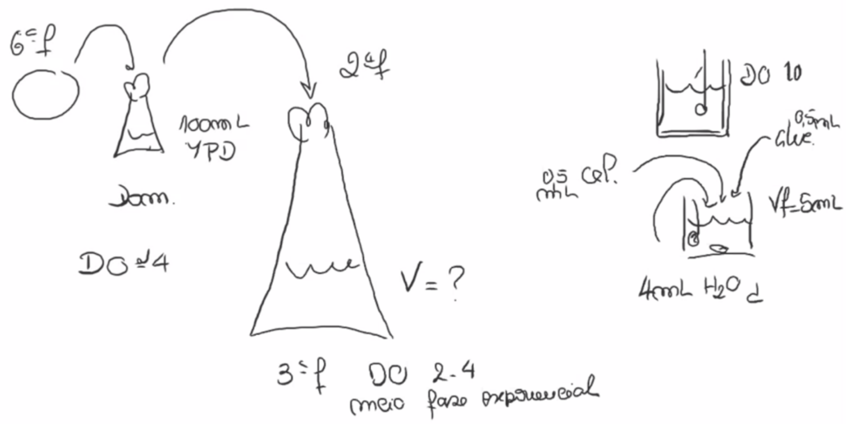
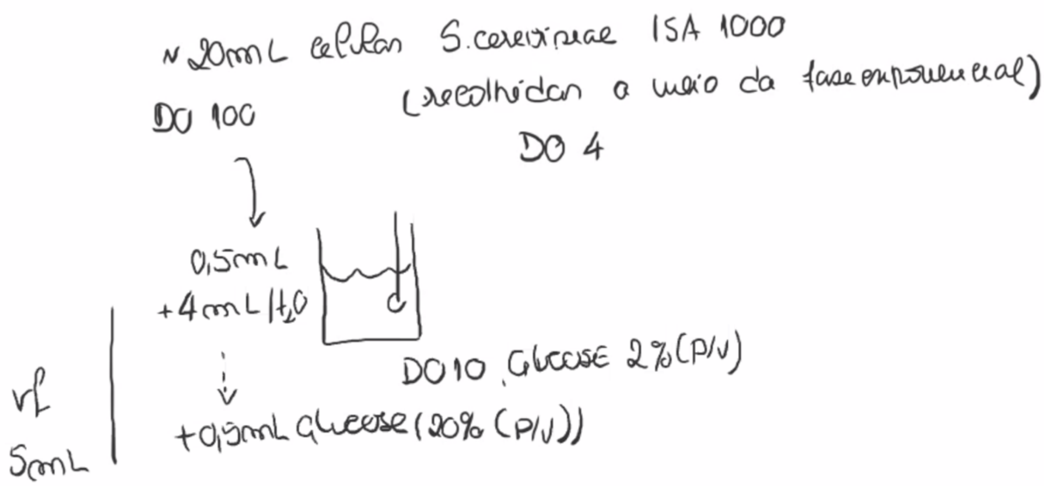
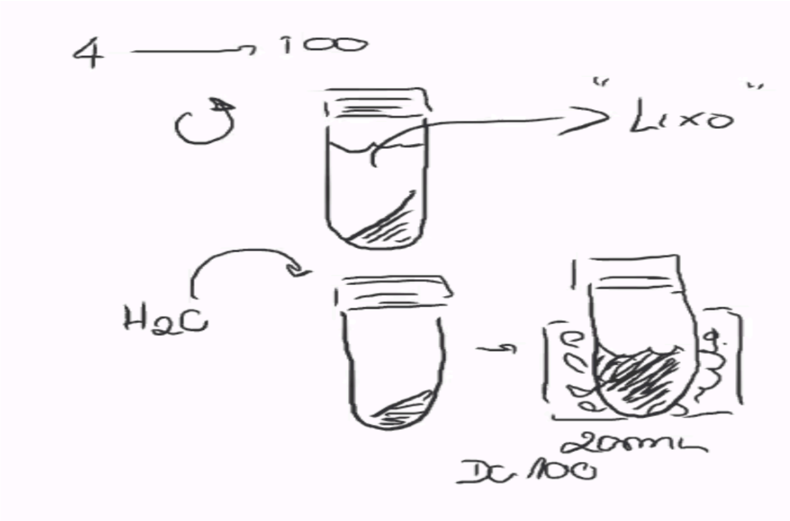
Esquemas nas Aulas Práticas Catarina Microbiologia Funcional 2021

1. 9 fev 21 – Apresentação

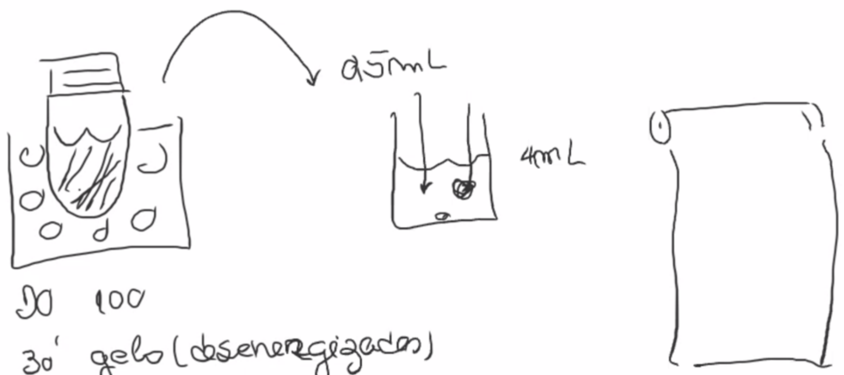
2. 16 fev 21- Atividade ATPase





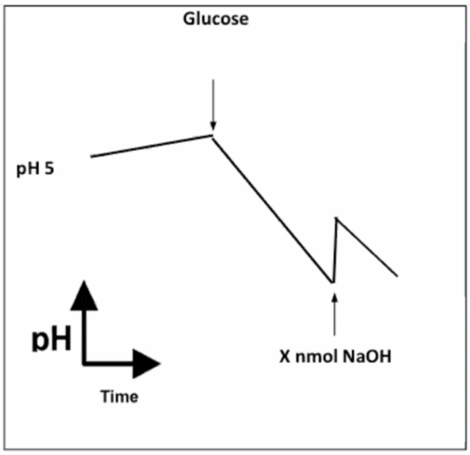


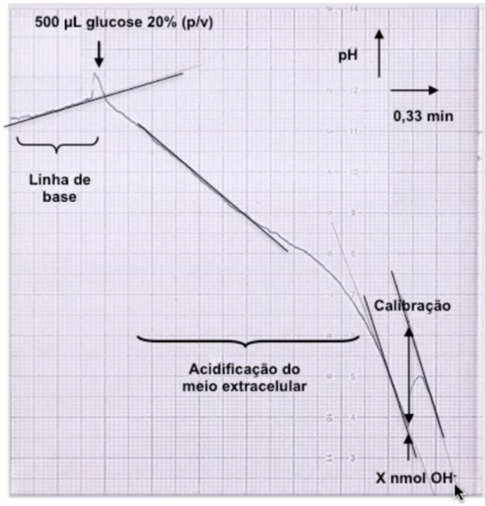
DO 100
30' gelo (desenergizadas)
s/ ATP (reservas)

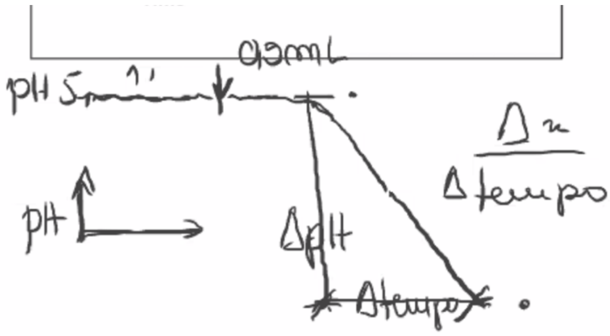


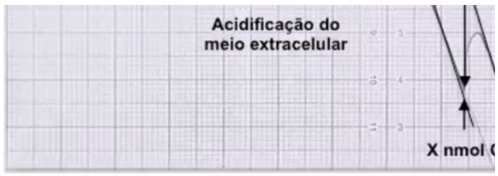
DO 100
30' gelo (desenergizadas)
s/ ATP (reservas)

Medir a atividade da H^+-ATPase









$$\frac{\Delta pH}{\Delta \text{tempo}}$$

3. 23 fev 21- Transporte Maltose

$10\ mM = 10\ mmol\ /$

$1M = 1mol/L \equiv 1mmol/mL$

$1mM = 1mmol/L = 1\mu mol/mL$

$= 10^3 nmol/mL$

$= 1nmol/\mu L$

$1mol = 10^3 mmol = 10^6 \mu mol$

$= 10^9 nmol$

$1nmol = 10^{-3}\mu mol = 10^{-6} mmol$

$= 10^{-9} mol$

$1L = 10^3 mL = 10^6 \mu L$

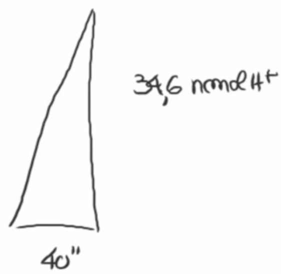
$1\mu L = 10^{-3} mL = 10^{-6} L$

$x = \frac{10^2}{10^6} = 10^{-4}$ mmol
$\downarrow$
10^2 nmol $\Leftarrow y$

1 mmol $\longrightarrow$ 10^6 nmol
10^{-4} mmol $\longrightarrow$ y

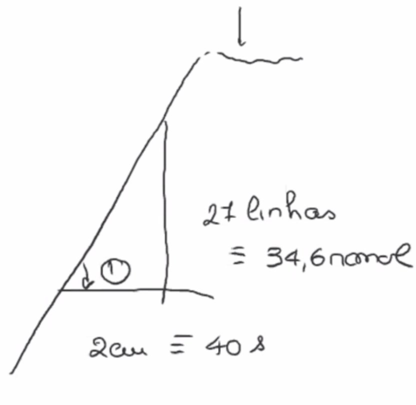
3 cm $\longrightarrow$ 1 min = 60 s
2 cm $\longrightarrow$ x

x = 40''

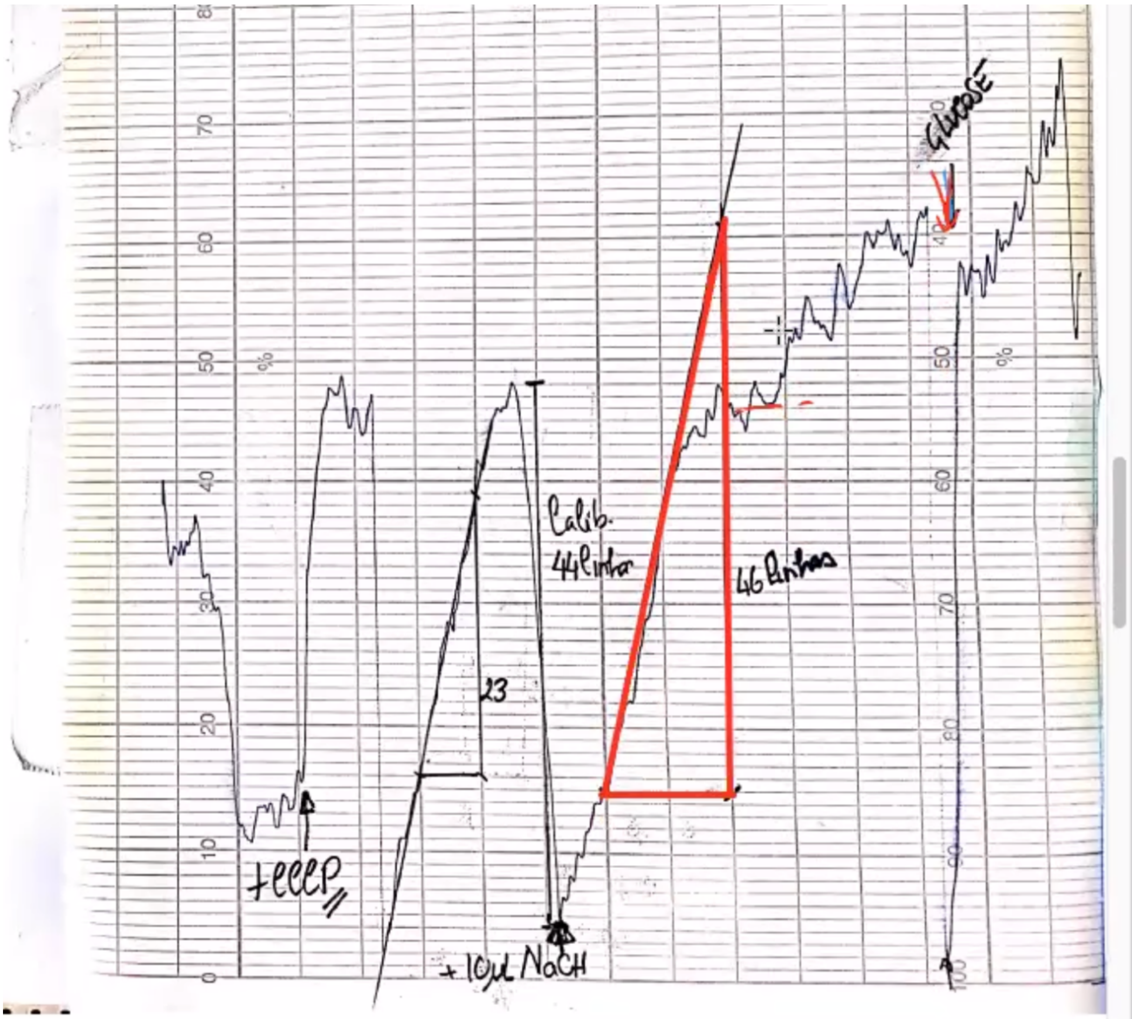


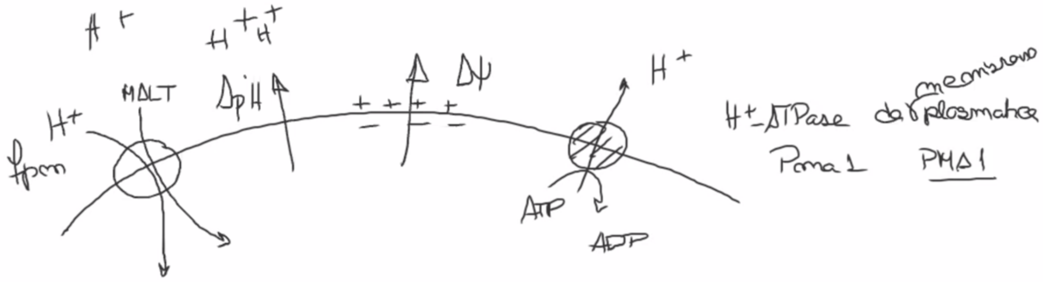
velocidade de H^+-ATP = $\frac{34,6}{40''}$ = 0,865 nmol/s
= 51,9 mmol/min
= 0,0519 µmol/min
= 3,114 µmol/h
= $3,114 \times 10^{-3}$ mmol/h
= 0,00311 mmol/h

① ~ 51 mmol/min



0,5 mL cel DO = 100 ≡ 38 mg
necessário = DO = 10
$\downarrow$
7,6 mg/100 µL

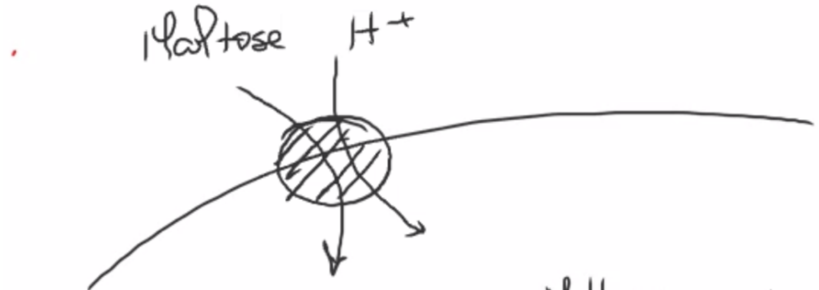




Km
Vmax } Parâmetros

alcalinização d uma susp. cuv. ñ tamponizada de células após um pulso de maltose a ≠ conc.

4. 2 mar 21- **Respiração Resistente ao Cianeto**



Maltase ≡ α-glucosidase

α 1,4 - glucosídicas

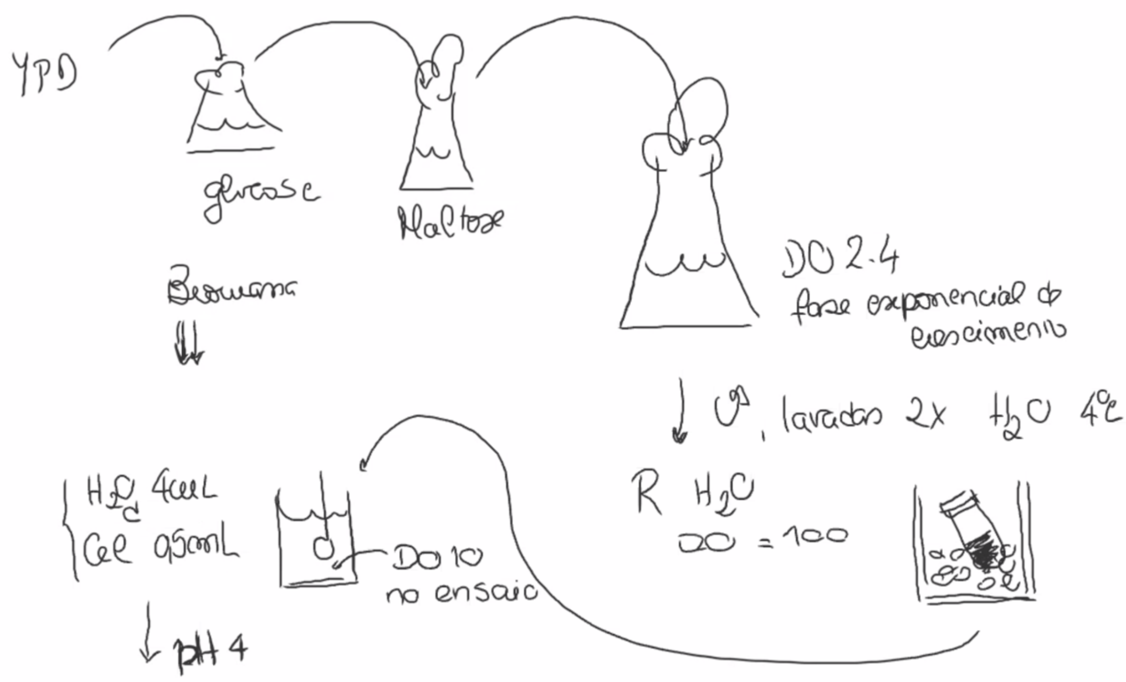
<u>S. cerevisiae</u>

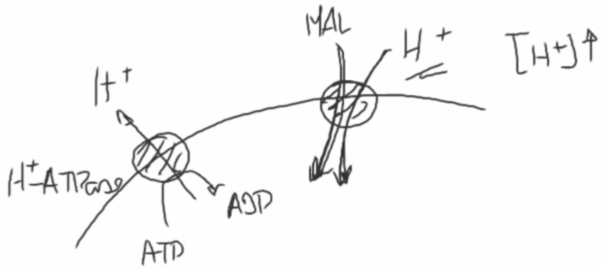
glucose ← Repressão pela glucose
frutose

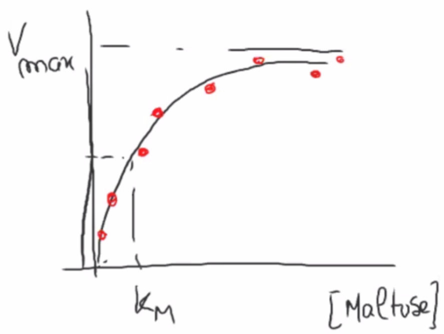
- glucose → <u>Mig1</u> (factor de transcrição)

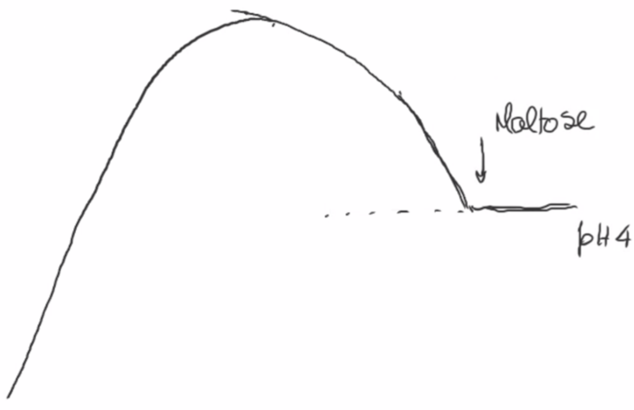
  fica retido no núcleo
  Regula negativamente os genes MAL
  Reprime a expressão dos genes associados à utilização de maltose

- Maltose - indutor

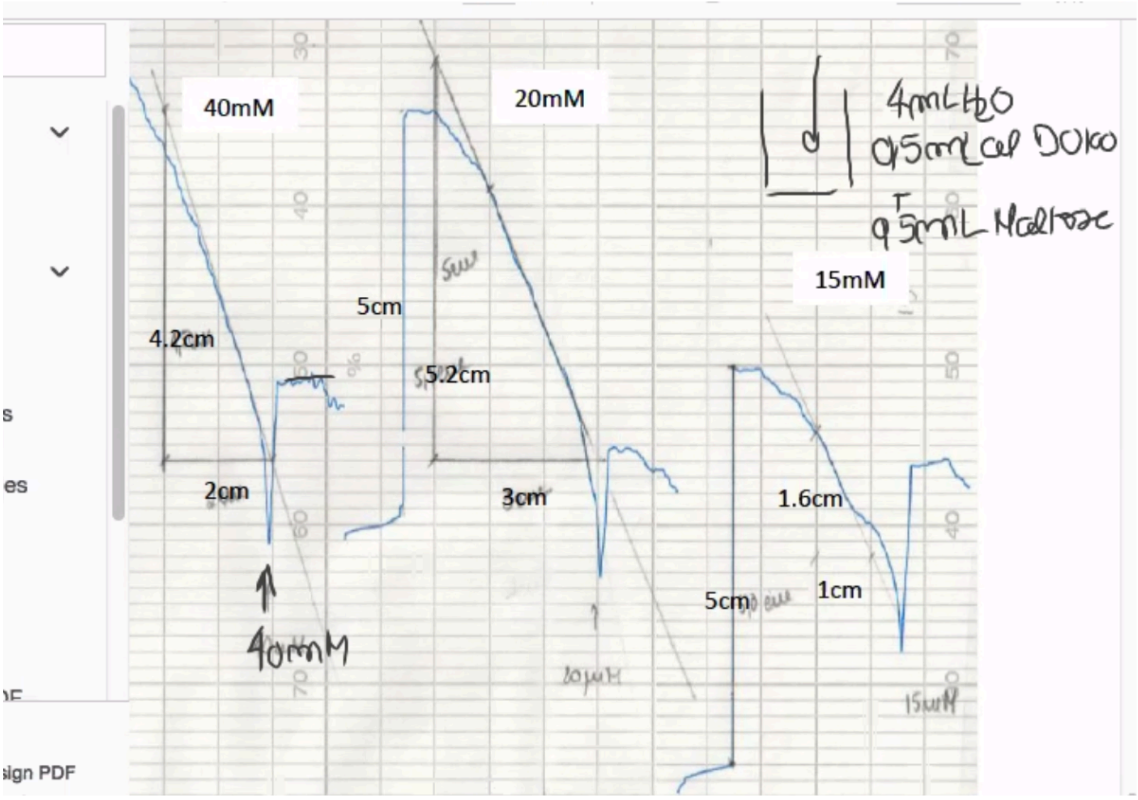


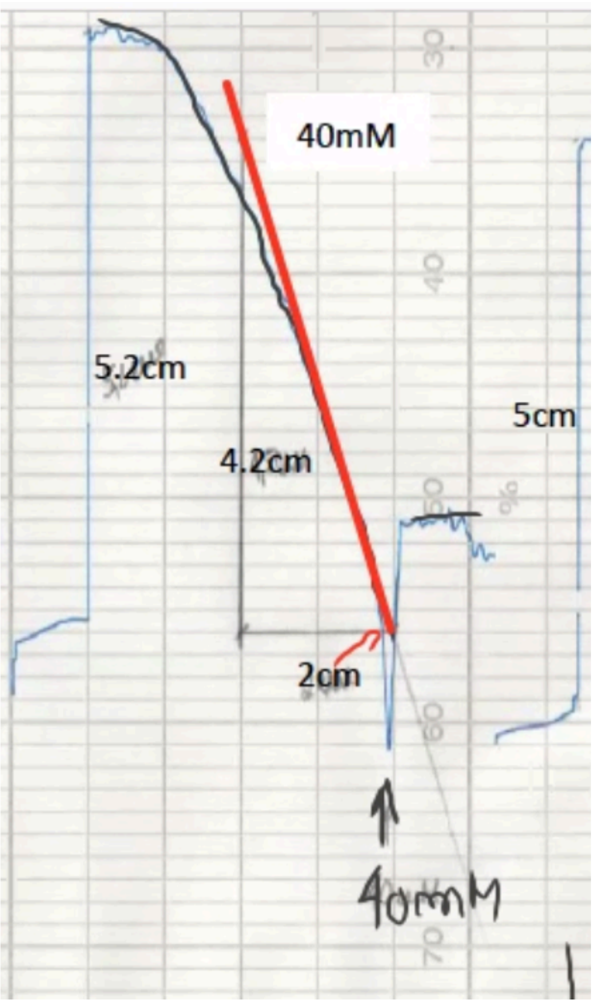






$$v = V_{max} \times \frac{[S]}{K_M + [S]} = \frac{V_{max} \times [S]}{K_M + [S]}$$


40mM
20mM
15mM
4.2cm
5cm
5.2cm
2cm
3cm
1.6cm
1cm
5cm
40mM
sign PDF


40mM
5.2cm
4.2cm
5cm
2cm
40mM

100mM

INSTITUTO SUPERIOR D AGRONOMIA
Universidade de Lisboa

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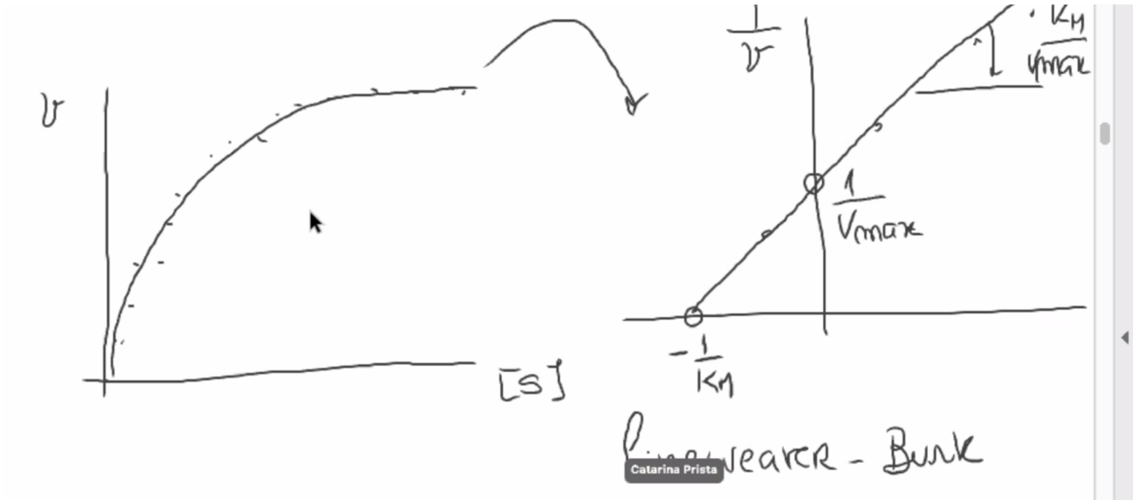
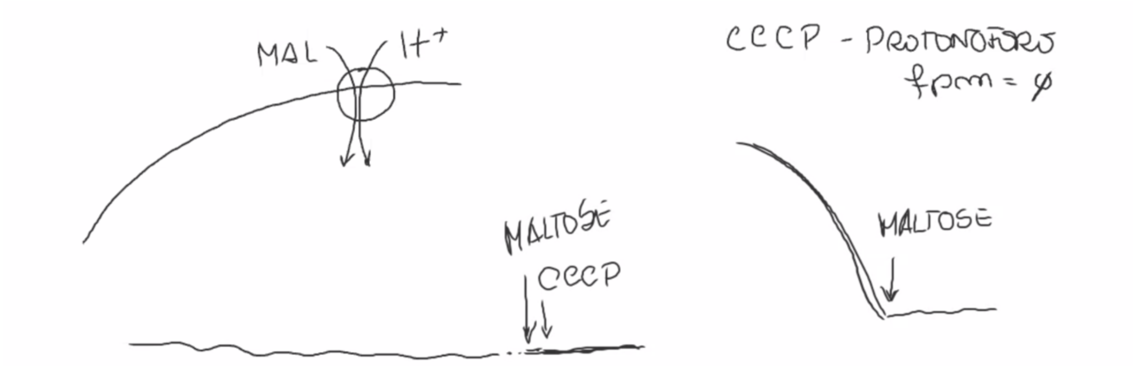
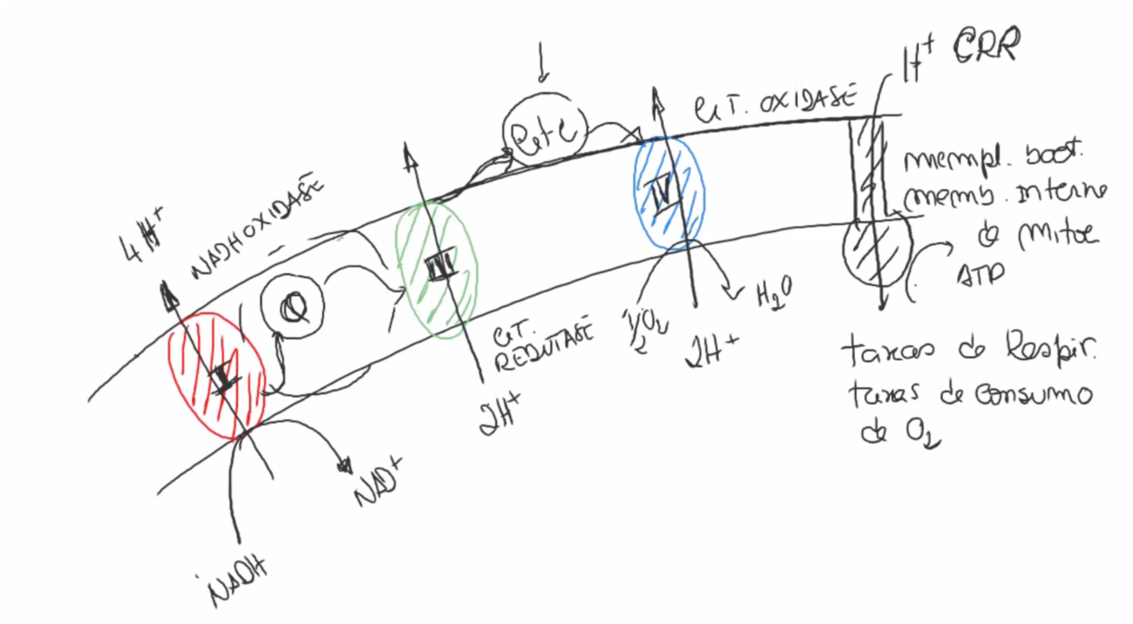
4mL H_2O
0,5mL cel (DO 200)
+
0,5mL Maltose

DO 10

1,6mL H_2O
0,2mL cel (DO 100)
+
0,2mL Maltose


v
[S]
1/v
KM/Vmax
1/Vmax
-1/KM
Catarina Prista
weaver - Burk

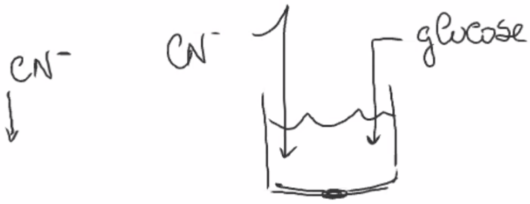

MAL
H+
CCCP - PROTONÓFORO
fpm = ϕ
MALTOSE
CCCP
MALTOSE

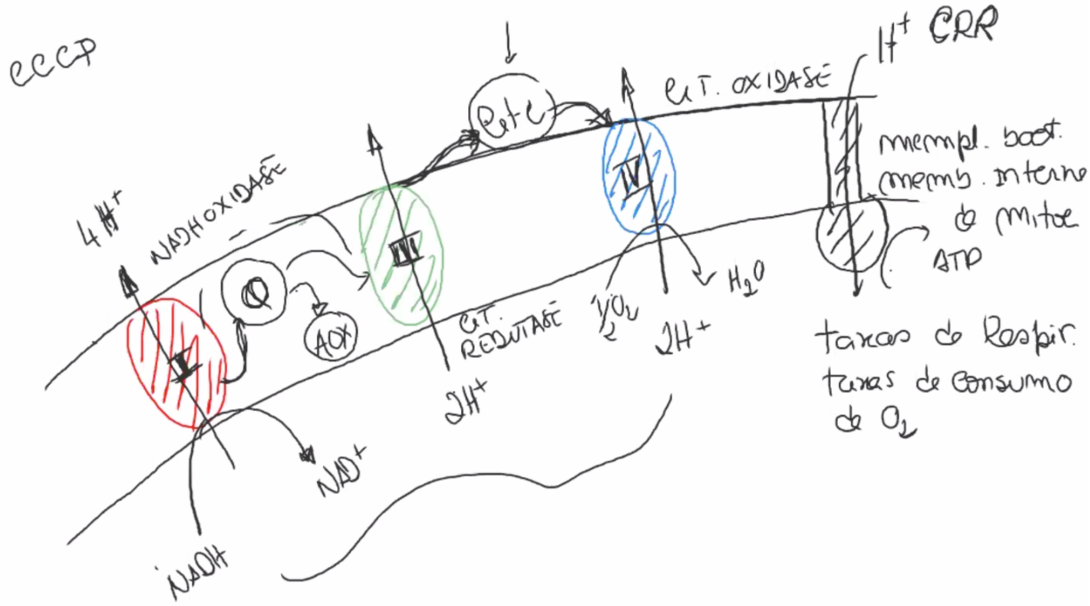

H+ CRR
cit. oxidase
cit c
4H+
NADH oxidase
Q
III
IV
I
cit. redutase
1/2 O2
H2O
2H+
2H+
memb. bact.
memb. interna de mitoc.
ATP
taxas de respir.
taxas de consumo de O2
NAD+
NADH

Debaryomyces hansenii → CRR – no CRR

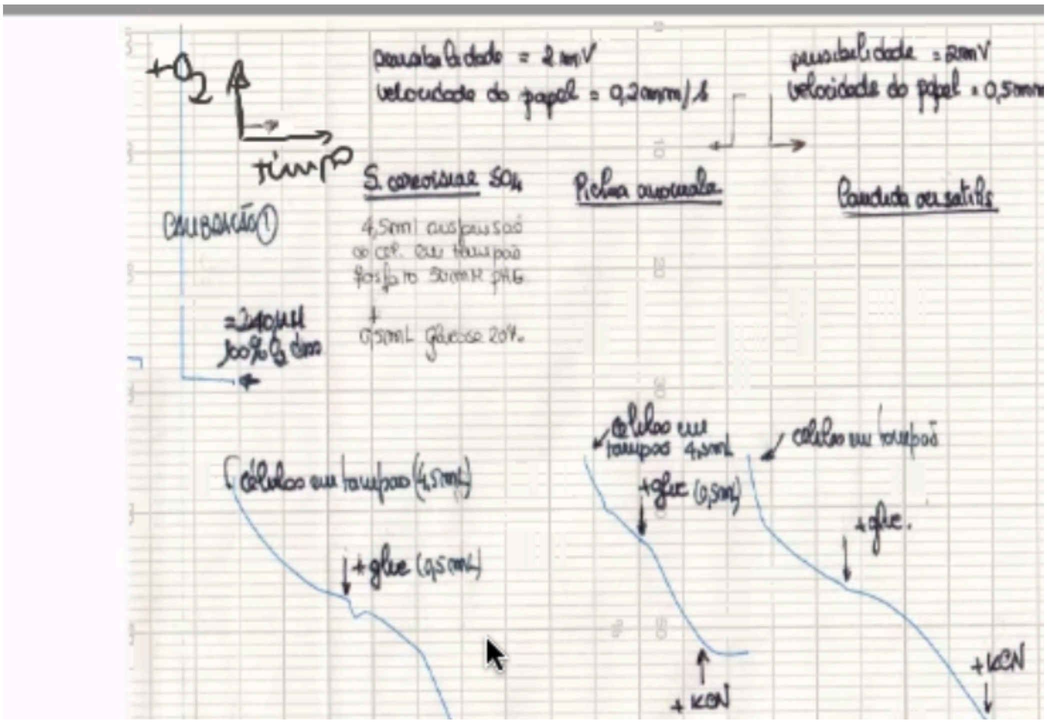
Pichia membranifaciens → CRR – no CRR

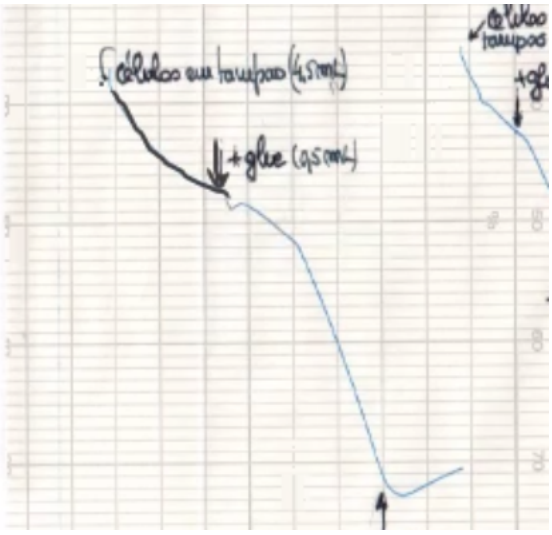
Saccharomyces cerevisiae → no CRR



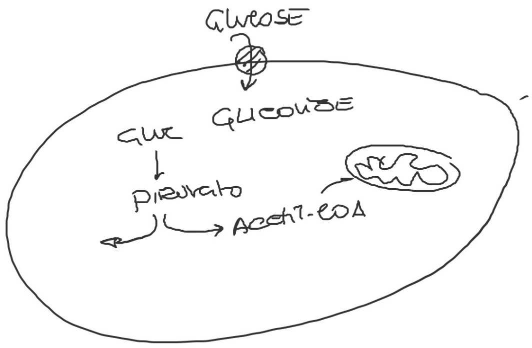


taxas de Respir.
taxas de consumo de O_2


+O2
tempo
velocidade do papel = 0,2mm/s
sensibilidade = 2mV
velocidade do papel = 0,5mm
4,5ml suspensão
0,5mL glicose 20%
células em tampão (4,5mL)
+ glic (0,5mL)
células em tampão 4,5mL
+glic (0,5mL)
+ KCN
células em tampão
+glic.
+KCN


células em tampão (4,5mL)
+ glic (0,5mL)

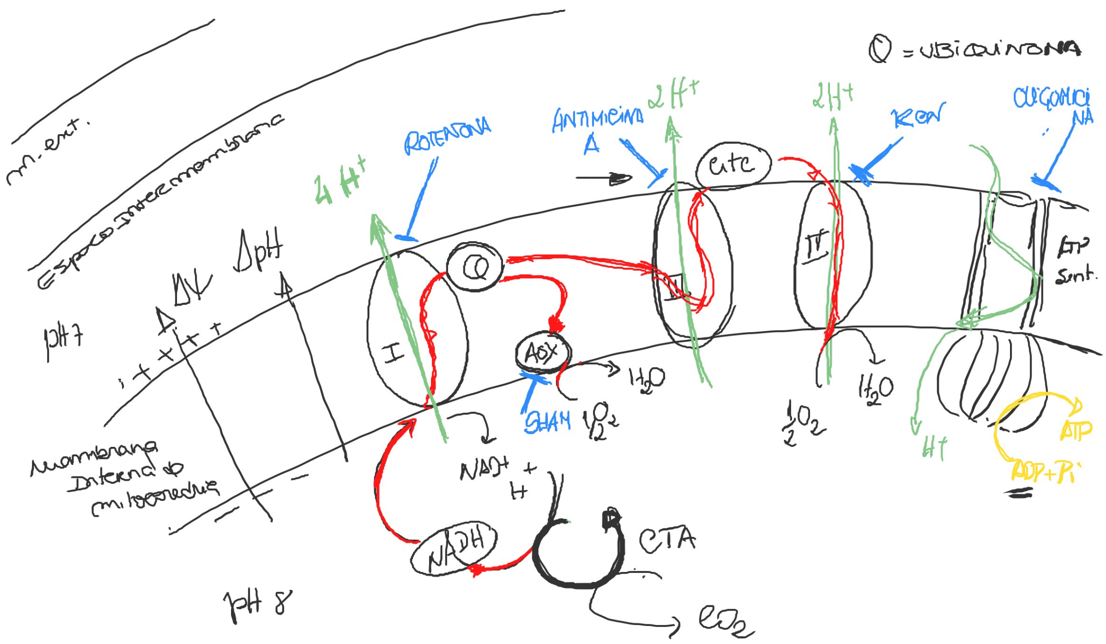
5. 9 mar 21- **Respiração Resistente ao Cianeto**



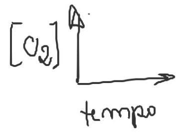
$[O_2]_{max}$ solução saturada aq. – 240 µM (100% saturada de O_2)

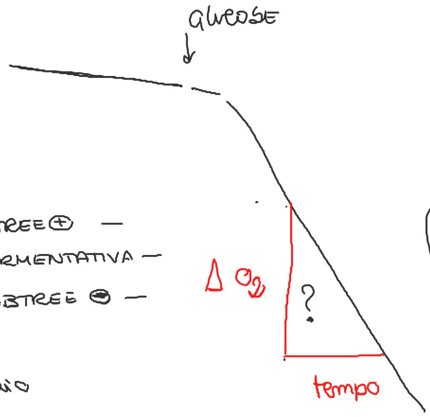
(à Patm)

$PV = nRT$



Taxa de consumo de O_2



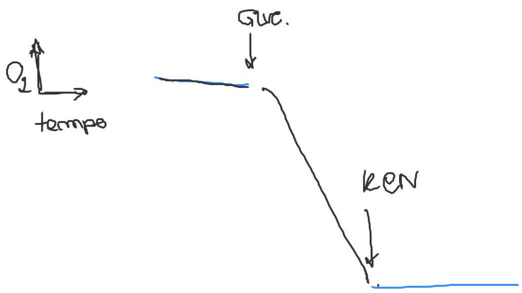


$$\frac{\Delta O_2 / \text{tempo}}{\text{Biomassa}}$$

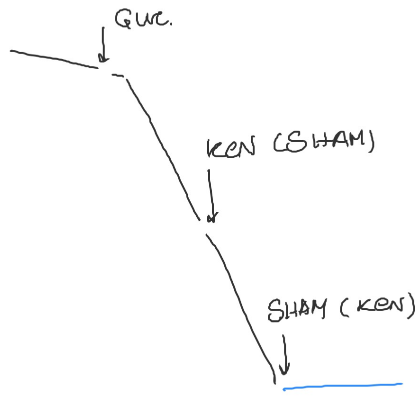
taxa específica de consumo de O_2

S. cerevisiae — CRABTREE ⊕ —
C. versatilis — Ñ FERMENTATIVA —
P. anomala — CRABTREE ⊖ —

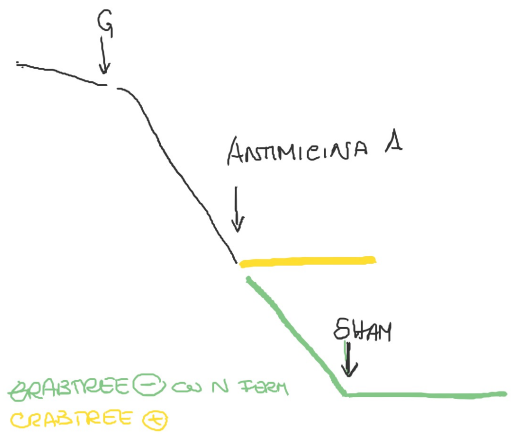
Biomassa no ensaio

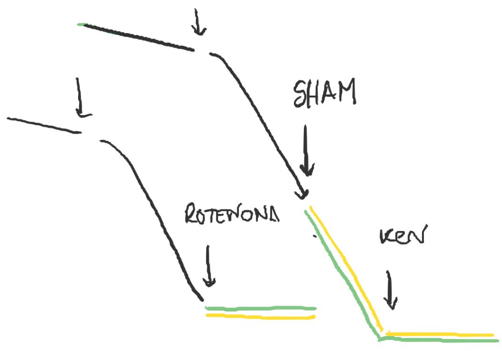


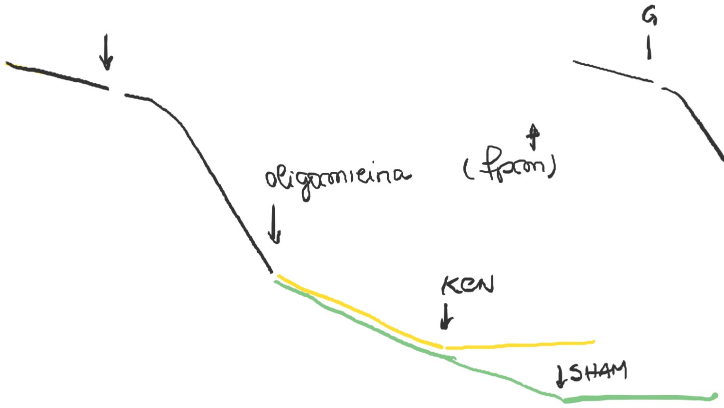
CRABTREE ⊕
CRR ⊖

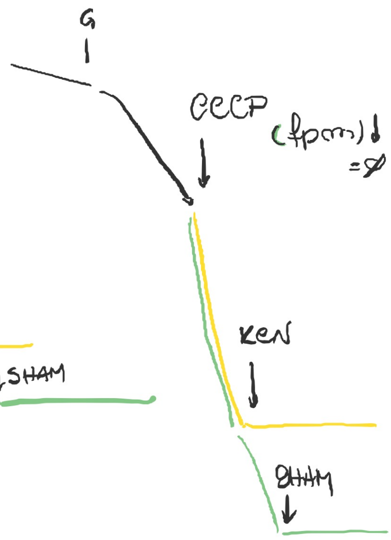


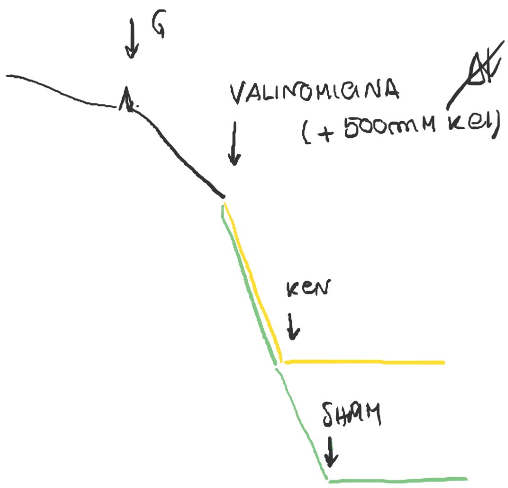
CRABTREE ⊖
Ñ FERMENTATIVAS
CRR ⊕

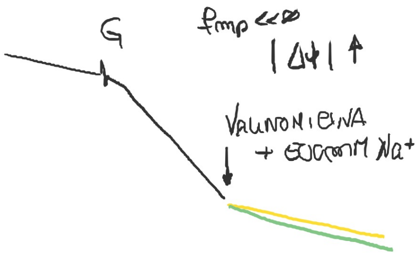

G
ANTIMICINA A
SHAM
CRABTREE ⊖ cω N FERM
CRABTREE ⊕

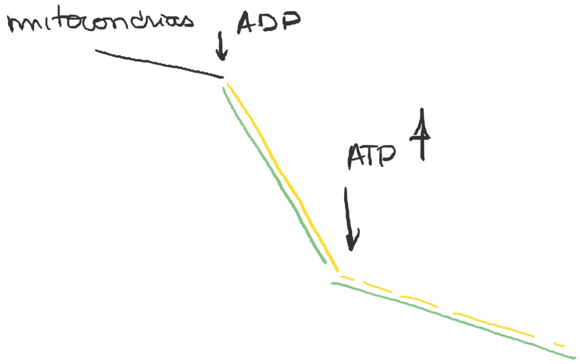

SHAM
ROTENONA
KCN


oligomicina
(fpcm) ↑
KCN
SHAM

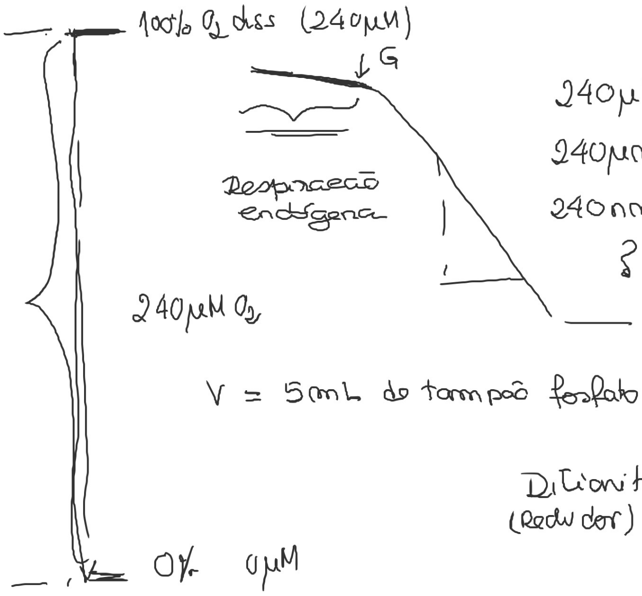

G
CCCP
(fpcm) ↓
= ∅
KCN
SHAM



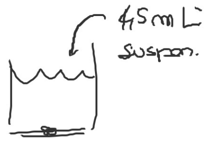




$$\uparrow \frac{ATP}{\uparrow ADP} \sim \text{const.}$$



240µM
240µmol / L
240nmol / mL
?   5mL
1200nmolO_2



DO = 10

V = 5mL de tampão fosfato

Ditionito
(Redutor)

$O_2 \rightarrow H_2O$