conversion that solve the equation.

Table S.13 – Modeling of a real FBR

Real Reactor Model		
v_w.W/Q (g Suc.L^{-1})	S_0.x-Km.ln(1-x) (g Suc.L^{-1})	TRS Conversion to equal the two expressions
-	-	-
-	-	-
23.327	23.327	0.599
12.374	12.374	0.367
6.998	6.998	0.222
4.733	4.733	0.154
2.859	2.859	0.095
2.238	2.238	0.075
1.840	1.840	0.062
1.430	1.430	0.049
1.129	1.129	0.038
1.100	1.100	0.037
0.955	0.955	0.033
0.852	0.852	0.029

W (mass of immobilized cells; see Immobilization section in Students Protocol)

Overall conversion data analysis

Globally, the conversions obtained for the FBR are lower than the ones obtained for the CSTR and PFR, since the flow rates used to achieve bed fluidization were much higher (10-100 $mL.min^{-1}$) than those used for CSTR and PFR operation (1-5 $mL.min^{-1}$). The highest conversions are observed in the PFR where the substrate concentration varies with the length of the reactor. For first order kinetics, a higher (average) concentration of substrate is generally achieved in PFR what turns out this reactor kinetically more favourable than the CSTR for the same enzymatic reaction (*see* [3,4]).

However, direct comparison of absolute values of conversion obtained for PFR and CSTR in this experiment should be made with care as the total amount of cells in the reactor was not exactly the same. The mass of cells/g alginate (which affects mass transfer across gel beads) was kept constant in all 3 reactors (500 mg cells g $Alginate^{-1}$) but, due to differences in the geometry of the three types of reactors, the amount of gel (and consequently of cells) was not exactly the same in all cases (for details please see Students Handout 2).

Estimation of the minimum fluidization velocity for the FBR

In a FBR, the fluidization of the solid-phase, in this case the alginate beads, occurs when the superficial velocity, U, of the liquid phase (feed) exceeds the terminal velocity of the particles, which are then suspended in the fluid. The minimum fluidization velocity, U_{mf}, characterizes each FBR and represents the minimum superficial velocity needed to obtain the fluidization of the solid phase (*see* [4-6]).

In this lab exercise, students can also estimate the U_{mf}, by applying the empirical correlation of Richardson and Zaki. This equation predicts that the superficial velocity of the fluid phase in a porous bed depends on its voidage, ε, and that the dependence of U on ε is linear in a *ln U vs ln* ε plot, where U_i and n are empirical parameters:

$$U = U_i \varepsilon^n \Leftrightarrow \ln U = \ln U_i + n \ln \varepsilon$$

The superficial velocity, *U,* is given by the ratio between the feed volumetric flow rate (Q) and the reactor cross-sectional area, *A (U=Q/A).* The void fraction (or voidage, ε_0) for the resting bed (before fluidization) is given by the ratio between the working volume and the total volume under these conditions:

$$\varepsilon_0 = \frac{V_0}{h_0 A}$$

where V_0 and h_0 represent the working volume and height of the resting bed, respectively. For high flow rates (Q_n), when bed fluidization occurs and the bed reaches a height h_n at stationary state, the void fraction, ε_n, is calculated by the following equation :

$$\varepsilon_n = \frac{h_n A - (h_0 A - V_0)}{h_n A}$$

where $h_n A$ is the total volume of the bed for a given flow rate and ($h_o A$-V_o) represents the volume of solid-phase (alginate beads).

The experimental data (bed height for different flow rates) and the calculation of U and ε are presented in Table S.14. The representation of *ln U vs ln ε* for several flow feeds for the FBR studied is represented in Figure S.2.

A minimum flow rate for bed fluidization of 33.4 mL.min^{-1} was estimated, corresponding to a minimum superficial velocity (U_{mf}) of 7.95 cm.min^{-1} and a ε_0 of 0.357.

Table S.14 – Values of bed heights measured in the stationary state for different flow rates and calculation of the Richardson-Zaki parameters.

h (cm)	Vtotal (mL)	ε	lnε	U (cm.min^{-1})	Q (mL.min^{-1})	lnU
6.0	25.2	0.357	-1.0297	7.969	33.47	2.076
6.4	26.9	0.397	-0.9231	9.914	41.64	2.294
6.8	28.6	0.433	-0.8375	12.631	53.05	2.536
7.1	29.8	0.457	-0.7837	14.210	59.68	2.654
7.5	31.5	0.486	-0.7222	15.719	66.02	2.755
7.8	32.8	0.506	-0.6822	17.769	74.63	2.878
8.3	34.9	0.535	-0.6249	19.460	81.73	2.968
8.6	36.1	0.552	-0.5951	21.650	90.93	3.075
9.1	38.2	0.576	-0.5515	23.810	100.00	3.170

Cross-sectional area = 4.2 cm^2 (estimated in the preparatory session)
h_0 = 6.0 cm; V_0 = 9.0 mL (measured at end of operation, after allowing the bed to rest until h_0)
(h_0A-V_0) = 16.2 mL (volume of alginate beads)

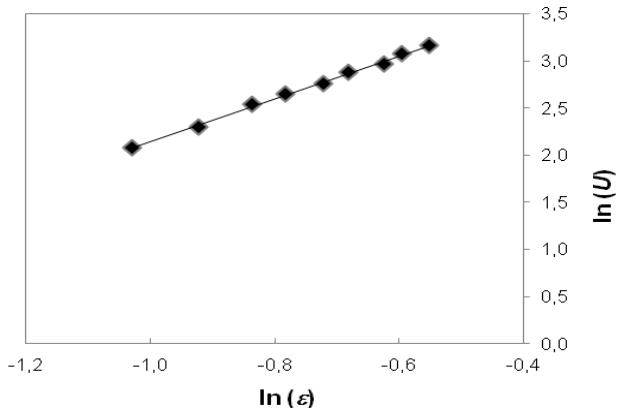

Figure S.2 - Linear flow velocity as a function of voidage: (◆) experimental data; (—) linear regression: ln(U) = 2.28ln(ε) + 4.42, R^2 = 0.998.

COMMON PROBLEMS

This experience is easily undertaken by Second-year/Upper-Division Undergraduate students with former laboratory experience. Main problems found are, sometimes, small variations in the inlet and outlet flow rates in some reactors (CSTR and PFR) which difficult the attainment of stationary (or steady) state. Such problems are easily surmounted by repositioning the tubes in the peristaltic pump. Also, in the immobilization procedure care must be taken to avoid air bubbles inside the beads. Air-containing alginate beads float in solution and should be removed before introduction in the reactors to avoid clogging of tubes (namely in FBR).

As this exercise is repeated in more than one lab session (or shift), if in one lab session, for a given type of reactor, the profile of experimental conversion *vs* the feed flow rate does not follow the expected behavior (which is common because *e.g*. the reactor was not in steady state when the sample(s) was(were) removed from the oulet stream or due to errors in the DNS analysis, *see* Supporting Information) students can obtain (share) the experimental profile of that type of reactor (to model) from the results obtained in a different lab shift.

[Note: Statistical analysis of values of experimental conversion for each flow rate, for a given type of reactor, implies that in the several lab sessions the reactor is operated at exactly the same feed flow rate values].

VERSATILITY

Although yeast cells and invertase are particularly suitable for teaching laboratory experiments, this laboratory activity can easily be adapted to other cell-contained enzyme systems that can be immobilized in calcium alginate.

COMPLEMENTARY STUDIES

An extension of this activity may be the study of operational stability of invertase. For this, one reactor (*e.g*., CSTR or PFR, at a low flow rate) is left in continuous operation after the operation class and groups are scheduled for sampling and analysis (of TRS by DNS) along a week to 10 days. It is observed that the enzyme is highly stable in operation and activity is approximately constant over this period. If a longer period of sampling is established, the half-time life of the enzyme can also be calculated.

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(Suggestions. Alternative books/papers may also be used/found by the instructors)

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APPENDIX 1

Table A1 – CAS-numbers and link to safety data of reagents used in the laboratory activities.

Compound	**Producer**	**CAS number**	**Material Safety Data Sheet**	**Classification**
Saccharomyces bayanus	Lallemand Inc. (Montreal, Canada)*			
D-Sucrose	BDH-Prolabo (VWR International, Radnar, PA/USA)	57-50-1	MSDS	Not hazardous
sodium alginate		9005-32-7	MSDS	Not hazardous
sodium-potassium tartrate		304-59-6	MSDS	Not hazardous
D-Glucose	Merck (Darmstadt, Germany)	50-99-7	MSDS	Not hazardous
3,5-dinitrosalicylic acid	Merck (Darmstadt, Germany)	609-99-4	MSDS	Acute Tox. 4; Skin Irrit. 2; STOT SE 3; H302, H315, H335
sodium hydroxide	Merck (Darmstadt, Germany)	1310-73-2	MSDS	Met. Corr. 1; Skin Corr. 1A; H290, H314
sodium acetate	Panreac (Castellar del Vallès, Spain	127-09-3	MSDS	Not hazardous
calcium chloride	Panreac (Castellar del Vallès, Spain	10043-52-4	MSDS	Eye Irrit. 2; H319
acetic acid	Panreac (Castellar del Vallès, Spain	64-19-7	MSDS	Flam. Liq. 3; Skin Corr. 1A; H226, H314

* Common baker yeast available at the supermarket can also be used.

(To see the safety data click simultaneously in MSDS and CRTL key)

Disposable of all reagents should be made according to recommended/common laboratory safety rules. Alginate beads containing yeast cells are processed as “solid residues” (go for incineration).