

# INDEX

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[illegible]

# Experiment-1

## Cell disruption by mechanical methods

★ Introduction: Cell lysis is an essential step in production of intracellular content when the desired product is not released out of the cell. It is the breaking of the cell wall or cell membrane to obtain the cellular content in order to isolate the product of interest.

There are several methods by which cells can be disrupted. Depending on the type of the cell and the proposed use of the product & choice of lysis buffer differs. Broadly, the cell disruption methods can be categorized into two types: mechanical and non-mechanical. Mechanical methods involve the use of mechanical forces to break the cell wall; they are often considered to be harsh on the cell and is used on cells that are ~~produced~~ protected by a sturdy cell wall like plant cells. The examples for this method are french press, bead mills, etc. These methods are generally used for industrial purposes as the chemical and enzymatic disruption involve an extra separation step and they are costly to be applied for large volumes. Additionally, they may change the properties of the desired intracellular product.

## \* Material Required

- (1) Cell Suspension
- (2) Lysis buffer.
- (3) Glass beads, pipettes
- (4) Vortex
- (5) Centrifuge
- (6) Falcon tubes

## \* Procedure

- (1) First take 10ml of the cell suspension in a 15ml falcon tube
- (2) Then add 20 glass beads to it
- (3) Vortex the sol<sup>n</sup> for 10min, then take a sample of 1ml in 2ml MCT.
- (4) Vortex the remaining for 10min more and take another sample
- (5) Then Repeat the above step once more
- (6) Centrifuge the samples at 4°C at 8000rpm for about 15 min
- (7) Then take the Supernatant in 2ml MCT
- (8) measure the absorbance at 280nm
- (9) If the absorbance is too high ( $>1$ ) then dilute the sample by 10 folds and again measure above step.

\* Lab Readings → lysis by glass beads

①  $0.219 \times 10$   
 $0.221 \times 10$   
 $0.223 \times 10$  } Abs after 10 min

②  $0.317 \times 10$   
 $0.307 \times 10$   
 $0.309 \times 10$  } Abs after 20 min

③  $0.333 \times 10$   
 $0.329 \times 10$   
 $0.329 \times 10$  } Abs after 30 min

Ultrasonification

④  $0.474 \times 10$   
 $0.477 \times 10$   
 $0.479 \times 10$  } Abs after 10 min.

→ Lysis

# \* Observations!

## Readings:

→ NaOH (Incubation time 10 minutes)

① 2N

$$0.637 \times 4$$

$$0.631 \times 4$$

$$0.620 \times 4$$

② 1N

$$0.755 \times 2$$

$$0.768 \times 2$$

$$0.762 \times 2$$

③ 0.5N

~~$$0.805 \times 2$$~~ 
$$0.496 \times 2$$

$$0.452 \times 2$$

$$0.454 \times 2$$

→ SOS

① ~~4%~~ 4%

$$\rightarrow 0.790$$

$$\rightarrow 0.773$$

$$\rightarrow 0.774$$

② 2%

$$\rightarrow 0.858$$

$$\rightarrow 0.854$$

$$\rightarrow 0.859$$

③ 1%

$$\rightarrow 0.934$$

$$\rightarrow 0.928$$

$$\rightarrow 0.935$$

→ lysosomes

① ~~0.5~~ 1

$$\rightarrow -0.617$$

$$\rightarrow -0.613$$

$$\rightarrow -0.613$$

② 0.5

$$\rightarrow -0.639$$

$$\rightarrow -0.643$$

$$\rightarrow -0.643$$

③ 0.25

$$\rightarrow -0.357$$

$$\rightarrow -0.353$$

$$\rightarrow -0.353$$



Exp-3

\* Readings

①

At $t$ <sup>(min)</sup>	$V$ (ml)
10 min	40
20 min	80
30 min	140
40 min	175
50 min	200
60 min	230
70 min	260
80 min	290
90 min	310

Recd  
11/9/22

## Experiment - 4

### Vacuum Filtration of yeast solution

#### → Introduction

Filtration separates insoluble solids from a liquid slurry. This is achieved by forcing a solid-liquid mixture through a filter medium that retains the solids while allowing the liquid to pass through. Darcy's law defines the ability of a fluid to pass through a porous material. It defines the relationship b/w the velocity of the flow and the pressure difference as shown in eqn. It is analogous to Ohm's law where the discharge rate is equivalent to current, pressure difference to the potential and  $1/k$  to the resistance. This resistance has two portions,  $R_m$  and  $R_c$ , that refers to the resistance from the filtration medium and cake formed.

$$v = \frac{k \cdot \Delta P}{\mu l}$$

\* Vacuum filtration is a method of filtration where the pressure is maintained due to the suction of air beneath the filter paper. It generates an additional force along with gravitational force and hence increases the rate of filtration.

## \* Material Required

- ① Cell suspension      ② measuring cylinder
- ③ water      ④ Conical flask (500ml)
- ⑤ Vacuum filter.

## \* Procedure

- ① first of all set up the filtration unit and make sure that there is no leakage in the system
- ② Then take the yeast solution and fill the filter chamber to 300ml mark.
- ③ Switch on the vacuum pump & the filter.
- ④ finally take 15 readings of volume of the volume of cell suspension remaining in the filter chamber b/w 0 → 25 min

Also for  $R_m$  &  $\alpha$ ,

$$\frac{At}{V} \geq \frac{KV}{A} + B$$



\* Readings

Pressure = 600 g/cm<sup>2</sup>

35

Time	Volume
5 min	200
10 min	160
15 min	130
20 min	100
25 min	80
30 min	70
35 min	60
40 min	50
45 min	45 (added 200 ml)
50 min	170
55 min	160
60 min	150
65 min	140
70 min	130
75 min	120

Atm

$$\text{Area} = \pi \times (2\text{cm})^2$$

$$\text{Diameter} = 4\text{cm}$$

# Ex-5! - Readings

<u>Conc.</u>	<u>T.V.</u>	<u>Sucrose</u>	<u>water.</u>
0%.	30 ml	0 ml	30 ml
15%.	10 ml	2.5 ml	7.5 ml
20%.	10 ml	3.33 ml	6.67 ml
25%.	10 ml	4.16 ml	5.83 ml
30%.	20 ml	10 ml	10 ml
35%.	10 ml	5.83 ml	4.166 ml
40%.	10 ml	6.67 ml	3.33 ml
45%.	10 ml	7.5 ml	2.5 ml
60%.	10 ml	10 ml	0 ml

## Experiment - 6

Dye-I Blue dye  $\rightarrow$  515 nm

Sample	Abs		
100%	1.703	1.702	1.693
75%	1.256	1.254	1.247
50%	0.859	0.861	0.859
25%	0.352	0.354	0.357
12.5%	0.247	0.248	0.246

Purple dye  $\rightarrow$  590

Sample	Abs		
100%	2.345	2.326	2.317
75%	1.651	1.640	1.640
50%	1.162	1.163	1.166
25%	0.607	0.606	0.607
12.5%	0.328	0.328	0.324

Dye-I (Coomassie blue)

Sample			
blue $\rightarrow$ 2 ml chloroform	1.323	1.322	1.323
$\rightarrow$ 4 ml	1.036	1.033	1.029
<del>Dye</del> $\rightarrow$ 6 ml	0.751	0.751	0.760
$\rightarrow$ 8 ml	0.598	0.603	0.594

## Dye-2 (Purple) [Crystal Violet]

Sample	Abs		
2ml	1.289	1.265	1.281
4ml	0.111	0.116	0.113
6ml	0.058	0.037	0.038
8ml	0.042	0.031	0.028

# Exp #7

06/10/20

Taking 40ml of dye  
like made 400 ml

100% ~~1.257~~ 1.255, 1.257, 1.254

75% 1.189, 1.193, 1.187

50% 0.730 - 0.729

25% 0.149 0.139 0.139

10% 0.141 0.140 0.142

0.100

	O.D		
10 min	0.054	0.055	0.052
20 min	0.034	0.035	0.034
30 min	0.033	0.032	0.032
40 min	0.079	0.079	0.08
	<del>0.051</del>	<del>0.079</del>	0.0
50 min	0.051	0.049	0.051
60 min	0.075	0.076	0.078
70 min	0.010	0.011	0.011