UNDERGRADUATE PROJECT

OPTIMIZATION OF ETHANOL PRODUCTION IN YEAST FERMENTATION PROCESS BASED ON MIXING TIME

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OVERVIEW ON FERMENTATION PROCESS

- Glucose $\xrightarrow[\textit{(S.cerevisiae)}]{\text{yeast}}$ ethanol + carbon dioxide + energy

- For efficient production of ethanol, once fermentation process should have:
    - good ethanol tolerance
    - rapid fermentation rate (i.e., high productivity)
    - high fermentation efficiency
    - temperature tolerance
    - maintenance of high cell viability and etc.

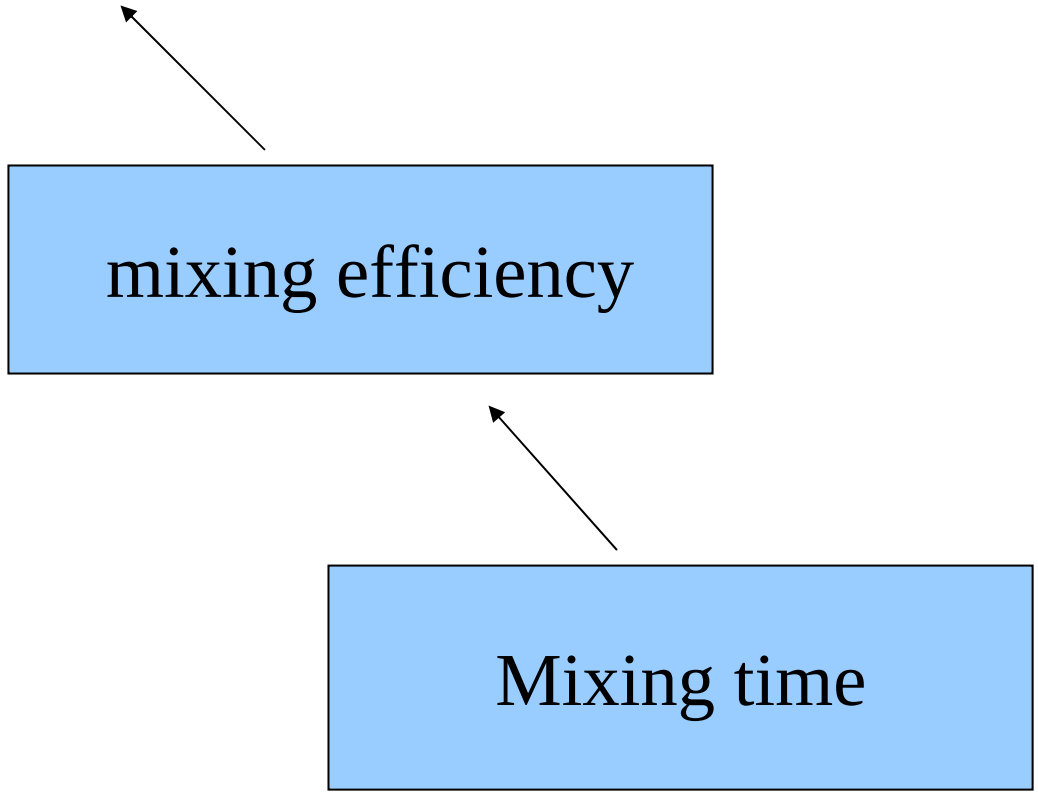
(Slapack *et al.*, 1987; Russell *et al.*, 1987)

Crucial Factor to be considered for Yeast Growth and Ethanol Production

- Substrate Concentration
- Temperature and pH
- Dissolved Oxygen
- Agitation and aeration rates

Why aeration and agitation rates are very important?

- Optimum hydrodynamic regime

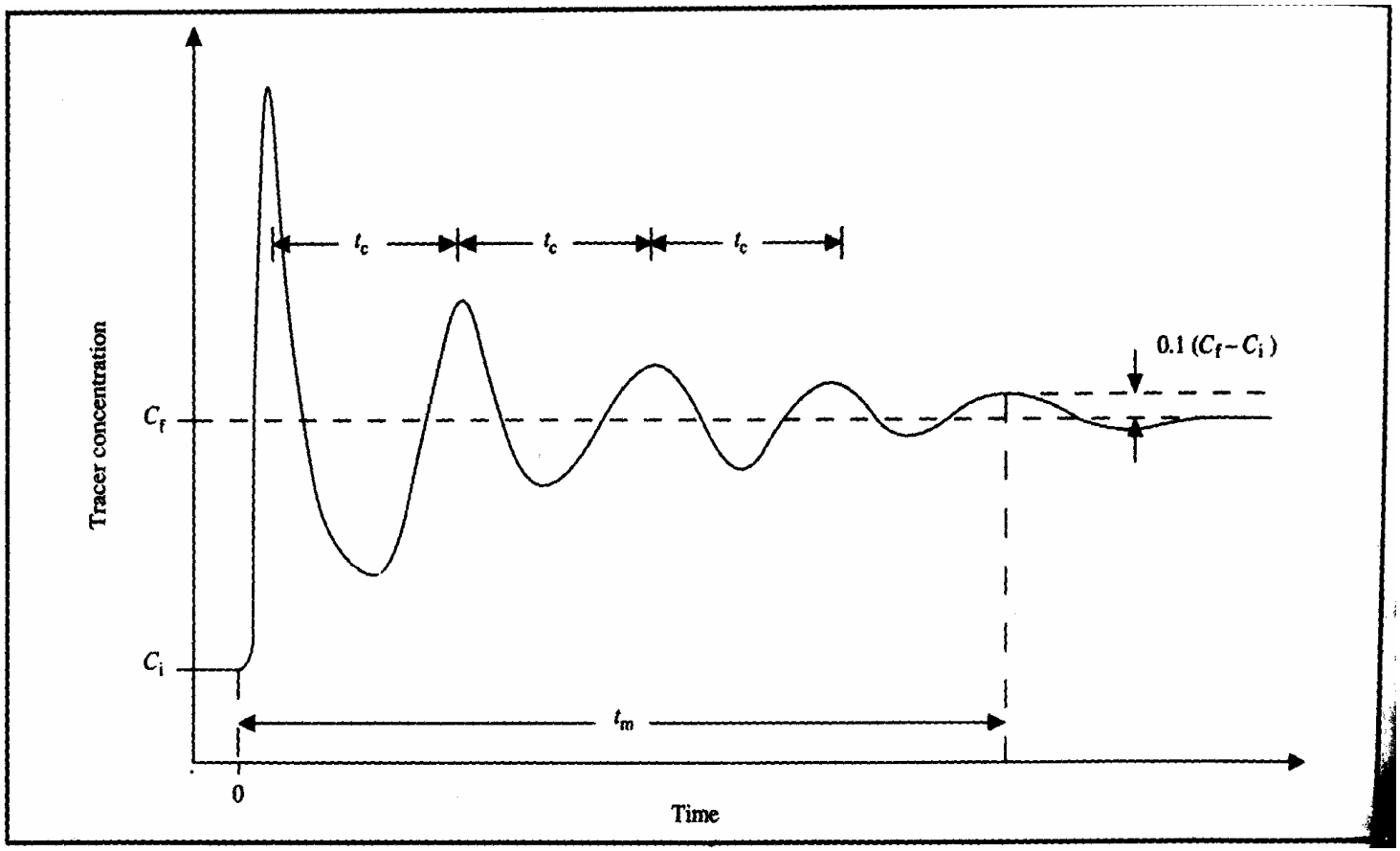


OVERVIEW ON MIXING TIME

- Mixing time (tm) is

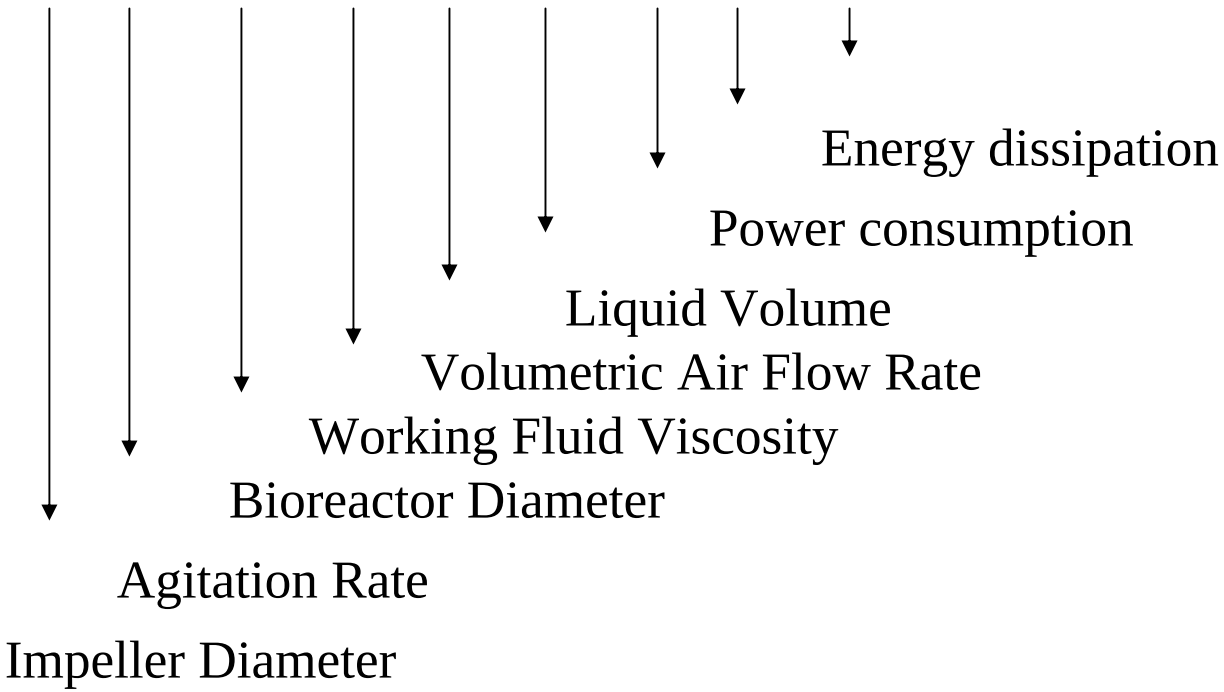
‘the length of time to detect last inhomogeneities in once mixing process after a step change in composition’

[D.Hadjiev *et al.*, 2006]


Tracer concentration
t_c
t_c
t_c
0.1 $(C_f - C_i)$
C_f
C_i
t_m
0
Time

Correlation for mixing time:

$$t_m = f\left(d/D,\ N,\ \eta,\ V_a/V,\ P/P_a,\ \varepsilon_t\right)$$



A GOOD MIXING CAN PROVIDE

- Better Oxygen Transfer
- High Productivity
- Avoid Excessive Foaming
- High mixing efficiency
- Optimum hydrodynamic regime

HOW MIXING TIME CAN BE USED TO OPTIMIZED FERMENTATION PROCESS

- Tm = length of time to detect last inhomogeneities in once mixing process
- So, the lowest tm means faster the system homogenized
- When the system is well homogenized, theoretically the mass and oxygen transfer will also give the highest rate
- Then, max mass and oxygen transfer to the microbe will surely make them to produce max product

[Hassan K.Sreenath *et al.*, 2001]

PROBLEM STATEMENT

- Development of BIOMASS in reactor

  -VISCOSITY, μ

  - OXYGEN MASS TRANSFER, POWER CONSUMPTION, SCALING UP

  -LOW YIELD & FINAL PRODUCT [CONCENT.]

- WHEN μ VARY WITH TIME:
  - Severe MIXING PROBLEMS occur.
  - SEGREGATION, STAGNANT, AND COMPARTMENTIALIZATION

- NEED HIGH SOLID IN FINAL PRODUCT:

  -LARGE NUMBER OF NON NEWTONIAN processes

  -resulted in many problems related with BIOREACTOR such as SHEAR, FOAMING, SCALE UP, POWER CONSUMPTION.

- Hydrodynamic properties vary with medium and impeller configuration

Problem Statement

- Predict experimental values of mixing time and do a comparison to the experimental values obtained
- Used the mixing time determination to optimized the ethanol production of yeast fermentation

Related studies on mixing time

References	Area Focused	Tracer Method	System
[1] (2006)	The effect of geometry, agitation speed and gas flow rate on the mixing time	top injections of 2M NaOH	Distilled water and genuine wastewater
[2] (2002)	The dependence between the mixing time, the rheological characteristics of broths, the fermentation conditions and the biomass concentration, for a stirred bioreactor.	a solution of 2N KOH as tracer	Water and simulated fermentation broths consisted of (CMCNa) having the apparent viscosity in the domain of 8.25 – 268.7 cP.
[9] (2006)	The effect of the density and the volume of the tracer pulse on the mixing time for two impeller combinations in the presence of gas.	NaCl solution as a tracer with density varied from 1054 to 1178 kg/m3. The amount of the tracer pulse was varied in the range of 0.25–1.75% of the bulk liquid volume.	Tap water at 30 ◦C.

Research Objective

- Predict the mixing time in Saccharomyces cerevisiae fermentation &
- Next, optimized the ethanol production of Saccharomyces cerevisiae based on mixing time

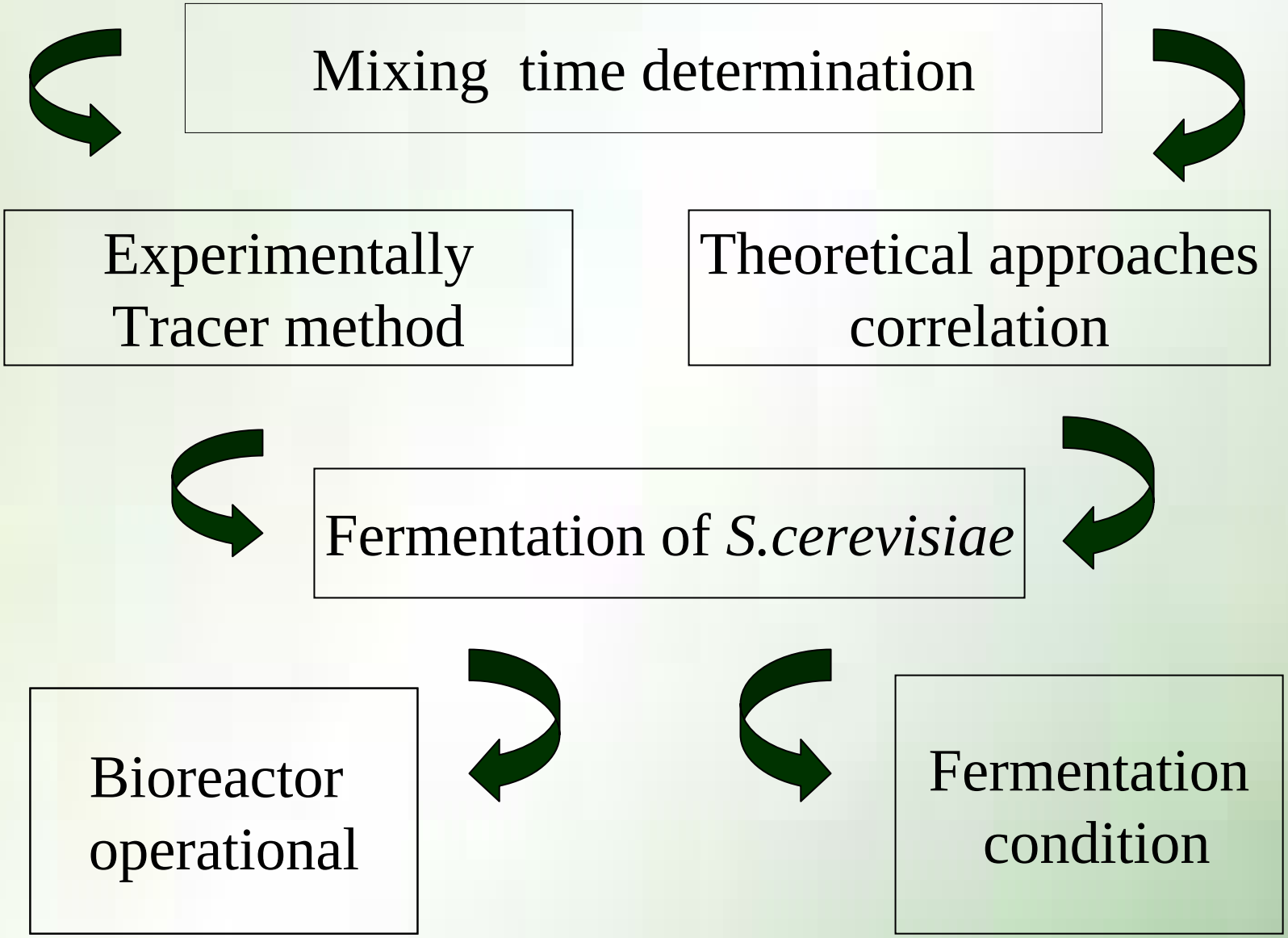
Significance of work

- A fast & uniform blending is necessary and important
- Could provide a clear picture on mixing in bioreactor
- Can be used to optimize any fermentation process

Scope

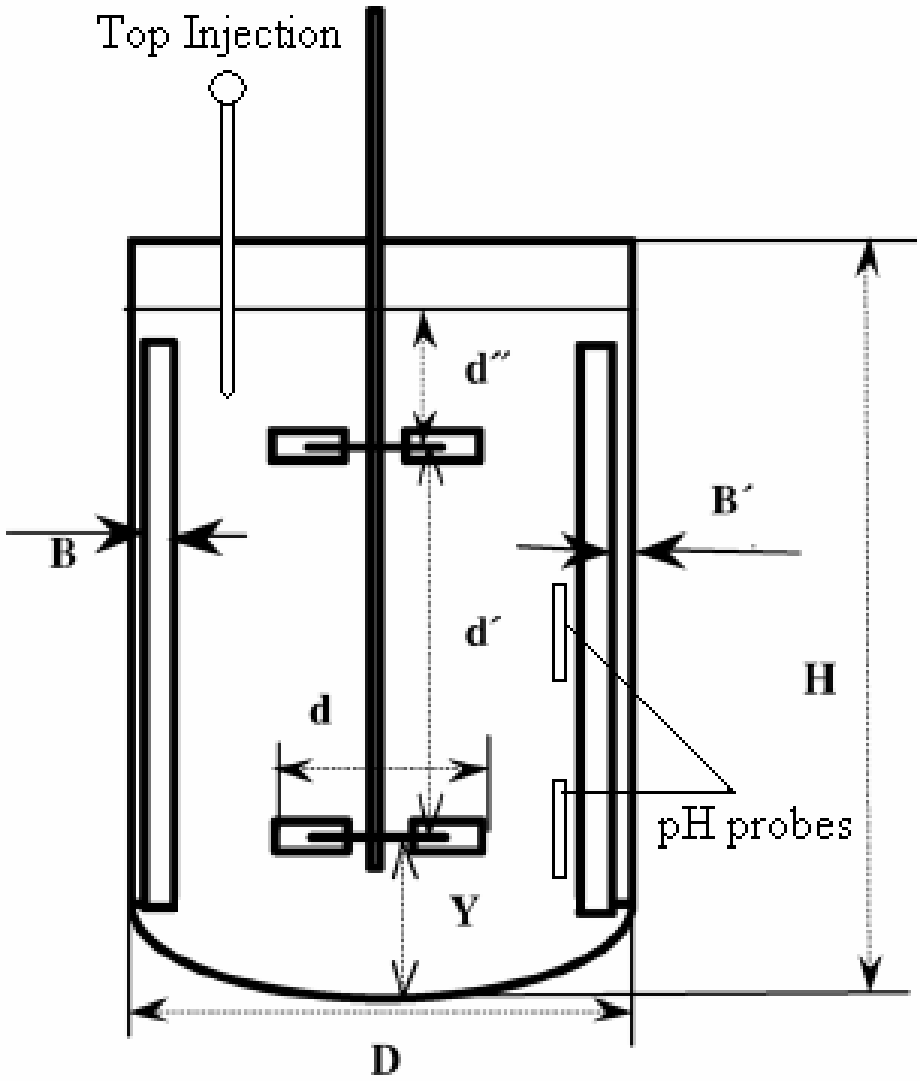
- Determination of mixing time using tracer method [L.Manna; 1997]
- Perform yeast fermentation by employing optimum medium / process condition [Jones *et al.*, 1981]

Methodology



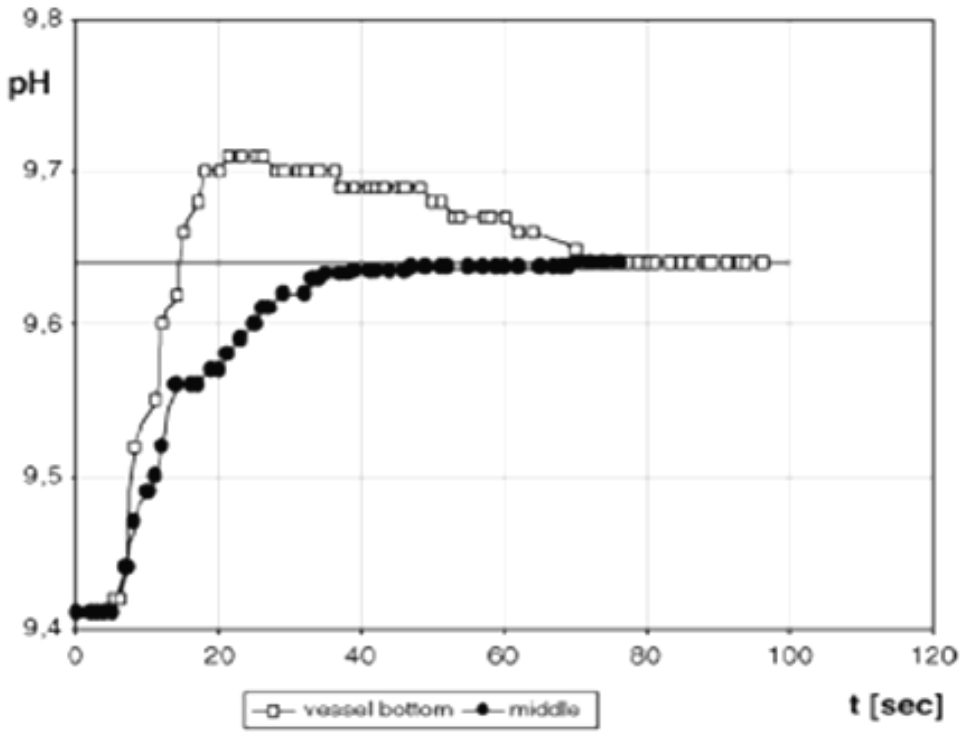
Tracer method

- Tracer = 2M NaOH (0.5 ml)
- Medium = extract yeast 9 g/L
- Agitation = 50 – 400 rpm
- Aeration = 1.0 – 6 L/min



[D.Hadjiev *et al.*, 2006]

OBSERVATION – TRACER METHOD



Example concentration variations obtained by the technique employed
(N = 200 rpm, Q= 5 l/min, d' = o.5d, Y/d = 1)

- Mixing time is the time when the two profile settle as shown in this figure.
- Graph for each impeller configuration are plotted before a comparison between impeller configuration can be made.

Main theoretical approaches

$$Ntm = 6.318619\ (Re^{0.275})\ (Fr^{0.275})\ (Fg^{-0.04})$$

$$Re = (N \times D^2) / v$$

$$Fr = (N^2 \times D) / g$$

$$Fg = Q_g / (N \times D^3)$$

- Approaches by Dimiter Hadjiev *et al.*, 2006

The Stages of a Fermentation Process

- The formulation of media
- The sterilization of the medium, fermentor and equipment
- The production of an active, pure culture
- The growth of the organism in the production fermentor
- The removal of the product
- The disposal of effluents produced by the process

(Stanbury *et al.*, 1995)

Ethanol Analysis

- Using HPLC
- Determination of ethanol concentration through std curve
  - » Ethanol concentration vs peak height

using an Aminex HPX-87H column (300 mm by 7.8 mm) and the following conditions: a temperature of 50C, with 5 mM H2SO4 as eluant (flow rate of 0.5 ml min)1) and a dual detection (refractometer and UV at 210 nm).

[Nicholas Perera et al., 2001]

For the fermentation condition;

- pH 4 – 4.5
- Temperature 28 – 35^{0}C
- Dissolved Oxygen > 40%
- Medium
  - extract yeast
  - glucose
  - magnesium sulphate
  - calcium chloride
  - ammonium sulphate
  - potassium phosphate
  - ferum sulphate
  - glycerol

Jones *et al.*, 1981

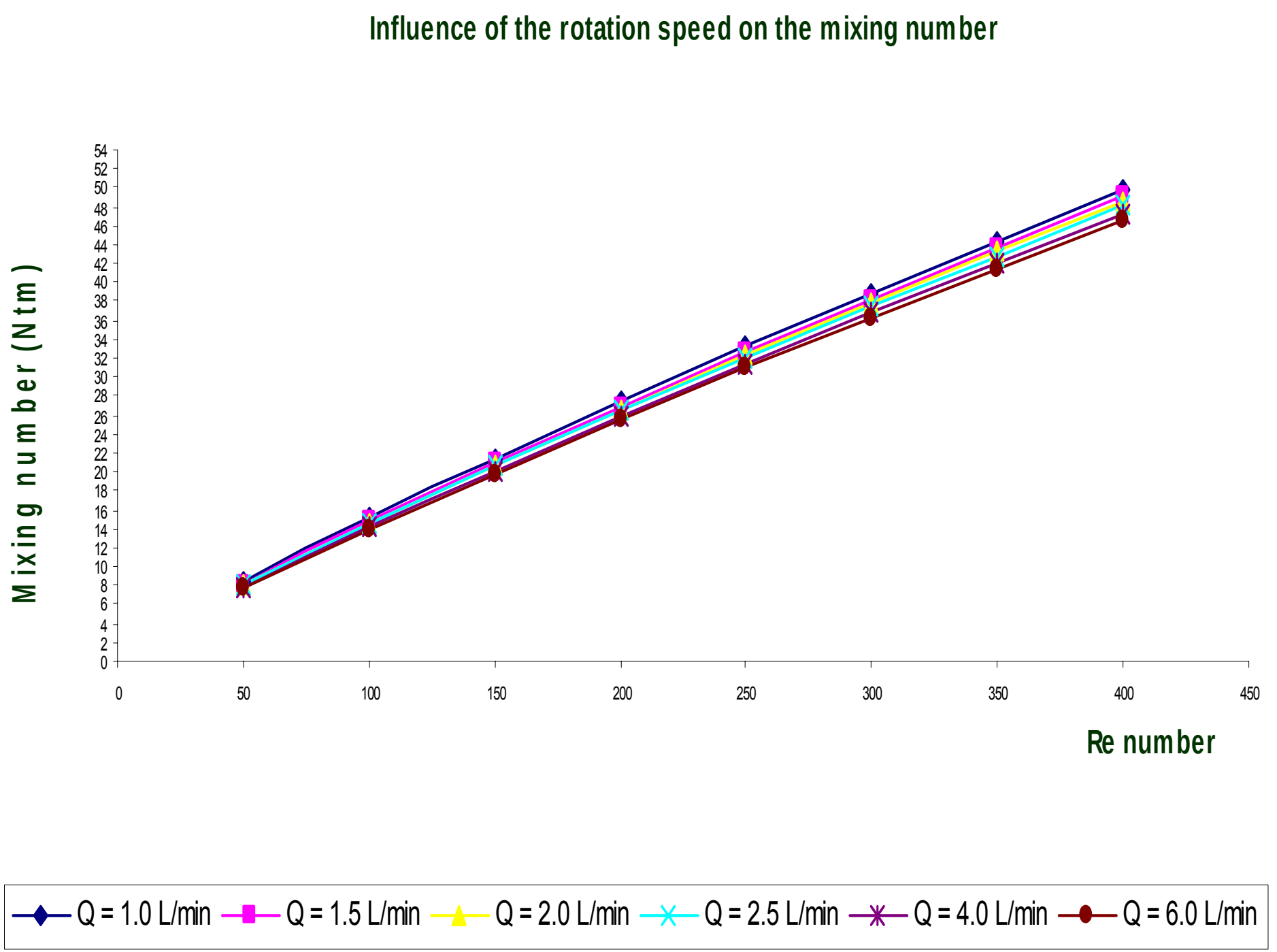
For the operational condition of bioreactor

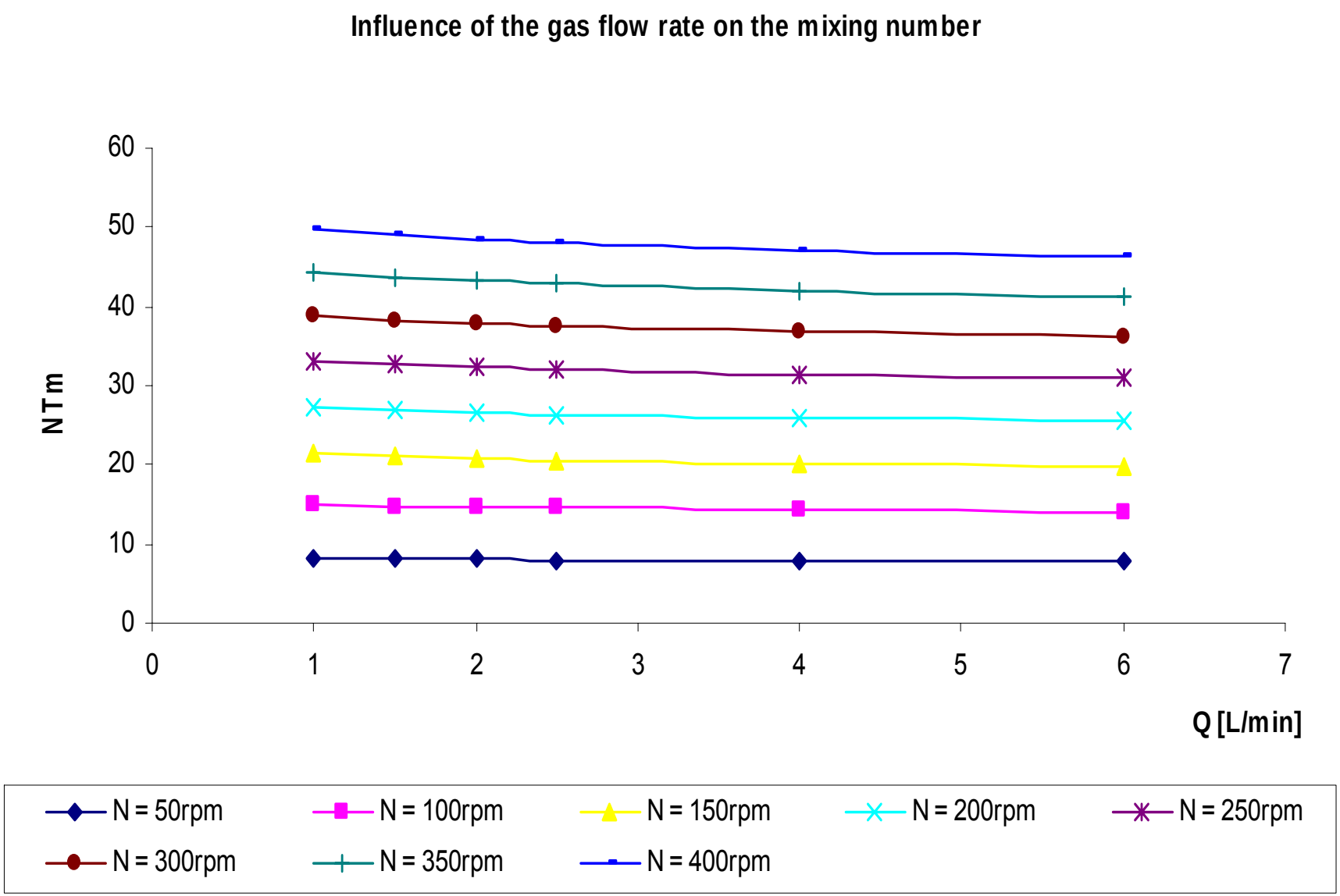
- Impeller type ➢ Turbine
- Number of impeller ➢ 2
- Impeller diameter ➢ 64 mm
- Number of blades ➢ 6
- Number of baffles ➢ 4
- Impeller speeds
- Air volumetric flow rate

} Values from tm determination

Corneliu Oniscu *et al.*, 2001

THEORETICAL MIXING TIME
(by using correlation)


Influence of the rotation speed on the mixing number
Mixing number (Ntm)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
52
54
0
50
100
150
200
250
300
350
400
450
Re number
Q = 1.0 L/min
Q = 1.5 L/min
Q = 2.0 L/min
Q = 2.5 L/min
Q = 4.0 L/min
Q = 6.0 L/min


Influence of the gas flow rate on the mixing number
NTm
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
Q [L/min]
N = 50rpm
N = 100rpm
N = 150rpm
N = 200rpm
N = 250rpm
N = 300rpm
N = 350rpm
N = 400rpm

A common feature of a bioreactor is the need to provide oxygen to the liquid nutrient medium in which the microorganisms are living.

This is achieved by passing air through the liquid. The air is fed into the bottom of the bioreactor.

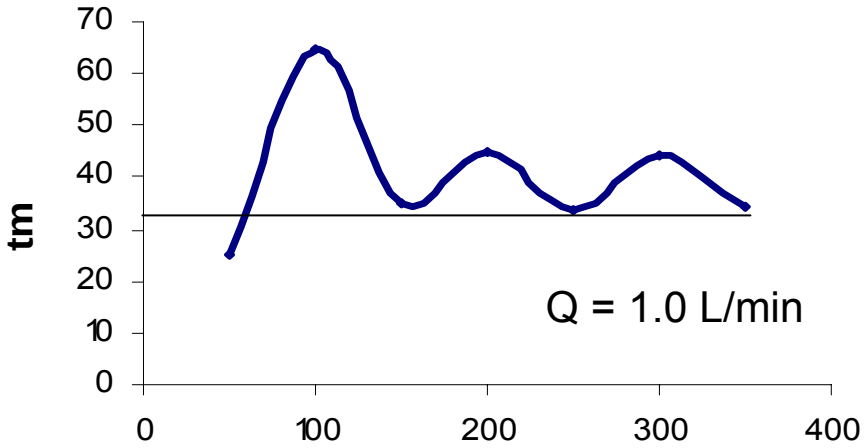
One way of increasing the rate of oxygen availability is by mechanical mixing, usually referred to as agitation. Agitators are rotated at high speeds in the liquid.

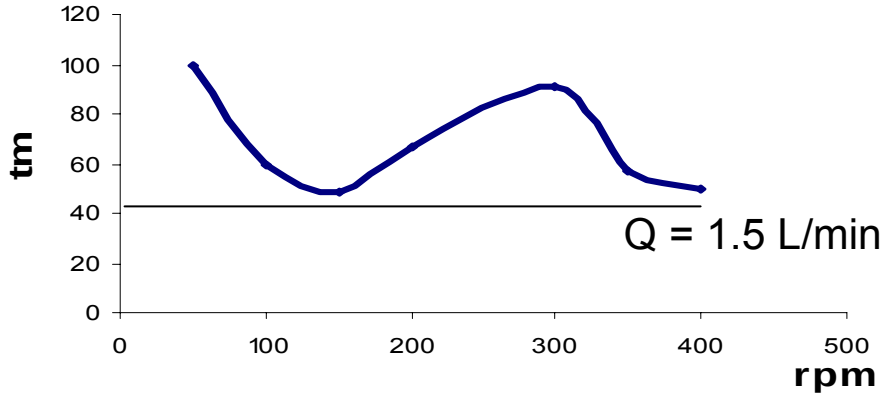
The power provided by the agitator to the liquid breaks up the air into small bubbles. The greater bubble surface area increases the rate at which oxygen dissolves from the air into the liquid.

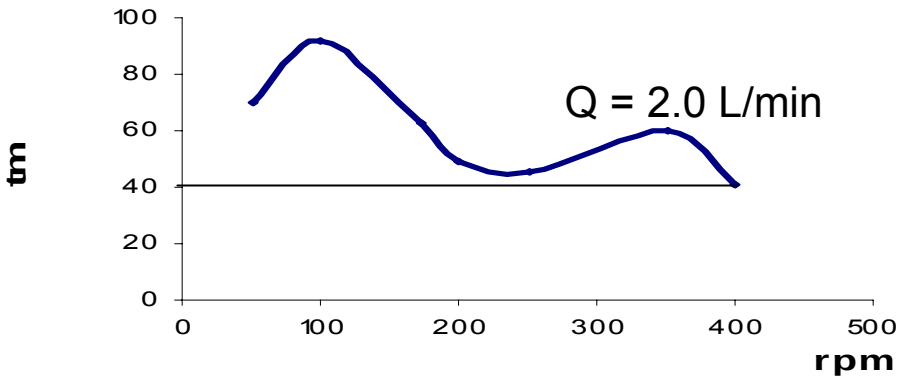
The faster the rate of oxygen supply, the greater the number of microorganisms which can be grown in the bioreactor.

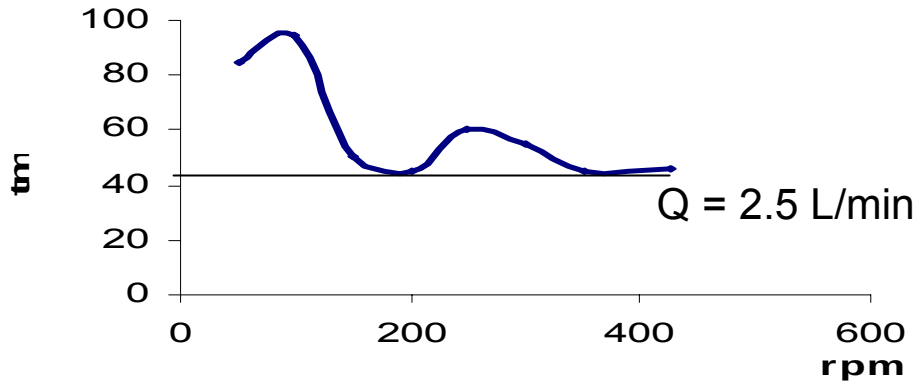
BUT…

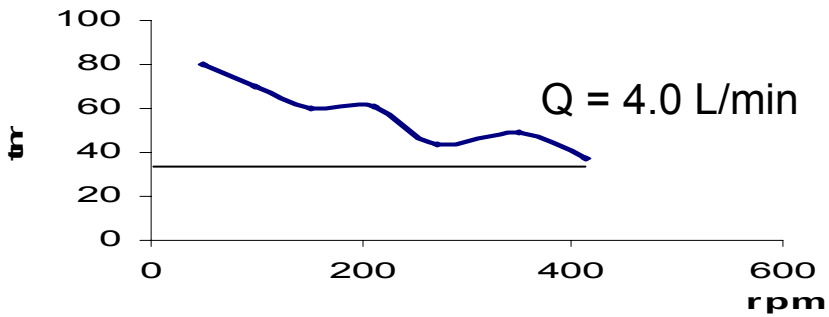
EXPERIMENTAL RESULTS…

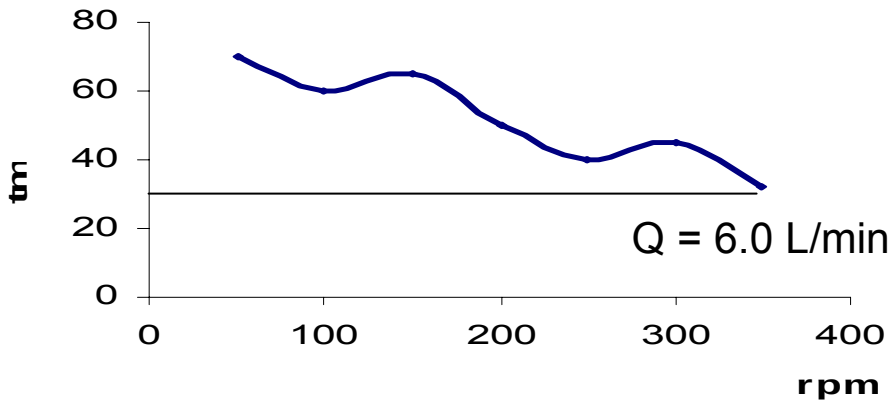

tm
70
60
50
40
30
20
10
0
0
100
200
300
400
Q = 1.0 L/min


tm
120
100
80
60
40
20
0
0
100
200
300
400
500
rpm
Q = 1.5 L/min


tm
100
80
60
40
20
0
0
100
200
300
400
500
rpm
Q = 2.0 L/min


tm
100
80
60
40
20
0
0
200
400
600
rpm
Q = 2.5 L/min


tm
100
80
60
40
20
0
0
200
400
600
rpm
Q = 4.0 L/min


tm
80
60
40
20
0
0
100
200
300
400
rpm
Q = 6.0 L/min

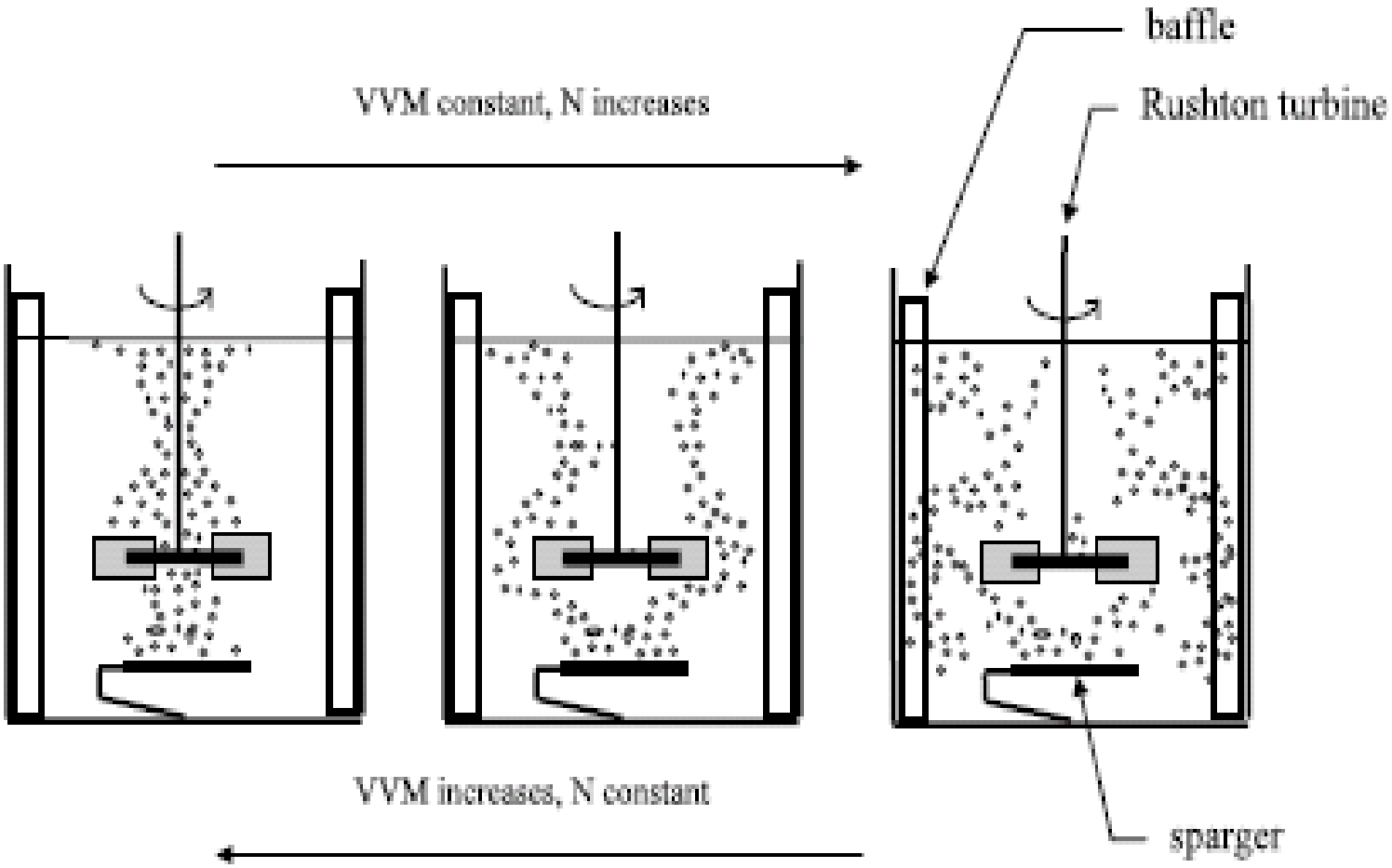
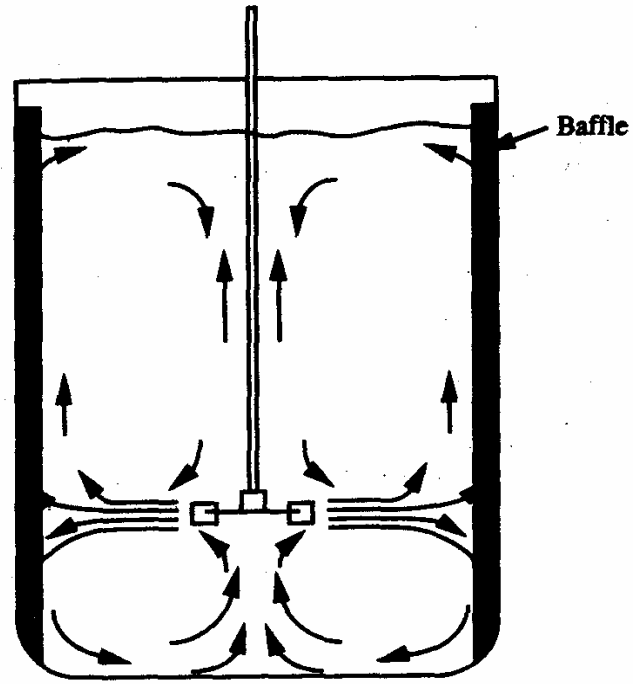


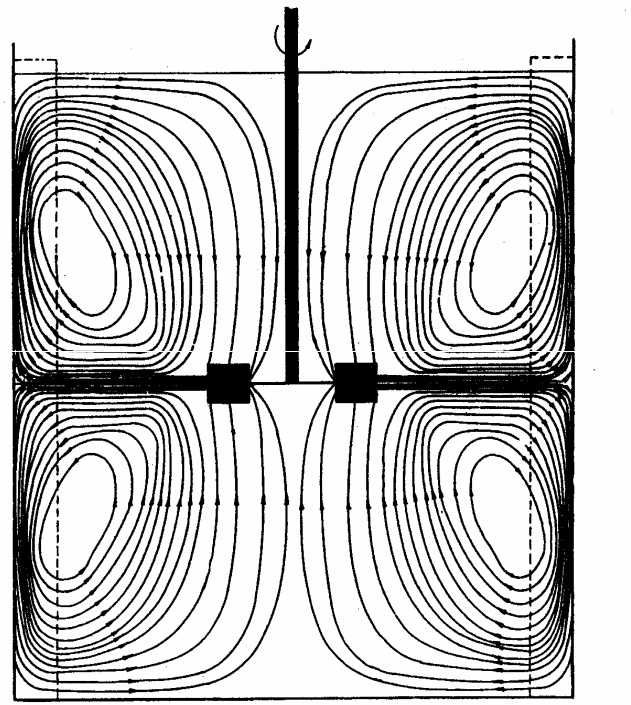
Fig. 7a Fig. 7b Fig. 7c

Fig. 7. Qualitative flow pattern of the liquid–gas phases in the stirred reactors.



Side view

(a)



aeration,Q (L/min) / agitation,N (min^{-1}) Theoretical value	1.0	1.5	2.0	2.5	4.0	6.0
50	8.2562	8.1234	8.0305	7.9591	7.8109	7.6852
100	15.0381	14.7963	14.6276	14.4992	14.227	14.0003
150	21.3552	21.0118	20.7722	20.5868	20.2034	19.8784
200	27.3887	26.9483	**26.6411**	26.4032	25.9115	25.4947
250	33.2205	32.6863	**32.3136**	32.0251	**31.4287**	30.9231
300	38.8951	38.2697	37.8333	37.4955	**36.7972**	36.2053
400	49.8848	49.0827	**48.5231**	**48.0899**	47.1942	46.4351

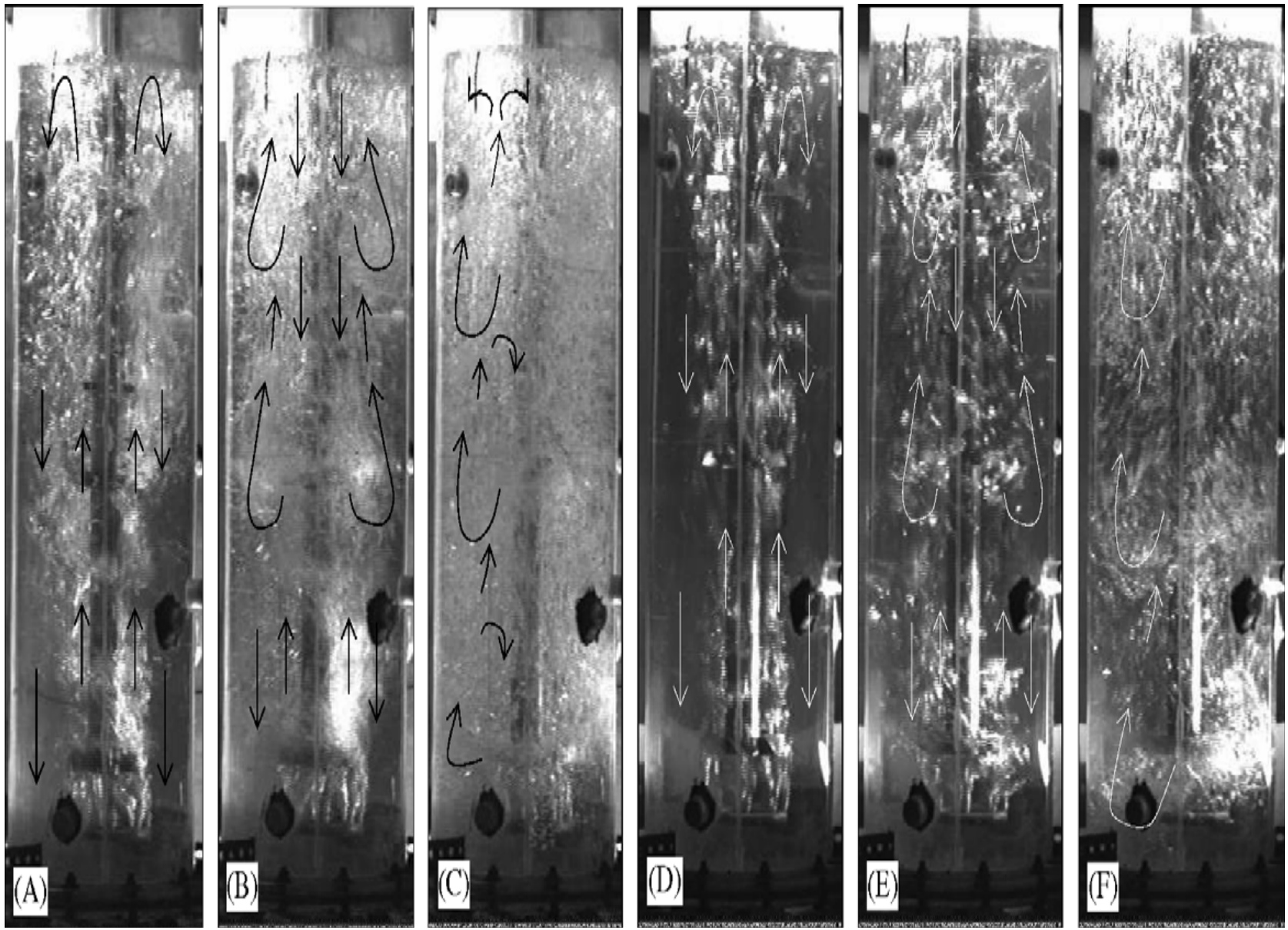
aeration,Q (L/min) / agitation, N (min^{-1}) Exp value	1.0	1.5	2.0	2.5	4.0	6.0
50	25	80	70	85	80	70
100	65	35	80	95	70	60
150	35	**25**	50	50	60	65
200	45	45	**30**	45	75	50
250	60	80	**35**	60	**35**	40
300	55	50	75	55	**35**	**45**
400	75	**50**	**45**	**50**	70	foaming

Based on the attained mixing time,

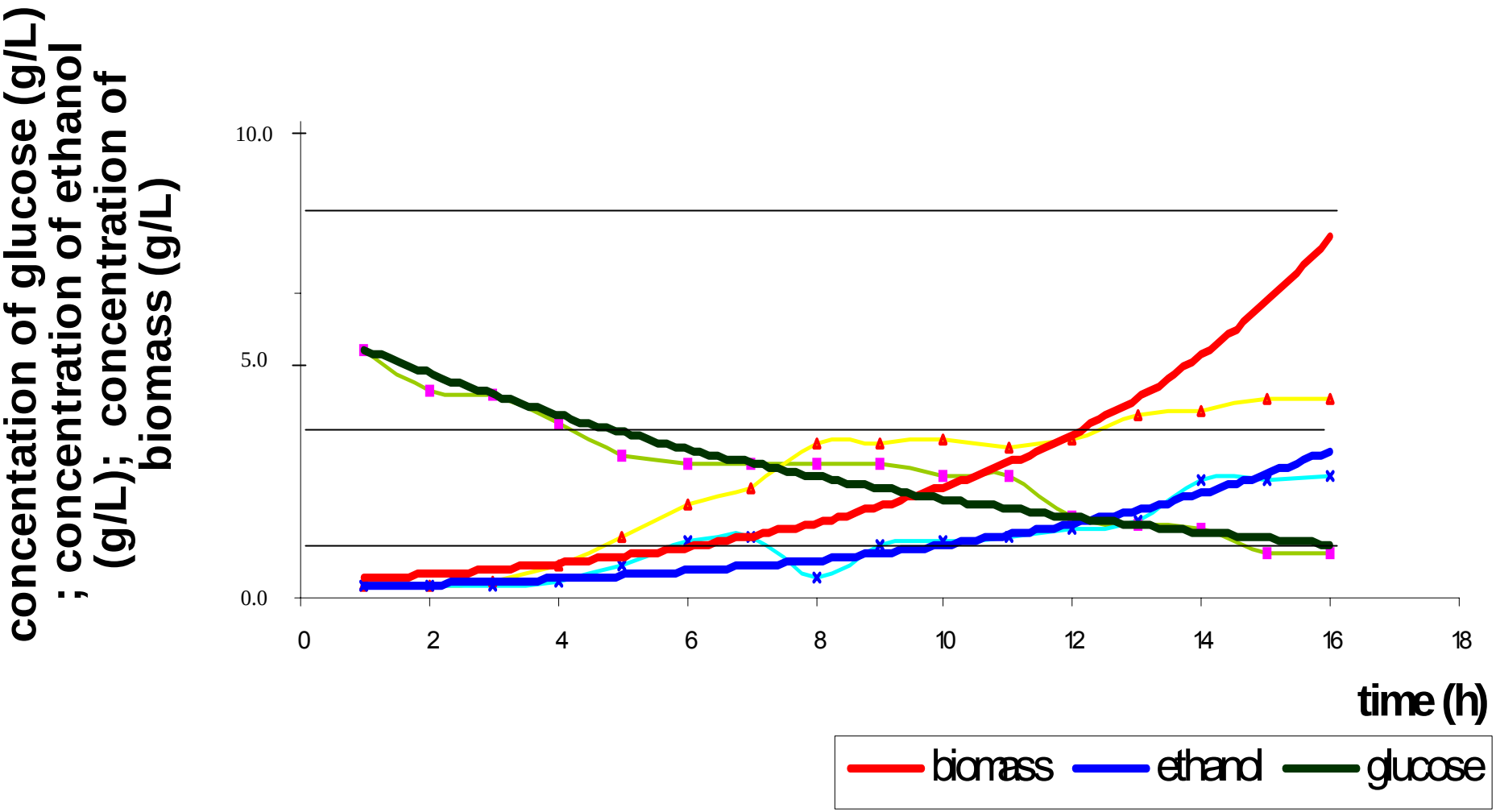
N = 350 - 400 rpm

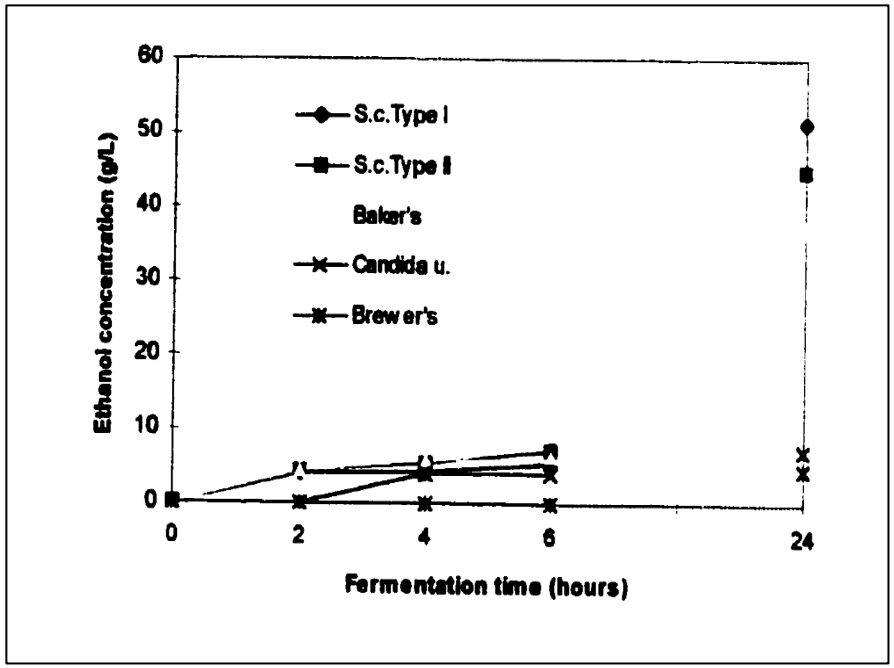
Q = 1.0 – 2.0 L/min

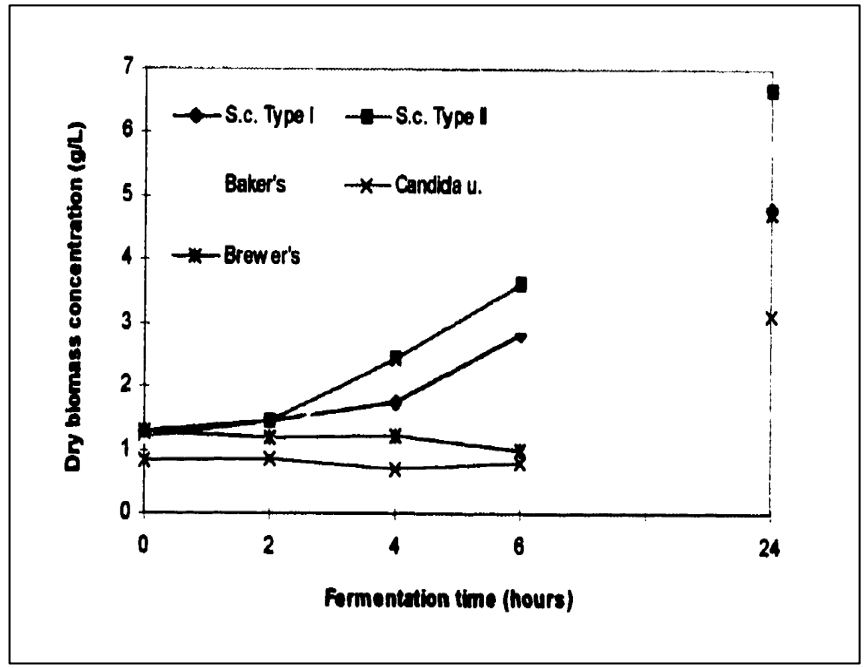
(as long as DO > 40%)

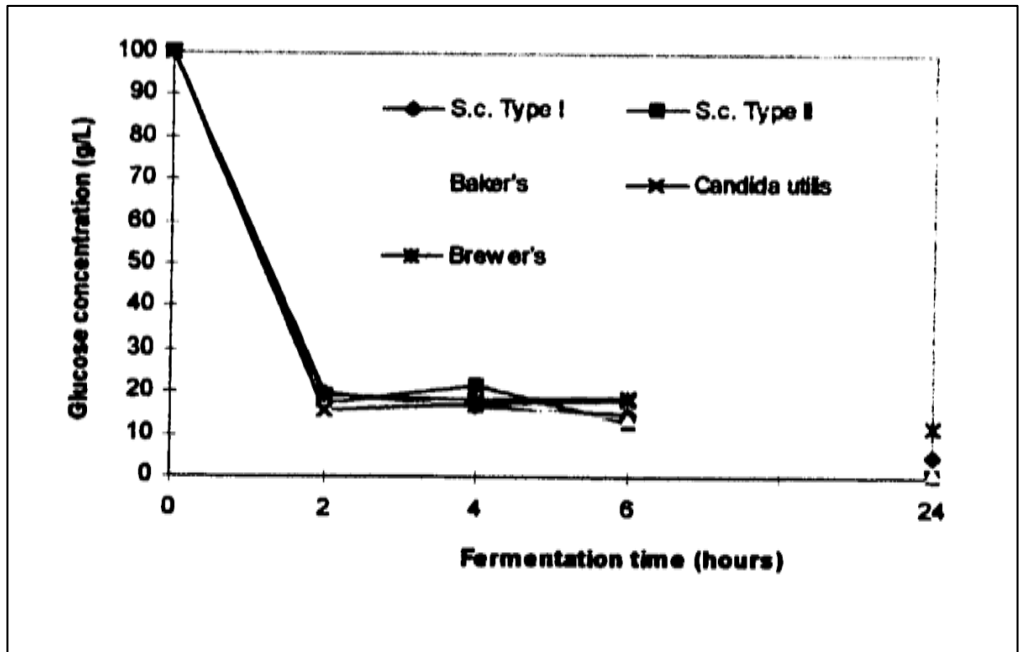

(A)
(B)
(C)
(D)
(E)
(F)

After 16 hours fermentation…




Ethanol concentration (g/L)
60
50
40
30
20
10
0
S.c.Type I
S.c.Type II
Baker's
Candida u.
Brewer's
0
2
4
6
24
Fermentation time (hours)


Dry biomass concentration (g/L)
7
6
5
4
3
2
1
0
S.c. Type I
S.c. Type II
Baker's
Candida u.
Brewer's
0
2
4
6
24
Fermentation time (hours)


Glucose concentration (g/L)
100
90
80
70
60
50
40
30
20
10
0
S.c. Type I
S.c. Type II
Baker's
Candida utilis
Brewer's
0
2
4
6
24
Fermentation time (hours)

So, how do I know that ethanol production has been optimized…?

- Compared to previous study, with the same fermentation condition, but the agitation is at 200 rpm…

The productivity of

- Glucose (g/L.h)
- Biomass (g/L.h)
- Ethanol (g/L.h)

200 rpm	400 rpm
0.0625	0.0125
0.1870	0.5625
0.3125	0.3500

Therefore,

- The yeast fermentation process at 2-L scaled has been successfully optimized with

$$[P] = 0.35 \text{ g / L / h}$$

$$[X] = 0.5625 \text{ g / L /h}$$

- For the fermentation process the mixing time values can be used as a guideline to determine the operating condition for yeast fermentation

However…

- For better estimation results of mixing time can be done by using more complex models
  - Compartment models
  - Networks-of-zones models
  - Numerical CFD models

But…

It require complex calculations which are time-consuming