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Review article

YEAST A SINGLE CELL PROTEIN: CHARACTERISTICS and METABOLISM

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INTRODUCTION

Most of the developing countries of the world are facing a major problem of malnutrition. Due to rapid growth in the population, food and feed scarcity are prevalent leading to a deficiency of protein and essential nutrients amongst human beings and livestock. It is therefore important to take necessary measures to stem this trend by increasing protein production and making it available and more affordable to the population by utilizing methods available for the production of alternative sources of nutrients. The increased world demand for food and in particular protein has engineered the search for non-conventional protein sources to supplement the available protein sources. Since the early fifties, intense efforts have been made to explore these alternate and non-conventional protein sources. In 1996, new sources mainly yeast, fungi, bacteria and algae have been used to ferment biomass in the form of biological waste to produce single cell proteins. Microbial biomass has been considered an alternative to conventional sources of food and feed. Large scale production processes for SCP production reveal interesting features.

• The wide variety of methodologies, raw materials and micro-organisms that can be used for this purpose

•High efficiency in substrate conversion

•High productivity, in terms of the fast growth rates of the micro-organisms

•freedom from seasonal factors (Roth, 1980; Parajo *et al*., 1995)

Yeast was the first micro-organism whose importance as animal feed supplement was recognized almost a century ago. During World War 1, Germany replaced half of the imported protein sources with yeast. Pruteen was the first commercial single cell protein used as an animal feed additive. Various micro-organisms used for the production of SCP are bacteria (Cellulomonas, *Alcaligenes)*, algae (*Spirulina, chlorella*), molds (*Trichoderma, Fusarium,Rhizopus*) and yeasts (*Candida, Saccharomyces*). These micro-organisms can utilize a variety of substrate like agricultural wastes and effluents, industrial wastes, natural gases such as methane and other decomposing pollutants (Miller and Litsky, 1976).

Description

Single cell protein has the potential to be developed into a very large source of supplemental protein that could be used in livestock feeding. In some the tropics single cell protein could become the principal protein source that is used for domestic livestock, depending upon the population growth and the availability of plant feed protein sources. This could develop because microbes can be used to ferment some of the vast amount of waste materials such as straws; wood and wood processing wastes; food, cannery and food processing wastes; and residues from alcohol production or from human and animal waste. Producing and harvesting microbial proteins is however not without costs. A variety of micro-organisms and substrates are used to produce single cell proteins. Yeast is suitable for single cell protein production because of its superior nutritional quality (Miller and Litsky, 1976). Supplementing cereals with single cell proteins especially yeast make them as good as animal proteins (Huang and Kinsella, 1986). The necessary factor considered for use of SCP is the absence of toxic and carcinogenic compounds that originate from the substrates, synthesized by the microorganisms or formed during processing.

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High nucleic acid content and low cell wall digestibility are two of the most important factors limiting nutritional and toxicological value of yeast for animal or human consumption (Alvarez and Enriquez, 1988). As constituents of nucleic acid, purine compounds in human diets are mostly metabolized to yield uric acid whose concentration may lead to gout or renal stones. However, nucleic acid is not a toxic compound and can only cause physiological effects at higher levels like any other essential dietary compound taken in large quantities. It has been calculated that 45kg of yeast will produce 250 tons of protein in 24 hours. Algae grown in ponds can produce 20 tons (dry weight) of protein, per acre, per year. Bacteria are usually high in protein (50- 80%) and have a rapid growth rate (Nasseri *et al*., 2011). The major disadvantages are as follows:

• Bacterial cells have small size and low density, which makes harvesting from the fermented medium difficult and costly.

•Bacterial cells have high nucleic acid content relative to yeast and fungi. To decrease the nucleic acid level, additional processing steps have to be introduced and this increases cost.

•The public misconception on the harmful nature of bacteria.

Yeast have advantages such as their large size, making them easier to harvest, lower nucleic acid content, high lysine content and ability to grow at lower hydrogen ion (pH) concentrations. However the most important advantage is its traditional use in fermentation, which makes acceptable to the general public. Disadvantages include lower growth rates, lower protein content (45-65%) and lower methionine content than in bacteria. Filamentous fungi have advantage since they are easily harvested, but have their limitations in lower growth rates, lower protein content and acceptability. Algae have disadvantages of have high cellulose walls which are not digested by human beings. They also contain heavy metals. Presently, world wide sophisticated technologies are used for mass production and processing of photoautotrophic microalgae. The annual world production of all microalgae species is estimated to be about 10,000 tons per year (Becker, 1994, 2007; Richmond, 2004). The algal biomass as sun dried or in compressed form is the predominant product in micro-algal biotechnology (Radmer, 1996). More than 75% of the annual microalgal biomass production is used for the manufacture of powders, tablets, capsules and pastilles. This biomass is harvested from natural waters or artificial ponds or photo bioreactors and subsequently separated from the growth media followed by drying (Renaud, *et al*., 1994). The two major species cultivated for this purpose are the unicellular green algae, *Chlorella* and more recently, filamentous blue-green algae *Cyanobacterium Spirulina* (Raja, *et al*., 2008). The production of SCP from various microbes, particularly from fungi and bacteria has received considerable attention, in contrast, only a few studies have dealt with the feasibility of using SCP from microalgae (Mahasneh, 1997).Algal proteins are of high quality and compare favorably with conventional plant proteins. However, due to high production costs as well as technical difficulties, cultivation of algal protein is still undergoing research (Rasoul-Amini, 2009). The celluloid cell wall, which represents about 10% of the algal dry matter, poses a serious problem in digesting/utilizing the algal biomass, since it is not digestible by humans and non-ruminants. Hence, effective treatments are necessary to disrupt the cell wall to make the protein and other constituents accessible for digestive enzymes. Several authors have studied the effect of different post-harvesting treatments on the digestibility of various algal species (Becker, 2007). Yeast cells have been considered as an essential food for the larval stages of fish and shell fish because of their particle size, high protein content (SCP) and relatively low production costs (Kim, *et al*., 1998). However, poor digestibility might be an important constraint in the use of this SCP as a food source in seed production of aqua cultural organisms, since yeast has a complex and thick cell envelope. The external mannoprotein layer of the yeast cell wall is probably the major barrier to digestion (Rumsey, *et al*., 2007; Kim and Chung, 2001). Several methods have been developed to improve the digestibility of SCPs: mechanical disruption, autolysis and enzymatic treatment (Curran *et al*., 1990). The substrates which have been used for SCP production by yeasts include sorghum hydrolysate, sulfate waste, prawn-shell wastes, dairy wastes, methanol, molasses, starch and plant-origin liquid waste (Bhalla, *et al*., 2007).

An accurate method to evaluate the quality of proteins is the determination of the Protein Efficiency Ratio (PER), expressed in terms of weight gain per unit of protein consumed by the test animal in short term feeding trials. Estimation of the biological value (BV) is a measure of nitrogen retained for growth and maintenance. Another parameter, which reflects the quality of protein, is the Digestibility Coefficient (DC) and also the Net Protein Utilization (NPU), which estimated by calculating BV x DCV. The NPU is a measure of the digestibility of the protein and the biological value of the amino acid absorbed from the food. SCP is generally evaluated in terms of Kjeldhal nitrogen x 6.25 (the standard factor relating amino nitrogen to protein content). About 10-155 of the total nitrogen in fungi and yeasts is in the form of nucleic acids (Riviere, 1977). World wide large scale development of SCP production processes has contributed greatly to the advancement of present day biotechnology. Research and development of SCP production processes has involved work in the fields of microbiology, biochemistry, genetics, chemical and process engineering, food technology, agriculture, animal nutrition, ecology, toxicology, medicine and veterinary science. Single Cell Proteins have application in animal nutrition such as: fattening of calves, poultry, pigs and fish breeding. SCPs also have application in foodstuff as: aroma carriers, vitamin carrier, emulsifying aids and improve the nutritive value of baked products. Production of SCPs is carried out by using selected strains of microorganisms which are multiplied on suitable raw materials which are added to the growth medium for cell mass production, this is then followed by a separation process. The major raw materials are substances containing mono- and disaccharides. Large scale production of microbial biomass as single cell protein has the following advantages: the microorganisms have a high rate of multiplication, they have high protein content, they can utilize a variety of carbon sources as energy source, microbial strains with high yield and good composition can be produced and cultured in large quantities under laboratory conditions and microbial biomass production is independent of seasonal and climatic changes. There are also some disadvantages in using microbial biomass as single cell protein: many microorganisms produce substances which are toxic to humans and animals, indigestion may sometimes occur after ingestion of SCPS as diet supplement, the high nucleic acid content can sometimes be undesirable if taken in large quantities and production of single cell proteins is a very expensive process. Further research and development will ensure usage of microbial biomass as SCP or as diet supplement in developing nations.

YEASTS

Yeasts are unicellular fungi. The precise classification is a field that uses the characteristics of the cell, ascopore and colony. Physiological characteristics are also used to identify species. One of the more well known characteristics is the ability to ferment sugars to produce ethanol. Budding yeasts are eukaryotic micro-organisms classified in the kingdom fungi of the phylum *Ascomycetes;* class *Saccharomyces* (also called *Hemiascomycetes*). The true yeast is separated into one main order *Saccharomycetales*. Yeasts are characterized by a wide dispersion of natural habitats, they are commonly found on plant leaves and flowers, soil and salt water. Yeasts are also found on the skin surfaces and in the intestinal tracts of warm-blooded animals, where they may live symbiotically or as parasites. The common "yeast infection" known as candidiasis is caused by the yeast-like fungus *Candida albicans*. In addition to being the causative agent in vaginal yeast infections *Candida* is also a cause of diaper rah and thrush of the mouth and throat. Yeast multiply as single cells that divide by budding (e.g. *Saccharomyces*) or direct division (fission, e.g. *Schizosaccharomyces*), or may grow as simple irregular filaments (mycelium). In sexual reproduction, most yeast form asci, which contain up to eight haploid ascospores. These ascospores may fuse with adjoining nuclei and multiply through vegetative division or, as with some yeasts, fuse with other ascospores. The awesome power of yeast genetics is partially due to the ability to quickly map a phenotype producing gene to a region of the Saccharomyces genome. For the past two decades S. cerevisiae has been the model system for much of molecular genetic research because the basic cellular mechanisms of replication, recombination, cell division and metabolism are generally conserved between yeasts and larger eukaryotes, including mammals. The most well–known and commercially significant yeasts are the related species and strains of *Saccharomyces cerevisiae*. These organisms have long been utilized to ferment the carbohydrates of rice, wheat, barley and corn to produce alcoholic beverages and in the baking industry to expand, or raise dough. *Saccharomyces cerevisiae* is commonly used as baker's yeast and for some other types of fermentation.

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Yeast is often taken as a vitamin supplement because it is 50% protein and is a rich source of B vitamins such as niacin, folic acid, riboflavin and biotin. In brewing, *Saccharomyces carlsbergensis*, named after the Carlsbergensis Brewery in Copenhagen, where it was first isolated by Dr. Emil Christian in 1883, is used in the production of several types of beers including lager beer. *Saccharomyces carlsbergensis* is used for bottom fermentation, while *Saccharomyces cerevisiae* (baker's yeast) is used for the production of ales and conducts top fermentation, in which the yeast cells rise to the surface of the brewing vessel. Yeast in the baking industry is used to ferment the sugars present in the flour or is added to the dough. This fermentation gives of carbon dioxide and ethanol. The carbon dioxide is trapped within tiny bubbles and results in the dough expanding. The fermentation of wine is initiated by naturally occurring yeasts present in the vineyards. The bubbles in sparkling wine are trapped carbon dioxide. One yeast cell can ferment approximately its own weight of glucose per hour. Under optimal conditions *Saccharomyces cerevisiae* can produce up to 18% by volume of ethanol. Only the mono, di- and trisaccharides are fermentable. The rate of fermentation is not the same for all sugars. There is a sequential utilization of the fermentable sugars. There is the immediate fermentation of sucrose followed by fructose, then glucose, followed by maltose and finally maltotriose. The rate of fermentation depends on the physiological state of the yeast and also certain constituents of the growth medium.

Characteristics

The capacity of yeasts to ferment sugar in different media into ethanol, carbon dioxide and biomass as end products is not the only factor which determines the suitability of yeast for industrial use. The critical factor could be the capacity of the yeasts to produce quantitative proportions of secondary metabolites such as acetic acid, esters, higher alcohols and undesirable ketones during fermentations. The production of these metabolites is linked essentially to the assimilation of amino acids in the growth medium which do not necessarily meet the needs for protein synthesis of the yeast. The yeast is thus in a medium rich in monomers that make up the cell constituents and are in a constant phase of anabolism. At the beginning of fermentation, when the fermentation intensity is high, the presence of these anabolic pathways results in the accumulation of acetyl- CoA which is converted into esters. This would mean that secondary metabolism depends fundamentally on the relationship between fermentation intensity and the metabolism of nitrogen, which means that the technological conditions of fermentation have to take cognizance of the aptitudes of the different cells. Another important factor in the choice of yeast for industrial use is its ability to flocculate. This process which is quite pronounced is the spontaneous ability of the yeasts to form an aggregation of cells during the growth cycle of the cells. This process of aggregation of cells termed flocculation is widespread among living cells and it conditions several aspects of the growth cycle of single and multi-cellular organisms. Within the same yeast specie, it is possible to distinguish between cells that can flocculate and those that cannot flocculate. As early as 1955, Helm and Thorne were able to differentiate four principal classes of yeasts.

1) Yeasts that are completely dispersed during each stage of fermentation. These yeasts are non flocculent or are described as being powdery.
2) Yeasts that don't flocculate initially, but form light aggregates towards the end of fermentation
3) Yeasts that initially disperse but flocculate towards the end of fermentation.
4) Yeasts that flocculate right from the beginning of fermentation.

The ability of yeasts to flocculate is a genetic characteristics and its phenotypic manifestation depends on several genes (Lewis *et al*., 1976). Several factors affect flocculation. These include the carbon source, the nature and quantity of nitrogen sources and also the presence of some mineral elements. The nutritive elements can be divided into two categories: inhibitors or activators. The same nutritive component can produce opposing effects depending on its concentration, the yeast or the presence of other products in the growth medium. The multiplicity of these factors and the complexity of their interactions make the process of flocculation difficult to understand, since the same yeast can manifest different aptitudes during a complete fermentation cycle.

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The yeast can change its degree of fermentation, becoming more and more flocculent or inversely losing this property. The physiological state of the yeast is another determining factor. Helm and Thorne (1955) observed that for the same yeast cell, rate of fermentation is linked to the concentration of nitrogen in the yeasts. These authors also observed that no relationship exists between the rate of fermentation and the process of flocculation. At equal temperatures, the yeast used for bottom fermentation (*S. carlsbergensis*) has higher fermentation intensity than yeasts used for top fermentation (*S. cerevisiae*). It is also known that initiation of fermentation is faster with yeasts that are flocculent. Wh the yeasts start fermenting poorly, it is said that the yeast is degenerating. Identification of the causes of degeneration is sometimes difficult. Degeneration can be caused by poor selection of the yeast cells, genetic modification of the cells a deficiency in one of the enzymes involved in fermentation, it may be caused by the limitation of some growth factor which is essential for yeast growth or it may simply be difficulty in adaptation to certain undetected change in the growth medium. Degeneration results in slower, incomplete fermentations, a biomass that is not compact enough or a change in the size and shape of the cells. Generally a frequent cause of degeneration is the loss of respiratory power, which might be the absence of cytochrome- oxidase and succinate- dehydrogenase which are enzymes involved in the respiratory phase of fermentation. Viability of the yeasts is another determining factor to consider for the industrial use of yeasts. Viability of the yeasts reduces when the concentration of ethanol or the temperature increases in the growth medium.

Metabolism: Metabolism refers to the sum total of all enzyme controlled oxidation- reduction reactions taking place within the living cells. Metabolism is a highly coordinated process in which many sets of inter-related multi-enzyme systems participate, exchanging both matter and energy between the cell and its environment.

Metabolism has four specific functions:

a) To obtain chemical energy from nutrient molecules or from absorbed sunlight
b) To convert exogenous nutrients into the building blocks or precursors of macro-molecular cell components
c) To assemble such building blocks into protein, nucleic acids, lipids and other cellular components
d) To form and degrade bio-molecules required in specialized functions of the cells.

Metabolism can be divided into two major phases, catabolism and anabolism.

Catabolism is the degradative phase of metabolism in which relatively large and complex nutrient molecules (Carbohydrates, lipids and proteins) coming either from the environment of the cell or from its own nutrient storage depots, are degraded to yield smaller simpler molecules such as lactic acid, acetic acid, CO_2, ammonia or urea. Catabolism is accompanied by release of the chemical energy inherent in the structure of the organic nutrient molecules and it is then conserved in the form of the energy transferring molecule, adenosine tri-phosphate (ATP). Anabolism on the other hand is the building up or biosynthetic phase of metabolism. It is the enzymatic biosynthesis of molecular components of cells such as nucleic acids, proteins, polysaccharides and lipids, from their simple building block precursors. Biosynthesis of organic molecules requires an input of chemical energy, which is furnished by the ATP generated during catabolism. Catabolism and anabolism take place simultaneously and concurrently in cells, but they are independently regulated. Because metabolism proceeds in a stepwise manner through many intermediates, the term intermediary metabolism is often used to denote the chemical pathways of metabolism. The intermediates of metabolism are also called metabolites. Each catabolic or anabolic pathway consists of a sequence of consecutive enzyme catalyzed reactions. Sometimes there are as many as 20 steps. The number of steps is usually very many because a specific fixed amount of free energy is required to form a molecule of ATP from ADP and phosphate during catabolism. Also there is a maximum limit to the amount of free energy that can be delivered by a molecule of ATP in a biosynthetic pathway.

Yeast Metabolism: The basic mechanism of metabolism of the different cells is identical, even though the production of metabolites may differ. Metabolism represents an ensemble of very complex enzymatic reactions which start when the substrates penetrate the cells followed by several modifications to their basic structure under the influence of certain factors.

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These enzymatic reactions are also necessary for the development of the cells, budding for the production of other cells and the processes of oxidation- reduction which give rise to alcohol and carbon dioxide from the fermentable sugars present in the medium. It is well established that most yeasts use sugars as their main carbon and energy sources, however there are some yeasts which can utilize non-conventional carbon sources. Yeasts are capable of assimilating simple nitrogenous sources to form amino acids and proteins. On penetration of the cell wall, the substrates (carbon and nitrogen) undergo various transformations (Fig. 1).

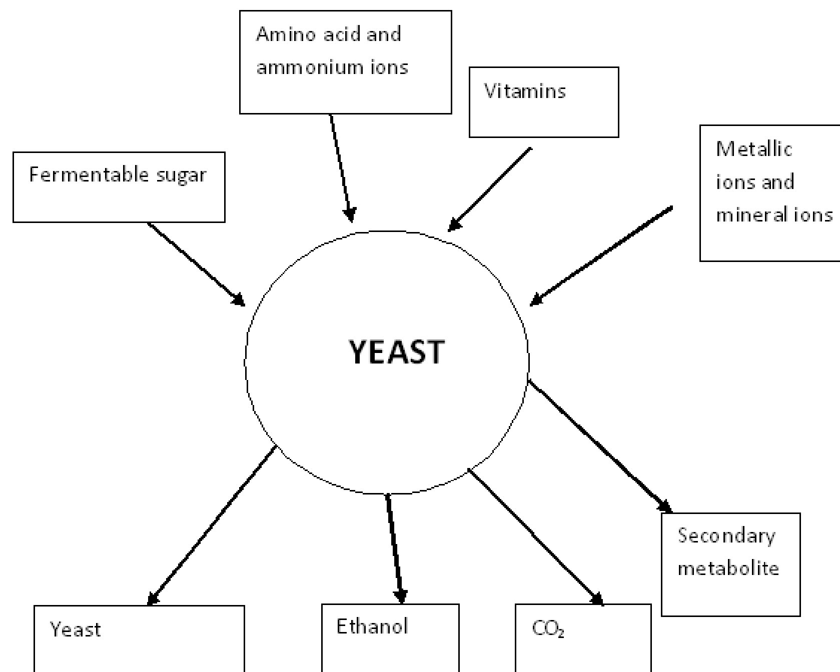

Fig.1 Utilization and transformation of diverse substrates in the growth medium.

Sugar metabolism in yeast The simple sugars, glucose and fructose are the main substrates used directly in the Embden-Meyerhof-Parnas pathway commonly known as glycolysis (fig. 2). Other sugars like maltose or maltotriose have to be initially hydrolyzed by the enzyme α-glucosidase to simpler sugars. During aerobic fermentation, respiration allows for total oxidation of the carbon substrate through the Krebs cycle and oxidative phosphorylation. On the other hand, during anaerobic alcoholic fermentation, degradation of the substrate stops at the end of glycolysis with the production of two molecules of a three carbon compound called pyruvate. This step represents an incomplete oxidation of glucose and provides very little energy. The sugars are essentially oxidized to ethanol and carbon dioxide. They can also be accumulated in the cells as reserve or include in the polysaccharides of the different structures of the cells. However, all the micro-organisms use the Krebs cycle for the synthesis of amino acids and lipids necessary for growth. The major source of energy production in yeasts is glucose and the general pathway for conversion of glucose to pyruvate is glycolysis, whereby production of energy in form of ATP is coupled to the generation of intermediary metabolites and reducing power in the form of NADH for biosynthetic pathways.

There are two principal pathways involved in the metabolism of pyruvate. One involves fermentation with release of very small amounts of energy and the other is respiration with release of large amounts of energy (Fig.2). In the presence of oxygen and absence of repression, pyruvate enters the mitochondrial matrix where it undergoes oxidative decarboxylation to acetyl- CoA by the pyruvate dehydrogenase multi enzyme complex.

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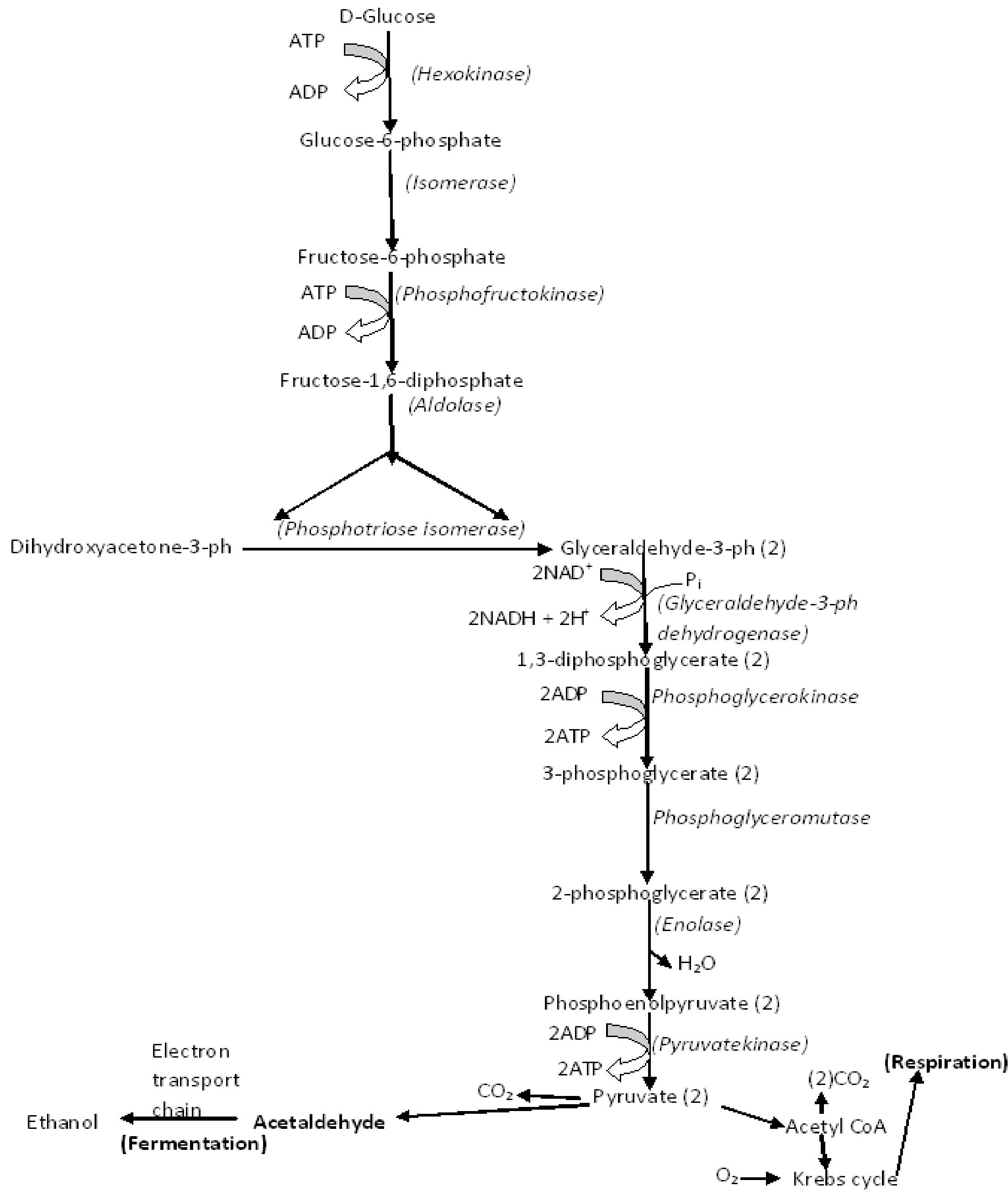

Fig 2 Glucose metabolism

This reaction links glycolysis to the Krebs cycle also known as the citric acid cycle or tricarboxylic acid cycle. Here acetyl CoA is completely oxidized to give two molecules of CO_2 and reducing equivalents in form of NADH + H^+ and $FADH_2$ + H^+. The Krebs cycle (citric acid cycle) is an amphibolic pathway as it combines both catabolic and anabolic functions. Some of the compounds necessary for the operation of the citric acid cycle; oxaloacetate and α-ketoglutarate are replenished by fixation of CO_2 to pyruvate and this reaction is catalyzed by the enzymes pyruvate carboxylase which is ATP dependent and phosphoenolpyruvate caboxykinase.

Replenishment of these compounds can also be carried out by the glyoxalate cycle. This is a shortcut across the citric acid cycle and is important when yeasts are grown on two-carbon sources such as acetate or ethanol. During alcoholic fermentation of sugars, yeasts re-oxidize NADH + H^+ to NAD^+ in a two step reaction from pyruvate, which is first decarboxylated by pyruvate decarboxylase followed by the reduction of acetaldehyde, which is catalyzed by alcohol dehydrogenase, at the same time, glycerol is generated from dihydroxyacetone phosphate to ensure production of this important compound. An alternative mode of glucose oxidation is the hexose phosphate pathway also known as the pentose phosphate cycle, which provides the cell with pentose sugars and cytosolic NADPH, necessary for biosynthetic reactions, such as the production of fatty acids, amino acids and sugar alcohols. The first step in this pathway is the dehydrogenation of glucose-6-phosphate to 6-phosphogluconate with the generation of one molecule of NADPH by the action of the enzyme glucose -6-phosphate dehydrogenase. Subsequently, the 6-phosphogluconate is decarboxylated by the action of phosphogluconate dehydrogenase to give ribulose-5-phosphate and a second molecule of NADPH. Besides generating NADPH, the other major function of this pathway is the production of ribose sugars which serve in the biosynthesis of nucleic acid precursors and nucleotide co-enzymes. The oxidation-reduction carriers, NAD and FAD, which become reduced during the breakdown of sugars to NADH and $FADH_2$, respectively, are re-oxidized in the electron transport chain located in the inner mitochondrial membrane. The energy released during transfer of electrons is coupled to the process of oxidative phosphorylation, which is effected by ATP synthase, an enzyme complex which is also located within the inner mitochondrial membrane and designed to synthesize ATP from ADP and inorganic phosphate.

Regulation of Biochemical Pathways: Biochemical pathways in yeasts are regulated at various levels:

i) Enzyme synthesis; induction, repression and de-repression of gene expression.
ii) Enzyme activity; allosteric activation, inhibition, or inter-conversion of isoenzymes.
iii) Cellular compartmentalization; localization of particular pathways to the cystol, mitochondria, peroxisomes, or the vacuole.
iv) Transport mechanisms; internalization, secretion, movement of compounds between various cellular compartments.

Respiration versus Fermentation: Yeast can be categorized into several groups according to their modes of energy production; either through fermentation or respiration (Table 1).These processes are however regulated by environmental factors which include the availability of oxygen and the carbon source. Yeasts can adapt to a variety of available substrates. It is important also to note that within a particular yeast cell, the prevailing pathways will depend on the actual growth conditions. For example, glucose can be utilized in several different ways by *Saccharomyces cerevisiae*, depending on the presence of oxygen and other carbon sources. Yeasts are chemo-organotrophs as they use organic compounds as a source of energy and do not require sunlight to grow. Carbon is obtained mostly from hexose sugars, such as glucose and fructose or disaccharides such as sucrose and maltose. Some species can metabolize pentose sugars like ribose (Barnett, 1975), alcohols and organic acids. Yeast species require oxygen for aerobic cellular respiration (obligate aerobes) or are anaerobic, but also have aerobic methods of energy production (facultative anaerobes). Unlike bacteria, there are no known yeast species that grow only anaerobically (obligate). Yeasts grow best in a neutral or slightly acidic pH environment. The optimum temperature for growth varies with the yeast specie ranging from 28°F to 113°F. The cells can also survive freeing under certain conditions; however the viability decreases over time.

In general, yeasts are grown in the laboratory on solid growth media or in liquid broths. Common media used for the cultivation of yeasts include potato dextrose agar (PDA) or potato dextrose broth, Wallerstein Laboratories nutrient agar (WLNA), yeast peptone dextrose agar (YPD).

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Table 1 Respiration versus Fermentation

Type of respiration	Example	Respiration	Fermentation	Growth pattern
Obligate respiration	Rhodotorula spp. Cryptococcus spp.	yes	no	Aerobic
Anaerobic respiration	Candida spp. Kluyveromyces spp. Pichia spp.	yes	Anaerobic in pre-grown cells	aerobic
Aerobic fermentation	S. pombe	limited	Aerobic and anaerobic	aerobic
Facultative fermentation	S. cerevisiae	limited	Aerobic and anaerobic	facultative
Obligate fermentation	Torulopsis	no	anaerobic	anaerobic

Catabolite Repression: This is a process that occurs when glucose or an initial product of glucose metabolism represses the synthesis of various respiratory and gluconeogenic enzymes. Catabolite inactivation results in the rapid disappearance of such enzymes on addition of glucose. In Catabolite repression, enzyme activity is lost by dilution with cell growth. Although the enzymes are still present in the medium, they are no longer synthesized due to gene repression by signals derived from glucose or other sugars.

Glucose Repression: In yeasts this process describes a long –term regulatory adaptation of the cells to exclusive breakdown of glucose to ethanol and CO_2. When *S. cerevisiae* is grown in an aerobic medium with high concentrations of glucose, fermentation will account for the bulk of glucose consumed. In batch cultures, when the levels of glucose decline, the cells gradually become de-repressed, resulting in the induction of the synthesis of respiratory enzymes. This then results in the oxidative consumption of ethanol, as the cells enter a second phase of growth referred to as the **diauxic shift**.

Catabolite Inactivation: This process is more rapid than repression and is thought to be due to deactivation of a limited number of key enzymes by glucose; this includes fructose 1,6-biphosphate. The process of inactivation is carried out primarily by enzyme phosphorylation, followed by slow vacuolar degradation of the enzyme. It has been established that cAMP plays a central role in regulating Catabolite repression and inactivation in *Saccharomyces cerevisiae*.

The Pasteur Effect and the Crabtree Effect: The Pasteur Effect and the Crabtree Effect are two mechanisms that control the pathways of energy catabolism and have opposing effects that tend to keep the energy charge of the cells relatively constant. This energy charge can be defined by the following equation:

$$\frac{ATP + \frac{1}{2} ADP}{ATP + ADP + AMP}$$

The Pasteur Effect is defined as the inhibition of fermentation by respiration, while the Crabtree effect is the repression of respiration by fermentation. The Pasteur Effect involves the integration of a series of feedback mechanisms working in sequence; NAD specific isocitrate dehydrogenase is inhibited by ATP and activated by AMP. High energy charges activate the inhibition of this enzyme with the accumulation of citrate. Citrate and ATP inhibit phosphofructokinase which causes the accumulation of hexosephosphate which in turn inhibits the utilization of high sugar concentrations (membrane transport, phosphorylation). Very high energy charges thus result in the considerable slowing down of the process of glycolysis. However it has been shown that this regulation, in which phosphofructokinase plays an important role, depends on the ammonium concentration of the yeasts, the ammonium reducing the inhibitory effects of citrate and ATP and the activating effects of AMP on this enzyme.

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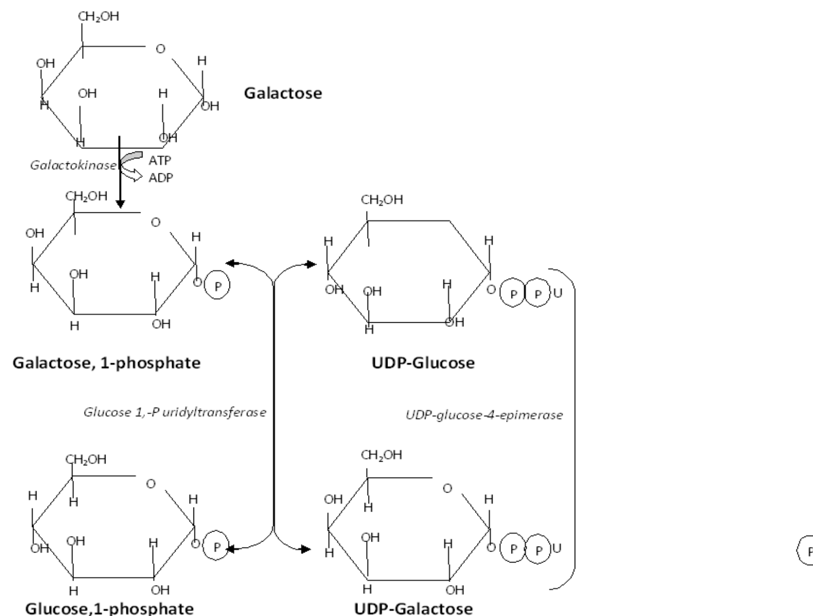
This means that glycolysis is a poorly regulated pathway in the presence of nitrogen sources. On the other hand, in the absence of nitrogen, this regulation becomes functional, with respiration strongly inhibiting fermentation. In the presence of nitrogen sources and high concentrations of sugar, yeast growth can be sufficiently rapid. Under these conditions, it has been shown that the synthesis of respiratory enzymes does not take place, even in the presence of oxygen which is an inducer of the synthesis of respiratory enzymes. Sugar catabolism during high growth rates represses the synthesis of respiratory enzymes. This regulation is referred to as the Crabtree effect (Crabtree, 1929; De Decken, 1966). This effect can be explained as a regulation of the energy charge of the cell in which glycolysis is a pathway poorly regulated in the presence of phosphofructokinase and assures a rapid synthesis of ATP.

Other sugars and non conventional carbon sources: Galactose is a non conventional substrate for yeast growth, which can however be used as the sole carbon source when glucose is absent from the growth medium. In yeast cells that are supplied with glucose, the *GAL* genes are repressed. These genes are however activated in the absence of glucose. The three enzymes involved are shown in figure 3. Yeasts also use other non conventional carbon sources such as biopolymers, pentoses, alcohols, polyols, hydrocarbons, fatty acids and organic acids. Other disaccharides found in nature can also be used as non conventional carbon sources. Examples include: maltose, sucrose, melibiose and lactose. The corresponding hydrolases are used to breakdown these disaccharides into their corresponding monosaccharide.

Pentose sugars can be fermented to ethanol by only a few yeasts species, although many types of yeast can grow aerobically on pentoses. Many types of yeast have the capability of metabolizing ethanol or methanol, an approach used in the production of yeasts of biotechnological interest. Examples of methanol utilizing (methylotropic) yeasts include *Hansenula polymorpha*, *Pichia pastoris*, several *Candida* species and *Torulopsis sonorensis*. These organisms first metabolize methanol by an oxygen dependent oxidase converting it to formaldehyde, which is then converted to dihydroxy acetone (DAH) by a DAH synthase. **Glycerol** functions as a compatible solute in osmoregulation in osmotolerant yeasts that are capable of growing in high sugar or salt environments. Many types of yeast can grow on glycerol as a sole carbon source under aerobic conditions; however glycerol is a non-fermentable carbon source for many yeast species, including *Saccharomyces cerevisiae*. Glycerol has to first of all undergo isomerization then conversion by glycerol kinase to glycerol, 3-phosphate, which is then transformed into dihydroxy acetone phosphate by glycerol,3-phosphate dehydrogenase.



. **Fig 3 Metabolism of Galactose**

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Gluconeogenesis and Carbohydrate Biosynthesis: The growth of yeasts on non-carbohydrate sources as sole carbon sources necessitates the synthesis of sugars required for macromolecular biosynthesis, especially that of complex polysaccharides. Like in other organisms, Gluconeogenesis, the conversion of pyruvate to glucose is energy demanding. Here ATP acts as the energy provider while $NADH^+ + H^+$ provides reducing power. Structural polysaccharides in yeasts include mannans, glucans and chitin. Synthesis of these components involves polymerization reactions and use sugar nucleotides as substrates which are formed via activation by uridine-triphosphate (UTP) or guanidine-triphosphate (GTP) depending on the substrate. A major activity in yeasts is the synthesis of storage carbohydrates such as glycogen and trehalose. Glycogen is formed by sequential addition of glucose units, employing glycogen synthase for the α-1,4-linkage of the skeletal chain and branching enzymes for the formation of α-1,6-branches. Degradation of glycogen to glucose 1-phosphate is carried out by the enzyme glycogen phosphorylase while cAMP is known to be involved in the regulation of glycogen metabolism.

An unconventional storage disaccharide found in yeasts is trehalose (α,α-1,1-diglucose. This compound is present in quite high concentrations in resting and stressed cells. Trehalose-phosphate is synthesized in yeasts from glucose-6-phosphate while UDP-glucose is synthesized by trehalose6-phosphate synthase and is converted to trehalose by a phosphatase.

Fatty Acid and Lipid Metabolism: Fatty acids available to yeasts for catabolism include those derived from microsomal alkane oxidation or extracellular lipolysis of fats or those exogenously supplied in the growth medium. The fatty acids are catabolized by β-oxidation in the peroxisomes; this differs from the system in the mitochondria in the use of catalase for the re-oxidation of $FADH_2$ and NADH. The series of reactions leading to the synthesis of long-chain fatty acids, starting from acetyl-CoA is achieved by a multi-enzyme complex called the fatty acid synthase. The subsequent formation of unsaturated fatty acids, which are needed for membrane integrity, involves an oxidative desaturation by fatty acid desaturase.

Nitrogen Metabolism: Yeasts are capable of utilizing a range of different inorganic and organic sources of nitrogen for incorporation into the structural and functional nitrogenous components of the cell, such as amino acids, peptides, proteins, polyamines, nucleic acids and vitamins. Yeasts can live on ammonium ions as the sole source of nitrogen, since they posses genes encoding enzymes for the biosynthesis of all amino acids. The ammonium ions that are either supplied as nutrient or are derived from the catabolism of other nitrogenous compounds can be directly assimilated into a couple of amino acids, notably glutamate and glutamine, which can then serve as donors of the amino group of the other amino acids. The major route for the assimilation of ammonium ions is through the reaction of the NADPH-dependent glutamate dehydrogenase, which forms glutamate from α-ketoglutarate and ammonium. Urea is widely used by yeasts as a nitrogen source. In Saccharomyces cerevisiae, urea aminohydrolase, which is ATP dependent, hydrolyses urea to ammonium and carbonate.

The role of oxygen: Oxygen has a profound influence on the activities of yeasts, especially on its growth pattern. Insufficient oxygen causes slow growth rate, low biomass production, reduced viability and slow fermentations. Some yeast enzymes do not function except in the presence of oxygen which cannot be replaced by other hydrogen acceptors. This applies to those oxygenases involved in the synthesis of unsaturated fatty acids and sterols which are important constituents of the cellular membranes. In the absence of these lipids, the cells cannot reproduce and their viability reduces. Sterols and unsaturated fatty acids are considered as important growth factors during anaerobic growth. The quantity of oxygen available is determined by its solubility in the growth medium and the period of aeration. In the absence of respiration, oxygen is used essentially for the synthesis of sterols and unsaturated fatty acids.

The quantity of oxygen required by the yeasts is determined by several factors. Studies by Kirsop (1974) revealed that these factors are linked to the variations of the nutritive value of the growth medium (presence of unsaturated fatty acids, sterols, assimilable nitrogen and the concentration of adjutants).The method of propagation, the rate of seeding and the quality of yeast used also have a role to play in the requirement for oxygen.

Propagation techniques: The process of propagation from stocks maintained at 4^oC on inclined agar medium to large quantities in the industry is described as a progressive fermentation of increasing volumes of the growth medium, such that at the last stage there is sufficient quantity of yeast that can be used for the inoculation of the large industrial fermentation tanks. Propagation starts with the stock that is maintained on the inclined agar medium at 4^oC which is then transferred to 10ml of the sterilized growth medium and allowed to grow for 48 hours at 25^oC. The cells are then transferred a larger vessel, and at each transfer the volume of the growth medium is increased by a factor of 10 with the temperature being reduced gradually until the optimum fermentation temperature is attained, depending on the end product desired. The aim of this process is to maintain the cells at the exponential phase of growth where the concentration of yeasts is high. In order to satisfy the demand for yeast used for the fermentation of large volumes of the growth media, it is necessary to repeat the latter stages of propagation. This is done by conserving a part of the preculture, and adding fresh growth medium. However this method requires an absolute sterile environment. In industry, this phase is carried out in propagation chambers composed of three reactors of increasing volumes, cleanly maintained and separated from the rest of the factory. The vessels are composed of stainless steel and are equipped with chambers which allow for temperature control

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The useful physiological properties of yeasts have led to their use in the field of biotechnology. Fermentation of sugars by yeasts is the oldest and largest application of this technology. Many types of yeasts are used for making many foods: baker's yeast in bread production; brewer's yeast in beer fermentation; yeast in wine fermentation (Rao,*et al*., 2004)

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