Chapter 2
BACKGROUNDS AND LITERATURE REVIEWS

2.1 Pathway of ethanol generation

Glucose from feed stock is transported by glucose transporter from outside of cell to cytoplasm. In cytoplasm, there are many enzymes that have weak interaction, called supramolecular structure. The advantageous structure leads to the rapid reaction of reforming glucose to pyruvate, Glycolysis.

2.1.1 Glycolysis – Embden-Meyerhoff-Parnas pathway (EMP)

Glycolysis is the most commonly used series of reactions for oxidizing glucose to pyruvate. It is an essential part of many organisms catabolism even in typical fermentation. There are three main stages of reformation glucose to pyruvate, activation of glucose, hexose splitting and energy extraction.

Activation of glucose

Glucose is a relatively stable molecule. In order to degrade it, it must first be destabilized by adding high energy phosphates. In the first step a phosphate is donated from ATP (phosphoenolpyruvate – the source of the phosphate) to glucose to form glucose-6phosphate by hexokinase enzyme. The molecule is isomerized to fructose-6-phosphate by phospoglucose isomerase enzyme and a second phosphate is added. Fructose-1,6-bisphosphate, formed by phosphofructokinase-1, is ready to be split and is easier to attack than glucose is.

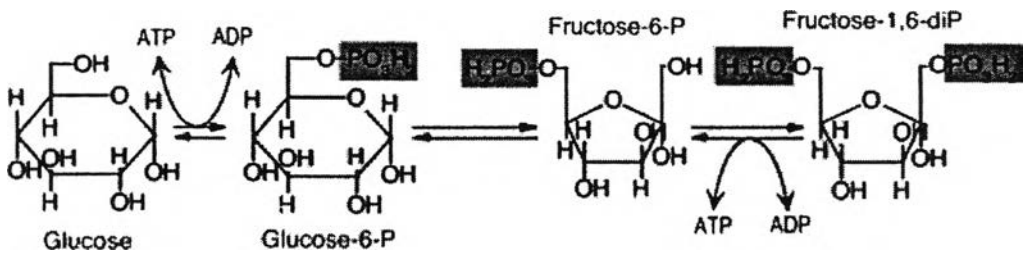

Figure 2.1 Activation of glucose by phosphorylation with ATP

Source: Paustian, 2003

Hexose Splitting

Fructose bisphosphate aldolase then breaks the phosphate loaded fructose into two 3 carbon compounds: glyceraldehydes-3-phosphate and dihydroxyacetonephosphate. This is the crucial step in the glycolysis pathway, converting the 6 carbon glucose molecule to two 3 carbon molecules that will eventually become pyruvate

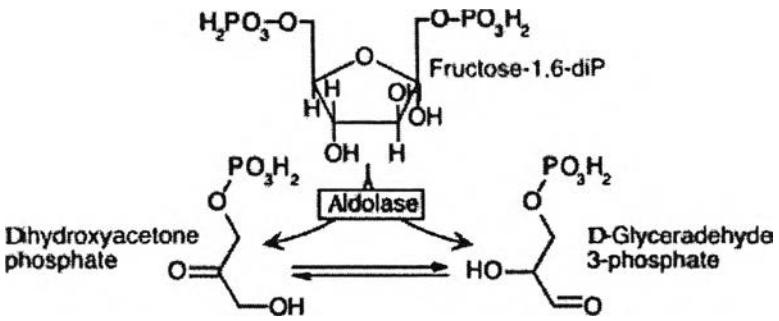

Figure 2.2 Splitting of Fructose by aldolase
Source: Paustian, 2003

From the last reaction, there are two products, dihydroxyzcetone phosphate and glyceradehyde-3-phosphate. It is found that only glyceradehyde-3-phosphate will be decomposed to the next step of glycolysis pathway. However, dihydroxyzcetone phosphate, the isomer of ketose-aldose with glyceradehyde-3-phosphate, can be transformed rapidly to glyceradehyde-3-phosphate by triose phosphate isomerase enzyme.

Energy Extraction

Inorganic phosphate is added to glyceradehyde-3-phosphate to make 1,3-bisphosphoglycerate by using glyceraldehydes-3-phosphate dehydrogenase. No energy is required; in fact, electrons are transferred from glyceradehyde-3-phosphate to NAD+. The inorganic phosphates are later transferred to ADP to make ATP and 3-phosphoglycerate by phosphoglycerate kinase. Then, 3-phosphoglycerate will be transferred functional group by phosphoglycerate mutase enzyme. Next, water is removed by using enolase enzyme. These are preparation steps to be high energy phosphate substance to synthesis other ATP at the last reaction. The

final reaction, pyruvate will be produced by transfering phosphate group to ADP by pyruvate kinase.

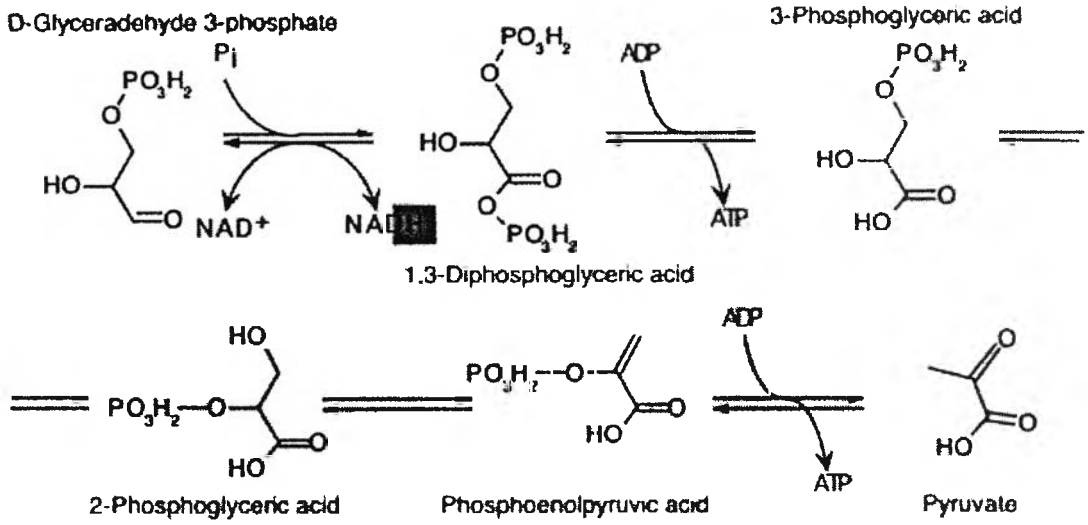

Figure 2.3 Extraction of Energy (half of pathway). Note the two highlighted reactions that yield energy

Source: Paustian, 2003

2.1.2 End Product Formation

After the formation of pyruvate, pyruvate is reduced to ethanol and carbon dioxide (CO_2). In anaerobic yeast cell culture, it is called alcohol fermentation. There are two reactions for this step. First, CO_2 is removed from pyruvate to form acetaldehyde by pyruvate decarboxylase enzyme. Secondly, the reduction of acetaldehyde changes to ethanol and NAD+ by NADH with alcohol dehydrogenase enzyme.

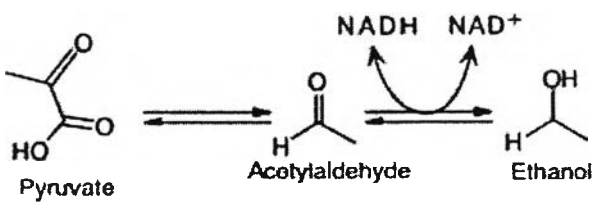

Figure 2.4 Oxidation of NADH. Acetaldehyde is reduced to ethanol. This is the final step in yeast fermentation of glucose to ethanol

Source: Paustian, 2003

Theoretically, the maximum conversion efficiency of glucose to ethanol is 51 percent on a weight basis and this alteration can be summarized as following equation.

$$\underset{(C_6H_{12}O_6)}{\text{Glucose}} \longrightarrow \underset{(CH_3CH_2OH)}{2\,\text{Ethanol}} + \underset{(CO_2)}{2\,\text{Carbon dioxide}} + \text{Heat} \qquad \text{------- (2.1)}$$

2.2 Batch fermentation

Batch fermentation is a system that is fermented by microorganism within close fermentor by limiting nutrient. Generally, kinetic in fermentation consists of biomass growth, substrate consumption, and product formation. For desired production in batch fermentation, it is necessary to control the environment of fermentation, like physical and chemical factors, such as pH level, temperature, and carbon or nitrogen source. Suitable nutrients are consumed by microbe to live and produce new substances. The time course of batch fermentation, as shown in Figure 2.5, has three main relations that are cells, substrates and products change which occur in fermentation.

The process dynamic of cells can be divided into five phases, an initial lag phase, a growth phase, a production phase, a stationary phase, and a declining or death phase. The initial lag phase is due to regulatory phenomena of the microorganisms as an adaptation from the preculture to the conditions in the fermentor. The time of adaptation is depending on many factors such as age of microbe, concentration of inoculums, and vitamin or minerals that can transfer into cell. These factors are necessary for pathway activities of enzymes in cell metabolism. For example, the increasing of magnesium in nutrient broth for *E. aerogenes* culture affects the adaptation time that shown in Figure 2.6 [Shuler and Kargi, 2001]. Magnesium is activated substance for phosphatase enzyme activity in those bacteria. In the same hand, if inoculums undergo in new carbon concentration substrate, adaptation time will change for new enzyme creation to degrade the carbon nutrient level. Further, cells in inoculum, for short adaptation time, must be prepared in young age, in log growth phase, and have enough amounts to subsequent growth (around 5-10% by volume). For industrial scale, lag phase must be controlled in the shortest adaptation time. Afterwards, exponential

growth can usually be observed. In both phases substrate is in excess. In a later stage of the process, many factors that are not suitable in fermentation environment lead to stationary phase, for example:

- Not enough nutrients for cell growth.
- Not enough oxygen for cell respiration. For high cell concentration, the viscosity of broth increases and lowers the oxygen transfer rate.
- High toxic concentration level from the accumulation of product such as ethanol or product that affects pH level as citric acid.
- Run out of growth factors such as vitamin or ion metal.
- Not enough area per unit of cell accommodation according to high cell concentration.

This can be followed by a declining phase in which the cell mass decreases due to lysis or endogenous metabolism.

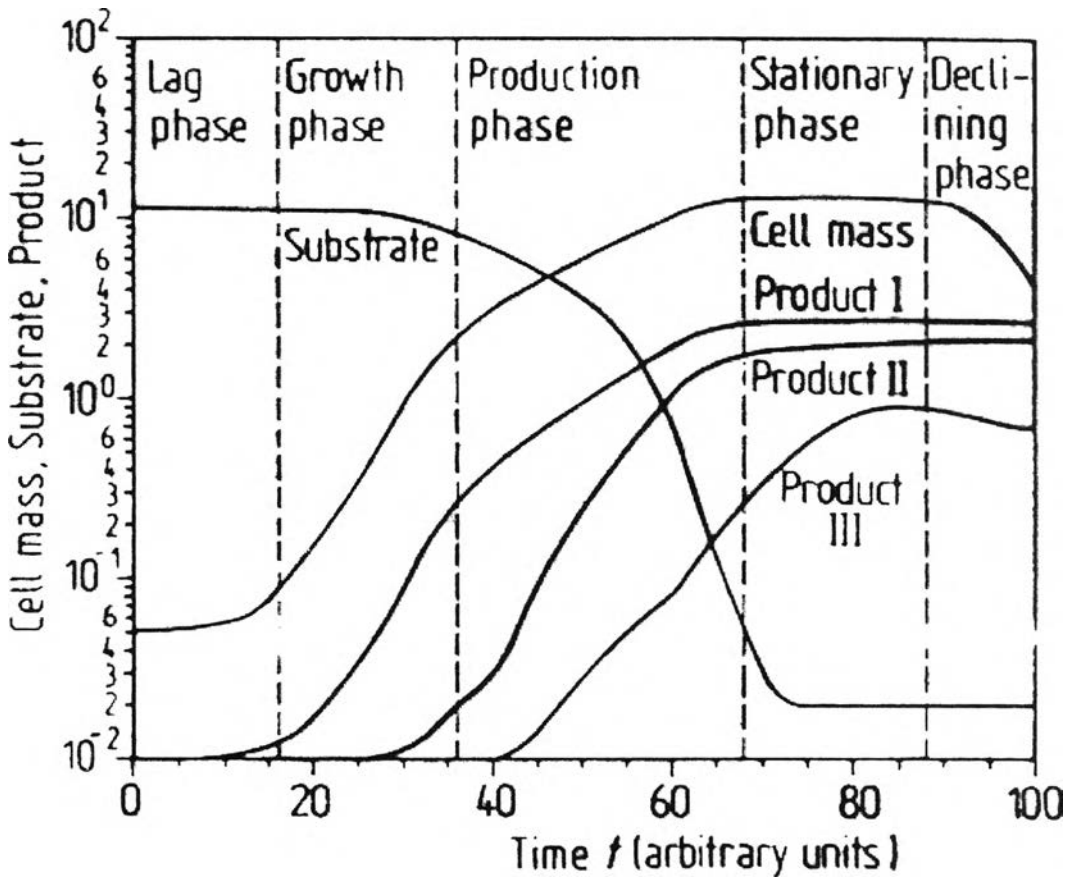

Figure 2.5 Principal time course of cell mass, substrate and product concentration for different types of fermentation

Source: Kehm et al., 2001

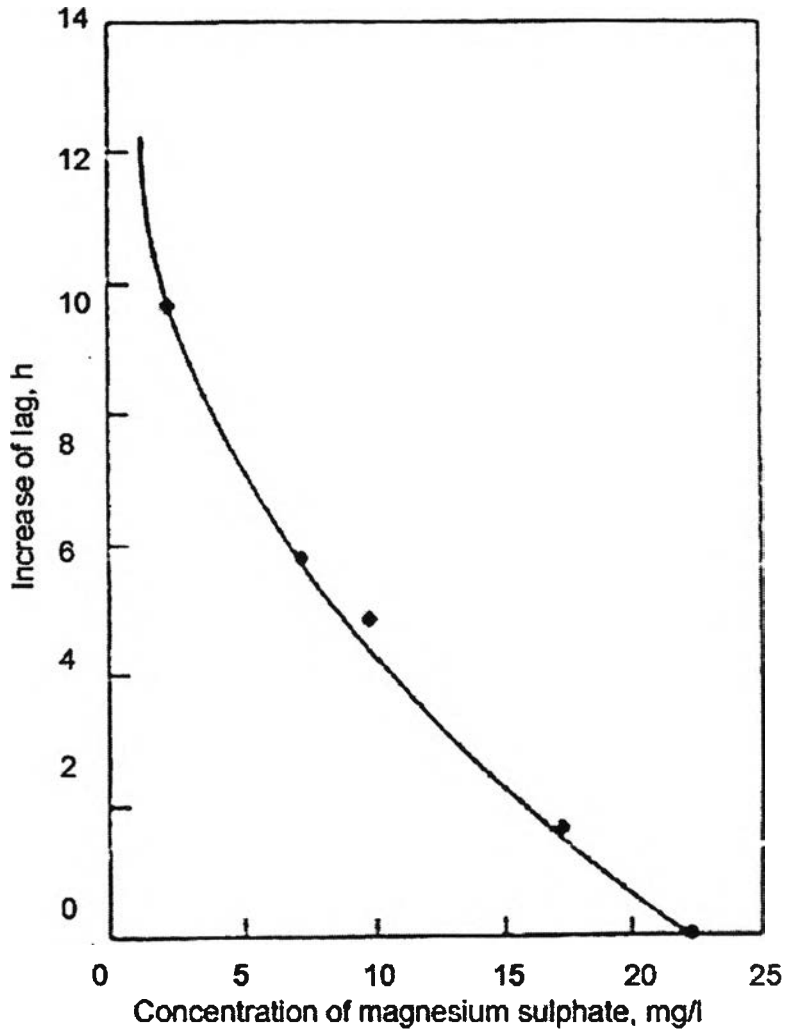

Figure 2.6 Effects of Mg2+ concentration on lag phase of *E. aerogenes*
Source: Shuler and Kargi, 2001

Whole phases have a unique metabolism; however, each phase needs the energy that comes from decomposing of substrate. The utilization of substrate can descript for growth, production, and cell maintenance. From mass balance theory, it can representatively write in mathematic equation 2.2.

$$\left\{\begin{matrix}\text{rate by which the substrate}\\ \text{is consumed by microorganisms}\end{matrix}\right\} = \left\{\begin{matrix}\text{rate of substrate utilize}\\ \text{for cell growth}\end{matrix}\right\} + \left\{\begin{matrix}\text{rate of substrate utilize for}\\ \text{maintenance}\end{matrix}\right\} + \left\{\begin{matrix}\text{rate of substrate utilize}\\ \text{for metabolite production}\end{matrix}\right\}$$

$$v_{Si} = \frac{r_{Si}}{C_X} = \frac{\mu}{Y_{X/Si,G}} + m_{Si} + \sum_j \frac{r_{Pj}}{C_X . Y_{Pj/Si}} \qquad \text{------- (2.2)}$$

Specific growth rate (μ) is proportionality coefficient in the differential equation related to the growth rate of cells to cell number as shown in equation 2.3 [Slator, 1916].

$$\frac{1}{C_X}\frac{dC_X}{dt} = \mu \qquad \text{------- (2.3)}$$

m_{Si} is a maintenance term of *i* substrate; an example of maintenance values is shown in Table 2.1.

Table 2.1 Maintenance parameter for cell alive

Organism	m_S (g substrate consumed·g cell^{-1}·h^{-1}
Penicillium chrysogenum	0.022
Aspergillus nidulans	0.029
Aerobacter aerogenes (aerobic)	0.094
A. aerogenes (anaerobic)	0.473

Source: Scragg, 1988

Yield coefficients, $Y_{X/S}$ and $Y_{P/S}$, are the relation between cell-substrate and product-substrate respectively. Yield of cell ($Y_{X/S}$) refers to the proportion of cell mass production to substrate utilization.

$$\text{Yield of cell} = \frac{\text{cell concentration change}}{\text{substrate concentration change}}$$

$$Y_{X/S} = \frac{\Delta X}{-\Delta S} \qquad \text{------- (2.4)}$$

Yield of production ($Y_{P/S}$) refers to the proportion of product accumulation to substrate utilization.

$$\text{Product yield} = \frac{\text{product concentration change}}{\text{substrate concentration change}}$$

$$Y_{P/S} = \frac{\Delta P}{-\Delta S} \qquad \text{------- (2.5)}$$

Substrate could be used by cell not only for production of cell mass but also used for production of metabolic products and maintenance of cell. Therefore growth yield obtained from experiment data will be an observed growth yield.

The cell yield from carbon source utilization is around 1.0 ± 0.4 gram cell per gram carbon source [Shuler and Kargi, 2001]. From Table 2.2, $Y_{X/S}$ of some microbe may be not constant according to a function of time or physical and chemical factors that affect the microbe properties.

Table 2.2 Summarized of cell growth and substrate utilization yield in aerobic organisms by different carbon source

Organism	Substrate	$Y_{X/S}$		
		g/g	g/mol	g/g-C
Enterobacter aerogenes	Maltose	0.46	149.2	1.03
	Mannitol	0.52	95.2	1.32
Candida utilis	Glucose	0.51	91.8	1.28
Penicillium chrysogenum	Glucose	0.43	77.4	1.08
Pseudomonas fluorescens	Glucose	0.38	68.4	0.95
Rhodopseudomonas spheroids	Glucose	0.45	81.0	1.12
Saccharomyces cerevisiae	Glucose	0.50	90.0	1.25
Enterobacter aerogenes	Ribose	0.35	53.2	0.88
	Succinate	0.25	29.7	0.62
	Succinate	0.25	29.7	0.62
	Glycerol	0.45	41.8	1.16
	Lactate	0.18	16.6	0.46
	Pyruvate	0.20	17.8	0.49
	Acetate	0.18	10.5	0.43
Candida utilis	Acetate	0.36	21.0	0.90
Pseudomonas fluorescens	Acetate	0.28	16.8	0.70
Candida utilis	Ethanol	0.68	31.2	1.30
Pseudomonas fluorescens	Ethanol	0.49	22.5	0.93
Klebsiella sp.	Methanol	0.38	12.2	1.01
Methylomonas sp.	Methanol	0.48	15.4	1.28
Pseudomonas sp.	Methanol	0.41	13.1	1.09
Methylococcus sp.	Methane	1.01	16.2	1.34
Pseudomonas sp.	Methane	0.80	12.8	1.06
Pseudomonas methanica	Methane	0.56	9.0	0.75

Source: Shuler and Karge, 1992

Product formation from microbe could be classified [Gaden, cited in Kehm et al., 2001] in to three types as shown in Figure 2.5.

1. Growth-associated production. In this case, growth and product formation are fully associated. The product arises from an essential growth process. Therefore, the product increases proportionally to the growth rate, and there is no separate production phase. The product is called primary metabolites such as ethanol fermented anaerobically by yeast and gluconic acid fermented by gluconobacter.
2. Mixed growth-associated. This model is an overproduction of primary metabolites, but the interrelation follows more complicated kinetics with separate growth and production phases. For instant, amino acid, lactic acid and xanthan pullan, polysaccharides production, these are extra cellular products.
3. Nongrowth-associated products. Product is generated in stationary phase, called secondary metabolite. Most antibiotic and vitamin process can be grouped into this type.

2.3 Mathematical modeling

Mathematical modeling for cell activities is an important part in design and fermentation process development. It can be specified by depending on complexity contemplation. Considering of the mechanism in cells, chemical and biochemical reactions, included transport phenomena and energy transfer in cells and also phase and component in fermentation, specification of mathematic model in fermentation must concern nearly real conditions. However, ideal situation for creating reality model is unreachable so the assumptions are made. It can divide the model into two types, structured and unstructured types.

For decades, the expression of cell kinetic has been developed in many ways. The best model is close to realistic condition; however, it has much complexity in the calculation. If the internal state of the cells should be considered, this leads to so-called structured models. The models can be structured on the basis of biomass components such as concentration of metabolites, enzymes, or RNA, or by population-related variables, describing

different morphological types of cells or cell aging. Models with a structure on the population level are also called segregated biological models.

To choose the approximated real state model, it depends on the creators which level of simplicity that they want to express the system. In the unstructured models, they take the cell mass as a uniform quantity without internal dynamics whose reaction rate depends only upon the conditions in the liquid phase of the reactor. Therefore, the models only contain kinetics of growth, substrate uptake, and product formation. Thus, in this model, the biological reaction depends directly and solely on macroscopic variables that describe the conditions in the fermentor. The only biological state variable is the cell mass concentration, C_X. Nevertheless, many phenomena in biotechnological process can be covered by this type of model. Besides the cell mass, other variables that show great variation during the fermentation have also significant influence on microbial behavior. The kinetics during the fermentation and mathematical model will be discussed in the next section.

2.4 Microbial Growth Kinetics

A functional relationship between the specific growth rate (μ) and an essential compound's concentration is normally formed in the differential equation during growth.

$$\frac{dC_X}{dt} = \mu(C_S)C_X \qquad \text{------- (2.6)}$$

A well known unstructured model (equation 2.7) was proposed by Monod in 1942.

$$\mu = \frac{\mu_m . C_S}{K_S + C_S} \qquad \text{------- (2.7)}$$

Monod model represents microbial growth rate, μ as a function of limiting substrate concentration, C_S. Here, μ_m is the maximum specific growth rate achievable when $C_S >> K_S$ and the concentrations of all other essential nutrients are unchanged. K_S is saturation constant, characterized as the affinity of microbial cells to substrate concentration. This value is shown by the limiting nutrient concentration at which the specific growth rate is half of its

maximum value as shown in Figure 2.6. This model shows the effect of depletion of nutrients but not including the effect from the accumulation of toxic metabolites which can cause deceleration and subsequent cessation of bacterial growth in a batch culture.

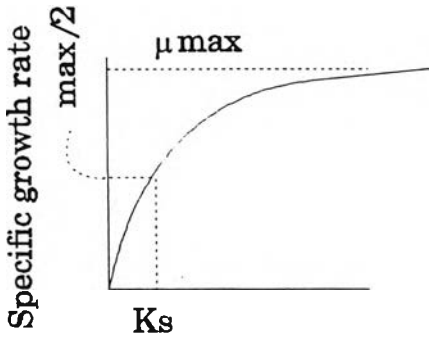

Figure 2.7 Relationship of μ and C_S in Monod kinetic

This model is similar to Michaelis-Menten equation in expression of enzyme activities. This equation can be applied to cell growth because almost reactions in cell are catalyzed by enzymes. By using the material balance equation, the calculation interpolates the instant residual substrate concentration in the course of microbial growth and absolute yield according to equation 2.8.

$$C_S = C_{S0} - (C_X - C_{X0})/Y \qquad \text{------- (2.8)}$$

In batch fermentation, the accumulation of cells from microbial growth is

$$\frac{dC_X}{dt} = \mu(C_S)C_X = \mu_m \frac{C_S}{K_S + C_S} C_X \qquad \text{------- (2.9)}$$

By substitution of C_S by C_X and separating the variables, we can obtain the following relationship.

$$\mu_m t = (1+P)\ln\frac{C_X}{C_{X0}} - P\ln(Q - \frac{C_X}{C_{X0}}) + P\ln(Q-1) \qquad \text{------- (2.10)}$$

where

$$P = \frac{YK_S}{YC_{S0} + C_{X0}} = \frac{YK_S}{C_{Xm}} \text{ and } Q = \frac{YC_{S0} + C_{X0}}{C_{X0}} = \frac{C_{Xm}}{C_{X0}}$$

Equation (2.10) describes the S-shaped growth dynamics of a batch culture. The equation includes the three parameters, Y, μ_m, and K_S, which can be constant values of particular organism such as *E. coli* grown on glucose at 30°C, Y = 0.23, μ_m = 1.35h^{-1} and K_S = 4 mgl^{-1}. Therefore, dynamic pattern of microbial growth can be predicted from the information on growth characteristics and cultivation conditions.

After the development of Monod equation, many other kinetic equations have been proposed. Almost these unstructured models are modified in the division of the Monod kinetics. Generally, batch fermentation can be set and written in mathematic equations by growth, substrate utilization, and production as in the following equations.

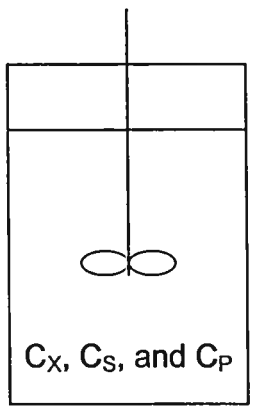

Figure 2.8 Batch fermentation

- Cell mass balance in batch fermentation

$$\left\{\begin{matrix}\text{rate of biomass} \\ \text{concentration change}\end{matrix}\right\} = \left\{\begin{matrix}\text{rate of biomass concentration} \\ \text{change caused by exponential growth}\end{matrix}\right\}$$

$$\frac{dC_X}{dt} = \mu(C_X, C_S, C_P, T)C_X \qquad \text{------- (2.11)}$$

- Substrate utilization in batch fermentation, integrated by using coefficient yield from cell growth

$$\left\{\begin{matrix}\text{rate of substrate}\\ \text{concentration change}\end{matrix}\right\} = -\left\{\begin{matrix}\text{rate by which the substrate}\\ \text{is consumed by mico - organisms}\end{matrix}\right\} - \left\{\begin{matrix}\text{rate by which the substrate}\\ \text{is utilized by production}\end{matrix}\right\}$$

$$\frac{dC_S}{dt} = -\frac{1}{Y_{X/S}}\mu(C_X, C_S, C_P, T)C_X - m_S C_X - \sum_j \frac{r_{Pj}}{Y_{Pj/S}} C_X \quad \text{------- (2.12)}$$

- Production in batch fermentation, integrated by using coefficient yield from cell growth

$$\left\{\begin{matrix}\text{rate of product}\\ \text{concentration change}\end{matrix}\right\} = \left\{\begin{matrix}\text{rate of product}\\ \text{synthesis}\end{matrix}\right\}$$

$$\frac{dC_P}{dt} = \frac{1}{Y_{X/P}}\mu(C_X, C_S, C_P, T)C_X \quad \text{------- (2.13)}$$

The setting of system equations can be suppressed by growth kinetics. The model formation depends on whether the data fitting is appropriate or not. Other expressions for specific growth rates rebuilt after Monod kinetics are written in normalized term as summarized in Table 2.3.

Table 2.3 Growth Kinetics for a Single Substrate

Name	Year	Normalized Kinetics τ
BLACKMAN	1905	$\min(1, K_B \cdot C_S)$
MONOD	1942	$\frac{C_S}{K_M + C_S}$
TEISSIER	1942	$1 - e^{-K_T \cdot C_S}$
MOSER	1958	$\frac{C_S^R}{K_M^R + C_S^R}$
CONTOIS	1959	$\frac{C_S}{K_C \cdot C_X + C_S}$
POWEL [a]	1967	$\frac{C_S - K_1 \cdot \tau(C_S)}{K_M + C_S - K_1 \cdot \tau(C_S)}$
MASON and MILLES	1976	$\frac{C_S}{K_M + C_S} + K_D \cdot C_S$
VAVILIN	1982	$\frac{C_S^R}{K_M^{R-P} \cdot C_{S0}^P + C_S^R}$

[a] this kinetics is given in implicit form

Source: Kehm et al., 2001

All above kinetics should be taken to consider for the globally observed behavior of the culture, which need not have a close relation to microkinetics of the biological reaction; one can give an interpretation of the special form of certain kinetics. The equations of Moser and Vavilin are similar to the Monod kinetics except that the reaction for the substrate is not of first order. The Vavilin equation, in which C_{S0} is the initial substrate concentration, has an application in processes with toxic substrates. The Blackman equation can be interpreted such that at high substrate concentrations not the substrate uptake, but another metabolic reaction, is rate limiting. The Contois kinetics considers an effect of cell concentration on the growth due either to inhibition by the cells themselves or to diffusional limitation for substrate by a limitation constant which is proportional to the cell concentration. The equations of Powel and Mason and Milles account for additional diffusion-driven flux of substrate into the cell. A plot of the $\mu(C_S)$ characteristics of the kinetics is given in Figure 2.9. In practice, because of measurement errors; it is difficult to discriminate between the different kinetics, especially when using only

batch culture data. Therefore, the Monod kinetics is generally a good choice for the model. In following section, the focus will be on factors such as substrate uptake kinetics, including limitation, inhibition effects, and product formation that have an influence on cell kinetic model.

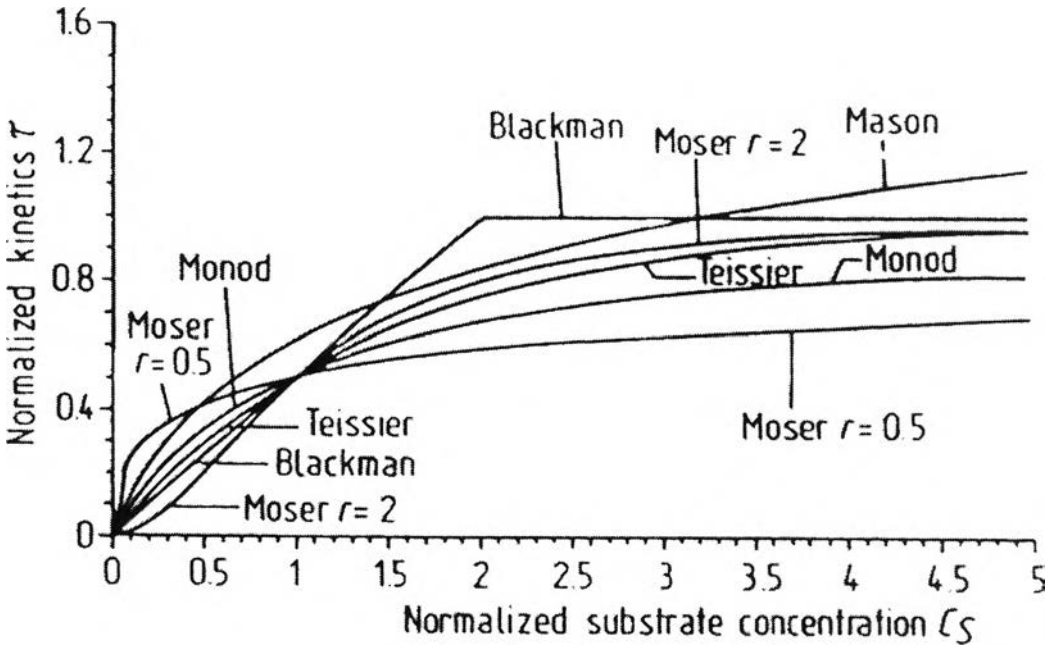

Figure 2.9 Normalized $\mu(C_S)$ characteristics of substrate uptake kinetics τ versus substrate concentration C_S, normalized to the half-saturation constant
Source: Kehm et al., 2001

2.5 Factors affect unstructured model

By categorizing factors that effect on cell growth, the models can be classified into two main influences. The first one is achieved from substances in fermentation, and next consequences which come from operating condition. Also, there are effects from substance concentration in fermentation, which are nutrients, gases and products that may limit or inhibit cell growth.

2.5.1 Substance limiting effect

For limiting substrate, microbial reactions usually show saturation at high substrate concentrations, that is, the reaction rate approaches a maximum value. On the other hand, the reaction rate equals zero if no substrate is available. There are many proposed kinetic model for limiting

substrate such as Monod, Moser, Teissier. For example, the kinetic study under oxygen limiting of *Aspergillus awamori* in submerged cultivations from whole wheat flour [Koutinas et al., 2002].

2.5.2 Substance inhibition effect

Beside substrate limitation, inhibition by substrates and products is quite often found in biotechnological processes. Both have been examined by many authors [Han and Levenspiel, cited in Kehm et al., 2001]. Table 2.4 gives a list of normalized inhibition kinetics. Most of the applied kinetics are extensions of the Monod equation and have been derived from enzyme inhibition kinetics [Dixon and Webb, cited in Kehm et al., 2001a]. These kinetics forms cannot predict zero growth for a finite inhibitor concentration. Therefore, other empirical equations have been proposed for the description of this behavior. The last five equations in Table 2.4 predict a zero growth rate at $C_I = K_I$. Product, substrate, and cell inhibition can be obtained by choosing the variable C_I as C_P, C_S, or C_X, respectively. The competitive type only applies for substrate inhibition. The given equations can be generalized for multiple inhibitions by combining the normalized kinetics to a product with several factors.

Table 2.4 Inhibition Kinetics for a Single Inhibitor

Name	Year	Normalized Kinetics τ
HALDANE (competitive type)	1965[a] (1930)	$\frac{C_S}{K_M + C_S + \frac{C_S^2}{K_I}}$
WEBB (competitive type)	1963[a]	$\frac{C_S \cdot \left(1 + \frac{C_S}{K_{I1}}\right)}{K_M + C_S + \frac{C_S^2}{K_I}}$
IERUSALIMSKY (non-competitive type)	1965[a]	$\frac{C_S}{K_M + C_S} \cdot \frac{1}{1 + \frac{C_I}{K_I}}$
YANO et al. (competitive type)	1969[a]	$\frac{C_S}{K_M + C_S \cdot \left(1 + \sum_N \left[\frac{C_S}{K_I}\right]^N\right)}$
EDWARDS	1970	$\frac{C_S}{K_M + C_S} \cdot e^{-C_I \cdot K_I^{-1}}$
YANO and KOYA (generally non-competitive type	1973	$\frac{C_S}{K_M + C_S} \cdot \frac{1}{1 + \left[\frac{C_I}{K_I}\right]^N}$
WAYMAN and TSENG	1976[a]	$\frac{C_S}{K_M + C_S} + K_I \cdot \min(C_{SI} - C_{S0})$
Teissier type		$e^{-C_I \cdot K_I^{-1}} - e^{-K_T \cdot C_S}$
GHOSE and TYAGI DAGLEY and HINSHELWOOD	1952[a]	$\left[1 - \frac{C_I}{K_I}\right] \cdot \frac{C_S}{K_M + C_S}$
CHEN et al.	1976	$\left[1 - \frac{C_I}{K_I}\right] \cdot \frac{C_S}{K_M + C_S - C_2 \cdot C_S^2}$
BAZUA and WILKE	1977[a]	$\left[1 - \frac{C_I}{K_I}\right]^{1/2} \cdot \frac{C_S}{K_M + C_S}$
LEVENSPIEL	1980[a]	$\left[1 - \frac{C_I}{K_I}\right]^N \cdot \frac{C_S}{K_M + C_S}$
HAN and LEVENSPIEL	1987	$\left[1 - \frac{C_I}{K_I}\right]^M \cdot \frac{C_S}{K_M \left[1 - \frac{C_I}{K_I}\right]^M + C_S}$

[a] cited in HAN and LEVENSPIEL (1987)
Source: Kehm et al., 2001

ต้นฉบับ หน้าขาดหาย

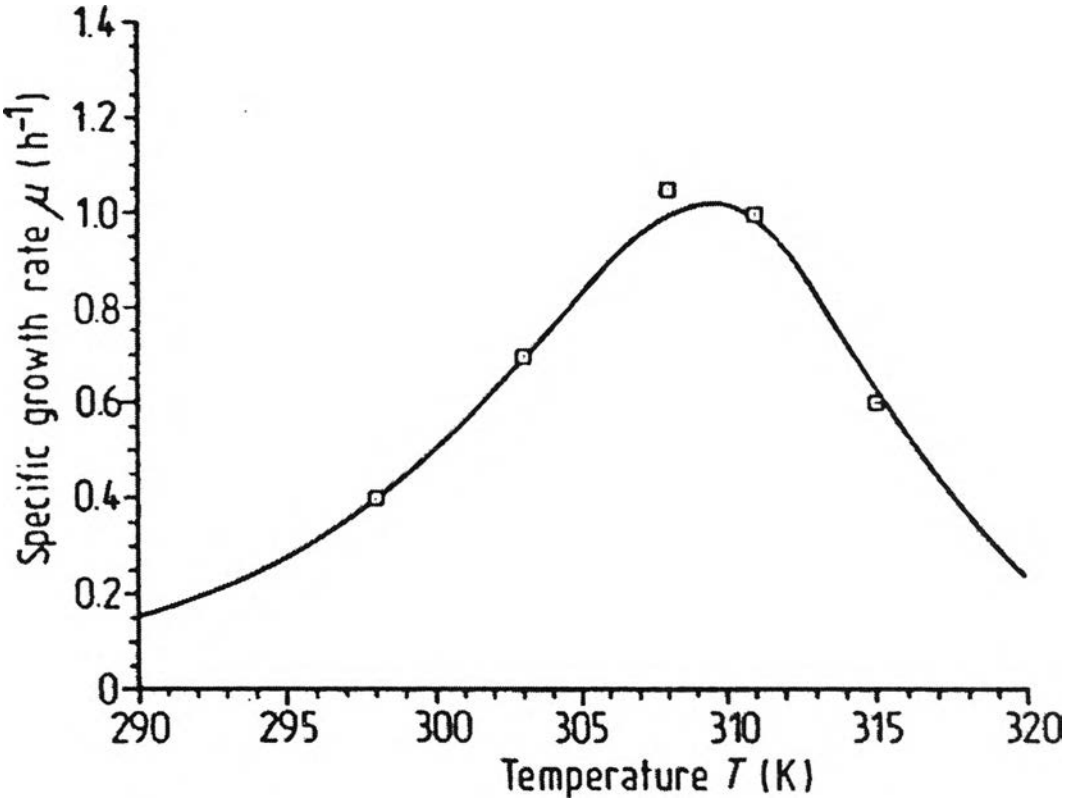

Figure 2.10 Dependency of the maximum specific growth rate of *Klebsiella pneumoniae* on the temperature
Source: Esner et al., 1980

2.5.4 pH level effect

pH level in fermentor is a vital process parameter as it has a significant effect on cell growth rate, viability and product synthesis. Experiments with synthetic lactose media indicated that cell growth rate and lactic acid production were substantially affected by the fermentation pH. The experiments shown that the optimal pH for *Lactobacillus plantarum* ATCC (21018) with lactose as substrate was in the range 5-6, expressed in Figure 2.11 [Fu and Mathews, 1999]

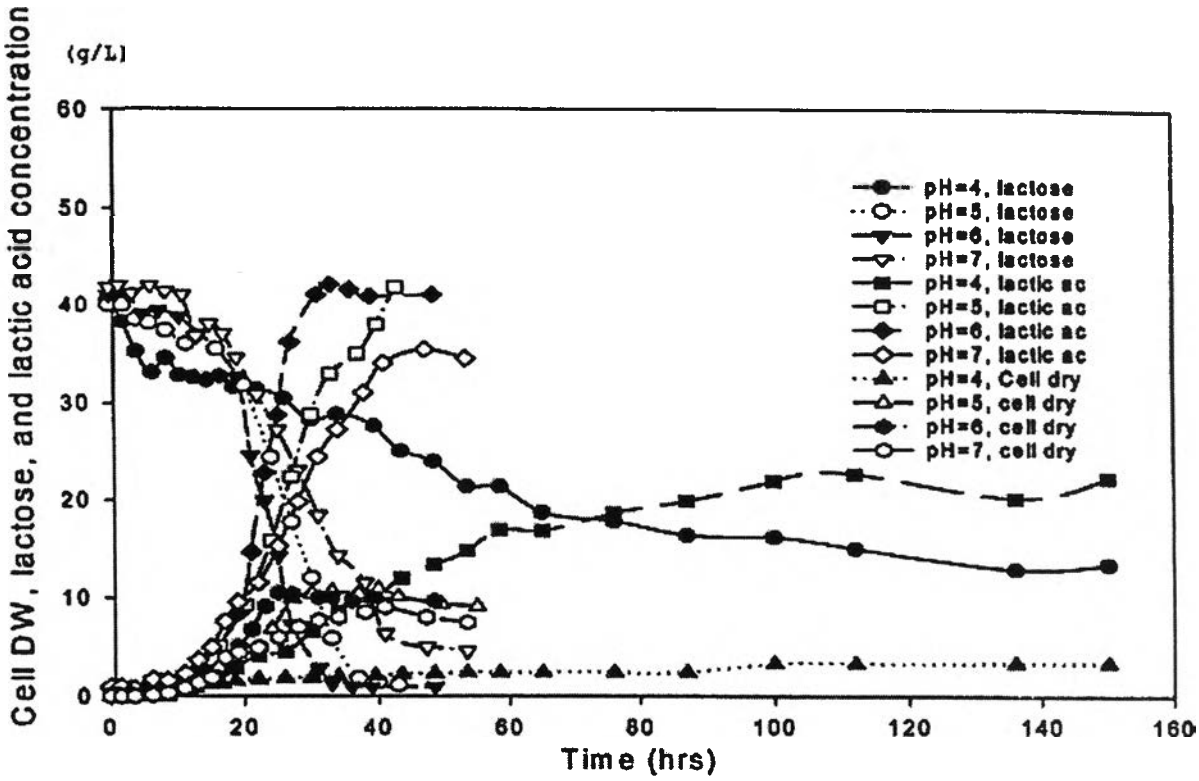

Figure 2.11 Effect of pH on lactose batch fermentation under anaerobic conditions

Source: Fu and Mathews, 1999

2.5.5 Power consumption effect

The mixing of broth in fermentor is a crucial character for transporting substance into cells. For good mixing for fermentation, the power consumption must be concerned not only the transport phenomena to the mixture, but also the establishment of growth rate or cell characteristic that is affected by that energy. Appropriate power consumption for cell growth had been studied by the effect of agitation conditions on biotin production. It was concluded that cell had been changed morphology as shown in Figure 2.12 when agitation conditions was changed. Biotin production was found to be strongly correlated with cell length and liquid transfer coefficient [Büchs, Lotter and Milbradt, 2001].

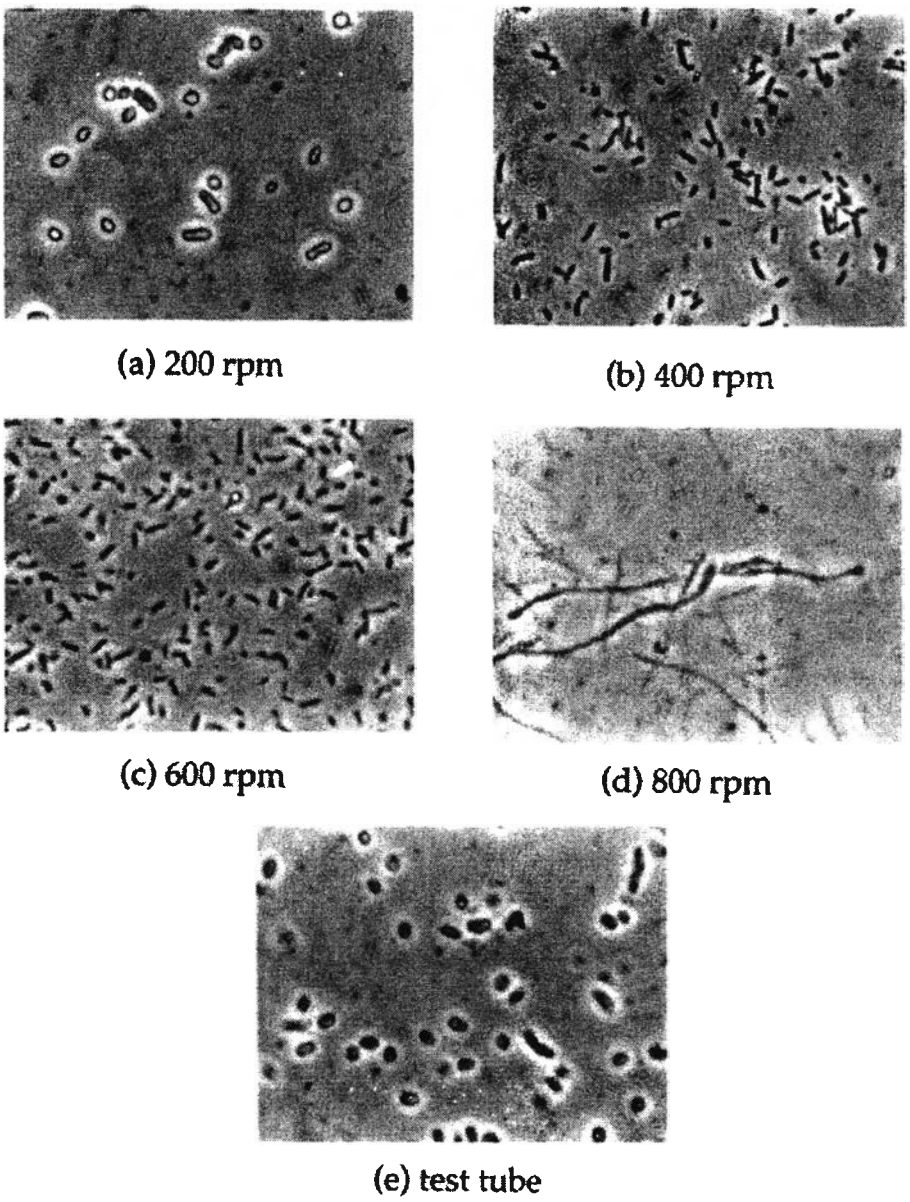

Figure 2.12 Photographs of cells grown under different culture conditions after 120 hours cultivation. (a-d), Cells grown in a jar-fermentor with turbine-blade impeller rotating at 200, 400, 600 and 800 rpm, respectively. (e) Cells grown in a test tube culture.

Source: Büchs, Lotter and Milbradt, 2001

2.6 Literature reviews

A graphical method based on batch-culture was applied for the prediction of multistage continuous ethanol fermentation by *Saccharomyces cerevisiae* NRRL Y-132 using a cheap nitrogen source, and bagasse hydrolysate as substrates. Batch and continuous experiments carried out at

different sugar levels indicated substrate and product inhibition on growth as well as on fermentative activity of yeast cells. Production yield was found to vary with sugar concentration in both of batch and continuous culture. An optimum reduced sugar concentration in feed was 258 g/liter (or glucose concentration of 180 g/liter) [Tyagi and Ghose, 1980].

A kinetic model accounts for substrate limitation, substrate inhibition, ethanol inhibition and cell death was developed using experimental data from 5L bioreactors of continuous anaerobic ethanol fermentation from cheese whey [Ghaly and El-Taweel, 1997]. By using *Candida pseudotropicalis* with lactose as substrate, lactose utilization and cell and ethanol productions were significantly affected by hydraulic retention time and initial substrate concentration. The model could predict the time profiles of cell, lactose and ethanol concentrations with high accuracy (R = 0.96-0.99).

The experimental result in batch fermentation by immobilize Saccharomyces cerevisiae ATCC 9763 in Ca-alginate gel beads was tested with eleven different kinetic models to relate biomass and ethanol production and glucose utilization. It is found that Monod and Hinshelwood models were more appropriate to describe the batch growth and ethanol production of immobilized *S.cerevisiae* at low (2-4%) and high (8.10%) initial glucose concentrations, respectively [Birol, *et al.*, 1998].

A kinetic segregated biomass model for beer production is proposed by taking into account of five responses: biomass, sugar, ethanol, diacetyl and ethyl acetate [Andrés-Toro, *et. al.*, 1998]. By separating biomass into three parts: lag, active and dead, only the active cell fraction was accounted for fermentation activities. The kinetic model, with the parameter values calculated as a function of temperature, was able to predict with a very high accuracy of the non-isothermal experimental data.

Batch fermentation of lactose to lactic acid was conducted using *Lactobacillus plantrarum* under aerobic and anaerobic conditions [Fu and Mathews, 1999]. Synthetic lactose medium was used as the culture medium and the effects of pH and substrate concentration on cell growth and lactic acid production were investigated. The pH conditions had been studied and accounted in a Monod's model.

Yeast metabolism and the kinetics of industrial batch fermentation of *Saccharomyces cerevisiae* were simulated [Sainz, *et. al.*, 2003]. Five differential equations describe the evolution of the main metabolites and biomass in the wine fermentation, while a set of linear algebraic equations models the pseudo steady-state microbial metabolism. This systemic stimulation of cell metabolism provides valuable information of the response on specific pathways to extracellular conditions. This continuity conduces to the potentially developed model.