CHAPTER II

LITERATURE REVIEWS AND THEORETICAL CONSIDERATIONS

2.1 Literature reviews

2.1.1 Endomycopsis fibuligera

Yeasts are generally considered to be unable to ferment starch. This lack of diastatic activity is the reason for the use, in fermentation industries, of barley malt and fungal diastase preparations for the conversion of starch substrates to sugars which may be attacked by the yeasts. If there should exist in nature yeasts which possess active amylase systems, they might be of much scientific and industrial interest. Lindner (1907) who originally isolated from spoiled bread a filamentous yeast which he named E. fibuligera, reported that this organism fermented sucrose strongly, glucose less strongly, raffinose and lactose weakly, and maltose and dextrin not at all. According to the method stated in the Yeast edited by Lodder (1970) E. fibuligera was classified as the following :

Class Ascomycetes (Ascomycotina)
Order Endomycetales
Subfamily Saccharomycoideae (Saccromycetoideae)
Genus Endomycopsis

E. fibuligera was identified as a strain of food yeast. E. fibuligera propagated by multilateral buffing and mycelial-formation. The yeast cell were short to long-oval (4-7 μm). Branched, septate mycelium was formed and oval blastospores were produced at the end and laterally. Spherical asci containing

two to four hat-shaped ascospores are laterally produced on the mycelium. The literature regarding the utilization of starch substrates by yeasts and the physiological properties of yeasts of the species E. fibuligerais sparse. Although strains of the species E. fibuligera have been known many years, their ability to attack starch has not been recognized. Lynferd J. W. and his collaboration were the first scientists who realized a research on the capacity of starch hydrolysis and fermentation by the yeast E. fibuligera. Under certain conditions, E. fibuligera was found to be a potent diastatic agent. The ratio of alpha to beta amylase was high (Lynferd J. Wickerham 1944).

2.1.2 Candida utilis

C. utilis is particularly desirable on account of its high protein content, its vitamins of the B-complex, and its ability to utilize hexoses, pentoses, acetic acid, and certain other constituents which may be found in the acid hydrolyzates or enzyme hydrolyzates from wood or cellulosic or starch materials. Therefore, the production of animal feed, usually using C. utilis as the producer organism, is carried out on a large scale in several countries. Studies concerning the metabolism of growing C. utilis under aerobic conditions have been published in many articles in Bio. Zeitschrift.

2.1.3 Mixed Culture process

The controlled mixed cultures of microorganisms is an important approach in the development of biotechnological processes. One of the advantages of such systems is that they make it possible to realize a two stage process in one reactor. This makes mixed cultures suitable for product formation from natural high-molecular weight substrates (e.g., starch and cellulose) without a previous hydrolysis step. In many cases the mixed culture contained E. fibuligera as the amylolytic microorganism in combination with either yeasts or bacteria, which use the products of starch hydrolysis. With this process, starch materials that could not be utilized directly, can be

converted economically into microbial protein or other needed products.

Kurt Jarl (1969) described process of conversion of starch to single cell protein on a mixed culture of an amylolytic yeast, E. fibuligera a:3 and an aerobic yeast, C. utilis NRRL Y 900, was defined as a Symba Yeast Process. The enzyme activity of E. fibuligera will convert the starch into lower saccharides, predominantly glucose, which the fast growing C. utilis then is able to use for biosynthesis of cell substance. Since the growth rate of E. fibuligera is moderate, the process yields of product substantially of C. utilis yeast. In a symbiotic cultivation, the proportion of inocula are of special importance to the course of growth and the composition of the resulting population. Thus in the Symba process the ratio E. fibuligera : C. utilis at the start can be critical. However, if a relatively high amylase activity is introduced with the inoculum, the proportions of the two initial cell densities will be of lesser importance. The C. utilis inoculum can thus be chosen relatively freely; its growth will be dominant any how provided the initial cell density is of the same order of magnitude as E. fibuligera.

The restricted growth of E. fibuligera in the symbiotic culture requires that its population be strengthened when starting a larger batch. This can be done by running pure E. fibuligera batches in parallel with the symbiotic propagation. In this manner, the amylase activity and so the hydrolysis and growth rates are kept at an adequate level.

The yield of yeast product in the Symba process depends on the raw material, especially on its content of unassimilatable substances. With whole potatoes as raw material a product with 40 % raw protein obtained in a yield of 60 % (dry weight). In another application, conversion of cassava starch into yeast substance, a yeast product containing 50 % raw protein in a yield of up to 46 % based on total available cassava substance (dry weight) was obtained (Jarl 1969).

Symba process was developed in Sweden during the late 1960s, but little technical information is available on this process. The comparison of amino acid composition of E. fibuligera, C. utilis and their mixture is shown in Table 2.1.

Table 2.1 Amino acid composition of Endomycopsis fibuligera and Candida utilis

No.	Amino acids	E. fibuligera[1] %	C. utilis[2] %	Symba Yeast[3] %
1	Arg	4.23	6	4.6
2	His	1.95	3	2.0
3	Lys	7.41	9	6.3
4	Tyr	0.90	4	4.8
5	Try	1.23	1	1.3
6	Phe	6.94	4	5.4
7	Cys	2.95	1	1.0
8	Met	1.37	1	1.5
9	Thr	3.58	6	5.4
10	Leu	8.48	9	7.5
11	Iso-leu	4.51	5	4.3
12	Val	6.48	6	4.2
13	Asp	-	10.3	-
14	Glu	-	13.8	-

1. Rossi, J., and Clementi Journal of Food Technology 1979, 20, 319-330.
2. Bui, K. and Galzy, P., Food Yeast Technology 1990.
3. Symba Yeast process, Swedish Sugar Company 1969.

Sales and Menezes (1977) studied the growth and biomass production by mixed cultures of amylolytic yeasts (E. fibuligera, E. capsularis) with C. utilis on a dextrinised cassava medium highest protein concentration in

biomass (56.7%) was achieved with mixed culture of C. utilis and E. fibuligera, on a medium supplemented with urea 3.0 g/l. The potential for production of single cell protein by fermentation of cassava is considered for food or feed.

Nga,Tan and Lee (1976) realized the laboratory investigation, two yeasts were grown on cassava starch. Seventeen amino acids in the dry weight of starch medium before and after yeast was grown in it. Crude protein content rose from 0.7% to 4.76%.

Rungrot (1980) studied on the fermentation of cassava by mixed cultures of two yeasts, Rhizopus nigrican and S. cerevisiae. The yeast protein of cassava rose from 0.9% to 12.04 %

Witchuporn (1980) used cassava power 7% and added 0.5% of $(NH_4)_2 SO_4$ for the experiment of mixed cultures. Rhizopus oryxae MB 67 and S. cerevisiae was used for the fermentation. The highest dry weight was 32.6 g/l, with protein 47.9% of dry weight.

Margareta and Milan (1983) studied on the production of ethanol from starch used a mixed culture of E. fibuligera and Z. mobilis. Zymomonas mobilis used instantly the glucose formed for ethanol production. The ethanol production released the glucose inhibition of hydrolysis of non-glucose reducing sugars.

Milan Dostalek (1986) studied the interaction between two microorganism, E. fibuligera and Rhodosporidium toruloides. In his work, a mixed culture of these two microorganism was used for production of lipid from starch. E. fibuligera NRRL Y76 was used as the amylolytic microorganism and R. loruloides CBS 14 as a lipid producer. pH was keep constant at 5.5 by automatic cultivation, temperature was 30°C in all cultures. The highest lipid concentration 9.7 g/l, and highest concentration of lipid in biomass (36.5%) were obtained in cultures with an initial nitrogen concentration of 0.5 g/l.

2.1.4 Cassava

Cassava is the product of a tropical root crop. Cassava has been regarded on one of the world most important crops that can be used for human consumption, for animal feed and in various industries. According to the Food and Agriculture Organization (FAO), the world production of cassava in 1990 should amount up to 146.8 million tons. The export of cassava products (in the form of chips, pellets and starch) in 1989 rose to 11.94 million tons, with Thailand serving as the largest exporter of cassava products. Thailand's total export alone was about 10.25 million tons at a low price (45 US$/t or 1,125 Baht/t). The Table 2.2 showed the statistical report on Thailand cassava production during fifteen year.

Table 2.2 Cassava Crop Surveys in 1976-1990

	1976	1980	1985	1990
Total Root Yield (million tons)	10	10.48	16.9	21.8

Source : Thai Tapioca Trade Association

Tapioca has been used extensively in the animal feed industry. It has high starch percent in the composition that is one of the most important feed ingredients cassava mixed in animal feed compound. The composition of different cassava root product is shown on Table 2.3. It can be seen that the ratio of protein and vitamins is very low.

Protein enrichment of starch materials such as cassava is attractive. In an application, conversion of cassava starch into yeast substance, a yeast product containing 50 % raw protein in a yield of up to 46 % based on total available cassava substance (dry weight) was obtained (Jarl 1969).

Table 2.3 The composition of different cassava root product by Lim (1968).

Composition (%)						
Comp.	Fresh*	Fresh	chips	flour	Meal	Pellet
Moisture	63.8	80	19.7	14.9	11.2	14
Protein	0.96	0.4	1.9	0.3	2.6	2.3
Fibre	0.85	1.6	3.0	0.1	5.6	2.4
Starch	27.6	17.6	70.5	84.4	73.9	70
ash	1.44	0.3	2.1	0.2	6.1	4.5
other	0.04	-	-	-	-	-

* Department of Agriculture, Bangkok, Thailand

2.1.5 Molasses

Molasses is the by-product from the saccharose extraction process from sugar-cane or sugar beet. The composition of molasses (Table 2.4) makes it a prime substrate for the production of feed and food yeast. The number and amounts of additives required for supplementation are limited (sulfuric acid and ammonia for pH regulation). Molasses is the main substrate for the production of Baker's yeast S. cerevisiae (Peppler1967; Burrows 1979). There are well-established processes, and molasses is becoming a scarcer and more expensive commodity, and its quality is also becoming poorer since the sugar industry is now better able to remove residual saccharose. It is quite probable that molasses will be used in the near future only for the production of expensive items.

It can be seen that molasses has many kind of vitamins and growth factor which it need for yeast fermentation. These vitamins and growth factor may be use in mixed culture for SCP production instead of using yeast extract which is very expensive.

Table 2.4 Composition of molasses

Composition		Beet	Cane
Dry matter	%	74 - 78	75
Sugar total	%	48 - 52	48 - 56
Invert	%	0.2 - 1.2	15 - 20
Fermentable	%	5 - 47	46 - 52
N- containing comp	%	6 - 8	3
Betain	%	3 - 4	-
Organic acid	%	6 - 8	-
Ash	%	10 - 12	10 - 15
Na	%	0.3 - 0.7	0.1-0.4
K	%	2 - 7	1.5- 5
Ca	%	0.1 - 0.5	0.4-0.8
Cl	%	0.5 - 1.5	0.7- 3
P	%	0.02-0.07	0.6- 2
pH		7 - 9	5 - 6
Vitamins (ppm)			
Biotin		0.04-0.13	1.3-3.2
Inositol		6000-7000	6000
Pantothenic acid		50 - 100	54 - 64
Thiamine		1.3	1.8
Nicotinic acid		30 - 45	30 - 80

2.2 Theoretical considerations

2.2.1 Requirements of the yeast growth

Study on the physiology and chemistry of yeast growth are realized for

many years ago. Although the growth requirements of yeast are not complex. But the mode of yeast growth is extremely sensitive to changes in the growth conditions. Especially the nature and concentration of the carbon substrate and the availability of oxygen are critical indetermining the yield of cells and the nature of the products of the fermentation. The important of oxygen and glucose in the regulation of the growth characteristic in yeast has been recognized for many years.

2.2.1.1 Nutrition of yeast

Although the nutritional requirements of yeast are not very demanding, they do vary with the mode of growth and they must be controlled precisely if maximum growth rates and optimum yields are to be obtained. The general requirements of yeast nutrition are described on Table 2.5.

Carbon source

Organic compounds such as glucose have a nutritional role as a source of energy. In combination primarily with hydrogen, oxygen and nitrogen, they make up the major structural units of yeast; approximately 50% of dry weight of yeast is carbon. The carbon sources are many kind of sugar such as glucose, fructose, sucrose and raffinose. It employed also industrially impure sources of carbohydrates for example: starch, beet, molasses.

Nitrogen source

Nitrogen is also the important constituent of a growth medium after carbon. Nitrogen is used in organic combination in amino acids, nucleotides and certain vitamins. Inorganic nitrogen sources are ammonium nitrogen in the form of NH_4NO_3, NH_4CL and $(NH_4)_2SO_4$. The last one provides the dual nutritional benefit as a sources of sulphur, is commonly employed as a nitrogen source in industrial fermentations. Amino acid, most amino acids can serve as

a sole source of nitrogen, although the maximum growth rate that they can promoted varies with the particular amino acid. Urea provides a good nitrogen source, although addition of a biotin supplement is required.

Table 2.5 Medium composition for Growth of yeast, Justin (1989).

Composition	Weight per litre
Glucose	10 g
$(NH_4)_2SO_4$	3 g
Mineral salt	
KH_2PO_4	2-3 g
KCl	120 mg
NaCl	60 mg
$CaCl_2.6H_2O$	135 mg
$MgCl_2.6H_2O$	520 mg
$FeSO_4(NH_4).6H_2O$	35 mg
$MnSO_4.4H_2O$	5 mg
$ZnSO_4.7H_2O$	2.3 µg
$CoSO_4.7H_2O$	2.3 µg
$Na_2MoO_4.2H_2O$	3.3 µg
H_3BO_4	7.3 µg
KI	1.7 µg
$NiSO_4.6H_2O$	2.5 µg
Vitamins	
Aneurine hydrochloride	5.0 mg
Pyridoxine hydrochloride	6.25 mg
Nicotinic acid	5.0 mg
D-Biotin	.23 mg
Ca-pantothenate	6.25 mg
Myoinositol	125 mg

A good nitrogen source must be:

a) Be rapidly taken up into the cell.

b) Undergo conversion once in the cell with the minimum intervening steps to form glutamate or ammonia or both.

c) Have no toxic side effects on the cell.

Inorganic Nutrients

Phosphate is assimilated as the dihydrogen orthophosphate ion, and any phosphate esters present in the medium are broken down by an acid phosphatase present in the yeast cell wall. Sulfur can be assimilated as sulfate, sulfite, or thiosulfate ions and as methionine, but other sulfur amino acids are not assimilated.

Metal

The metal requirements of yeast are given in Table 2.5. Potassium is required as an effector for several glycolytic enzymes but also plays a key role in the maintenance of cation balance in the cell (Mailrella et al., 1984). Magnesium is required as a cofactor for over 100 enzymes and also for the stability of nucleic acids and cell membranes. Iron, zinc, and manganese are also important as enzyme cofactors. The requirement for iron, which is present in some enzymes, such as cytochromes, is greater in aerobically grown yeast, whereas the requirement for zinc, which is required for alcohol dehydrogenase activity, is greater in anaerobic conditions.

Growth factors

Growth factors are also required either as an absolute requirement or in promotion of growth and optimal cell yield. Growth factors in Table 2.5 shows

the vitamin requirement common to most strain of E. fibuligera.

Oxygen Requirement

Efficient yeast propagation is highly aerobic. The material balance equation presented by Trivedi et al (1986) shows that the respiratory is very close to unity. The oxygen is usually air, and the low solubility of oxygen in water requires that aeration of the nutrient medium be as effective as possible. Aeration is a large portion of total process costs, and efficient filtration of input air is necessary for maintaining aseptic conditions and avoiding microbial contamination of the fermenters. At normal feed rates the air supply rate can reach 1 volume of air per unit volume of liquid in the fermenter per minute. Yeast yields have been significantly improved through oxygen enrichment of the broth (Matsumura et al. 1982).

200 g sucrose +132 g NH_3 +100.44 g O_2 +7.5 g ash = 100 g yeast solids +
140.114 g CO_2 + 78.12 + H_2O

The productivity of fermenters for the production of cell mass is limited by their oxygen transfer characteristics. The substrate concentration should be as high as possible depending on the oxygen transfer required for its utilization. Each given fermenter has a limited maximum biomass production capacity, per unit volume, per unit time. The measurement of oxygen transfer in industrial scale fermenters is therefore very important.

pH

The initial pH is generally adjusted from 4 to 6 depending upon the raw materials used and conditions of fermentation. The proper pH ranges depending also on the type of organisms being grown, pH 4.5 to 6 for yeast, pH 6.0 to 7.5 for bacteria, or pH 4.5 to 7 for molds.

Temperature

The temperature normally used for food and feed yeast production is 30 ^{o}C. Where it is difficult to maintain the temperature at the optimum, special strains of yeast that grow well at higher temperature (35^{o}C) may be used.

2.2.2 Fermentation apparatus

Biomass of yeast is produced normally in batch fermenters the final fermentation vessels generally of the order of 100-200 m^3 in size. It is fabricated from stainless steel or other corrosion resistant materials. The fermenter is equipped with an aeration system, cooling system, sugar and nutrient metering system, sampling ports, and foam and pH controlling devices.

Efficient aeration is a crucial aspect of biomass production. It is important to appreciate at the out set that simply ensuring sufficient aeration, i.e. rate of oxygen transfer to the fermentation medium greater than rate of oxygen utilization by the biomass, is not the only measure of the adequacy of an aeration system, since in many cases the aeration system, since in many cases the aeration system is also used for mixing of the fermentation contents. Effective aeration and complete mixing is significant. Since the product relies on a carefully controlled composition and activity. Generally, three major types of aeration systems are used in the SCP's yeast industry.

a. air spargers without mechanical agitation
b. agitated vessels (conventional stirred bioreactors)
c. proprietary aeration systems.

Air spargers without mechanical agitation consist of a horizontal tube with side tubes provided with a large number of small holes. Rosen (1977) describes a typical system consisting of horizontal tube with 24 side tubes provided with 30000 holes of diameter 1.5 mm. The provision of a large number of small holes does not necessarily guarantee good mass transfer since

small bubbled formed in a tiny orifices may eventually coalesce to form large bubbled in a medium if there is inadequate mixing or ineffective surfactants. Power input from the gas phase is also important. If the power input from the gas phase is insufficient to generate turbulence in the liquid phase, then the bubbles size will increase with liquid height in the fermenter, due to coalescence. However, if the liquid is in turbulent motion, bubble break up will also occur. The coalescence and break up bubble equilibrium will determine the mean bubble size. The gas flow distribution and the height:diameter ratio of fermenter will also have a considerable effect on the extent of liquid turbulent motion for a given gas phase power input. So for engineering calculation, differences in theoretical properties of fermentation liquids from ideal experimental conditions and scale up condition must be taken into account with respect to the application of any correlation to a particular fermentation process.

The provision of air to air sparged systems is generally of the order of 0.8-1.4 volume of air per fermenter volume per minute. Anyway, such data are not meaningful for any comparative purposes (i.e. different fermenter geometry and different aeration systems. Because they give no information regarding bubble size, gas hold up, actual dissoled oxygen concentration, etc. Specific energy consumption is of the order of 150 Wh/kg yeast (28 % dry solids). Agitated vessels are used by some companies also. The major reason for using these systems are to attempt to promote more extensive mixing for the liquid and gas phases at larger scale, and to ensure effective power input by turbulent liquid motion.

In yeast production good mixing is essential as previously stated. The impeller speed is provided above a critical value in small tanks, both the liquids and gas phase are considered to be perfectly mixed. For large tanks this is not often the case industrially (Oosterhuis and Kossen 1983). The actual behaviour of the two phases should be established, because of either well mixed or plug flow behaviour of the gas phase may lead to gas absorption rate predictions

which differ by a significant order of magnitude. Modeling of both liquid and gas phase behaviour has been carried out at both laboratory (see Moo Young and Blanch 1981) and industrial scale (Oosterhuis and Kossen 1983).

The major propriety aeration systems are typified by those of Vogelbysch, Frings. The basic of the Vogelbusch aeration system is the development of a special centrifugal pump suitable for pumping homogeneous gas and liquid mixtures of a specific density of 0.3-0.95 at a hydraulic efficiency of 0.65-0.75, depending upon the application. The hydraulic part consists of a multistage impeller with a spiral casing. The gas separated in the pump is removed from the impeller by a system of bored holes. The gas is removed from the pump by a special constriction in the pump casing. The rear of the impeller is equipped with a special separation stage. It is in conjunction with a specific casing cover design and a throttling flag in the pressure pipe, ensures that only gas without liquid leaves the pumps.

Fermentation medium (liquid and gas) is pumped from the bioreactor using this pump for circulation of reaction liquid and simultaneous discharging of a controlled part of the waste air. A pressure line is then used to supply an aeration installation with the pump exit reaction liquid. A tube heat exchanger is installed in the pump pressure line to remove the heat involved during fermentation. The aeration installation involves injection of air into the medium, generally introduced by pumping the medium past air inlet holes.

In the Vogelbusch system the aeration installation is placed at the top of a long shaft, the outlet of which is on the level of the surface of the liquid to be aerated. The air flow is self priming. The air sucked in is finely dispersed, and a largely homogeneous mixed jet leaves the shaft end. The aerated jet penetrates nearly to the bottom of the fermentation vessel by virtue of its energy content.

SCP's production

An excellent reviews concerning the production of SCP's yeast have been published recently (Reed 1982). Baker's yeast (S. cerevisiae) fermentation are carried put under highly aerobic conditions with incremental feeding of molasses. Molasses is received at about 80° Brix with a fermentable sugar concentration of 50-55%. It is diluted (to about 40° Brix) and clarified for using batch fermentation.

Nitrogen sources used are ammonia ammonium sulphate and urea; phosphorus sources are orthophosphates phosphoric acid. Mineral (magnesium and trace minerals) and vitamin (biotin and thiamin) supplements are added. The fermentation is carried out at a pH of between 4 and 6 , at a temperature of 30°C for periods of up to 24 h.

When the fermentation is completed (yeast solids of the order of 40-60 g/l) the yeast cells are concentrated by centrifugation to a yeast cream of 15-20% solids. The cream is cooled and then filtered using either a filter press or a rotary vacuum filter. The yeast press cake is then extruded and sold as compressed baker's yeast in blocks in wax paper or it is crumbled and sold in bulk. In the production of active dry yeast (ADY) the press cake is extruded in the form of fine strands. Active dry yeast contain 90-95% yeast solids compared with approximately 30 % in compressed yeast. It has a longer shelf-life but less baker activity.