EFFECT OF SUBSTRATE CONCENTRATION ON PRODUCTION OF SORBITOL

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ABSTRACT

This research was conducted to study the effect of substrate concentration in production of sorbitol in fermentation process by *Candida tropicalis* (IFO 0618) that was purchased from America type Culture Collection (ATCC). The growth of the bacteria can be calculated using Monod Equation. Monod equation showed the relationship of specific growth rate to substrate concentration often assumes the form of saturation kinetics. Increasing in substrate concentration resulted in increasing the growth rate of the bacteria while changes in other nutrient was not affected the growth of the bacteria. In order to study the effective condition for the maximum growth of glucose, different substrate concentration of cellulose was used. Other parameter such as temperature, pH value, and time was constant. The pH 6 was maintained by sodium acetate buffer. This study was performed in 16 hours and the samples were collected every 2 hours to get their optimum density using UV- Vis Spectrophotometer. The commercial cellulose act as the food sourced for this research. YPD medium is the medium used for the growth and maintaining the cell of the *Candida tropicalis* . The medium was autoclaved before used for further process. In addition, all the preparation steps of bacteria and nutrient medium was done in laminar flow space to avoid any contamination. The production of sorbitol can be done by several steps; cultivation of yeast, inoculum preparation, solid state fermentation and lastly analysis. The analysis of sorbitol was done by determining the dry cell weight, determining kinetic parameter (μ_{max}) of the microorganism (*Candida tropicalis*) by Monod equation and HPLC analysis of the sorbitol. The highest substrate concentration showed the highest dry cell weight (g/L) obtained while there were low dry cell weight (g/L) obtained from the low of substrate concentration. The increasing of the dry cell weight showed that *Candida tropicalis* was able to react with cellulose to form sorbitol. Concentration of sorbitol was differing in every HPLC results as the substrate concentration was manipulated in this study.

ABSTRAK

Kajian ini telah dijalankan untuk mengkaji kesan kepekatan substrat dalam proses penapaian kepada kepekatan sorbitol. Pertumbuhan bakteria boleh dikira dengan menggunakan Persamaan Monod. Peningkatan kepekatan substrat akan menyebabkan peningkatan kadar pertumbuhan bakteria manakala perubahan nutrien yang lain tidak akan menjejaskan pertumbuhan bakteria. Dalam usaha untuk mengkaji keadaan yang berkesan untuk pertumbuhan maksimum bakteria, kepekatan substrat (selulosa) yang berbeza daripada telah digunakan. Parameter lain seperti suhu, nilai pH, dan masa adalah tetap. pH 6 dikekalkan dalam proses penampaian untuk memberi keadaan yang baik kepada pertumbuhan bakteria. Kajian ini dilakukan selama 16 jam dan sampel telah dikumpulkan setiap 2 jam. Setiap sampel dianalisis menggunakan UV-Vis Spektrofotometer. Selulosa komersial digunakan dalam kajian ini sebagai sumber makanan untuk pertumbuhan bakteria yang dikaji. Medium YPD merupakan medium yang digunakan untuk pertumbuhan bakteria. Medium itu diautoklaf sebelum digunakan untuk proses seterusnya. Di samping itu, semua langkah-langkah penyediaan bakteria dan medium nutrien telah dilakukan di dalam ruang aliran lamina untuk mengelakkan sebarang pencemaran. Penghasilan sorbitol boleh dilakukan dengan beberapa langkah; penyediaan inokulum, penapaian keadaan pepejal dan akhir sekali analisis. Analisis sorbitol dilakukan dengan menentukan berat sel kering, menentukan parameter kinetik (μ_max) daripada mikroorganisma *Candida tropicalis* dengan persamaan Monod. Kromatografi Cecair Prestasi Tinggi telah digunakan semasa process analisis kepekatan sorbitol. Kandungan substrat yang tertinggi telah menghasilkan jumlah sel kering yang tinggi. Peningkatan jumlah berat kering sel menunjukkan bahawa Candida tropicalis mampu bertindak balas dengan selulosa untuk menghasilkan sorbitol. Kepekatan sorbitol adalah berbeza dalam setiap keputusan Kromatografi Cecair Prestasi Tinggi apabila peratus kepekatan substrat telah dimanipulasi dalam kajian ini.

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LIST OF ABBREVIATIONS

SmF	Submerged State Fermentation
SSF	Solid State Fermentation
Uv- Vis	Uv –Vis Spectrophotometer
HPLC	High Performance Liquid Chromatography
μ_{max}	Maximum specific growth
μ	Specific growth of bacteria
K_s	Monod constant
S	Substrate concentration
g/g	Gram per gram
g/L	Gram per Litter
M	Mole

1 INTRODUCTION

1.1 Background

Microbial fermentation is one of the important manufacturing process used in food, pharmaceutical, fuel cosmetic and others. Last decade, there has been an increasing of commercial product using this process. Fermentation process can be carried out in batch, fed batch or continuous. Batch fermentation was the common process in fermentation. Generally there are two type of fermentation process. They are submerged fermentation (SmF) and solid state fermentation (SSF). Submerged fermentation involved the production of biomass in liquid media. The selected microorganism will be growth in closed vessel with high concentration of oxygen. This type of fermentation occurs in aerobic conditions and sometimes uncontrollable. Examples of product from SmF were wine making, cheese, yogurt and brewing, (Chisti, 1999).

Solid state fermentation can be defined as the fermentation process with the low amount of water content or water free. The microorganism will grow on the moist solid surface. An example of products from SSF includes industrial enzyme, fuel, and nutrient enrichment. This type of fermentation was used in production of food in Asia (Holker et al., 2005). SSF also been used in production of enzyme (González et al., 2003). Both processes of fermentations will require food source (substrate) such as glucose. Sorbitol is an alcohol sugar that produces from fermentation of glucose.

1.2 Motivation

According to findings by the National Health and Morbidity Survey (NHMS) in 2012, about 2.6 million Malaysians were found to be suffering from diabetes last year. One of the most common causes of diabetes in Malaysia is high sugar consumed. The Health general director Datuk Seri Dr Hassan Abdul Rahman described the findings was worrying despite concerted efforts by the health ministry over the years (3rd National Diabetes Conferences 2012). Those diabetes patients must get proper treatment and care, such as strict control of blood sugar levels, treatment for raised blood pressure and foot n eye care. Alternative sugar was needed in order to help those diabetes patients. Sorbitol is one of the alternative sugars that will give the same sweet taste as other

complex sugar (Silveira et al., 2002). Sorbitol can be produce by chemical synthesis and bioprocess method. The production of sorbitol by chemical will involve other chemical substances. In other hand, the synthesis of sorbitol by bioprocess will not involve other chemical substance. Based on this comparison the production of sorbitol by bioprocess is better than the chemical synthesis. Other than that, the world market demand for sorbitol is constantly growing (Bizzari et al., 2008) have reported that there has been a growth rate around 1–2% per year since 1997.

1.3 Problem Statement

Production of sorbitol can be done by submerged fermentation (SmF) but there are some problems can cause from SmF. Submerged fermentation needs water and it can cause contamination. Besides, these types of fermentations need a large bioreactor to proceed. Submerged state fermentation will need some further unit operation to concentrate the product. Because of these weaknesses, the solid state fermentation was chosen in this study to replace the submerged state fermentations. Solid State Fermentation (SSF) has more advantages compare to the submerged fermentation (SmF)(Holker et al., 2005). SSF process was simpler and requires smaller volume than SmF. Besides, SSF can reduce cost due to the simple grow of bacteria. Product form from the SSF also higher than the SmF and they are more concentrated. In addition, SSF used little amount of water which is considerable and can reduce the chances of contamination (Asther et al., 2002). This process could be also effectively used at smaller level which makes them suitable for any areas. The biomass that produces from SSF also will be easy to separate compare to SmF. Although SSF shows more advantages compared to SmF, high conversation of product by bacteria in SSF is still new. The growth of the bacteria must be study prior to fermentation process and it will be easily being study by using Monod Equation (Liu, 2006).

1.4 Objective

This research was conducted to study the effect of substrate concentration on production of sorbitol prior to fermentation process.

1.5 Scope of study

This research was conducted to determine the growth of bacteria *Candida tropicalis* (IFO 0618) by using the Monod Equation. Different amount of substrate concentration (commercial cellulose) was used to determine the optimum growth of the microbial. Other parameters including temperature, pH, and time interval will be constant. Microorganism that involved in this research was *Candida tropicalis*, YDP Agar and YDP Broth that used for isolation and medium for cultivation was formulated by Rafiqul 2012. The bacteria was then cultivated in aerobic condition in YDP medium for 24 hours at 30ºC in incubator (Ladero et al., 2007). The optical density of inoculums will be examined using UV-Vis Spectrophotometer. Setting the optical density at UV-Vis spectrophotometer equal to 600nm (OD_{600}). The values of OD_{600} should be less than 1.0 or (0.1-0.7) (Sabu et al., 2008). Growing of the microorganism can be predicted by Monod Equation. Solid State Fermentation (SSF) process was chosen in this research because it will produce higher amount if biomass compare to the Submerged State Fermentation (SmF). The controlled of parameter involved which is different substrate concentration (g/g) of cellulose will be easily to control in SSF compared to the SmF. The product (sorbitol) will be analysed using HPLC.

2 LITERATURE REVIEW

2.1 Monod Equation

According to Shuler et al., 1979, in previous research, several expressions have been developed for predicting specific growth rates of microbial cultures as a function of the concentration of a single rate-limiting substrate. Such models were nonsegregated, nonstructured and apply strictly to balanced growth situations only. The most widely expressions that been used were Monod, Contois Teissier, and Moser equations (Monod, 1949), (Teissier 1936), (Moser 1958), (Contois 1959). Each of them has been used successfully for certain microorganism under certain growth condition (Chiu et at., 1972).Selection of these equations should be made carefully to get the best data and fulfil the objective of the study.

Liu Y,(2006) was claimed that Monod equation was one of the best methods to describe microbial growth. This equation shows a functional relationship between the specific growth rate and an essential substrate concentration. Monod equation was developed from a curve fitting exercise, which is an example of an empirical correlation. (Bailey et al.,1986) and (Roels, 1983). Monod relationship can provide the most generally satisfactory curve fitting of the growth data (Gaudy et al., 1980) .The Monod equation was purely empirical compare to the other models for microbial growth such as Grau equation, Hill or Moser equation (Grady et al., 1999 ;Monod, 1949; Roels, 1983 ; Shulter et al., 2002) .In the present study, thus attempted to derive a general equation for microbial growth according to the thermodynamics of a microbial growth product. In Monod equations, there was no relationship between two constants (Monod, 1949). A tremendous quantity of experimental data of microbial growth had been interpreted and modelled by using the Monod equation which is strictly empirical for the past half century.

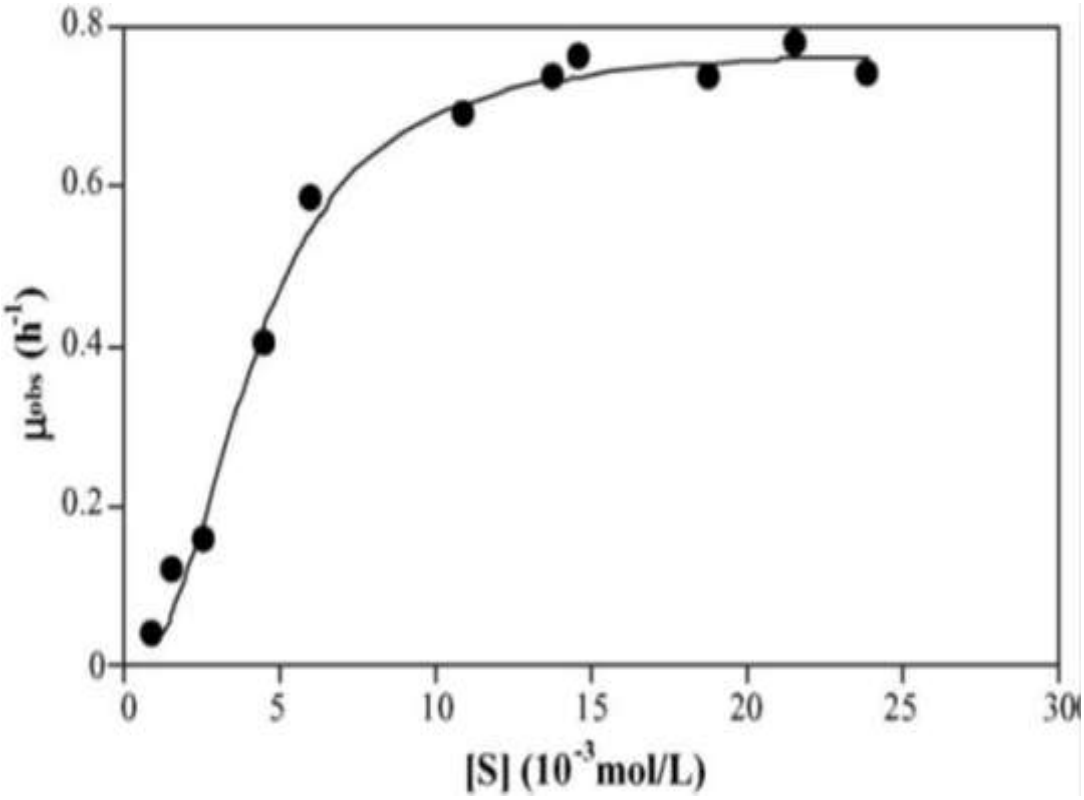

Figure 2-1: Relationship between μ_{max} and [S] substrate concentration.

According to Kayombo et al (2002), Monod equation was the most commonly used model to relate the microbial growth with substrate utilization. (Monod,1949). In pure cultures and continuous reactor systems, the relationship between the bacterial growth and the substrate availability in the system with a single growth-limiting substrate could be expressed empirically. Where μ is a specific growth rate (day-1), μ_{max} is the maximum growth rate (day-1), S is the substrate concentration (mg/l), and $K_s$is the half saturation coefficient (mg/l). The specific growth rate depends on the concentration of the limiting nutrient, which can be a carbon source or other factors needed by the microorganisms for growth (Grady, 1999).

The growth characteristics of micro-organisms include several growth phases: lag phase, accelerating log phase, the exponential growth phase and the stationary phase. The kinetics of batch fermentation involves all these phases (Moser, 1981). Most widely method that used in order to study the growth kinetics of the microorganism were Monod, Teissier and Levenspiel (Ahmad et al., 1993) .The specific growth rate, μ, is a measurement of how quickly the cell population is growing. The higher the value of μ will show the greater the value of the growth. A relationship between the specific

growth rate, μ, and the substrate concentration, S, was proposed by Monod (Monod, 1979). The Monod equation states that:

$$\mu = \frac{\mu_{max} S}{K_s + S}$$

S is the concentration of the substrate (g/L), $\mu_{max}$is the maximum specific growth rate (h^{-1}) and K_s, is the saturation constant (g/ L), K_s, is evaluated where the specific growth rate has half its maximum value. The value of μ was strongly dependent on S and the higher range; μ will become independent of S. If an organism has a very high affinity for the limiting substrate (a low K_s, value), the growth rate will not be affected until the substrate concentration has declined to a very low level with a corresponding short decelerating growth phase for the culture. However, if an organism has a low affinity for the substrate (a high K_s, value) the growth rate will be deleteriously affected at a relatively high substrate level. Thus the deceleration phase for the culture would be relatively long, and the yield of biomass will be reduced (Ahmad et al., 1993).

Monod model was one of the microbiological model that were usually been used in kinetic study of the microorganism. This model describes the microbial growth in batch culture (Strigul et al., 2009). Nobel Laureate J. Monod was suggested The Monod model in 1942 and the model had been used for 60 years and been one of the most frequently used in microbiology. (Mono, 1949; Prit, 1975; Koch, 1997; Kavarova et al., 1988). Most of the chemostat model growth were based on the Monod Equations (Prit, 1975; Smith et al., 1995).One of the important applications of Monod model was the evaluation of biodegradation kinetics in environmental system (Blok, 1994; Blok et al., 1996). It was assumed that the growth conditions for all cells were similar for all cells in a simple batch culture (Pirt, 1975). A typical growth curve was divided into six phase, 1) lag phase, 2) accelerations,3) exponential, 4)decelerating, 5) stationary and 6) death phase or declining phase (Monod,1949). In most cases some of the microorganisms do not show this typical growth and might interested in only particular aspect of growth. Duration of the lag phase was the main parameter to characterize the growth inhibition (Dette et al., 2005).

In additions, Monod model were used widely especially in describing biodegradation kinetics in batch growth process (Blok, 1994; Dette et al., 2005). In biodegradation kinetics, only 3 phases were recognizing, 1) exponential phase, 2) stationary phase and 3) decelerating phase. It was assumed that lag and accelerating phase and declining phase growth phase do not exist. This assumption has been satisfied by other researches in many practical cases. The cells that used to inoculate batch cultures were often taken from another actively growing culture and the growth will start in exponential phase. However, if the lag phase was observed, Monod's model parameter can't be obtained directly from the experimental data. In that case, it is important to estimate the duration of the lag phase in preliminary experiment and take times time for the phase ends as the starting moment of the Monod experiment. In biodegradation studies, the declining growth phase was usually not been considered, but can be analysed separately using the negative exponential model (Dette et al., 2005).

Typically, in Monod equations model the stationary phase was not observed for a long time and sometimes it occurs only for a short time, followed by a decline in microbial mass. It was important to identify the microbial biomass at the stationary phase because the value can determined the yield coefficient. In determining Monod parameter values, the starting point of the stationary phase and the biomass should be determine by ignoring any decline in biomass (Strigul et al., 2009).

Different types of models have been proposed to study the growth kinetics of a microbial population that grow with a single limiting substrate. A statistical analysis had been done by Senn et al., (1994) shown that several tested models, the three model of Monod (1942), Shehata & Marr (1971) and Westerhoff et al., (1982) described their experimental data equally well. Monod model had described the relationship between μ and s (substrate concentration) by a type of saturation kinetics: μ is specific growth rate, $\mu_{max}$is the maximum specific growth rate at saturating substrate concentration (h^{-1}), S is the concentration of the substrate (g/L)and K_s, is the substrate affinity(g/ L), i.e. the substrate concentration at which the cell grow at half maximum specific growth rate. The advantage of Monod model is all the parameter, μ, μ_{max}, K_s, and S have a biological meaning and they are experimentally accessible (Wick et al., 2002).

In addition, The Monod model showed that in certain cases, populations of cells cultivated at longer generation times can grow as quickly as populations of cells grown at shorter generation times. This is due to a stronger selection pressure per generation can compensate for fewer generations per time interval. This model also explains the apparent clock-like behaviour of the selection-driven evolution described previously. (Dykhuizen & Hartl, 1981; Hartl & Dykhuizen, 1979).

In other paper, microbial growth kinetics is a relationship between the specific growth rate (μ) of a microbial population and the substrate concentration (s). Microbial growth kinetics is an important tool in all field of microbiology, be it genetics, ecology, physiology, and biotechnology. (Kovari et al., 1998).

The concepts in microbial in microbial growth kinetics have been nominated by the relatively simple empirical model proposed by Monod during the last half century. The Monod model introduced the concept of a growth controlling limiting substrate. Monod model was used first in a stoichiometric sense to indicate that certain amount of biomass can be produced from a particular amount of nutrient or element or substrate in a culture medium. The availability of this nutrient determines the cell density which can be achieved (Liebig's law). Seconds, this model was also used to indicate the microbial growth rate (μ) was dictated by the low concentration of a particular substrate (s). In most applications of Monod model, it has turned out that growth or degradation phenomena can be described satisfactory with four parameter, the two kinetics parameter and two stoichiometric parameters. Monod model relates the growth rate to the concentration of a single growth controlling substrate (μ) via two parameters, the maximum specific growth rate (U_{max}) and the substrate affinity (K_s). Since the growth is a result of anabolic and catabolic enzyme activity, the growth associated product formation can also be quantitatively described on the basic of growth model (Kovari et al., 1998).

According to Klecka et al., 1985, the analysis from the reactor performance data by using a mathematical model based on Monod equation indicated that the biodegradation of pentachlorophenol was the first order with respect to the substrate. Recent concern has been expressed over the use of mathematical model based on Monod kinetics for describing the biodegradation of inhibitory substrate in wastewater treatment system.

This model may be valid for describing the kinetics of substrate removal at low specific growth rate.

Apart from that Hu et al., 2001 was claimed that process modelling was useful in order to predict and describe the performance of anaerobic digestion system. Monod models have been widely used to describe the process kinetics of anaerobic digesters (Andrew 1971). Although there has been some success in applying Monod model to the anaerobic system, some researchers found it was difficult to apply them for their systems (Grady et al., 1972).

2.1.1 Advantages of Monod

The advantage of Monod kinetics is minimizing the number of model parameters needed to characterize the problem (Borden et al., 1984; Borden and Bedient, 1986). The multiple-term Monod expression was commonly used when it is unknown which of the species (substrate, nutrient such as assimilatory nitrogen, or electron acceptor or all three simultaneously) is rate-limiting to avoid unnecessary analysis in the numerical solution to find the limiting species. Furthermore, Monod function contains zero order, first order and mixed order regions. Monod function reduces to zero order decay when the substrate concentration far exceeds the half-saturation constant of the substrate. On the other hand, the Monod function simplifies to a first order decay when the half-saturation constant of the substrate far exceeds the substrate concentration. If the Monod function cannot be simplified to either zero or first order reaction, then the contaminant advection dispersion mass transport equation is non-linear due to the coupling with the growth dynamics of the microorganisms (MacQuarrie et al., 1990).

A simple comparison was done by Bedient et al., (1994) showed that Monod function was the most conservative followed by the instantaneous reaction model and it was the first-order decay model. The first order decay model can under predict the concentration because the amount of electron acceptor available may not be stoichiometrically sufficient to support the predicted reduction. Therefore, it is important to recognize that the first order expression does not incorporate the electron acceptor limitation, and thus care should be taken when using this expression Bekins et al., (1997). Compared zero and first order approximations to the Monod kinetic model. The results suggested that, the first-order model may not be valid for substrate concentrations above the half-

saturation constant and either the zero order models or the Monod model should be used. It was also suggested that Monod kinetics are capable of capturing both zero and first order regions of the biodegradation process.

In light discussion made by Rashid et al., (1998), it was proved that Monod functions were an important and valid representation of biological decay under a variety of geological and biochemical conditions. Therefore, an efficient numerical algorithm that can solve the non-linear, coupled Monod functions accurately can provide a contribution in field-scale applications of biodegradation models.

Kinetic model was substantially capable to predict the product formation in biopolymer synthesis. Mathematical model gains from kinetic study facilitate data analysis and provide useful strategy for solving problems in fermentation process (Sharifzadeh et al.,2011). The information from fermentations process kinetics has a potential to improve the process performance. Kinetic model also allow the researchers to predict the cell growth. One of the most widely used models to describe cell growth, known as the unstructured model, describes the single component as the sole source of energy for prediction of cell growth is Monod model. Monod models were successfully applied to describe the batch growth kinetics. The Monod kinetic model used for production of biopolymer described by; μ is the specific growth rate (h^{-1}), S is substrate concentration (g/L) and μ_{max} and K_s were defined as maximum specific growth rate and Monod constant (g/L). The growth of the microorganism is well adjusted to its new environment at the end of the lag phase. After that, the cells multiply rapidly. The exponential phase or known as a log phase was the most active part which will be used to determine the kinetic parameters. The log phase was a period balanced growth, because all the components of a cell grow at the same rate (Divyashree et al., 2009).

Table 2.1: Summary of application of Monod Equation in the previous study

TYPE OF BACTERIA	DISCRIPTION PARAMETER INVOLVED	REFERENCES
Corynebacterium glutamicum	Study the growth of bacteria using Monod Equation. Different initial of glucose concentrations was used. Biomass production is L- glutamic acid	Khan et al., 2005
Lactobacillus coryniformis	Study the growth of bacteria in SSF in favourable conditions. Substrate – cellulose. Biomass production is Lactic acid.	Yanez et al., 2003
Heterotrophic bacteria and algae	Effect of substrate concentration on the growth of mixed culture in secondary facultative ponds (SFPs). The maximum growth rate was determined using Monod kinetic equation.	Kayombo et al., 2002
Lactobacillus helveticus	Estimation of growth and production using different factor; pH set point, yeast extract and initial whey permeate concentration	Schepers et al., 2001
Lactobacillus plantarum	Applicability of using brown seaweed as a source of nutrition for the growth of lactic acid bacteria. Growth of lactic acid bacteria was studied.	Shilpi et al., 2010
Aspergillus niger	SSF culture was compared with SmF in the production of feruloyl esterase. Raw material- sugar beet pulp.	Asther et al., 2002
Unicellular microorganism	A general formula for the growth rate of unicellular microorganism. Nutrients and inhibitors are considered as limiting substances	Bertolazzi, 2004
Lactobacillus plantarum	Growth of *Lactobacillus plantarum* in media containing hydrolysates of fish viscera	Horn et al., 2005
Microbial cell	Growth kinetics of suspended microbial cells: from single-substrate-controlled growth to mixed-substrate kinetics	Kovarova et al., 1998

Arthrobacter sp.	Kinetics of microbial growth of pentachlorophenol	Klecka et al., 1985
Escherichia coli	Evolution of the maximum specific growth rate with Monod kinetics	Wick et al., 2002
Microorganism	Kinetic study of anaerobic digestion-comparison between Monod and Contois	Hu et al., 2001
Microorganism	A simplified numerical algorithm for oxygen and nitrate-based biodegradation of hydrocarbons using Monod expressions	Rashid et al., 1998
Cupriavidus necator	Growth kinetic parameters and biosynthesis of polyhydroxybutyrate on selected substrates	Sharifzadeh et al., 2011

2.2 Contois Equations

Contois (1959) claimed that the specific growth rate was the function of the growth limiting nutrient in both effluent and input of substrate concentration. Chen and Hashimoto (1980), suggested that Contois equation model would be more suitable than the Monod type kinetic model to predict digester performance.

Hu et al., (2001) was claimed that in the equation proposed by Contois, (Contois 1959) the specific growth rate was considered as a function of the growth limiting nutrient in both input and effluent substrate concentration by using an empirical constant, which was related to microbial concentration. Besides, the Contois type model was found to be more suitable than Monod equation for predicting the performance of the anaerobic contact process at pilot-scale due to the effect of the variation of substrate concentration being considered in the Contois equation. In other opinion, Chen and Hashimoto (Chen et al., 1980) had developed kinetic models for substrate utilisation and methane production. They had suggested that the Contois models would be more suitable than the Monod type kinetic models to predict digester performance.

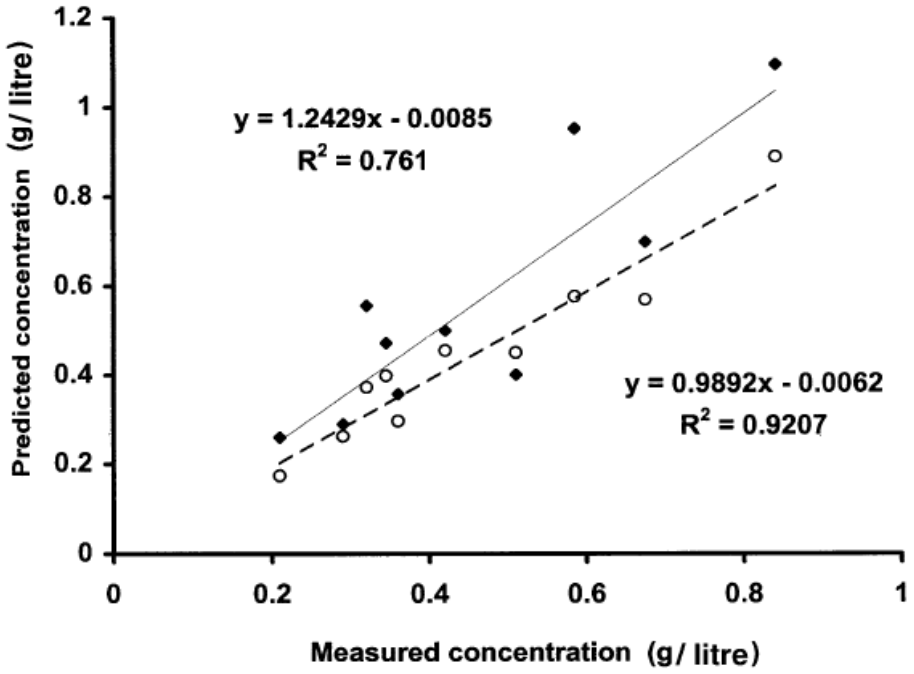

Figure 2-2: Comparison between the predicted and the actual effluent data from the full scale anaerobic contact process. (Contois type model (o); Monod type model ( ) with the modified μ_{max} values. (Hu et al., 2001)

According to Sharifzadeh et al., (2011) several kinetic model other than Monod also can be used in order to predict the cell growth rate. They were Contois, Westerhoff, Herbert, Moser and Tessier models. Contois model showed that the specific growth rate was inversely proportional to the cell concentration. Waterhoff proposed a linear model for the specific growth rate. On the other side, the Monod equation had been modified as the maintenance term was incorporated in Herbert model (Kovar et al., 1998) and the Tessier model represent exponential substrate consumption (Bailey et al., 1986).

Models	Non-linear models	Linear Models	Parameters	R^2
Monod	$\mu=\frac{\mu_{max}S}{K_S+S}$	$\frac{1}{\mu}=\frac{K_s}{\mu_{max}}\frac{1}{S}+\frac{1}{\mu_{max}}$	$\mu_{max}=0.17\,h^{-1}$ $K_s=86\,g\,l^{-1}$	0.976
Contois	$\mu=\frac{\mu_{max}S}{K_SX+S}$	$\frac{1}{\mu}=\frac{K_s}{\mu_{max}}\frac{X}{S}+\frac{1}{\mu_{max}}$	$\mu_{max}=0.17\,h^{-1}$ $K_s=93\,g\,l^{-1}$	0.972
Westerhoff	$\mu=a+b\ln S$	$\mu=a+b\ln S$	$a=0.015\,h^{-1}$ $b=0.146\,h^{-1}$	0.971
Herbert	$\mu=(\mu_{max}+m)(\frac{S}{K_s+S})-m$	$\frac{1}{\mu}=\frac{K_s}{\mu_{max}+m}\frac{1}{S}+(\frac{1}{\mu_{max}+m}-\frac{1}{m})$	$\mu_{max}=0.14\,h^{-1}$ $K_s=34\,g\,l^{-1}$ $m=-0.36\,h^{-1}$	0.889
Moser (n=2)	$\mu=\frac{\mu_{max}S^2}{K_s+S^2}$	$\frac{1}{\mu}=\frac{K_s}{\mu_{max}}\frac{1}{S^2}+\frac{1}{\mu_{max}}$	$\mu_{max}=0.1\,h^{-1}$ $K_s=54\,g^2\,l^{-2}$	0.936
Tessier	$\mu=\mu_{max}(1-e^{\frac{S}{K_S}})$	$\ln\left(1-\frac{\mu}{\mu_{max}}\right)=-\frac{S}{K_S}$	$\mu_{max}=0.12\,h^{-1}$ $K_s=28\,g\,l^{-1}$	0.939

Figure 2-3: Kinetic model applied for biopolymer production using glucose as carbon source.(Sharifzadeh et al., 2011)

2.3 Candida Tropicalis

2.3.1 Applications Of Candida Tropicalis

Latif et al., 2001 was claimed that strains of *Candida shehatae* and *Pichiastipitis* were now known as the best ethanol producing yeasts from xylose under microaerophilic conditions (Hinfray et al., 1995; Moniruzzaman,1995). However, the other substitute for ethanol production can be the formation of xylitol from lignocellulosic hydrolyzates by the yeasts such as *Candida guilliermondii* and *Candida tropicalis* (Barbosa et al., 1988; Horitsu et al., 1992).

According to Azuma et al., 200 there was a great deal of interest in microbial production of xylitol nowadays. Xylitol can be produced by a number of microorganisms, including the genus *Candida.* (Barbosa et al., 1998; Sirisansaneeyakul et al., 1995). Previous study was found that *Candida sp* was found to be an efficient xylitol producer from high concentration of xylose (Ikeuchi et al., 1999). A high substrate concentration was desirable for practical purposes. However, the purification and the concentration of D-xylose were necessary in order to get higher xylitol. On the other hand, in order to consider the cost, hydrolysates of cellulosic materials should be used without purification of D-xylose for xylitol production. In present study, xylitol production in media containing D-xylose (5-15%) and D-glucose (2%) was investigated. The xylitol production was increased via addition of salt regardless of the presence or absence of D-glucose. It was suggested that the addition of sugar was useful for higher production of xylitol whenever a hydrolysate cellulosic materials was used as a substrate.

In other researches that done by Weng et al., 2006, *Candida tropicalis* strain was used in biodegradation of free gossypol under solid state fermentation. Solid state fermentation (SSF) was used to produce microbial biomass, and was an attractive process to produce valuable products due to its low operating cost (Pandey et al., 200). Caffeine degradation in SSF was successfully carried out by fungi (Pandey et al., 2000). Therefore, it was an interested to explore the use of SSF as a process for BFG (biodegradation of free gossypol) by microorganism. The selection of suitable solid substrates for a fermentation process was an important factor. In general, abundant agro-industrial by products, such as wheat bran, rice straw, rice bran, wheat straw etc. can be used as solid supports or substrates for various fermentations. In this work, cottonseed meal was used as a substrate to evaluate the feasibility of BFG by *Candida tropicalis* under SSF, and to investigate its fermentation mechanism to provide practical guidelines for using fermented cottonseed meal as an animal feed protein source.

The identification of the physiological factors such as the defence of yeast against salt stress was an important goal of salt tolerance studies. Type of sugar metabolism used was one of the important factors in yeast physiology, fermentative or respiratory (Gancedo & Serrano, 1989). Glucose exerts a general modulation of metabolism and stress responses in yeast (Thevelein, 1994; Serrano, 1996). In researched study done by

Garcia et al., 1997 comparison was done between different type of strain which were *Saccharomyces cerevisiae* and *Candida tropicalis.* The results showed that the operation of general catabolite control in *Saccharomyces cerevisiae*, but not in and *Candida tropicalis*, correlates with a high capability for glycerol synthesis and osmotic tolerance but a low capability for sodium and lithium extrusion and cation tolerance.

Candida tropicalis was tested for xylitol production from corn fiber and sugarcane hydrolysates (Rao et a., 2004). Researched done by Rao et al., 2004 showed that fermentation of corn fiber and sugarcane bagasse hydrolysate had produced xylitol and xylose uptake. These uptake and xylitol production were very low even after hydrolysate neutralization and some treatments with activated charcoal and ion exchange resins. Initial xylitol production was0.43 g/g and 0.45 g/g of xylose utilised with corn fiber hydrolysate and sugarcane bagasse hydrolysate respectively.

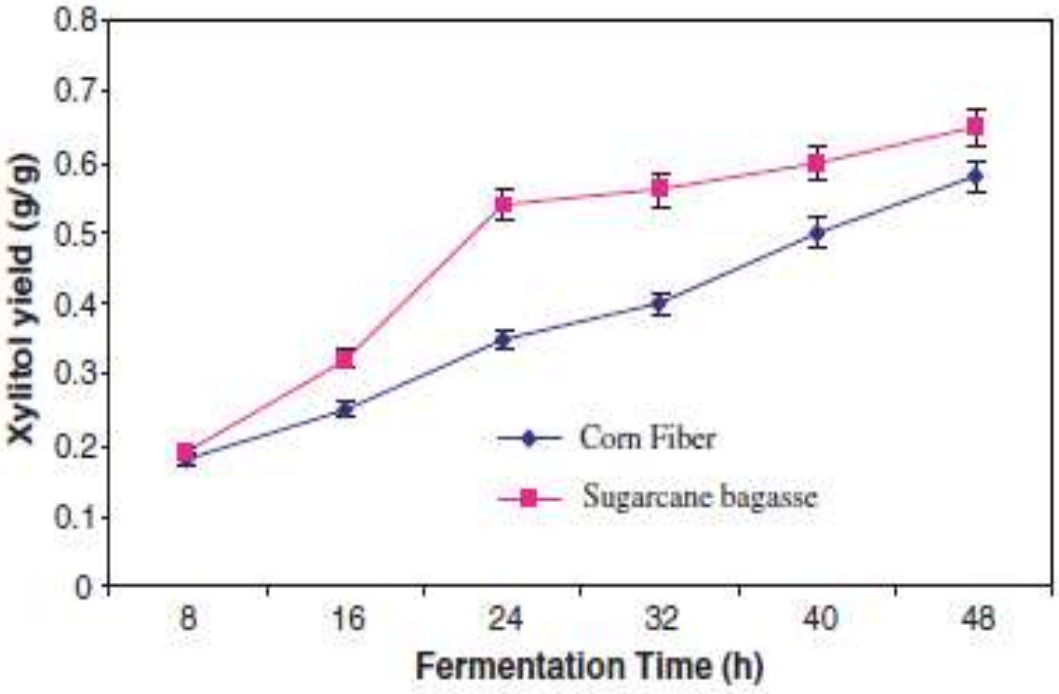

Figure 2-4: Xylitol yield with corn fibre and sugarcane bagasse hydrolysated by *Candida tropicalis* (Rao et al., 2004)