## Problem 2.1

A batch reactor containing 0.3% (w/v) glucose and a simple medium is inoculated with a culture grown in a medium of the same composition. Optical density (OD) at 420 nm as a function of time evolves as shown in the Table (column 1).

The same strain, this time cultivated in complex medium, is inoculated in another batch reactor containing a mixture of glucose 0.15% (w/v) and lactose 0.15% (w/v). The evolution of optical density, at 420 nm, as a function of time is represented in the Table (column 2). If there is a linear relationship between optical density and cell concentration where 0.175 (OD) corresponds to 0.1 mg cells per ml, calculate:

- a) The maximum specific growth rate,  $\mu_{max}$ ;
- b) The adaptation time,  $t_{lag}$ ;
- c) The growth yield coefficient, Yx/s, assuming complete substrate exhaustion in each case.
- d) Explain the shape of the curves in each case.

#### Problema 2.1.

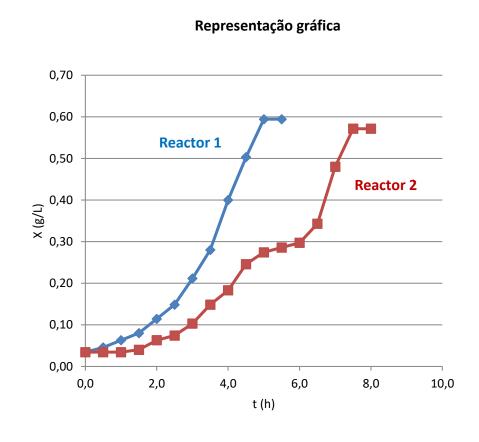
Reactor 1  $S_0 = 0.3\%$  (w/v) glucose

Reactor 2  $S_0 = 0.15\%$  (w/v) glucose + 0.15% (w/v) lactose

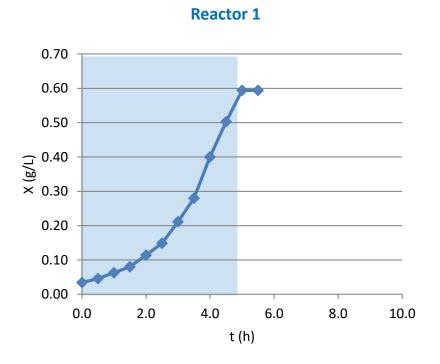
 $A = 0.175 \longrightarrow X = 0.1 \text{ mg/ml}$ 

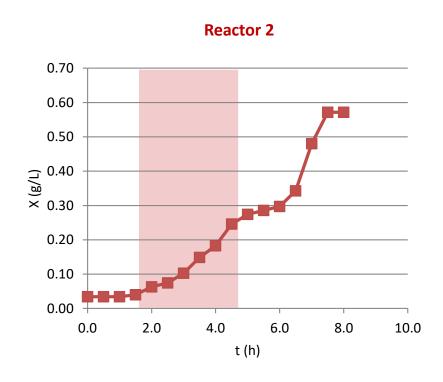
## 2.1.a) $\mu_{max}$

t (h)	DO (1)	X (1) (mg/mL)	DO (2)	X (2) (mg/mL)
0,0	0,06	0,03	0,06	0,03
0,5	0,08	0,05	0,06	0,03
1,0	0,11	0,06	0,06	0,03
1,5	0,14	0,08	0,07	0,04
2,0	0,20	0,11	0,11	0,06
2,5	0,26	0,15	0,13	0,07
3,0	0,37	0,21	0,18	0,10
3,5	0,49	0,28	0,26	0,15
4,0	0,35*	0,40	0,32	0,18
4,5	0,44*	0,50	0,43	0,25
5,0	0,52*	0,59	0,48	0,27
5,5	0,52*	0,59	0,50	0,29
6,0			0,52	0,30
6,5			0,30*	0,34
7,0			0,42*	0,48
7,5			0,50*	0,57
8,0			0,50*	0,57

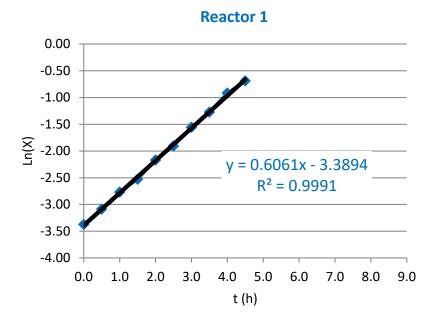


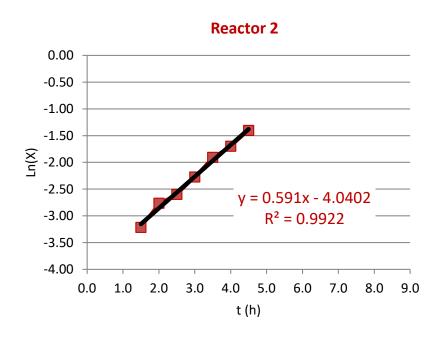
- $\Rightarrow$  Identify the cell growth phase
  - $\triangleright$  Reactor 1: 0 ~ 5h
  - ➤ Reactor 2: ~1.5 4.5h





⇒ Represent Ln(X) vs t ⇒ Slope

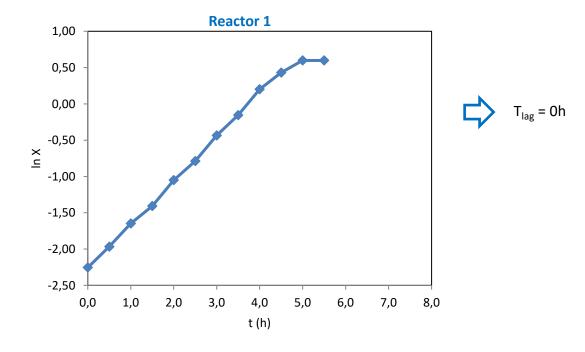




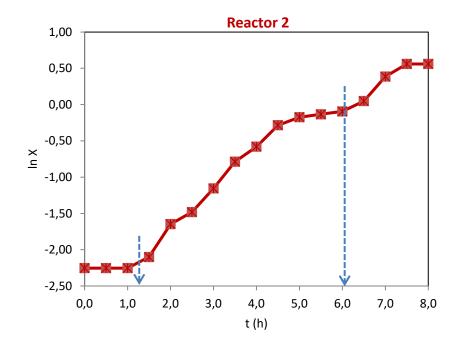
$$\mu_{max} = 0.61 \ h^{-1}$$

$$\mu_{max} = 0.59 \ h^{-1}$$

**2.1.b)** t<sub>lag</sub>



**2.1.b)** t<sub>lag</sub>



$$t_{lag} = 1.2h$$

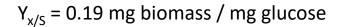
$$t_{lag} = ^5-6h$$

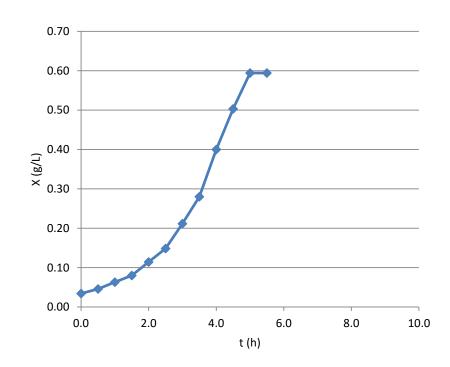
$$Y_{x/S} = \frac{\Delta x}{\Delta S}$$

# Reactor 1

$$\Delta$$
 x = 0.56 mg/mL

$$\Delta S = 0.3\%(w/v) = 3 \text{ mg/mL}$$



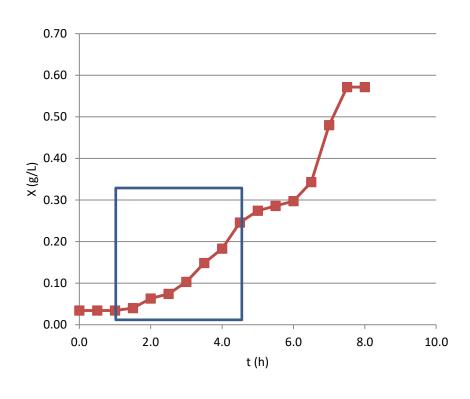


$$Y_{x/S} = \frac{\Delta x}{\Delta S}$$

Reactor 2

$$\Delta X (0-5h) = 0.24 \text{ mg/mL}$$

$$\Delta$$
 S<sub>1</sub> = 1.5 mg/mL



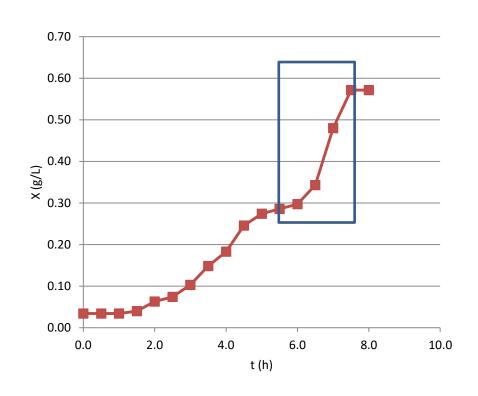
 $Y_{x/S}$  (1) = 0.17 mg biomass / mg substrato

$$Y_{x/S} = \frac{\Delta x}{\Delta S}$$

Reactor 2

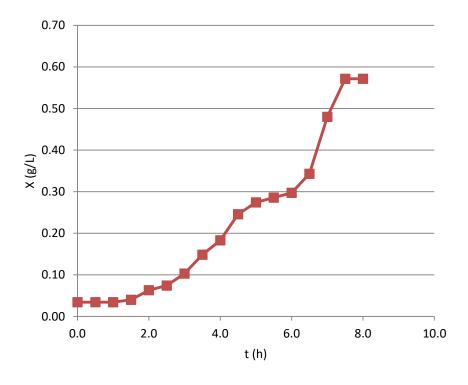
$$\Delta X (5.5-7.5h) = 0.29 \text{ mg/mL}$$

$$\Delta S_2 = 1.5 \text{ mg/mL}$$



$$Y_{x/S}$$
 (2)= 0.19 mg biomass / mg substrato

# 2.1.d) Reactor 2: diauxic growth



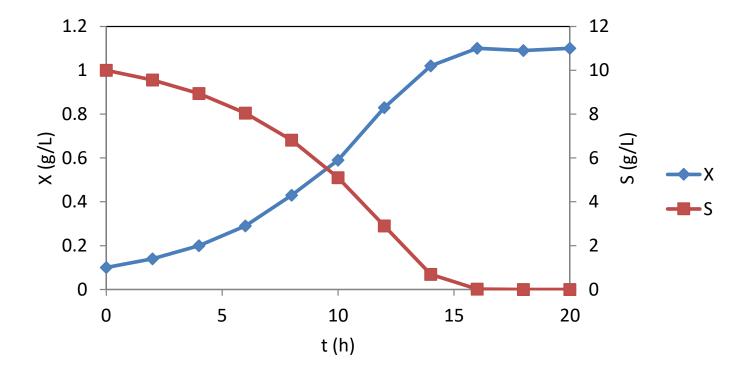
## Problem 2.2

A certain microorganism was cultured in a batch reactor for 20 h. Samples were taken every 2 hours to measure cell and substrate concentration. Assess the degree of substrate limitation over time and calculate the maximum specific growth rate as well as the Monod constant.

T(h)	X(g/L)	S(g/L)
0	0.10	10.00
2	0.14	9.56
4	0.20	8.94
6	0.29	8.05
8	0.43	6.81
10	0.59	5.10
12	0.83	2.90
14	1.02	0.69
16	1.10	0.02
18	1.09	0.00
20	1.10	0.00

ENGENHARIA BIOQUÍMICA I Folhas de Problemas 2

 $\Rightarrow$  X vs t e S vs t



$$\mu = \frac{dx}{dt} \cdot \frac{1}{x}$$

$$\mu_i = \frac{x_{i+1} - x_{i-1}}{t_{i+1} - t_{i-1}} \cdot \frac{1}{x_i} \longrightarrow \text{3 points rule}$$

$$\mu_i = \frac{x_i - x_{i-1}}{t_i - t_{i-1}} \cdot \frac{1}{x_i} \quad \longrightarrow \quad \text{Euler rulw}$$

			euler	3 ptos
t (h)	X (g/L)	S (g/L)		
0	0,10	10.00		
2	0,14	9,56		
4	0,20	8,94		
6	0,29	8,05		
8	0,43	6,81		
10	0,59	5,10		
12	0,83	2,90		
14	1,02	0,69		
16	1,10	0,02		
18	1,09	0		
20	1,10	0		

$$\mu = \frac{dx}{dt} \cdot \frac{1}{x}$$

$$\mu_i = \frac{x_{i+1} - x_{i-1}}{t_{i+1} - t_{i-1}} \cdot \frac{1}{x_i} \longrightarrow \text{3 points rule}$$

$$\mu_i = \frac{x_i - x_{i-1}}{t_i - t_{i-1}} \cdot \frac{1}{x_i} \quad \longrightarrow \quad \text{Euler rulw}$$

			euler	3 ptos
t (h)	X (g/L)	S (g/L)	μ (h-1)	μ (h <sup>-1</sup> )
0	0,10	10.00		
2	0,14	9,56	0,14	0,18
4	0,20	8,94	0,15	0,19
6	0,29	8,05	0,16	0,20
8	0,43	6,81	0,16	0,17
10	0,59	5,10	0,14	0,17
12	0,83	2,90	0,14	0,13
14	1,02	0,69	0,09	0,07
16	1,10	0,02	0,04	0,02
18	1,09	0	0,00	0,00
20	1,10	0	0,00	0,06

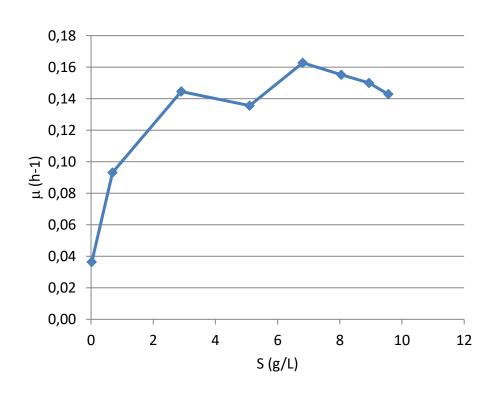
	euler	
S (g/L)	μ (h-1)	
10		
9,56	0,14	
8,94	0,15	
8,05	0,16	
6,81	0,16	
5,1	0,14	
2,9	0,14	
0,69	0,09	
0,02	0,04	

### **Monod model**

$$\mu = \frac{\mu_{\text{max}} S}{K_S + S}$$

### **Lineweaver-Burk**

$$\frac{1}{\mu} = \frac{K_S}{\mu_{\text{max}}} \frac{1}{S} + \frac{1}{\mu_{\text{max}}}$$



#### **Eadie-Hofstee**

$$\mu = -K_S \frac{\mu}{S} + \mu_{\text{max}}$$

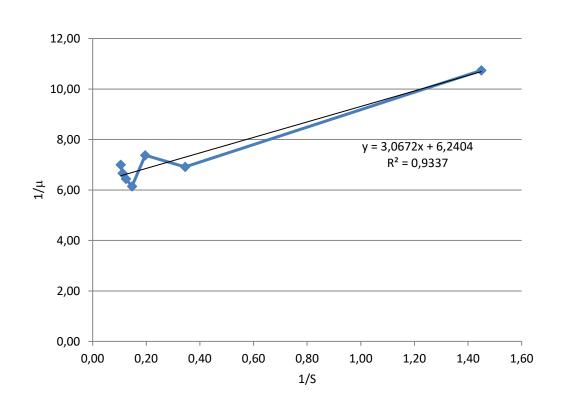
### **Hanes-Woolf**

$$\frac{S}{\mu} = S \frac{1}{\mu_{\text{max}}} + \frac{K_S}{\mu_{\text{max}}}$$

### **Lineweaver-Burk**

$$\frac{1}{\mu} = \frac{K_S}{\mu_{\text{max}}} \frac{1}{S} + \frac{1}{\mu_{\text{max}}}$$

1/S	1/μ
0,10	7,00
0,11	6,67
0,12	6,44
0,15	6,14
0,20	7,38
0,34	6,92
1,45	10,74
50,00	27,50



$$\frac{K_{\rm S}}{\mu_{\rm max}} = 3.0672$$

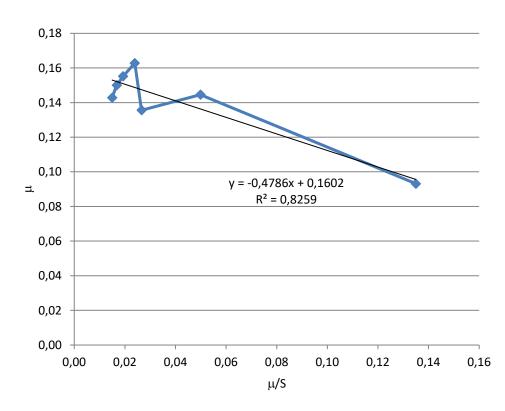
$$\frac{1}{\mu_{\rm max}} = 6.2404$$

$$\iff K_{s} = 0.4908 \text{ g/L}$$
 
$$\Leftrightarrow \mu_{\text{max}} = 0.1602 \text{h}^{-1}$$

### **Eadie-Hofstee**

$$\mu = -K_S \frac{\mu}{S} + \mu_{\text{max}}$$

μ/S	μ
0,01	0,14
0,02	0,15
0,02	0,16
0,02	0,16
0,03	0,14
0,05	0,14
0,13	0,09
1,82	0,04



$$-K_S = -0.4786 \text{ g/L}$$

$$\mu_{\text{max}} = 0.1602 \text{h}^{-1}$$

## Problem 2.3

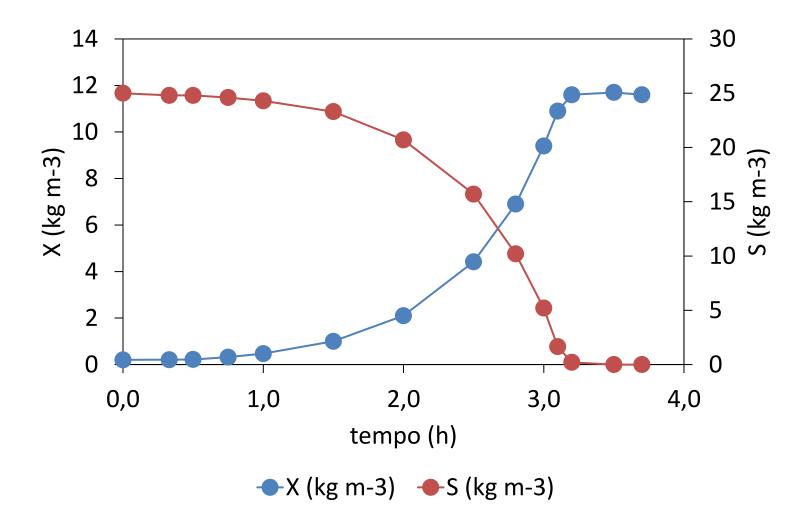
Escherichia coli grows in a batch reactor using glucose as a substrate. The following table shows cell concentrations as a function of substrate concentration.

a) Represent  $\mu$  as a function of time.

c) Calculate the apparentgrowth yield coefficient andthe real growth yield coefficient.

,	Тетро	Concentração Celular	Concentração Substrato
	(h)	$(kg m^{-3})$	$(kg m^{-3})$
	0.0	0.20	25.0
	0.33	0.21	24.8
	0.5	0.22	24.8
	0.75	0.32	24.6
	1.0	0.47	24.3
	1.5	1.00	23.3
•	2.0	2.10	20.7
	2.5	4.42	15.7
	2.8	6.9	10.2
	3.0	9.4	5.2
	3.1	10.9	1.65
	3.2	11.6	0.2
	3.5	11.7	0.0
	3.7	11.6	0.0

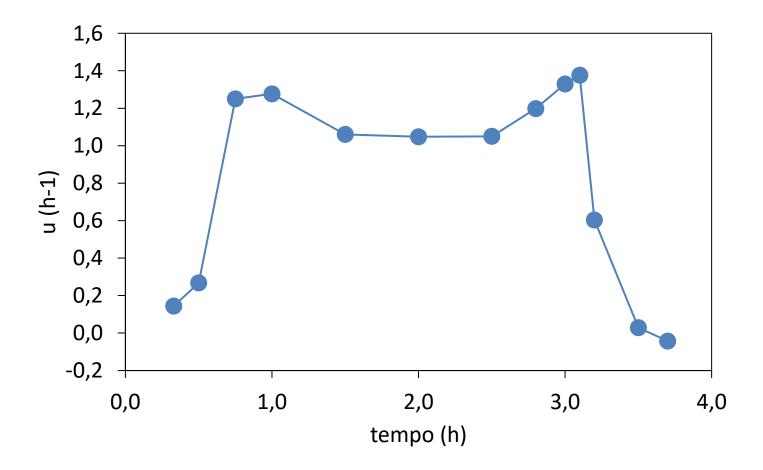
# 2.3.a) Growth profile: cell growth and substrate consumption



# 2.3.a) Calculate the specific cell growth rate (Euler)

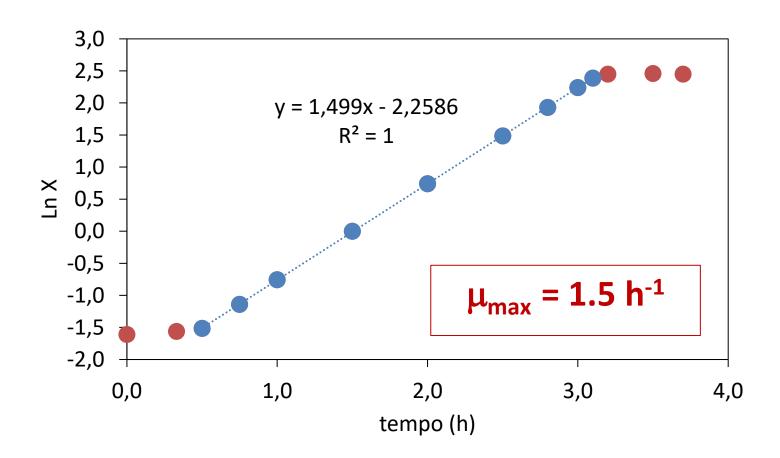
Tempo (h)	X (kg m <sup>-3</sup> )	S (kg m <sup>-3</sup> )	u (h <sup>-1</sup> )
0.00	0.20	25.00	
0.33	0.21	24.80	0.14
0.50	0.22	24.80	0.27
0.75	0.32	24.60	1.25
1.00	0.47	24.30	1.28
1.50	1.00	23.30	1.06
2.00	2.10	20.70	1.05
2.50	4.42	15.70	1.05
2.80	6.90	10.20	1.20
3.00	9.40	5.20	1.33
3.10	10.90	1.65	1.38
3.20	11.60	0.20	0.60
3.50	11.70	0.00	0.03
3.70	11.60	0.00	-0.04

# 2.3.a) $\mu$ as a function of time



# 2.3.b) 1. Calculate $\mu_{max}$

# Ln X vs time



# 2.3.c) Calculate the yield coeficients

a. Apparent

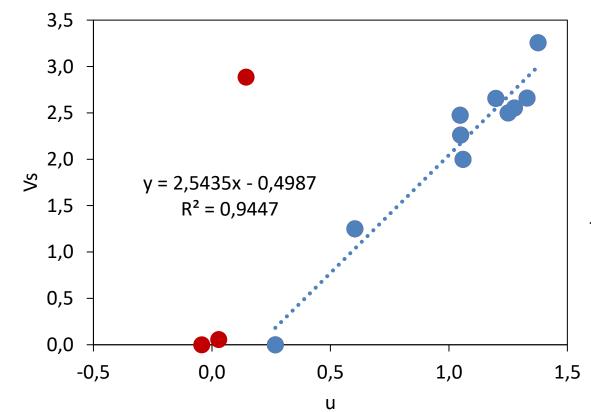
$$Y_{x/S} = \frac{\Delta x}{\Delta S}$$

$$Y_{x/s} = \frac{\Delta X}{\Delta S} = 0.46 \ kg_X/kg_S$$

# 2.3.c) Calculate the yield coeficients

a. Real

$$V_s = \frac{1}{Y'_{x/s}} \; \mu + m$$



$$Y'_{x/s} = 0.39 kg_X/kg_S$$

$$m=0.50 \text{ kg_S/kg_X h}^{-1}$$