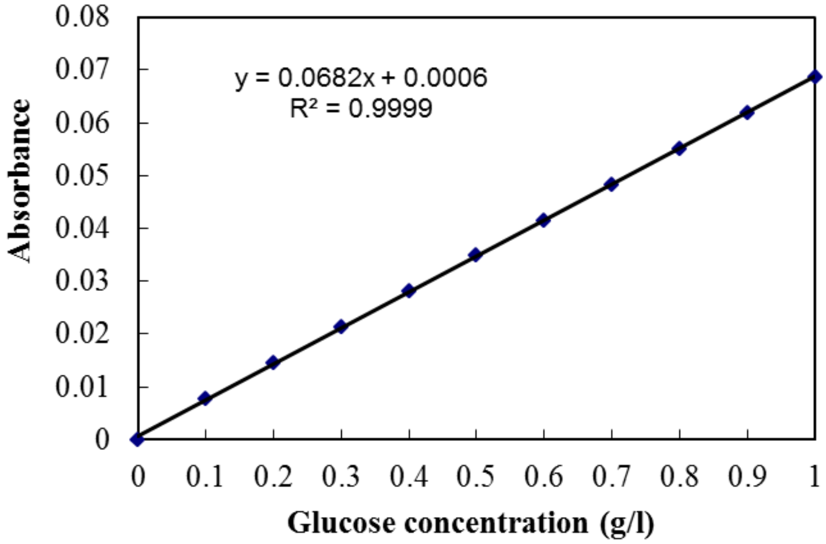
Appendix 1

Total sugar estimation using standard glucose curve



Appendix 2

Mathematical modeling of growth curve

Sigmoidal functions have been the most popular ones to fit microbial growth data, since these functions consist of three phases similar to the microbial growth curve. Models that describe the response of microorganisms to single set of conditions over time are primary models and those describing the effect of environmental conditions are the secondary models. Logistic and Gompertz are the primary models that describe the kinetics of the process with a few parameters while still being able to accurately describe the distinct phases of growth. Logistic model was published by Pierre-Francois Verhulst in 1838. Gompertz model was introduced in 1825 to describe the mortality in humans. Equation A2.1 and A2.2 are the original forms of the Logistic and Gompertz model where a, b and c are the modeling parameters.

$$y = \frac{a}{1+\exp(b-ct)} \qquad \text{.............................. A2.1}$$

$$y = a.exp[-exp(b-ct)] \qquad \text{.......................... A2.2}$$

Since bacteria grow exponentially the growth curve is plotted as log of relative population versus time. Sigmoid models were used to describe the increase in log of the bacterial cell density with time. Among them are the Logistic and Gompertz model. These models were not intended to describe growth of microorganisms, were adapted for bacterial growth and were reparameterized so that they include the relevant biological parameters.

Bacterial growth often shows a phase in which the specific growth rate starts at a value of zero and then accelerates to a maximal value (μ_m) in a certain period of time, resulting in a lag time (λ). In addition, growth curves contain a final phase in which the rate decreases and finally reaches zero, so that an asymptote (A) is reached. When the growth curve is defined as the logarithm of the number of organisms plotted against time, these growth rate changes result in a sigmoidal curve. In the sigmoidal curve, the parameters are defined such as the maximum specific growth rate μ_m i.e. the tangent in the inflection point; the lag time λ which is defined as the x-axis intercept of this tangent; and the asymptote A is the maximal value reached (Figure A2.1)

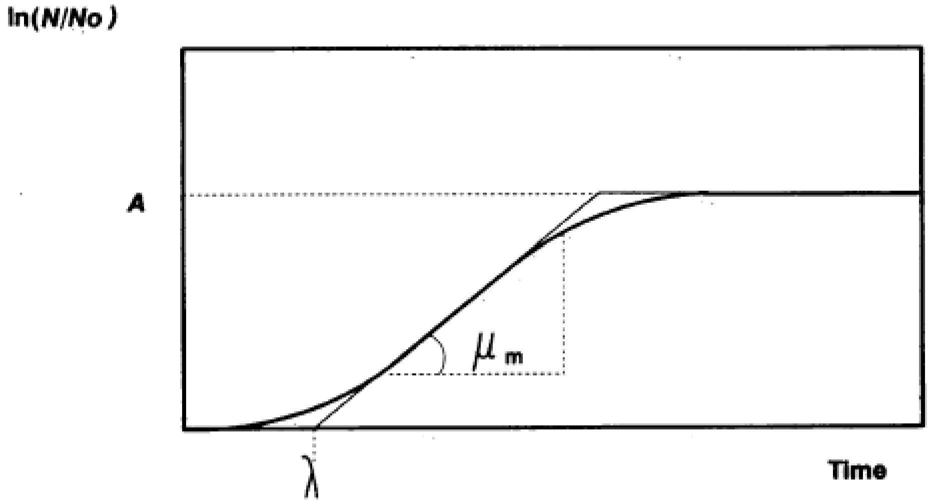


Fig. A 2.1: Growth curve

The mathematical model however consists of mathematical parameters (a, b and c) which do not give any meaningful idea about the bacterial growth. Therefore, these models are rewritten to substitute the mathematical parameters with meaningful biological parameters such as μ_m, λ and A. This is done by deriving an expression of biological parameters as the function of the parameters of the basic function and then further substituting in the equation.

Zwietering et al. (1990) discussed that if the data points are less, then the three parameter models are more useful, since the estimates have more degree of freedom which is important with less data points. Furthermore, it is also very important that these data points can be given a proper biological meaning. Therefore, all the growth models were rewritten to substitute the mathematical parameters with A, μ_m and λ. This was done by deriving an expression of the biological parameters as a function of the parameters of the basic function and then substituting them in the relevant equations.

Logistic model

The Logistic equation is written as

$$y = \frac{a}{1 + \exp(b - ct)} \quad \text{...A2.1}$$

In order to obtain the inflection point of the curve, the second derivative of the function with respect to t is calculated:

$$\frac{dy}{dt} = \frac{ac\,[\exp(b - ct)]}{[1 + \exp(b - ct)]^2} \quad \text{.. (A2.3)}$$

$$\frac{d^2y}{dt^2} = -1 + 2\exp(b - ct)\text{..(A2.4)}$$

At inflection point where t = t_i, the second derivative = 0, Therefore,

$$\frac{d^2y}{dt^2} = 0 \text{ .... (A2.5)}$$

$$\frac{d^2y}{dt^2} = -1 + 2\exp(b - ct) = 0 \text{ ....(A2.6)}$$

$$\ln e^{b-ct} = \ln 1$$

$$b - cti = 0$$

$$b = cti \text{ or } ti = b/c \text{ ....(A2.7)}$$

An expression for the maximum specific growth rate can be derived by calculating the first derivative at the inflection point.

$\mu_{max} = (\frac{dy}{dt})_{ti}$ = first derivative at inflection point where t = t_i . ...(A2.8)

Therefore from equation (A2.3)

$$\frac{dy}{dt} = \frac{ac\,[\exp(b - ct)]}{[1 + \exp(b - ct)]^2}$$

Substituting t = t_i and b = ct_i from equation (A2.8)

$$\frac{dy}{dt} = \frac{ac\,[\exp(0)]}{[1 + \exp(0)]^2}$$

$$\mu_{max} = \frac{ac}{4} \text{ ....(A2.9)}$$

$$c = \frac{4\mu_{max}}{a} \text{ .... (A2.10)}$$

$$b = \frac{4\mu_{max}\,\lambda}{a} + 2 \text{ (From the graph at inflection point) ....(A2.11)}$$

$$\lambda = \frac{(b - 2)}{c} \text{ ....(A2.12)}$$

Therefore substitution of the parameters derived above for b and c and as t $\rightarrow \infty$,y $\rightarrow$ a and A= a (Asymptotic value is reached), we further get the modified logistic equation with all mathematical parameters replaced by all biological parameters

$$y = \frac{A}{\left\{1 + \exp\left[\frac{4\mu m}{A}(\lambda - t) + 2\right]\right\}} \text{ ....(A2.13)}$$

Gompertz Model

$$y = a.\,exp[-exp(b - ct)] \text{..} A2.2$$

First Derivative

$$\left(\frac{dy}{dt}\right)_{t=ti} = acexp[exp(b - ct)]exp(b - ct) \text{ ....} (A2.14)$$

$$\frac{d^2y}{dt^2} = ac^2 \exp[-\exp(b - ct)] \exp(b - ct) \text{...............................} (A2.15)$$

At inflection point, the second derivative is equal to zero;

$$\frac{d^2y}{dt^2} = 0 \rightarrow t_i = b/c \text{ ....} (A2.16)$$

Further the expression for maximum specific growth rate can be derived by calculating the first derivative at the inflection point.

$$\left(\frac{dy}{dt}\right)_{t=ti} = \mu_m = \frac{ac}{e} \text{ ....} (A2.17)$$

The parameter c in the Gompertz equation can be substituted by $c = \mu_m e/a$

The tangent line through the inflection point is

$$y = \mu_m .\, t + \frac{a}{e} - \mu_m t_i \text{.............................} (A2.18)$$

The lag time is defined as t-axis intercept of the tangent through the inflection point

$$0 = \mu_m .\, \lambda + \frac{a}{e} - \mu_m t_i$$

$$\text{So the value of } \lambda = \frac{(b-1)}{c} \text{ ....} (A2.19)$$

The asymptotic value is reached for t tending to infinity

$$t \quad \infty,\ y \rightarrow a,\ A = a$$

The value of b in Gompertz equation can be substituted by $b = \frac{\mu_m e}{a} + 1$...... (A2.20)

Therefore the modified Gompertz equation with all biological parameters is obtained as

$$y = Aexp\left\{-\exp\left[\frac{\mu_m e}{a}(\lambda - t) + 1\right]\right\} \text{..} (A2.21)$$

Appendix 3

Cost Estimation

The cost estimation for the production of biosurfactant – surfactin has been done with the basis of 20 kg of surfactin production per year. Rice bran is the major raw material utilized for fermentation and subsequent biosurfactant production which is procured from the nearby local rice mill. The cost estimation is done considering Bharuch and Ankleshwar region as the main site for production.

The selection of equipments for the process are based on judicious consideration with the objective to keep the batch sizes small to facilitate media sterilization and downstream separations as well as to keep the production line intact in case some contamination seeps in the fermentation set up.

Fixed Capital Investment (FCI)

Purchase Equipment Cost (E)			
Sr No.	**Name of Equipment**	**Quantity**	**Total Cost in (Rs)**
1	Fermentor rack (4X300 L)	4	80000000
2	Centrifuges	2	12500000
3	Vacuum Dryer	1	150000
4	Seed Fermentor (25 L)	1	1812875
5	Precipitation Tanks (~250 L)	1	2500000
6	Foam Fractionation Assembly	-------	2000000
7	Sterile room with hepa filters	1	2500000
8	Packaging machinery	-------	5000000
9	Distillation Plant 150lt/hr	1	2000000
10	Diesel generator 350 KVA	1	6500000
11	Refrigerated storage (16000lt)	1	5000000
12	AHU(10 ton) and Dehumidifier	1	5000000
13	Laboratory equipments	-------	5000000
		Total (E)	**129962875**

Direct cost (D)

Sr No.	Component	% of Purchase Equipment Cost	Total cost in (Rs)
1	Purchase Equip Cost	100	129962875
2	Equipment installation	15	19494431.25
3	Instrumentation	15	19494431.25
4	Piping	10	12996287.5
5	Electrical	15	19494431.25
6	Building and service	40	51985150
7	Yard Improvement	5	6498143.75
8	Utilities	30	38988862.5
9	Insulation	5	6498143.75
10	Land	----	12500000
		Total (D)	**317912756.3**

Indirect cost

Sr No.	Component	Cost in (Rs)
1	Engineering & supervision (30%E)	38988862.5
2	Contingency (10%D)	31791275.63
	Total (Indirect cost)	**70780138.13**

FCI = Direct cost + Indirect cost 160374372.3

WORKING CAPITAL INVESTMENT

A) Raw material consumption per annum (Considering 20 Kg surfactin production per annum)

Sr No.	Raw material	Cost in (Rs) per year	Unit rate
1	Rice bran (4800 kg)	96000	Rs 20/kg
2	Nutrient media (10 kg)	30000	Rs 3000/kg
3	Agar powder (5 kg)	15000	Rs 3000/kg
4	Power	2000000	------
	Total	**2141000**	

B) Labor Charges per annum considering salary of people employed = 39500000

C) Maintenance and repair: 20% of FCI = 77738578.88

D) Transportation = 350000

Total working capital cost: (WCI) = A+B+C+D **=** 119729578.9

GENERAL EXPENSES

Sr No.	Particular	Cost in (Rs) per year
1	Administration expenses	12000000
2	R & D Expenses and QC	7000000
3	Hospital & Accidental	200000
	Total General Expense	**19200000**

a) Depreciation = 10% of (FCI-LAND)	=	37619289.44
b) Local taxes: = 2% of FCI	=	7773857.888
c) Insurance: = 1% of FCI	=	3886928.944
Fixed charges = a+b+c	**=**	**49280076.27**

TOTAL PRODUCT COST:

WCI + GEN EXPENSES + FIXED CHARGES = 188209655.1

INCOME SOURCE:

Market selling price of Surfactin = Rs 20 per mg

Total selling price or turn over for 20 kg product = 400000000

A) Gross Profit

Gross profit = Turn over - WCI - Fixed charges - General expenses

= Turn over - Total product Cost = 211790344.9

B) Income Tax = 30% Of Gross profit **=** 63537103.46

C) Net Profit = Gross profit – Income Tax = 148253241.4

% Rate of return = Net profit*100/ (FCI + WCI) = 29.16

Pay out period $= \dfrac{\text{FCI}}{[\text{ Net profit/yr + depreciation/yr}]}$

= **2.091**

Salient Features of Cost Estimation

- Total production of surfactin: 20kg per annum
- Total yield of surfactin : 6.0g/kg rice bran used as substrate
- Selling price of surfactin: Rs 20 per mg
- Total product cost : Rs 188209655.1
- Gross Profit: Rs 211790344.9
- Net Profit (Less income tax): Rs 148253241.4
- % Rate of return : 29.16
- Pay out period : 2.09