# EFFECTS OF TRACE ELEMENTS ON THE PRODUCTION OF BAKER'S YEAST (*Saccharomyces cerevisiae*)

**By**

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# ABSTRACT

Baker's yeast has been used widely in traditional bakeries to produce different kinds of baked products and for many biotechnological purposes. The principal raw materials used in producing baker's yeast are the pure yeast culture and molasses. Cane molasses and beet molasses contain 45 to 55 % sugar in the forms of sucrose, glucose, and fructose. Molasses, which is a rich carbon and mineral source, contains most of the nutrients and minerals required for baker's yeast production. Nutrients and trace elements (magnesium, copper and zinc) that are not present in sufficient quantities in molasesses are added to the fermentation medium.

In this study, the effects of $Ca^{2+}$, $Mg^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ on baker's yeast production were investigated. The method of Food Composition Laboratory (FCL), Human Nutrition Research Center, Beltsville, US, was used for the preparation of the fermentation samples prior to ICP-AES analysis. The concentrations of $Ca^{2+}$, $Mg^{2+}$, $Cu^{2+}$, $Zn^{2+}$ were determined in the raw materials entering the fermentors and the products. FCL digestion method was evaluated on precision and accuracy; linear standard curves were obtained for the studied concentration ranges of $Ca^{2+}$, $Mg^{2+}$, $Cu^{2+}$ and $Zn^{2+}$. Standard addition curves for each element in 45 brix molasses and dry yeast samples were constructed and spike recovery percentages were calculated. High spike recovery percentages were obtained for all elements in both dry yeast and molasses with the FCL method.

In industrial fermentations, uptakes of $Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ by the yeast cells were calculated as mg/kg dry yeast at 4h time intervals. The uptake rates were evaluated based on the available element concentration in the medium and the uptake of the elements. Results showed that, as the uptake of $Ca^{2+}$ increased with time, uptake of $Mg^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ decreased. The yeast cells used the available elements with decreasing uptake rates except $Ca^{2+}$. During the first hours of fermentation, uptake rates were high. The maximum uptake rates observed for each element in two different industrial fermentations were: 17.73-23.32 mg $Cu^{2+}$/ kg dry yeast cells , 195.64-413.46 mg $Zn^{2+}$ / kg dry yeast cells, 2106.46-2051.74 mg $Mg^{2+}$ / kg dry yeast cells.

Ion concentrations of biomass in industrial fermentations revealed that same amounts of Mg were taken by the cell from the fermentation medium under similar

conditions. This was verified by the studies done in lab-scale using pure culture of *S.cerevisiae*, grown on different concentrations of $Mg^{2+}$. It was found that uptake of $Mg^{2+}$ was constant and very close to the uptake values of industrial fermentations. 806.7 μM $Mg^{2+}$ was taken by 1 kg of dry yeast cells. This is in agreement with the stated $Mg^{2+}$ uptake concentration range 500-1000 μM of *Saccharomyces cerevisiae* in literature.

In the industry, magnesium is added in the form of $Mg.SO_4.7H_2O$ to the fermentation medium. $SO_4^{2-}$ ions coming from the $MgSO_4.7H_2O$ cause settling problems in the waste treatment system of the plant. Generally, molasses contain sufficient concentration of $Mg^{2+}$ ions required for baker's yeast production in the industrial scale. Since $Mg^{2+}$ has toxic effects at high concentrations and its addition is increasing the cost of the process and causing difficulties in the waste treatment of the plant, it may be concluded that addition of $Mg^{2+}$ as $MgSO_4.7H_2O$ to the fermentation medium should be done after determining the $Mg^{2+}$ concentration of molasses. Instead of using excessive amounts of $MgSO_4.7H_2O$, only the required quantity should be added to the fermentation medium.

Important Note!
In this thesis, biomass and yeast cake have been used to give the same meaning.

# ÖZ

Ekmek mayası geleneksel olarak çeşitli unlu mamullerin üretilmesinde ve biyoteknolojik amaçlar için kullanılmaktadır. Ekmek mayası üretiminde kullanılan en temel hammaddeler saf maya ve melastır. Yaygın olarak şeker pancarı ve kamışı melası olarak bulunan melasın kütlece %45-55'ini sukroz, glukoz ve fruktoz şekerleri oluşturur. Maya için gerekli olan besin maddelerinin çoğu, zengin bir besin ve mineral kaynağı olan melastan sağlanmaktadır. Melasın içinde bulunmayan ya da yeterli gelmeyen besin maddeleri ve eser elementler (magnezyum, bakır ve çinko) fermantasyon ortamına dışarıdan eklenmektedir.

Bu çalışmada $Ca^{2+}$, $Mg^{2+}$, $Cu^{2+}$, $Zn^{2+}$ iyonlarının ekmek mayası üretimi üzerine etkileri araştırılmıştır. Fermantasyon örneklerinin ICP-AES analizi öncesi hazırlanmasında, Food Control Laboratory (FCL), Human Nutrition Research Center, Beltsville, US, tarafından oluşturulan bir metot kullanılmıştır. Fermentörlere giren hammaddelerde ve ürünlerde $Ca^{2+}$, $Mg^{2+}$, $Cu^{2+}$, $Zn^{2+}$ konsantrasyonları tayin edilmiştir. FCL bozundurma metodunun doğruluk ve kesinliği değerlendirilmiştir; $Ca^{2+}$, $Mg^{2+}$, $Cu^{2+}$, $Zn^{2+}$ elementlerinin çalışılan konsantrasyonları için lineer standart eğriler oluşturulmuştur. Her bir element için 45 Brix melas ve kuru mayada standart ekleme eğrileri çizilmiş ve yüzde kazanım değerleri hesaplanmıştır. FCL metoduyla, kuru maya ve melasta bütün elementler için yüksek kazanım yüzdeleri elde edilmiştir.

Endüstriyel fermantasyonlarda, elementlerin maya bünyesine alınma miktarları hesaplanmıştır ve alınma hızları, ortamdaki element konsantrasyonlarına ve alınan element miktarlarına göre ifade edilmiştir. Sonuçlar, zamanla $Ca^{2+}$ un alımının arttığını, $Mg^{2+}$, $Cu^{2+}$ ve $Zn^{2+}$ alımlarının ise azaldığını göstermiştir. Maya ortamdaki $Ca^{2+}$ hariç diğer üç iyonu azalan hızlarla kullanmaktadır. Fermantasyonun ilk saatlerinde alım hızları yüksektir. Bunun sebebinin protein sentezinin, büyümenin ilk 6 saatinde olması kaynaklı olduğu sanılmaktadır. Her iki endüstriyel denemede maksimum alınmalar şu aralıklardadır; 17.73-23.32 mg $Cu^{2+}$/ kg kuru maya keki, 195.64-413.46 mg $Zn^{2+}$ / kg kuru maya keki, 2106.46-2051.74 mg $Mg^{2+}$ / kg kuru maya keki.

Endüstriyel fermantasyonlarda, biyokütlenin iyon konsantrasyonları, benzer koşullarda aynı miktarlardaki $Mg^{2+}$un hücre içine alındığını göstermiştir. Bu durum *S.cerevisiae*'nın saf kültürü kullanılarak yapılan laboratuvar fermentasyonlarında farklı $Mg^{2+}$ konsantrasyonları uygulanarak doğrulanmıştır. $Mg^{2+}$ alım miktarının sabit ve

endüstriyel fermantasyonlardaki miktarlar ile çok yakın değerlerde olduğu bulunmuştur. 806.7 μM $Mg^{2+}$, 1kg kuru maya keki tarafından alınmıştır. Bu, literatürde *S.cerevisiae* için 500-1000 μM aralığında belirtilen $Mg^{2+}$ konsantrasyon aralığındadır.

Endüstride $Mg^{2+}$, fermantasyon ortamına $MgSO_4.7H_2O$ formunda eklenmektedir. $MgSO_4.7H_2O$ dan gelen $SO_4^{-2}$ iyonları, fabrikanın arıtma sisteminde çökelti problemlerine sebep olmaktadır. Genellikle, melas, endüstriyel üretimlerde ekmek mayası üretimi için yeterli miktarda $Mg^{2+}$ içermektedir. $Mg^{+2}$ un yüksek konsantrasyonlardaki toksik etkileri ve dışarıdan eklemenin üretim maliyetlerini arttıracağı ve arıtmada problemlere yol açacağı düşünüldüğünde, $Mg^{2+}$ un fermantasyon ortamına $MgSO_4.7H_2O$ olarak eklenmesinin, melasın $Mg^{+2}$ konsantrasyonu belirlendikten sonra yapılması sonucu doğmaktadır. Fazla $MgSO_4.7H_2O$ kullanımı yerine, sadece gerekli miktarlar fermantasyon ortamına eklenmelidir.

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# CHAPTER I

# INTRODUCTION

Baker's yeast is a mass of viable cells of *Saccharomyces cerevisiae*, which is a unicellular fungus that multiplies asexually through budding. *S.cerevisiae* is a facultative anaerobe that can survive under both aerobic and anaerobic conditions.

When *S.cerevisiae* cells are supplied with glucose as the carbon source under anaerobic conditions, ethanol is produced as a result of fermentation. When *S.cerevisiae* cells are cultivated with molasses under aerobic conditions, commercial quantities of the yeast itself, known as the baker's yeast, is produced as a result of aerobic respiration.

Baker's yeast is used widely in traditional bakeries to produce different kinds of baked goods. Dough is inoculated with baker's yeast and incubated at the required temperature and time. The $CO_2$ produced during the incubation period of the baking process results in raised dough with specific taste and smell.

Historically, the first written record of the actual existence of bread dates to around 266 BC in Babylonia. The discovery of leavened bread was generally attributed to ancient Egyptians. Mixing of fermenting beer with the wheat flour was the development of sour-dough process. In the year of 1780, pressed distiller's yeast was produced by using 70% rye and 30 % kiln malt, fermented by pitching yeast. During 1840s, in Austria, corn was used and yield was increased. First scientific fermentation studies began with Pasteur at 1860. After 1870s, technical progress was achieved, an experimental laboratory was founded in Berlin. The improvements in baker's yeast technology continued. Finding aeration was effective for yeast fermentation and first dry yeast was produced in 1890. Molasses was introduced to the process as a new raw material in Germany in 1915. Modifications in the production procedure have continued until today. Air distribution systems were developed and replaced by filter presses. Further enrichment of the fermentation medium, resulted in yield increases (1).

Today, the major raw material used in producing baker's yeast is molasses. Molasses is rich in carbon and some important ions for yeast metabolism. Baker's yeast industry in Turkey is using molasses as the carbon source. Molasses contains 45 to 55

weight percent fermentable sugars, in the forms of sucrose, glucose, and fructose. Most of the nutrients and minerals required for the industrial production of baker's yeast are present in molasses. The nutrient and mineral requirements include nitrogen, potassium, phosphate, magnesium and calcium, with traces of iron, zinc, copper, manganese and molybdenum.

A complete understanding of the nutrition of the yeast is required to optimize its growth rate and metabolic activities. An essential but often neglected part of yeast cells is the ionic constituents of the medium. The uptake of metal ions into cells depend on their concentration in the growth medium and their bioavailability. Since these metal ions can affect the growth rate and thus the productivity of industrial baker's yeast productions, it is important to be able to determine their concentrations in all the raw materials and the fermentation medium (2).

Although baker's yeast production is one of the oldest productions in biotechnology, there are not many reports on the effect of trace elements on baker's yeast production. This study focuses on the effects of some cations like $Ca^{2+}$, $Mg^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ on industrial baker's yeast production. Hence, two industrial scale and four lab-scale fermentations were followed at Pakmaya Inc., commercial baker's yeast producer in İzmir, Turkey. The concentrations of the stated cations were analysed in raw materials such as molasses and water, products such as dry yeast and compressed yeast and samples of fermentation media.

The concentrations of $Ca^{2+}$, $Mg^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ in the samples stated above were determined by ICP-AES (Inductively Coupled Plasma). The method of analysis used was first verified on precision and accuracy within the sample matrices before concentration determinations of the cations.

# CHAPTER II

# BAKER'S YEAST TECHNOLOGY

In industrialized countries with European bread-eating habits, the average consumption of compressed yeast per year is 1.2 to 2 kg. These markets are largely saturated, and no major growth rates in the consumption of baker's yeast are registered (1).

In other countries, the consumption of bread, especially white bread, increases at the same rate as industrialization and the development of modern mass society. Accordingly, there is increased demand for baker's yeast, which is covered partly by imports (especially dry baker's yeast) partly by new yeast production plants.

Until several decades ago, countries with an established yeast industry, notably in Europe, had quite a large number of small and medium-sized plants. Since then, there has been a distinct trend towards concentration of business in small number of large-scale operations. In Table 2.1, the annual production of the regions in the world can be seen;

Table 2.1. 1982 production figures for baker's yeast (as compressed yeast with a D.M. of 29 to 30%)

| | **tons/year** |
|---|---|
| Western Europe(including Turkey) | 443,000 |
| Eastern Europe | 394,000 |
| North America and Central America | 330,000 |
| South America | 75,000 |
| Africa | 47,000 |
| Asia | 132,000 |
| Australia | 10,000 |

In Table 2.2, main yeast producers of the world were shown. Among these, Lasaffre in France and Pakmaya in Turkey are at top of the list.

Table 2.2. Main baker's yeast producers in the world

| Countries | Number of most important companies |
|---|---|
| Germany | 9 DHW / UNIFERM |
| U.S.A. | 5 Anheuser Busch |
| | Nabisco Brands(Fleischmen) |
| | Universal Foods (Red Star) |
| England | 3 Distiller's Co.,Ltd.(DCL) |
| France | 2 Lasaffre |
| Belgium | 2 Brüggeman |
| Netherlands | 1 Koninklijke Nederlandsche Gist-en Spiritus-Fabriek N.V., Delfth |
| Austria | 4 Mautner-Markhof |
| Sweden | 1 Swenska Jastfabriks AB |
| Italy | 4 Eridania |
| Japan | 5 Oriental Yeast |
| Turkey | Pakmaya |
| | Saf Maya |
| | Mauri Maya |

## 2.1. Baker's yeast

Historically, beer yeast or brewer's yeast (*Saccharomyces uvarum, Saccharomyces carlsbergensis*) was used for baking purposes, but it was subsequently replaced by distiller's yeast and baker's yeast (*S. cerevisiae*). Attempts have been made periodically to evaluate the baking properties of other yeast strains and cultures. For example, Mitchell (1957) evaluated the baking properties of some 75 cultures including *Candida arborae, C. pseudotropicalis, C. tropicalis, Hansenula subpelliculosa, Saccharomyces chevalieri, Saccharomyces chodati, S.diastaticus, S. ellipsoideus thermophilus, S.fragilis, S.italicus, S. intermedius, S. logos, S.marxianus, S.osmophilus, S. oviformis, Schizosaccharomyces pombe, Torula colliculosa, T. dattila,*

*Zygosaccharomyces lactis, Z. drosophilae*,etc. Except possibly for the last one, none of these species and cultures has been found to be superior to the *S.cerevisiae* (2).

There are at least 1000 separate strains of *Saccharomyces cerevisiae*. These strains encompass brewing, baking, wine, distilling, and laboratory cultures.

Taxonomically, the two species *S. uvarum (carlsbergensis)* and *S.cerevisiae* have been distinguished on the basis of their ability to ferment the disaccharide melibiose. Strains of *S.uvarum (carlsbergensis)* possess the MEL genes. They produce the extracellular enzyme α-galactosidase (melibiase) and are able to utilize melibiose, whereas strains of *S.cerevisiae* do not produce α-galactosidase and therefore unable to utilize melibiose. Recently, yeast taxonomists (Barnett, Payne, and Yarrow, 1983; Kreger-van Rij, 1984) consolidated *S.uvarum (carlsbergensis)* and *S.cerevisiae* into one species, *S.cerevisiae* (3).

There are hundreds of different species of yeast identified in nature, but the genus and species most commonly used for baking is *Saccharomyces cerevisiae*. The scientific name *Saccharomyces cerevisiae*, means a mold which ferments the sugar in cereal (*saccharo-mucus cerevisiae*) to produce alcohol and carbon dioxide (3,1).

### 2.1.1. Yeast Cell

Species: *Saccharomyces cerevisiae*

Genus: *Saccharomyces*

Subfamily: *Saccharomycetoideae*

Family: *Saccharomycetaceae*

Cell dimensions and specific values about *Saccharomyces cerevisiae* were shown in Table 2.3 (1);

Table 2.3. Cell dimensions of *Saccharomyces cerevisiae*

| | **Limits** | **Average Values** |
|---|---|---|
| **Size of cell** | 2 – 8 * 4- 12 μm | 5 * 8 μm |
| **Volume** | $0.8 - 2 * 10^{-10}$ $cm^3$ | $10^{-10}$ $cm^3$ |
| **Dry weight of cell** | $0.2 - 0.4 * 10^{-10}$ g | $0.3 * 10^{-10}$ g |
| **Number of cells per g** | $8 - 14 * 10^9$ | $10^{10}$ |
| **Surface per g** | | 2.4 $m^2$ |

Cell consists of a large variety of different biopolymers and macromolecules. Knowledge of their composition and quantity is essential for a metabolic and energetic analysis of the biomass growth. The biomass of *Saccharomyces cerevisiae* is commonly described as consisting of five groups of macromolecules; proteins, carbohydrates, lipids, RNA, and DNA. Together with water and metals these components give the molecular composition of the biomass (4).

In Figure 2.1, a typical yeast cell and the organelles are illustrated;

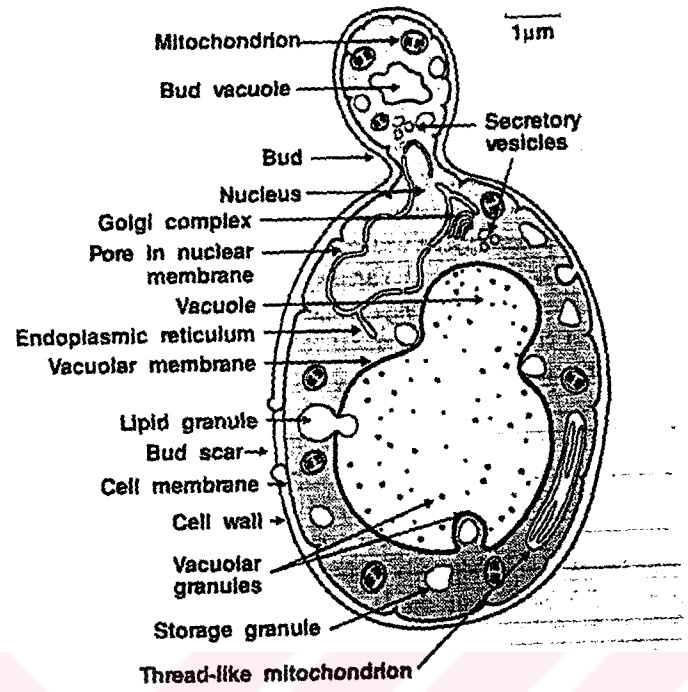



Figure 2.1. Main features of a typical yeast cell

### 2.1.1.1. Cell Wall

The cell wall of *Saccharomyces cerevisiae* is structured double layer which is 150-300 nm thick. Average chemical content:

30% glucan
40% mannan
1% chitin
15% protein
8% lipids
6% minerals etc.

The yeast cell wall is a multifunctional organelle of protection, shape, cell interaction, reception, attachment, and specialized enzymatic activity. Its cell wall constitutes 15-25 % of the dry weight of the cell and consists primarily of equal amounts of phosphomannan (31%) and glucans (29%). There are three glucans present in the wall. The major component is an alkali–insoluble, acid insoluble β(1-3)-linked

polymer which helps the wall maintain its rigidity. There is also an alkali-soluble branched glucan with predominantly β(1-3)-linkages but some β(1-6) linkages as well. Finally the cell wall also contains a small portion of predominantly β(1-6)-linked glucan. Chitin is almost always restricted to the bud scar and does not account for more than 4% of the cell material. Lipid is present at about 8.5 % and protein at about 13%. The carbohydrate portion of the mannoprotein on the yeast cell surface determines the immunochemical properties of the cell (3,5).

#### 2.1.1.2. Plasma Membrane

The plasma membrane acts as a barrier to separate the aqueous interior of the cell from its aqueous exterior. It consists of lipids and proteins, in more or less equal amounts together with a small amount of carbohydrate and is 30 nm thick. The carbohydrate portion of the membrane-bound glycoproteins is believed to extend only from the external surface of the membrane. The plasma membrane has a role in regulating the uptake of nutrients and in the excretion of metabolites (3,5).

#### 2.1.1.3. Nucleus

The cell nucleus is roughly spherical, about 2 μm in diameter. In resting cells it is usually situated next to a prominent vacuole (3,5).

#### 2.1.1.4. Mitochondria

Mitochondria of yeast cells are round or elongated structures composed of two distinct membranes, the outer and the inner. In aerobically grown yeast cells, there is an average of 30-50 mitochondria / cell. It is 0,05-3 μm in size. The cristae within the mitochondria are formed by the folding of the inner membrane (3).

### 2.1.1.5. Other Cytoplasmic Structures

Vacuoles are a part of an intramembranous system which includes the endoplasmic reticulum. The form and size of the vacuoles change during the cell cycle. Mature cells contain large vacuoles, which fragment into small vesicles when bud formation is initiated.

Ribosomes are small, optically invisible corpuscles with a diameter of approximately 20 nm whose the main function is protein synthesis. The most important organelle of yeast is nucleus and 1.2-2 μm in size which is the seat of the cell's genetic replication apparatus (3,5).

### 2.1.2.Yeast Metabolism

Within the cell, maltose and maltotriose are enzymatically hydrolysed to glucose. The simplest expression for fermentation is;

Glucose ⟶ 2 Carbon dioxide + 2 Ethanol + Energy
mol wt 180 2*44 2*46

This equation named after the French scientist Gay-Lussac, shows that glucose yields almost equal weights of carbon dioxide and alcohol plus energy for the cell's activities (6).

Under aerobic conditions, expression of the baker's yeast production;

Sucrose + Ammonia + Oxygen ⟶ Yeast + Water + Carbon dioxide +Energy
100g 5g 51g 48g 35g 73g 194kcal
(dry weight)

### 2.1.3. Nutrients for Growth

*Saccharomyces cerevisiae* yeast requires the following essential nutrients and growth promoters for the multiplication of its cells, in the presence of atmospheric oxygen;

1. A source of assimilable organic carbon and energy
2. Assimilable nitrogen composition
3. The essential minerals $PO_4^{-3}$, $K^+$, $SO_4^{-2}$, $Mg^{2+}$ and trace element ions
4. The growth promoters biotin, pantothenic acid and m-inositol (as exceptions, some strains also require aneurin and or pyridoxine)

A complete understanding of the nutrition of *Saccharomyces cerevisiae* is required in order to optimise its growth and metabolic activities.

The nutritional requirement for the growth of baker's yeast may be estimated from its elemental composition. Based upon the average values published by several authors, baker's yeast contains (on a dry solids basis) about 46 %carbon, 32 % oxygen, 8.5 % nitrogen, 6% hydrogen and 7.5 % ash. On the assumption that 200g of sucrose is required for the production of 100g of yeast solids under efficient growth conditions, the following material balance equation may be established;

$$200g\ \text{sucrose} + 10.32g\ NH_3 + 100.44g\ \text{oxygen} + 7.5g\ \text{ash} \longrightarrow$$
$$100g\ \text{yeast solids} + 140.14g\ CO_2 + 78.12g\ H_2O$$

### 2.1.3.1. C Sources

*Saccharomyces cerevisiae* is able to use various organic compounds as sources of carbon and energy. Carbon can be used aerobically or anaerobically as it was shown in Figure 2.2.

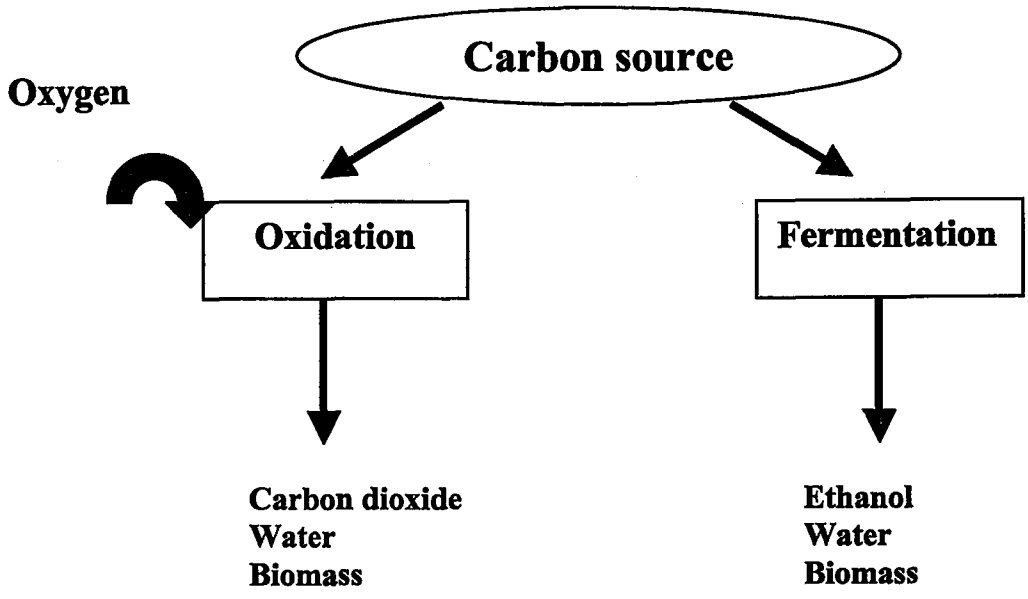



Figure 2.2. Utilization of carbon by yeast

However the speed of cell multiplication, cell yield and catabolism then differ; in particular some sources of carbon are oxidizably assimilable by the yeast, but not fermentable. Only a small portion of sugars is fermentable. Combined sources of carbon and energy useful to *Saccharomyces cerevisiae*;

Table 2.4. Useful carbon and energy sources for *Saccharomyces cerevisiae*

| | **Oxidatively assimilable** | **Fermantable** | | **Oxidatively assimilable** | **Fermentable** |
|---|---|---|---|---|---|
| Starch | - | - | D-erythritol | - | - |
| Dextrine | - | - | Mannitol | +- | - |
| Maltotriose | +- | +- | Glycerin | + | - |
| Maltose | + | + | Ethanol | + | - |
| Cellobiose | - | - | Methanol | - | - |
| Sucrose | + | + | Gluconic a. | - | - |
| Lactose | - | - | 5-keto | | |
| Glucose | + | + | gluconic a. | - | - |
| Fructose | + | + | Lactic acid | - | - |
| Galactose | + | +- | Citric acid | - | - |
| Arabinose | - | - | Succinic acid | - | - |
| Xylose | - | - | Acetic acid | - | - |
| Sorbite | +- | - | Paraffins | - | - |

The carbon source for baker's yeast propagation usually consists of assimilable sugars, such as glucose, fructose, mannose, galactose, sucrose, maltose and hydrolysed lactose. Ethanol has also been used, at least partially, as a substrate for yeast production. In order to be assimilated, these compounds must be transported into the yeast cells. Lactose can be used after it is hydolyzed. Monosaccharides are transported by carrier-mediated or facilitated diffusion (1).

### 2.1.3.2. N Sources

Nitrogen in various types of bounds can be assimilated by *Saccharomyces cerevisiae* yeast;

Table 2.5. Assimilation of N derivatives by *Saccharomyces cerevisiae*

| Source of N | Assimilation of N |
|---|---|
| $NH_4^+$ | + |
| $NO_3^-$ | - |
| Protein hydrolysate | + |
| **Simple aminoacids** | |
| Aspartic acid | + |
| Glutamic acid | + |
| **Amides** | |
| Asparagine | + |
| Glutamine | + |
| Urea | + |

The type of N assimilation with the least problems is with ammonium ions in the form of ammonium hydroxide solution or ammonium salts and when an anorganic source of N is replaced by amino acids, due to the amino acid's carbon content, another source of C is added which will influence the yeast yield. Amino acids have a regulatory effect on the yeast metabolism they accelerate or slow down the process (1).

The N is dosed in accordance with the desired concentration of raw protein in the crop yeast. Pure culture yeast and setting yeast should have the maximum concentration of raw protein, therefore, at least 8% N, in relation to the increase of yeast dry substance must be applied (7).

### 2.1.3.3. Minerals

An essential but often neglected part of the nutritional requirements of yeast is the ionic constituents of the medium. The chelation of metal ions by organic

components of the fermentation substrate, e.g. molasses or corn step liquor and other physicochemical attributes that can affect ionic availability such as pH or ionic strength. Limitation of trace elements may therefore be more common than is realized. In other cases, the removal of potentially toxic metal ions from solution by binding or complexation may even enable yeast growth and fermentation to proceed in the presence of total metal concentrations that would otherwise be toxic (1).

The essential mineral phosphorus is assimilated by yeast only in the form of the anion phosphate ($PO_4^{3-}$). Phosphorus will often have a toxic effect in linkages with different valences. If the quantity of phosphate is unsufficient, not all available N is absorbed, despite a possible surplus of N. Conversely, if there is a lack of N, not all available phosphate is assimilated, despite a possible surplus of phosphate.

The element sulfur exists in yeast mainly in reduced 2-valence form. The elements potassium and magnesium can only be assimilated in the form of the cations $K^+$ and $Mg^{2+}$. As for the trace elements, very little known about yeast's specific need of trace element. The concentrations of important trace elements in baker's yeast are shown in Table 6. There is evidence that certain antagonistic effects on one hand, the complex forming processes on the other, also play a part in regarding both quality and quantity, since the amounts of trace elements that are made available to the yeast during the technical production process through raw materials cannot be controlled (1,2,7).
The total ash content of baker's yeast varies from 4.7 to 10.5 % (1). The concentration of individual trace elements are shown in Table 2.6.

Table 2.6. The concentration of individual trace elements detected through analysis in various types of baker's yeast

| Trace element | Conc. (mg/kg) | Trace element | Conc. (mg/kg) |
|---|---|---|---|
| **Al** | 100-1000 | **Fe** | 90-170 |
| **Ba** | 150-200 | **Pb** | 8-400 |
| **B** | 200 | **Mn** | 5-70 |
| **Cr** | 10 | **Sn** | 200 |
| **Co** | 5-50 | **Zn** | 50-3000 |
| **Cu** | 20-40 | | |

(concentration in ppm: mg/kg yeast dry substance)

It can safely be assumed from a number of additional reports that yeast needs at least;

Fe 0.2 ppm in the nutritive broth

Cu 0.01 ppm in the nutritive broth

Zn 0.2 ppm in the nutritive broth (1)

When there is an overdose of trace elements, whether individually added or in a mixture, the yeast metabolism is poisoned.

In a nutritive broth made with molasses, however the inhibiting effects are usually lower by more than one decimal power, i.e. there is inhibition only when the concentration of trace elements is considerably higher. It seems that certain substances contained in molasses have a chelating (complex forming) effect on the metal ions, and thus exercise a certain protective function towards the yeast cells (7).

### 2.1.3.4. Growth Promoters

*Saccharomyces cerevisiae* yeast is heterotrophic with regard to growth promoters, i.e. it cannot reproduce without the help of certain growth promoter. Most strains of baker's yeast needs three growth promoters: D-biotin, D-pantothenic acid and m-inositol.

Most strains of baker's yeast have the growth promoters which are seen in Table 2.7;

Table 2.7. Requirements of growth promoter regarding quantity and quality, in most strains of baker's yeast

| **Growth promoters** | **mg/100g yeast dry substance** |
|---|---|
| D-biotin | 0.03 |
| D-pantothenic acid | 15 |
| m-inositol | 200 |

D-biotin can be partially replaced by high doses of aspartic growth promoter acid or by relatively high doses of unsaturated fatty acids (oleic, linoleic acid, kinolenic acid) (1).

### 2.1.4. Physical and Chemical Factors of Growth

Yeast cells survive only within certain limits, and an active yeast metabolism including the production of cells demands even more exacting limits. The most important physical-chemical factors for yeast multiplication in the technology of baker's yeast are;

1.Water supply/osmotic pressure

Metabolically active yeast cells in aqueous culture broth consist of roughly 70-75 % water. *Saccharomyces cerevisiae* multiplies in substrates with a water activity of 0.999-0.970, corresponding to an osmotic pressure of approximately 1-40 bar.

2.Static pressure /pressure impulses

Static pressures of up to 10 bar during yeast cultures in the fermentor are harmless; but at higher pressures, inhibited breathing and fermentation were sometimes observed, although these inhibitions could also be indirect effects of simultaneous increases in $CO_2$-partial pressure.

3.Temperature

The temperature has an extremely important effect on the metabolism and the multiplication of the yeast, as well as on the composition cell substance. For *Saccharomyces cerevisiae* growth, average values are given in Table 2.8;

Table 2.8. Temperature dependency of *Saccharomyces cerevisiae*

| | **Temperature (°C)** |
|---|---|
| **Minimum growth** | 2 °C |
| **Optimal growth (aerobic)** | 33 °C |
| **Maximum growth (aerobic)** | 38 °C |
| **Maximum fermentation** | 40 °C |

**4.**Concentration of hydrogen ions

The culture broth's pH value has a two fold effect on yeast metabolism;

1.) Direct impact of the concentration of hydrogen ions on the yeast cell. This impact is similar to that of trace elements; high concentrations (low pH value) have a toxic effect, low concentrations (higher pH value) cause deficiencies; medium concentrations are preferable;

2.) Indirect impact of the concentration of hydrogen ions by influencing the dissociation state of the nutrients and metabolites. The rate at which many nutrients can be absorbed by yeast cells depends on the dissociation state of these nutrients; a similar situation exists for the toxic impact of many substances.

In the industrial production of baker's yeast, the following pH ranges are;

Culture of pure yeast and setting yeast: pH 4.0-4.8

Culture of shipping yeast pH 4. 6 (beginning): pH 5.6-6.4

**5.**Redox potential

**6.**Surface tension

**7.**Concentration of oxygen in the broth

**8.**Concentration of carbon dioxide in the broth (1,2,7)

### 2.1.5. Proliferation of Yeast

Baker's yeast is unicellular budding fungi which normally multiplies through budding, which is called as asexual reproduction. Bud formation can be seen in Figure 2.3.

The cells are normally diploid that is their nucleus contains two sets of chromosomes. During budding, there is a complete division of this double set of chromosomes, each daughter cell that is vegetatively produced again contains a complete double set of chromosomes.

The first step in sexual reproduction is the formation of ascospores from the diploid cells. These four spores form within the cell which then loses its vegetative

character and becomes an ascus. Each ascospore contains only a simple set of chromosomes.

The ascus splits under right conditions and the individual spores are released. They germinate and produce daughter cells, which possess only a simple set of chromosomes. These are the haploids. Aside from the purely vegetative reproduction of haploid cells through budding, the cell can also mate. When two haplonts fuse, they form a zygote. The individual sets of chromosomes of the two haplonts associate and form the double set of chromosomes already familiar to the diplonts. The zygote then buds again like a regular diploid cell, and its daughter cells again form a diploid culture (1,2,3,6).

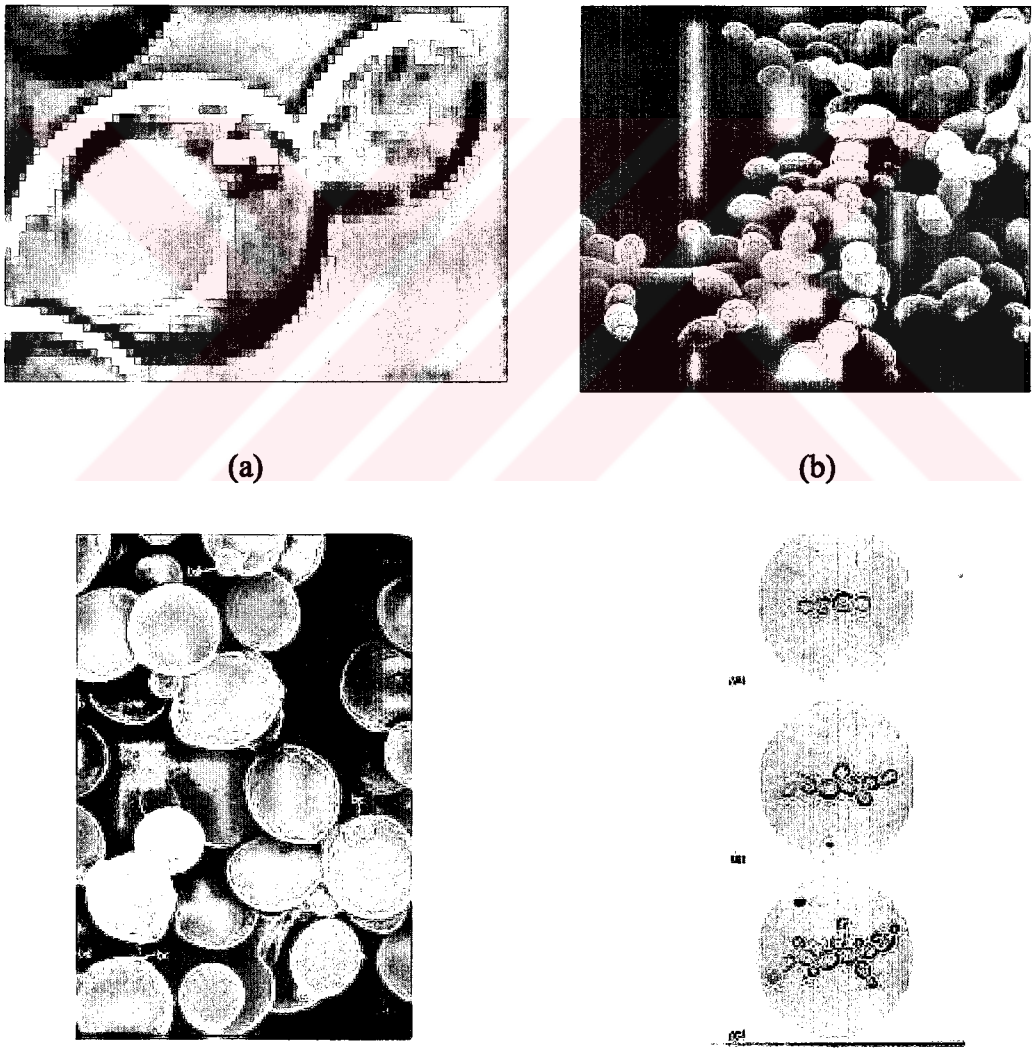



Figure 2.3. (a,b,c)SEM photographs of budding formation.
(d)Formation of double set of chromosomes

### 2.1.6. Oxygen Requirement

On the basis of material balance calculations, 1 g of oxygen is required for the production of 1 g of yeast solids under efficient growth conditions. Thus, oxygen, like other nutrients, must be supplied for efficient yeast growth. In commercial production of baker's yeast, oxygen is supplied by sparging an air stream through the fermentor broth. Under oxygen-limiting conditions, the amount of yeast which can be produced per unit fermentor volume and unit time is dependent upon the amount of oxygen which can be transferred from the gas phase to the liquid phase per unit volume and unit time. For example, for a volumetric productivity of 5 g of yeast solids $l^{-1}h^{-1}$ the amount of oxygen required is 5 g or 156 mmol of $O_2\, l^{-1}h^{-1}$ (2).

### 2.1.7. Kinetics of Growth

Cell multiplication occurs exponentially as long as it is not impeded by external conditions. One cell multiplication follows the other at constant time intervals, and the cells' volume increases in geometric progression. The constant time intervals of exponential cell duplication are called generation time $t_g$ (duplication time). Exponential cell multiplication is illustrated in Figure 2.3.

$t_g$ is generation time,
$X_0$ is cell weight
N number of generations,

| Time | Amount of cells |
|---|---|
| 0 | $X_0 = 1.X_0$ |
| $1.t_g$ | $2^1.X_0 = 2.X_0$ |
| $2.t_g$ | $2^2.X_0 = 4.X_0$ |
| $3.t_g$ | $2^3.X_0 = 8.X_0$ |
| $4.t_g$ | $2^4.X_0 = 16.X_0$ |
| ... | ... |
| $n.t_g$ | $2^n.X_0 = 2^n.X_0$ |

Figure 2.4. Scheme of exponential cell multiplication

The following simple exponential function expresses the amount of cells (X) present an arbitrary point in time (t= n. tg) of the multiplication.

$X=X_0.2^n$.....(1)

In this equation, n is the number of generations present up to the observation period t ($n = t / t_g$).

The generation period in exponential cell multiplication is constant. Therefore it characterizes the progression of multiplication. So, it characterizes the progression of multiplication (1).

The following straightforward connections exist between growth rate $\mu$ and generation time $t_g$:

$$\mu = \frac{\ln 2}{t_g} = \frac{0.69}{t_g} \quad \text{.....(2)}$$

$$t_g = \frac{\ln 2}{\mu} = \frac{0.69}{\mu} \quad \text{.....(3)}$$

The exponential factor $2^n$ can be replaced by exponential factor $e^{\mu \cdot t}$ and equation becomes;

$$\frac{X}{X_0} = e^{\mu . t} \quad \text{.....(4)}$$

$$\frac{dX}{dt} = \mu . X \quad \text{.....(5)}$$

In yeast technology, the modulus H is used instead of the expression $e^{\mu}$. The following connection exists between the two;

$H = e^{\mu}$.....(6)

$\mu = \ln H$.....(7)

so that, after integration of the modulus H, the basic equation;

$X = X_0 . H$.....(8)

## 2.2. Baker's Yeast Industry

Commercial baker's yeast is usually produced in a multiple stage process. The early stages, one or more, are batch fermentations in that all ingredients are in the fermentors before yeast inoculum is added. The latter stages are fed-batch fermentations, where the ingredients are added to the fermentors in a predetermined way at the begining and during fermentation (2).

The first stage of production consists of growing the yeast from the pure culture in a series of fermentation vessels. The yeast is recovered from the final fermentor using centrifugal action to concentrate the yeast solids. Next, the yeast product is subjected to one or more washings in another centrifugal separator (1).

### 2.2.1. Process Description

The first stage of yeast production consists of growing the yeast from the pure yeast culture in a series of fermentation vessels. The yeast is recovered from the final fermentor by using centrifugal action to concentrate the yeast solids. The yeast solids are subsequently filtered by a filter press or a rotary vacuum filter to concentrate the yeast further. Next, the yeast filter cake is blended in mixers with small amounts of water, emulsifiers, and cutting oils. After this, the mixed press cake is extruded and cut. The yeast cakes are then either wrapped for shipment or dried to form dry yeast.

#### 2.2.1.1. Raw Materials and the Preparation of Raw Materials

The principal raw materials used in producing baker's yeast are the pure yeast culture and molasses. Cane molasses and beet molasses are the principal carbon sources to promote yeast growth. Molasses contains 45 to 55 weight percent fermentable sugars, in the forms of sucrose, glucose, and fructose. Most of the nutrients and minerals are provided from the molasses that is a rich carbon and mineral source for baker's yeast. The average composition of beet and cane molasses can be seen in Table 2.9 (1).

Table 2.9. Average composition of beet & cane molasses

| % | Beet molasses | Cane molasses |
|---|---|---|
| Water | 16.5 | 20.0 |
| Sucrose | 61.0 | 32.0 |
| Glucose | - | 14.0 |
| Fructose | - | 16.0 |
| Invert sugar | 1.0 | - |
| Rafinose | 1.0 | - |
| | 63 % | 62 % |
| N substances Free & combined acid soluble rubber materials | 19.0 | 10.0 |
| $SiO_3$ | 0.1 | 0.5 |
| $K_2O$ | 8.0 | 8.5 |
| CaO | 0.26 | 1.5 |
| MgO | 0.16 | 0.1 |
| $P_3O_6$ | 0.06 | 0.2 |
| $Na_2O$ | 1.3 | 0.2 |
| $Fe_3O_2$ | 0.02 | }0.3 |
| $Al_2O_3$ | 0.07 | |
| | 11.5 % | 8.0 % |
| Soda carbonat residue | 1.5 | |
| Sulphate residue | 0.33 | 1.0 |
| Chloride | 1.5 | 0.6 |

The amount and type of cane and beet molasses used depend on the availability of the molasses types, costs, and the presence of inhibitors and toxins. Molasses is an inexpensive and commonly used source of sugars for producing baker's yeast (8). Usually, a blend consisting of both cane and beet molasses is used in the fermentations. Once the molasses mixture is blended, the pH is adjusted to between 4.5 and 5.0 because an alkaline mixture promotes bacteria growth. Bacteria growth occurs under the same conditions as yeast growth, making pH monitoring very important. The molasses mixture is clarified to remove any sludge and is then sterilized with high-pressure

steam. After sterilization, it is diluted with water and held in holding tanks until it is needed for the fermentation process.

Molasses has replaced grain mash as the primary energy substrate for yeast production since World War I. Beet and cane molasses are used separately or in combination.

Initially the term molasses referred specifically to the final effluent obtained in the preparation of sucrose by repeated evaporation, crystallization and centrifugation of juices from sugar cane and from sugar beets. Today several types of molasses are recognized and in general, any liquid feet ingredient that contains in excess of 43% sugars is termed molasses. The Association of American Feed Control Officials (AAFCO, 1982) describes the following types of molasses:

**Cane molasses:** is a by-product of the manufacture or refining of sucrose from sugar cane. It must contain less than 46% total sugars expressed as invert.

**Beet molasses:** is a by product of the manufacture of sucrose from sugar beets. It must contain not less than 48% total sugars expressed as invert.

**Citrus Molasses**: is the partially dehydrated juices obtained from the manufacture of dried citrus pulp. It must contain not less than 45% total sugars expressed as invert.

**Hemicellulose extract:** is a by product of the manufacture of pressed wood. It contains pentose and hexose sugars, and has a total carbohydrate content of not less than 55%.

**Starch Molasses:** is a by-product of dextrose manufacture from starch derived from corn or grain sorghums where the starch is hydrolysed by enzymes and/or acid. It must contain not less than 43% reducing sugars expressed as dextrose.

Raw beet and cane molasses enter the factory at 80 to 90 °Brix and contain 80 to 85 % solids of which up to 45-55 % may be sugar. Dilution is necessary to facilitate pumping, clarification and sterilization. Dilution is accomplished in one of several ways. The simplest is to weigh a given quantity of molasses and add to it a given quantity of water.

In literature, alternative carbon sources are being explained. In regions where molasses must be imported, transportation costs and custom duties add to its price and

make it expensive. The total or partial replacement of molasses with a cheap and locally available fermentable source of sugars, as cheese whey, can improve the industrial competitiveness. Whey is a waste by-product of the cheese industry and lactose is the main constituent of whey (9).

The use of whey in baker's yeast industry presents three important challenges: (1) *Saccharomyces cerevisiae* is unable to assimilate lactose (whey sugar) so to be used as substrate first it must be hydrolysed by $\beta$-galactosidase to glucose and galactose. (2) *Saccharomyces cerevisiae*'s metabolism of glucose can be either respiratory or fermentative. In baker's yeast production the respiratory metabolism is desired and it is achieved by controlling the availability of the hexose concentration under aerobic conditions. In practice, it is accomplished by fed-batch operation that allows the control of the key nutrient concentration (8). In table 2.10, experimental fermentation parameters can be seen;

Table 2.10. Experimental parameter values

| **Parameter** | **Molasses** | **Molasses/ Whey** | | | | |
|---|---|---|---|---|---|---|
| Total sugar fed(g) | 178.6 | 181.2 | 189.7 | 190.8 | 189.2 | 187.1 |
| Sugar from molasses(%) | 100 | 65 | 56 | 54 | 55 | 54 |
| Sugar from whey(%) | - | 35 | 44 | 46 | 45 | 46 |
| Enzyme dosage($U.g^{-1}$lactose) | - | 43.0 | 25.3 | 19.5 | 14.9 | 10.6 |
| Final biomass produced($g.l^{-1}$) | 32.3 | 33.7 | 35.3 | 33.5 | 33.2 | 35.0 |
| Final cell conc.($cells.ml^{-1}$) | $1.1*10^9$ | $1.5*10^9$ | $1.5*10^9$ | $0.4*10^9$ | $4.5*10^9$ | $1.8*10^9$ |
| Lactose hydrolysis(%) | - | 100 | 100 | 100 | 100 | 89.7 |
| Consumption of sugar(%) | 98.4 | 98.5 | 99.3 | 99.1 | 99.7 | 94.7 |
| Biomass yield(g biomass $g^{-1}$ sugar) | 0.490 | 0.451 | 0.445 | 0.410 | 0.407 | 0.491 |
| Ethanol yield(g ethanol $g^{-1}$sugar) | 0.063 | 0.046 | 0.129 | 0.080 | 0.077 | 0.052 |
| Glycerol yield(g glycerol $g^{-1}$sugar) | 0.003 | 0.004 | 0.003 | 0.004 | 0.003 | 0.004 |
| Overall biomass volumetric productivity($glh^{-1}$) | 2.84 | 2.71 | 3.01 | 2.86 | 2.65 | 2.83 |
| Specific growth rate ($h^{-1}$) | 0.109 | 0.096 | 0.159 | 0.121 | 0.102 | 0.116 |
| Maximum specific sugar consumption rate ($g.gl^{-1}$) | 0.53 | 0.62 | 0.75 | 0.71 | 0.72 | 0.59 |

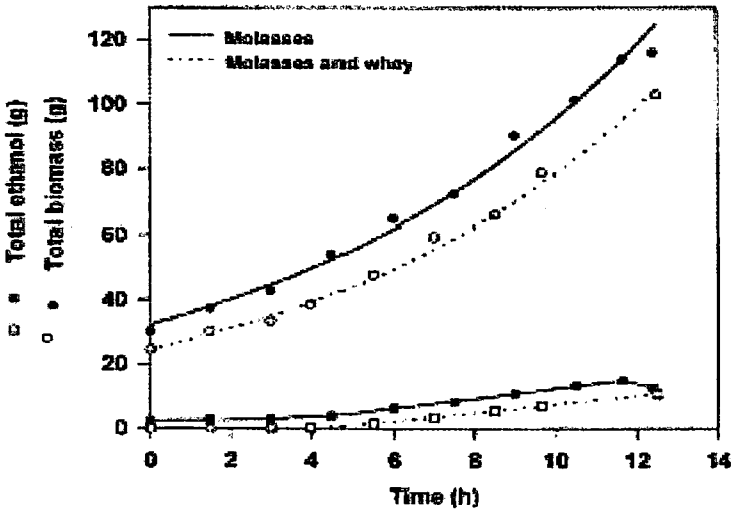



Figure 2.5. Total biomass and ethanol in the bioreactor during experiments using molasses and molasses/whey (54%–46%) feeds

Partial substitution of molasses by whey did not significantly affect the overall biomass volumetric productivity as it was seen in Table 2.10. Water used to dilute molasses in the commercial process was replaced with whey. The change in total biomass and ethanol produced uşing the molasses and molasses/whey by the time are shown in Figure 2.5 (8).

A variety of essential nutrients and vitamins are also required in yeast production which all cannot be provided from molasses. The nutrient and mineral requirements include nitrogen, potassium, phosphate, magnesium, and calcium, with traces of iron, zinc, copper, manganese, and molybdenum. Normally, nitrogen is supplied by adding ammonium salts, aqueous ammonia, or anhydrous ammonia to the feedstock. Phosphates and magnesium are added, in the form of phosphoric acid or phosphate salts and magnesium salts. Vitamins are also required for yeast growth (biotin, inositol, pantothenic acid, and thiamine).

### 2.2.1.2. Industrial Baker's Yeast Fermentation

Yeast cells are grown in a series of fermentation vessels. Yeast fermentation vessels are operated under aerobic conditions (free oxygen or excess air present) because under anaerobic conditions (limited or no oxygen) the fermentable sugars are consumed in the formation of ethanol and carbon dioxide, which results in low yeast yields. Overall production scheme of baker' s yeast industrially was illustrated in Figure 2.6.

#### 2.2.1.2.1. Laboratory Stage

The initial stage of yeast growth takes place in the laboratory. A portion of the pure yeast culture is mixed with molasses malt in a sterilized flask, and the yeast is allowed to grow for 2 to 4 days. The entire contents of this flask are used to inoculate the first fermentor in the pure culture stage.

#### 2.2.1.2.2. Pure Culture Stage

Typically, this stage consists of two pure culture fermentations. These are batch fermentations, where the yeast is allowed to grow for 13 to 24 hours. The pure culture fermentations are basically a continuation of the flask fermentation, except that they have provisions for sterile aeration and aseptic transfer to the next stage. The contents of the fermentor from the first pure culture stage are added to the next fermentation vessel, which already contains the nutrient-rich molasses malt. These fementations are a continuation of the flask fermentation, except that they have provisions for sterile aeration and aseptic transfer to the next stage.

The critical factor in this stage is sterility. Rigorous sterilization of the fermentation medium prior to inoculation is conducted by heating the medium under pressure or by boiling it at atmospheric pressure for extended periods. If a sterile environment is not provided, contaminating microorganisms can easily outgrow the yeast (8).

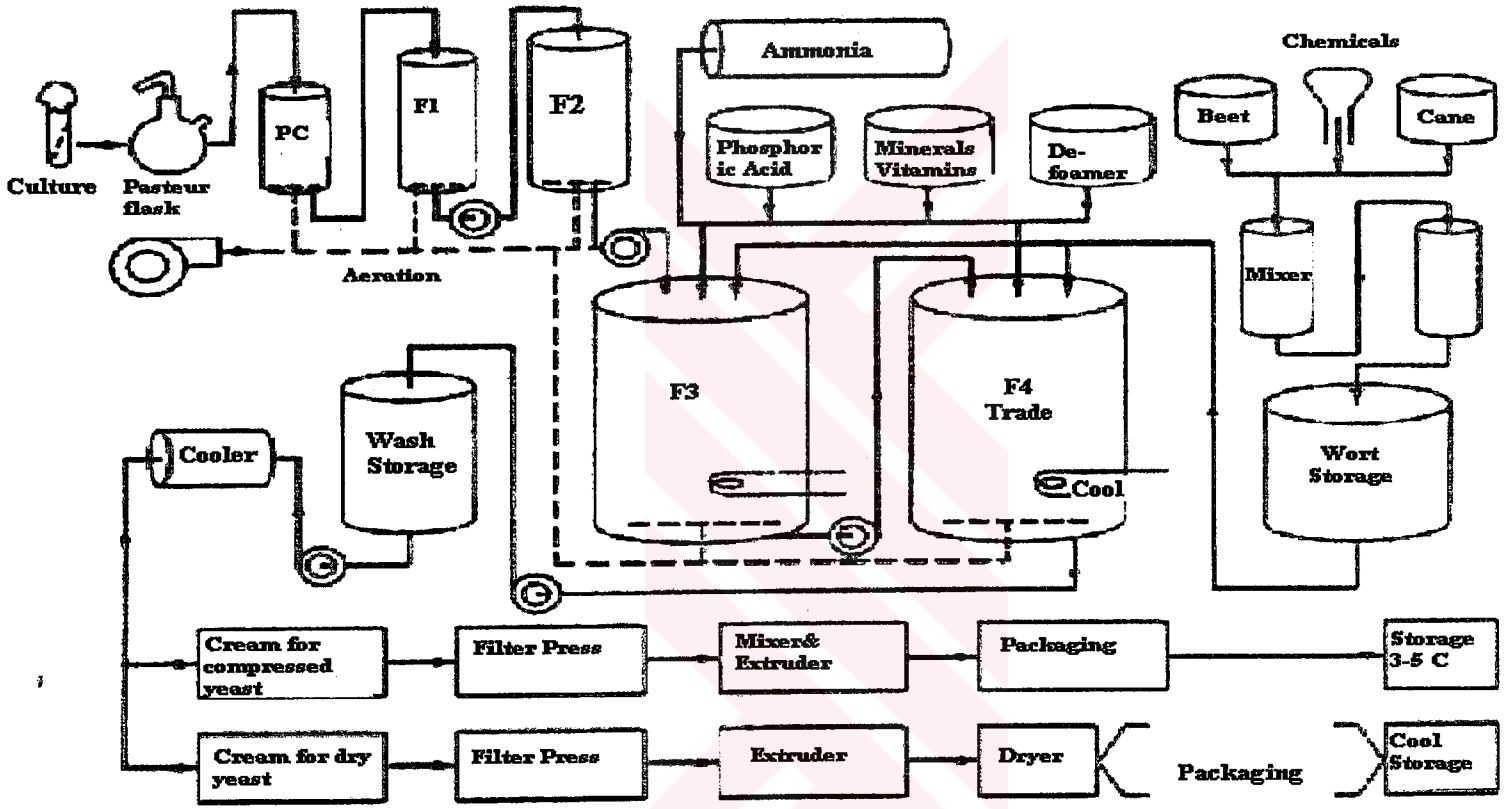



Figure 2.6. Industrial baker's yeast production

#### 2.2.1.2.3. Main Fermentation Stages

The majority of the yeast yield grows in the final fermentation stages. The main fermentation steps may take place in a two-stage or four-stage sequence, depending on company operations. The fermentors used in the final stages are usually constructed of stainless steel and vary considerably in size, ranging from 37,900 L to over 283,900 L. These vessels have diameters in excess of 7.0 meters and heights up to 14 meters. The larger vessels are associated with the final fermentation stages. The fermentation vessels are typically operated at 30 °C. Fermentors are usually equipped with an incremental feed system. The incremental feed system may be a pipe or a series of pipes that distributes the molasses over the entire surface of the fermentor liquid. The rate at which the molasses is fed is critical and may be controlled by a speed controller connected to a pump or by a valve on a rotameter, which delivers a certain volume of molasses at regulated time intervals. Nutrient solutions of vitamins are kept in small, separate tanks and are added through rotameters into the fermentor. The rate of this feed is not as critical as the molasses feed rate. However, if ammonia is used as a nitrogen source, additions must be made in a manner that avoids sudden pH changes. Nitrogen salts and phosphates may be charged in a shorter period of time than molasses.

Fermentors used in the final stages must also be equipped with heat exchangers to remove the heat produced from the production process and to cool the fermentor. The type of heat exchanger system depends on the size of the fermentation vessel. Because large volumes of air are supplied to the fermentation vessels during this stage of production, the fermentor size and the type of aeration system selected are interdependent. The different types of aeration systems include horizontal, perforated pipes; compressed air and mechanical agitation; and a self -priming aerator.

In the horizontal, perforated pipe system, air is blown through a large number of horizontal pipes that are placed near the bottom of the fermentor. With this aeration system, the only agitation of the fermentor liquid is carried out by the action of the air bubbles as they rise to surface. Typically, this type of aeration system requires from 25 to 30 cubic meters of air to produce 0.45 kg of yeast.

Large air supplies are required during the final trade fermentations, so these vessels are often started in a staggered fashion to reduce the size of the air compressors.

The duration of the final fermentation stages ranges from 11 to 15 hours. After all of the required molasses has been fed into the fermentor, the liquid is aerated for an additional 0.5 to 1.5 hours to permit further maturation of the yeast, making it more stable for refrigerated storage. The amount of yeast growth in the main fermentation stages described above increases with each stage. Yeast growth is typically 120 kilograms in the intermediate fermentor, 420 kilograms in the stock fermentor, 2,500 kilograms (5,500 pounds) in the pitch fermentor, and 15,000 to 100,000 kilograms in the trade fermentor (1,2,7).

### 2.2.1.3. Concentration, Separation and Filtering

Once an optimum quantity of yeast has been grown, the yeast cells are recovered from the final trade fermentor by centrifugal yeast separators. The centrifuged yeast solids are further concentrated by a filter press or rotary vacuum filter. Filter presses having frames of 58 to 115 cm are commonly used, and pressures between 860 and 1030 kPa are applied. A filter press forms a filter cake containing 27 to 32 percent solids. Rotary vacuum filters are also used for continuous feed of yeast cream. Generally, the filter drum is coated with yeast by rotating the drum in a trough of yeast cream or by spraying the yeast cream directly onto the drum. The filter surface is coated with potato starch containing some added salt to aid in drying the yeast product. The rotary vacuum filter forms cakes containing approximately 33 percent solids. This filter cake is then blended in mixers with small amounts of water, emulsifiers, and cutting oils to form the end product. The final packaging steps, as described below, vary depending on the type of yeast product.

### 2.2.1.4. Shaping and Packaging

The filter cake is then blended in mixers with small amounts of water, emulsifiers, and cutting oils to form the end product. The final packaging steps, as described below, vary depending on the type of yeast product.

In compressed yeast production, emulsifiers are added to give the yeast a white, creamy appearance and to inhibit water spotting of the yeast cakes. A small amount of oil, usually soybean or cottonseed oil, is added to help extrude the yeast through nozzles to form continuous ribbons of yeast cake. The ribbons are cut, and the yeast cakes are

wrapped and cooled to below 8°C (46°F), at which time they are ready for shipment in refrigerated trucks (7). Compressed baker's yeast has 27-32% dry matter content and preserved at 4-6 °C for 5- 6 weeks and at room temperature only for approx.10 days (1,7).

### 2.2.2. Active Dry Yeast Production

Dry baker's yeast is always made from fresh baker's yeast . Through special thermic procedures, the water content is decreased to 4-8% with very little damage to the yeast cells' vitality. In dry yeast production, the product is sent to an extruder after filtration, where emulsifiers and oils (different from those used for compressed yeast) are added to texturize the yeast and to aid in extruding it. After the yeast is extruded in thin ribbons, it is cut and dried in either a batch or a continuous drying system. Following drying, the yeast is vacuum packed or packed under nitrogen gas before heat sealing. The shelf life of active dry yeast and instant dry yeast at ambient temperature is 1 to 2 years (1).

### 2.2.3. Instant Dry Yeast Production

Instant dry yeast is produced from a faster-reacting yeast strain than that used for active dry yeast. The main difference between active dry yeast and instant dry yeast is that active dry yeast has to be dissolved in warm water before usage, but instant does not for providing the activation (7).

# CHAPTER III

# UTILIZATION OF METALS BY BAKER'S YEAST

The metal cations $K^+$, $Mg^{2+}$, $Ca^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ are known to directly influence fermentative metabolism in yeast, and therefore knowledge of their interactions is essential to manipulate their availability in industrial fermentations to optimal levels (10).

## 3.1. Main Mechanisms

The main mechanisms by which microorganisms immobilize, complex or otherwise remove metals from solution are as follows (12):

1. Volatilization
2. Extracellular precipitation
3. Extracellular complexing and subsequent accumulation
4. Binding to the cell surface
5. Intracellular accumulation

The interactions are summarized in Figure 3.1.

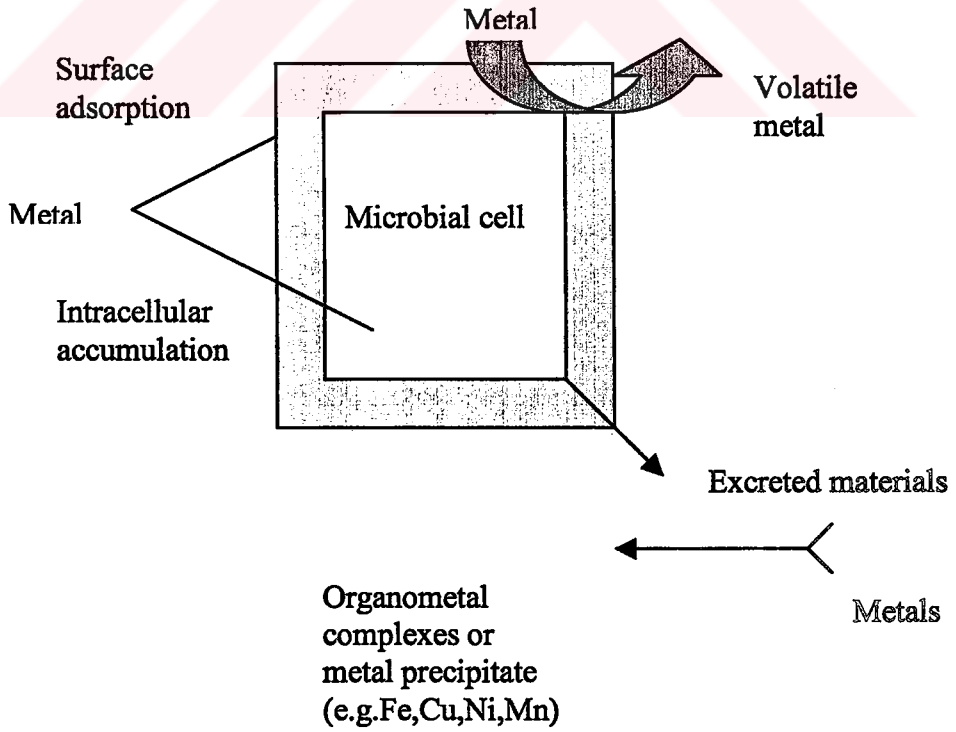



Figure 3.1. Schematic representation of the interactions which can occur between metals and the microbial cell.

### 3.2. Specific Uptake Mechanisms of *Saccharomyces cerevisiae*

The ability of microbial cells to deal with the wide range of metal ions required for metabolic functions is an interesting physiological phenomenon with both fundamental and applied aspects. The uptake of metal ions into cells depends on the concentration of particular ions in the growth environment and on their bioavailability. The latter depends on both the solubility and the properties of ion-complexing ligands. Translocation is controlled at the plasma membrane, which can distinguish among metal ions and provide a specific transport system into intracellular sites. Other metal cations are necessarily excluded from these sites. This precise control contrasts with a chemical situation where the competition between metal ions for a ligand is normally won by the metal ion that is the strongest Lewis acid. However, cells have the ability to select required cations and insert them into appropriate complexing sites. During the binding process there is a competition between foreign and native cations, and it is possible to arrange suitable conditions for the uptake of foreign cations by facilitating competition with the native ion and finally replacing it. It is desirable to study such interactions for a number of reasons. For example, the replacement of active ions may result in perturbation of normal biological function and may provide insight into the role of the native cation under normal physiological conditions. Studies into ionic interactions also promote understanding of the toxic effects of metals toward microorganisms (2,13).

Among the cationic yeast nutrients, potassium, magnesium, calcium, and zinc are involved in structural and enzymatic regulatory activities during growth and metabolism. Potassium is mainly involved in osmoregulation, charge balancing, and in regulation of divalent cation and phosphate uptake into the yeast cell.

In particular, knowledge of interactions among $K^+$, $Mg^{2+}$, $Ca^{2+}$, and $Zn^{2+}$, as well as their relative bioavailability in industrial growth substrates is important to manipulate optimally their levels in fermentations (13).

Having surveyed the scope and complexity of yeast ionic nutrition, it is clear that in order to fully understand the industrial implications of yeast ionic nutrition, it is logical to turn to a study of the physiological mechanisms involved in the uptake of ions by yeast. Of central importance to such a study are the transport properties of the yeast

plasma membrane, since this is the primary biological site for interaction with the external environment.

While the plasma membrane is permeable to water, $O_2$, $CO_2$, free ammonia, ethanol, and the un-ionized form of organic acids, a variety of systems can be utilized for the uptake of ions into the cytoplasm, and the relation to ions of major importance to the efficient growth and metabolism of *Saccharomyces cerevisiae.* Jones, R.P. et al. (1990) reported that these will include monovalent and divalent cations, e.g. $K^+$, $Na^+$,$Ca^{2+}$, $Mg^{2+}$, as well as inorganic anions, e.g. $Cl^-$, $H_2PO_4^-$, and $SO_4^{2-}$ (2).

Since, the transport systems associated with the vacuolar membrane (tonoplast) have a major role in the regulation of the intracellular ionic environment, as does the operation of specific ion binding proteins. The intracellular ionic composition of yeast is different from that of the external environment, while within the cell there are asymmetric distributions of ions, both of these features being largely dependent on the operation of various transport systems and subject to effects relating to the physical and chemical nature of the external environment. An understanding of ion transport mechanisms and their regulation by the ionic environment will enable the further optimisation of industrial media for yeast growth and metabolic functions.

Additional mechanisms are therefore required for the intracellular accumulation of essential ions from the exterior, and a variety of transport systems of varying specificity maybe utilized. Such systems, together with additional mechanisms for intracellular compartmentation and/or sequestration, act to regulate the intracellular ionic environment. Integral to the asymmetric distribution of ions within cells, and in relation to the external environment, is the operation of enzymatic pumps that act across biological membranes. Such a proton gradient ($\Delta\mu_H^+$) has electrical ($\Delta\psi$) and chemical ($\Delta$pH) components which are interconvertible and which can each drive transport of ionisable substances across membranes. In yeast cells, three classes of $H^+$-pumping ATPases have been identified, the mitochondrial ATPase is the synthesis of ATP via the mitochondrial respiratory chain. It is the plasma membrane and vacuolar ATPases that are intimately associated with transport of ions, and other nutrients, intracellular compartmentation, and also regulation of cell pH. Both intracellular and external pH can affect yeast ionic uptake and nutrition (14).

### 3.2.1.Transport of monovalent cations ($M^+$)

The transport of monovalent cations is linked to the action of the plasma membrane- bound $H^+$-ATPase that expels protons, thus creating a transmembrane electrochemical proton gradient ($\Delta\mu_H^+$), negative and alkaline inside that has both a chemical component ($\Delta pH$) and an electrical component ($\Delta\psi$). The nutrient transport, including monovalent and divalent cations, by glucose can be due to two processes. In one process, glucose acts as an energy source for the biosynthesis of both cytoplasmic and membrane transport proteins that have been repressed during prior growth or starvation; cycloheximide can block this type of stimulation. In the second process, the $H^+$ pump is activated, which increases $\Delta\mu_H^+$, thus stimulating transport. Substrates other than glucose can also energize cation uptake, as well as respiratory processes, particularly those which activate the $H^+$ pump. (14)

Proton extrusion by the plasma membrane $H^+$-ATPase is chemiosmatically linked to the uptake of particular nutrients by proton symport. In *Saccharomyces cerevisiae*, active transport of maltose and maltotriose is via proton symport, as is the transport of certain amino acids. These processes may depolarise the plasma membrane. Such effects may explain the dependency of intracellular $K^+$ concentration on carbon source.

$K^+$ ion is preferentially taken up by the monovalent cation transport system. This system is known as "the physiological $K^+$ carrier", although other monovalent ions, including $Rb^+$, $Na^+$, $Cs^+$, $Li^+$, and $NH_4^+$, maybe transported with lower affinities. $Ca^{2+}$ and $Mg^{2+}$ can also be taken up via the $K^+$ transport system, though the affinity for these cations is very low. The relative order of affinities of these cations for the monovalent cation transport system is;

$$K^+ > Rb^+ > NH_4^+ > Cs^- > Na^+ > Li^+ >> Mg^{2+} > Ca^{2+}$$

Thus, $Na^+$ is generally excluded from cells and is present at much lower internal concentrations than $K^+$. A considerable proportion of cellular $K^+$ can be replaced with $Na^+$ by repeated culture in $Na^+$ rich medium containing little or no $K^+$. The sum of cellular $K^+$ and $Na^+$ is generally constant, although it can vary under different phosphate, $Mg^{2+}$, or $NH_4^+$ contents of the medium. In $Na^+$ loaded cells, $K^+$ uptake is balanced by both $Na^+$ and $H^+$ efflux. Cell $K^+$ can also be partially replaced by $Rb^+$, $Cs^+$, $Li^+$, and $NH_4$ by cultural manipulations, the replacement by $NH_4^+$ being highly pH dependent.

The $Na^+$/ $K^+$ selectivity of yeast depends on the stage of growth, with a greater proportion of $K^+$ being accumulated in the logarithmic phase than in the stationary phase than in the stationary phase, where the intracellular concentrations of $Na^+$ and $K^+$ can be equal.

Several yeasts, including strains of *Saccharomyces cerevisiae*, can survive in high concentrations of NaCl. While some aspects of osmotolerance may rely on the exclusion of $Na^+$ in presence to $K^+$, it is clear that the intracellular ionic composition may have only a minor role in osmotolerance (14).

The kinetics of monovalent cation transport are complex, with more than one binding site involved in translocation, and may be affected by the surface and membrane potential.

Both the electrical component ($\Delta\psi$) and the chemical component ($\Delta$pH) of the proton gradient ($\Delta\mu_H^+$) can vary with the external and intracellular pH and the external ion concentration, thus affecting transport. Yeast cells appear to maintain a $\Delta\mu_H^+$ of approximately –150 mV at pH 4.5 to –90 mV at pH 7.0. Inhibition of $K^+$ uptake by high external $K^+$ concentrations maybe explained by its ability to depolarise the plasma membrane, $\Delta\mu_H^+$ decreases at pH values between 4.5 and 7.0 at an external $K^+$ concentration of 200 mM, the main component affected being $\Delta\psi$.

Heavy metal ions, e.g. $Cu^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Cd^{2+}$, $Mn^{2+}$, mercurials, and other organometallic compounds, can inhibit the yeast plasma membrane ATPase by means of various toxic binding interactions both specific and non-specific. For $H^+$ efflux, heavy metal inhibition generally increased with increasing metal concentration with a toxicity sequence of;

$$Cd^{2+} > Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+}$$

These metals also inhibited $K^+$ uptake with a toxicity sequence of;

$$Cd^{2+} > Cu^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Zn^{2+}$$

Since these toxic metals are supplied in the extracellular medium, it is most likely that they inhibit the transport mechanism responsible for outside to inside transport rather than vice versa. The difference in the toxicity series for the last three ions ($Zn^{2+}$,$Co^{2+}$ ,$Mn^{2+}$ ) maybe a reflection of their uptake affinities into the cell and hence their ability to promote inhibition of the $H^+$ efflux systems. Inhibition of $H^+/K^+$ exchanges across the cell membrane would be expected to lead to inhibition of a range of transport functions, including the uptake of divalent cations and other nutrients (14).

### 3.2.2.Transport of divalent cations ($M^{2+}$)

Divalent cations such as $Mg^{2+}$, $Ca^{2+}$, $Cu^{2+}$, $Zn^{2+}$, and $Mn^{2+}$ are essential for growth and metabolism and therefore need to be accumulated from the external environment. However, above certain concentrations, all divalent cations, particularly those of the so-called heavy metals, are toxic and may cause impairment of cell metabolism, including ionic nutrition, and ultimately cell death, the toxicity being related to the strength of the coordinating abilities and the coordination chemistry of the particular ion. Some divalent cations, such as $Ca^{2+}$, $Mg^{2+}$, may enter *Saccharomyces cerevisiae* as low-affinity substrates of the monovalent cation transport system, but this is probably of little not significant to the ionic well-being of the cell (14).

In *Saccharomyces cerevisiae*, energy-dependent transport of many divalent cations has been demonstrated with an apparent affinity series of $Mg^{2+}$, $Co^{2+}$, $Zn^{2+}$ > $Mn^{2+}$> $Ni^{2+}$ >$Ca^{2+}$> $Sr^{2+}$. However, differences in accumulation may not be due to differences in affinities for the transport mechanism, as some reductions in the net rate of $Ca^{2+}$ and $Sr^{2+}$ uptake maybe due to increased leakage or efflux of these cations. In addition, there maybe effects on the structural integrity of the membrane, depending on the metal and its concentration, that result in permeability changes (14,12).

As with monovalent cations, the uptake of divalent cations is inhibited or halted by metabolic inhibitors, low temperatures, and the absence of energy-yielding substrates. It follows that uptake thus depends on the metabolic state of the cell and may vary with different growth media and conditions. For maximal uptake rates, cells appear to require adequate $K^+$ and phosphate. It was originally thought that $H_2PO_4^-$ was required to activate the transport system. Phosphate causes an increase in the net negative charge of the cell membrane (9,14).

Divalent cation transport is dependent on plasma membrane $H^2$-ATPase activity, and a similar affinity sequence of divalent cations for stimulation of the ATPase as for transport has been demonstrated. It also depends on the membrane potential, and substances that depolarise the cell membrane, such as protonophoric uncouplers and high concentration of external $K^+$, inhibit uptake. Divalent cation transport is concentration dependent and frequently shows saturation with increasing external concentration.

For $Zn^{2+}$, an apparent second phase of low-affinity transport coincided with such effects in *Saccharomyces cerevisiae*, which suggested that altered kinetics were a result of increased membrane permeability. In fact, if affinity constants are compared for a range of potentially toxic divalent cations, e.g. $Zn^{2+}$, $Cu^{2+}$, $Cd^{2+}$, $Ni^{2+}$, $Co^{2+}$, it seems that where the concentration range used is high, the apparent affinity of the transport system is low.

Mutual interactions between divalent cations may result in inhibition or stimulation of uptake by additional means, often indirect, such as alteration of membrane permeability due to toxic effects. Although $M^{+}$ ions are able to inhibit $M^{2+}$ uptake, e.g. >100 mM $K^{+}$ inhibits the uptake of $Mg^{2+}$ (1.1 mM), their effect on $\psi$ is complicated because monovalent cations can also depolarise the cell membrane.

The internal environment of the cell does not reflect the external environment, with an exclusive preference for a high relative internal concentration of $K^{+}$ in preference to $Na^{+}$ and $Mg^{+}$ in preference to $Ca^{2+}$. Under the usual conditions, variation in external $K^{+}$ between 0.4 and 10 mM has no effect on internal $K^{+}$ levels, indicating the efficiency of the monovalent transport / antiport system (8,12,14,16).

### 3.2.2.1. Magnesium

Magnesium is essential to yeast growth and cellular activities, since it serves as enzyme activator for many enzymes (2). Magnesium is involved in many essential, physiological and biochemical functions in yeast cells, including growth, cell division, and enzyme activation. Yeasts have a very high growth demand for magnesium ions, and magnesium accumulation by yeast correlates closely with the progress of fermentation (17). It is an essential cation in nucleic acid synthesis and is a cofactor of more than 300 enzymes, including hexokinases, phosphofructokinase, phosphoglycerate kinase, pyruvate kinase, andenolase in glycolysis (13). $Mg^{2+}$ ions also required as cofactors for the activity of key glycolytic and alcohologenic enzymes and may also play a regulatory role at the level of pyruvate-metabolizing enzymes when cells are grown respirofermentatively (18).

$Mg^{2+}$ acts to stimulate both the specific growth rate and cell division (replication), while $Co^{2+}$ acts to inhibit growth and replication and exponential phase cells are most sensitive. $Mg^{2+}$ can also act as an antagonist to the effects of excess $Co^{2+}$.

Results obtained show that, by increasing the extracellular availability of magnesium ions, physiological protection may be conferred on temperature and

ethanol-stressed yeast cells with respect to culture viability and growth. Magnesium prevents stress-induced damage to yeast cells by protecting the structural and functional integrity of the plasma membrane (17).

In a study, which investigated the regulation of cellular $Mg^{2+}$ by *Saccharomyces cerevisiae*, the minimal concentration of $Mg^{2+}$ results in optimal growth of the yeast was about 30 μM and a half-maximum growth rate was attained at about 5 μM $Mg^{2+}$ as seen in Figure 3.2 (2).

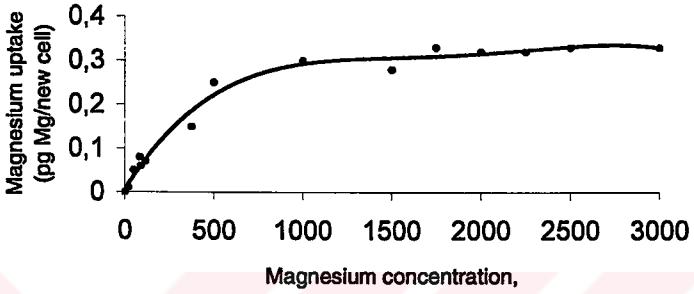



Figure 3.2. Magnesium uptake

### 3.2.2.2. Calcium

Calcium is believed to be non-essential for yeast growth but, the calcium requirements for yeast growth are debatable. The presence of $Ca^{2+}$ in fermentation media may also compete with essential divalent cations like $Mg^{2+}$ and cause growth inhibition at high concentrations (2). On the other hand, calcium are involved in membrane structure and function (21).

$Ca^{2+}$ and $Mg^{2+}$ are competitors / antagonists to the action of each other, and 10 times excess of $Ca^{2+}$ over $Mg^{2+}$ prevents cell growth, while a three times excess increases the lag phase, decreases the specific growth rate, but has little effect on the value of $Y_{x/x}$ (2,14).

In Figure 3.3, working model for $Ca^{2+}$ flow in growing yeast cells was illustrated (16).

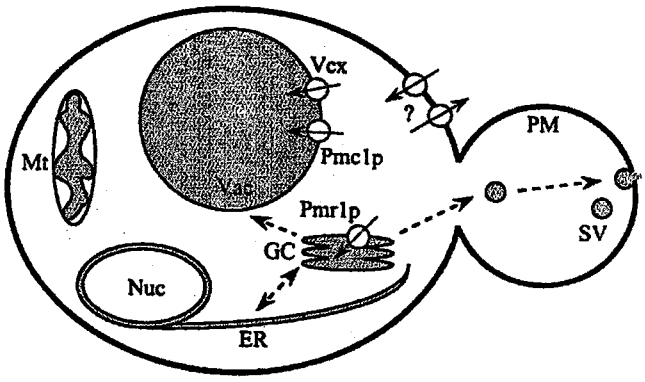



Figure 3.3. Working model for $Ca^{2+}$ flow in *Saccharomyces cerevisiae*.

*Solid arrows indicate $Ca^{2+}$ movement through membranes catalyzed by putative channels, pumps and the vacuolar $H^+/Ca^{2+}$ antiporte. Dashed arrows represent vesicle-mediated trafficking of proteins and presumed paths of $Ca^{2+}$ flow. Vac, vacuole; Mt, mitochondria; Nuc, nucleus; ER, endoplasmic reticulum; GC, golgi complex; SV, secretory vesicles; PM, plasma membrane*

### 3.2.2.3. Copper

Copper is an essential metal ion to many living organisms, including mammals, as it mediates a wide variety of important biochemical process. At elevated concentrations, copper is extremely toxic to host cells. The trace metal copper is widely used as a cofactor for a variety of metalloenzymes (22). Metallothionein, which is a protein, binds essential metal ions including copper and plays a role in copper sequestration (10). $H^+$ efflux rate and duration all being affected with increasing Cu concentration (5-100 μM) (11).

The electrochemical potential of $Cu^{2+}$ / $Cu^+$ is 268 mV, well within the physiological range. Copper easily interacts with radicals, best with molecular oxygen. Its radical character makes copper very toxic, and many organisms are more sensitive to copper than *E. coli*. Copper toxicity is based on the production of hydroperoxide radicals and on interaction with the cell membrane.

The most important function of copper is in the cytochrome c oxidase and related enzymes, which are oxygen-dependent terminal oxidases in the respiratory chain of many organisms. Two copper centres exist in the cytochrome c oxidase and they have different roles in the catalytic cycle. The CuA center is responsible for the uptake of electrons from the soluble cytochrome c which finally reduces molecular oxygen to

water; the resulting energy is used to pump protons across the cytoplasmic membrane (11).

Copper-transporting P-type ATPases have been found in a variety of organisms, in cyanobacteria and in eukaryotes; however, in *Saccharomyces cerevisiae*, the copper P-type ATPase does not transport copper across the cytoplasmic membrane. For uptake into the yeast cell, $Cu^{2+}$ is first reduced by the iron / copper-specific reductases FRE1p, FRE2p and FRE7p to $Cu^{+}$, which is transported into the cell by the CTR1p transporter. CTR1p is a novel protein with two related putative copper transporters (CTR2p, CTR3p) in yeast and a homologue in. In addition, $Cu^{2+}$ is accumulated by the CorA-related transporters ALR1p and ALR2p.

Inside the yeast cell, copper may be bound by various compounds, and a copper-bisglutathionato complex is likely to be formed. The metallothionins of yeast, CUP1p and CRS5p, probably store copper. For synthesis of cytochrome c oxidase, copper is delivered into the mitochondria by COX1p. ATX1p, CCSp and the copper P-type ATPase CCC2p are required for copper insertion into proteins of the trans-Golgi network. In *Saccharomyces cerevisiae*, a MIT system takes up $Cu^{2+}$, while CTRp systems transport $Cu^{+}$, which has been previously reduced by FREp systems. Copper is bound to glutathione (GSH) and metallothioneins (MT's). P-type ATPases transport copper into the trans-Golgi system, and may detoxify copper by efflux in mammalian cells.This is shown in Figure 3.4.

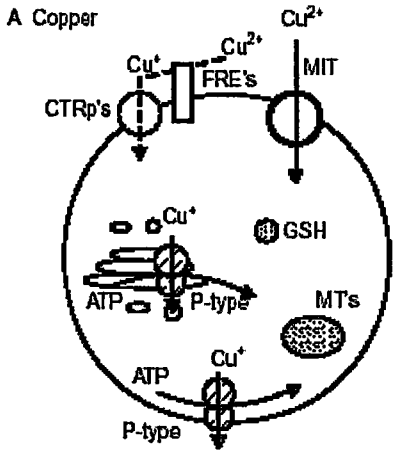



Figure 3.4. Protein families involved in heavy-metal metabolism in yeast.

$Cu^{2+}$ is possibly accumulated by the CorA-$Mg^{2+}$ transporter, and additionally by P-type ATPases under copper starvation (shown in *Enterococcus hirae*). The mechanism of resistance systems similar to the *Pseudomonas* Cop system is still elusive but, in gram-positive bacteria, P-type ATPases seem to detoxify copper via efflux. The copper-resistance systems of the Pseudomonas type usually encode four proteins (circles with A, B, C, or D), which bind copper in the periplasm or close to the outer membrane. This is shown in Figure 3.5.

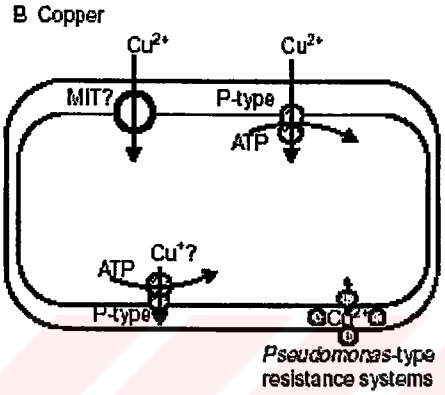



Figure 3.5. Copper accumulation inside the cell

$Cu^{2+}$ (10 ppm) is highly deleterious and reduces the final ethanol concentration. In contrast, the same concentration of $Fe^{2+}$ or $Pb^{2+}$ has no deleterious effect (14).

### 3.2.2.4. Zinc

Zinc is an essential micronutrient in yeast metabolism. It functions as a cofactor of essential enzymes such as alcohol dehydrogenase, lactic dehydrogenase, carbonic anhydrase, carboxypeptidase, glutamic dehydrogenase, lactic dehydrogenase that are directly involved in yeast production (2,13). Addition of $Zn^{2+}$ to the growth medium results in a higher rate of fermentation and protein autolysis (2).

Zinc occurs exclusively as the divalent cation $Zn^{2+}$. With its completely filled d orbitals, the zinc cation is not able to undergo redox changes under biological conditions. It is used to complex polypeptide chains, for example, when redox reactions are not desired, and, as a Lewis base, mainly to activate water.

Three transporter groups contribute to the observed zinc transport by those systems: the CorA MIT transporter transports zinc in *Saccharomyces cerevisiae* and CorA has been shown to be present in archaea and many bacteria but magnesium

transport by CorA was not inhibited by $Zn^{2+}$. A second type of potential chemiosmotically driven transporter forms the MgtE family which also seems to transport zinc. This protein is present in *Providencia stuartii* and a few other gram-negative and gram-positive bacteria; however, it is not as broadly distributed as CorA.

A moderate excess of $Zn^{2+}$ or $Pb^{2+}$ does not affect the rate of fermentation, although $Cd^{2+}$ and $Hg^{2+}$ are deleterious. $Zn^{2+}$ is able to reverse the effect of a moderate excess of $Cd^{2+}$, although high levels of $Zn^{2+}$ potentiate the effects of $Hg^{2+}$ and $Cd^{2+}$ (14).

In a study, it was found that zinc concentrations do not affect cell growth, but rather affect the specific rate of fermentation (21).

### 3.2.2.5. Other elements

Yeast cells are relatively impermeable to bivalent cations, but elements such as $Ba^{2+}$, $Zn^{2+}$, $Mn^{2+}$, $Sr^{2+}$, $Ni^{2+}$, $Co^{2+}$, $Fe^{2+}$ and $Cu^{2+}$, can equilibrate rapidly with the cell surface by combining with its phosphate and carboxyl groups. The binding of exogenous bivalent cations is rapid and reversible. Like $Mg^{2+}$ and $Ca^{2+}$, transport of these bivalent cations into yeast cells is mediated by an active transport system having the following affinities;

$$Mg^{2+} > Co^{2+} > Zn^{2+} > Mn^{2+} > Ni^{2+} > Ca^{2+} > Sr^{2+}$$

Among these trace elements *Saccharomyces cerevisiae* requires;
200 µg of $Zn^{2+}$
75 µg of $Fe^{2+}$
12-15 µg $Cu^{2+}$ per liter of medium for optimum growth (1).

High concentrations of $Cd^{2+}$, $Cu^{2+}$, or $Zn^{2+}$ and other metal ions can be toxic and induce the leakage of UV-absorbing metals and $K^+$ from yeast cells, rupture of site-specific lesions in the plasma membrane being the postulated mechanism for these ions

Potassium plays a central role in the regulation of yeast growth and fermentation under both aerobic and fermentative conditions. For example, during fermentation in the presence of dinitrophenol (DNP), high concentrations of $K^+$ inhibit the secretion of the by-product succinate, under aerobic conditions, a deficiency in $K^+$ (i.e. around 500 µM)

promotes ethanol production due to the resulting energetic demands for its uptake against a very high concentration gradient (14).

Phosphorus plays a central role in both energy metabolism and in the biosynthesis of membrane phospholipids. Intracellular concentrations of free $H_2PO_4^-$ usually range from 3 to 10 mM.

Phosphorus deficiency limits ethanol production, as does its excess. Molasses is typically deficient in phosphorus and hence usually requires supplementation. Uptake of phosphate and that of $K^+$, $Mg^{2+}$, and $Ca^{2+}$ are also linked, and the requirement for phosphorus will partly be determined by the concentrations of these species (14).

Magnesium and calcium are taken up by an active transport system at the expense of ATP. $Mg^{2+}$ was transferred into yeast cells by the same carrier as that for $K^+$ ions and that the uptake involved $H^+$ secretion. The activity of this carrier is dependent on the presence of oxygen.

The function of trace elements, in general, is as catalysts or activators in enzyme or vitamin systems.

# CHAPTER IV

# MATERIALS AND METHODS

## 4.1. Materials

### 4.1.1. Microorganism and Fermentation Samples

The yeast used in this study was an industrial strain of baker's yeast "*Saccharomyces cerevisiae*" provided by Pakmaya Inc.(Baker's yeast producer), İzmir, Turkey. Fermentation samples were also collected from Pakmaya periodically.

### 4.1.2. Chemicals

In the experimental study, ICP-AES grade 1000 ppm Calcium, Magnesium, Copper, Zinc and multielement stock solutions in 5% $HNO_3$ were used as standards. The fermentation samples were digested with nitric acid, hydrochloric acid, sulfuric acid and hydrogen peroxide. The chemicals used in this study are given in Table 4.1.

Table 4.1. The chemicals and their properties

| Chemical | Purity and properties | Producer |
|---|---|---|
| | | Merck |
| Nitric acid ($HNO_3$) | 65%, d=1.40 g/cm$^3$ | Merck |
| Hydrochloric acid (HCl) | 37%, d=1.19 g/cm$^3$ | Merck |
| Sulfuric acid ($H_2SO_4$) | | Merck |
| Hydrogen peroxide ($H_2O_2$) | 30% | Merck |
| Calcium (in 5%$HNO_3$) | ICP standard solution | Merck, Fluka |
| Magnesium (in 5%$HNO_3$) | ICP standard solution | Merck, Fluka |
| Copper (in 5%$HNO_3$) | ICP standard solution | Merck, Fluka |
| Zinc (in 5%$HNO_3$) | ICP standard solution | Merck, Fluka |
| $MgSO_4.7H_2O$ | | Merck |
| $ZnSO_4.7H_2O$ | | Merck |
| $CuSO_4.5H_2O$ | | Merck |
| Deionized water (WaterPro PS, model 90007-05) | 18.1 megaohm | Labconco |

## 4.2. ICP-AES Operating Conditions

The concentrations of elements in the fermentation samples were determined by Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES, Varian Liberty Series II, Axial

). The instrument operating conditions of ICP-AES are described in Table 4.2.

Table 4.2. ICP-AES operating conditions

| | |
|---|---|
| Argon gas flow | 15 L/min |
| Argon auxiliary flow | 1.50 L/min |
| PMT voltage | 650 V |
| Sample uptake | 30 sec |
| Rinse time | 10 sec |
| Spray chamber | Glass Cyclonic |
| Nebulizer | Glass Nebulizer |

Working conditions in ICP-AES were set up for the best performance and reliable results. Selected wavelengths are shown in Table 4.3 (28);

Table 4.3. Selected wavelengths for all samples

| **Elements** | **Wavelengths (nm)** |
|---|---|
| Ca | 317.933 |
| Mg | 279.079 |
| Cu | 324.754 |
| Zn | 213.856 |

In Appendix E, the standard peaks of $Ca^{2+}Mg^{2+}$,$Cu^{2+}$ and $Zn^{2+}$ ions at selected wavelengths are illustrated.

### 4.3. Digestion Methods

Although there are many different methods in literature for the digestion of samples prior to ICP-AES analysis, it is difficult to find a specific method of digestion for baker's yeast fermentation samples. Most of the methods are related to atomic absorption analysis of food materials.

In this study, samples were taken from the industrial and lab-scale fermentors. The samples were the raw materials entering the fermentors and the products. These samples were digested by FCL (The Food Composition Laboratory) method (30). Modified FCL method and simple digestion methods were also applied before ICP-AES analysis.

Filtered water samples were analyzed for Ca, Cu, Mg and Zn ion concentrations by ICP-AES according to the AOAC 993.14 method. $HNO_3$ concentration in water samples was adjusted to 1 % of solution (23).

Polypropylene volumetric flasks were used to prepare the solutions and all the plastic and glassware were soaked in 1:5 nitric acid solution for 24 hours, rinsed thoroughly with deionized water and dried in an oven at 70-80 °C (23,26).

#### 4.3.1. FCL Method

In literature, there was not a direct method for determining the elemental composition of molasses. Hence a method suggested by FCL for the determination of elements in food and biological samples by ICP-AES, was used (30). FCL (The Food Composition Laboratory) is in Beltsville Human Nutrition Research Center, US. Department of Agriculture, which is involved in the development of quality control procedures and commercial reference materials used in cooperation with National Institute of Standards and Technology (NIST), the International Atomic Energy Agency (IAEA), the National Research Council of Canada (NRCC), and Agriculture Canada.

As it is shown in Figure 4.1, 0.5-2 g of homogenized sample was placed into acid-cleaned tubes and 1 ml of concentrated nitric acid was added with 1-2 ml of 18 MΩ

deionized distilled water. Test tubes were placed in a water bath and heated at 80°C overnight. The next day the digests were treated with 1 mL of 30 % hydrogen peroxide added dropwise and heated at 100 °C for several hours, repeating the peroxide treatment until sample digests were clear. Digests were subsequently heated overnight at 80 °C and then 1 mL of HCl was added and they were heated for 3-4 h more. Sample digests were than filtered using ashless 7 cm No.41 filter paper (Whatman, Maidstone, England) and they were diluted to a final volume. Sample preparation blanks were analysed with each batch of samples and all data were blank corrected. Triplicate sample preparations of each sample were analysed to determine the elemental composition (30).

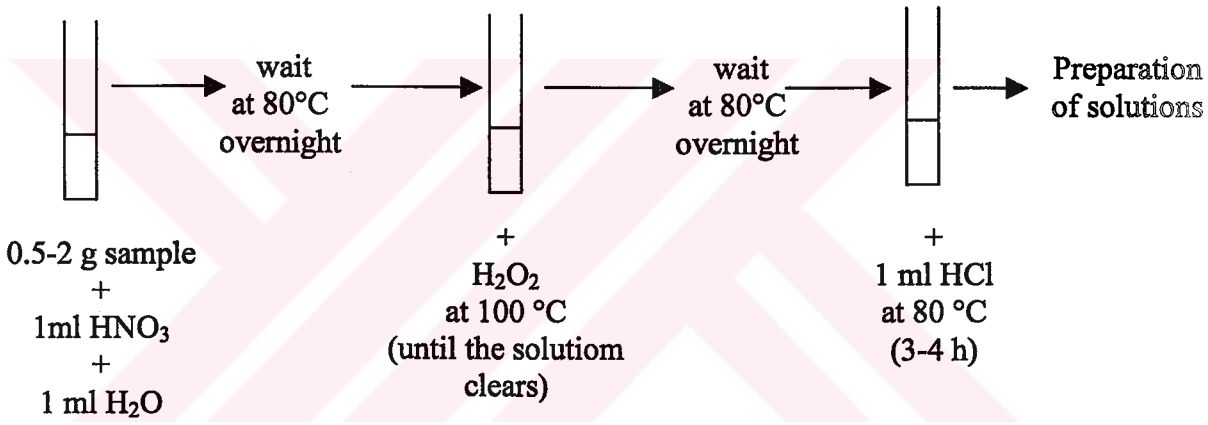



Figure 4.1. Schematic representation of FCL method

### 4.3.2. Modified FCL Method

As it is seen in Figure 4.1, 3 days are required to digest any sample with the FCL method. Therefore, the FCL method was modified to shorten the experimental time. 0.5-2 g molasses or dry yeast samples were weighed, 1 ml $HNO_3$ and 1 ml deionized $H_2O$ were added and test tubes were placed in a water bath and heated at 80°C for several hours. $H_2O_2$ was added at 100 °C until the solutions were clear. After peroxide addition the test tubes were used in the preparation of solutions without waiting for an extra day. The modified method is represented in Figure 4.2.

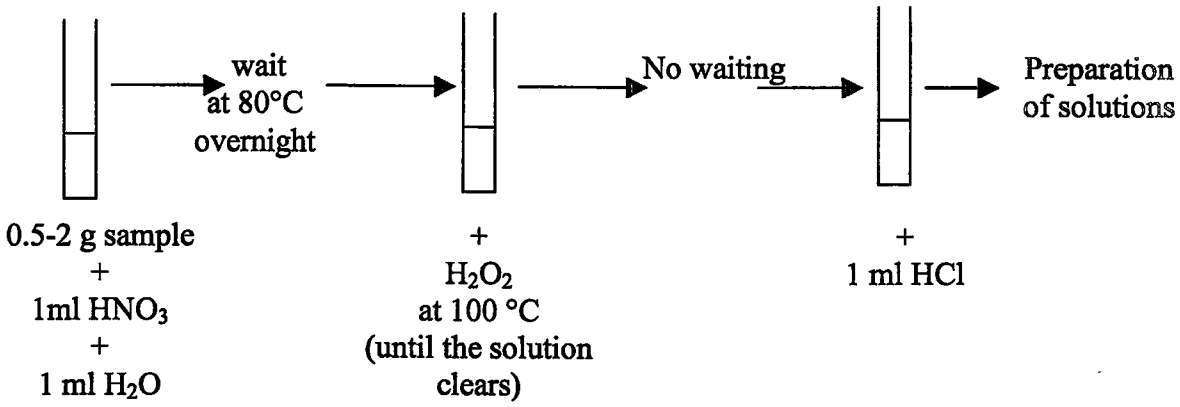



Figure 4.2. Schematic representation of modified FCL method

### 4.3.3. The Simple Digestion Method

In the simple digestion method, 0.5-2 grams of molasses or dry yeast samples were treated with 5 ml 65% $HNO_3$ at room temperature and waited for several hours, completed to 50 ml by adding deionized water. Solutions were then filtered through the Whatman no.41 filter paper (Maidstone, England) and further centrifuged at 10,000 rpm for 10 minutes if still not clear.

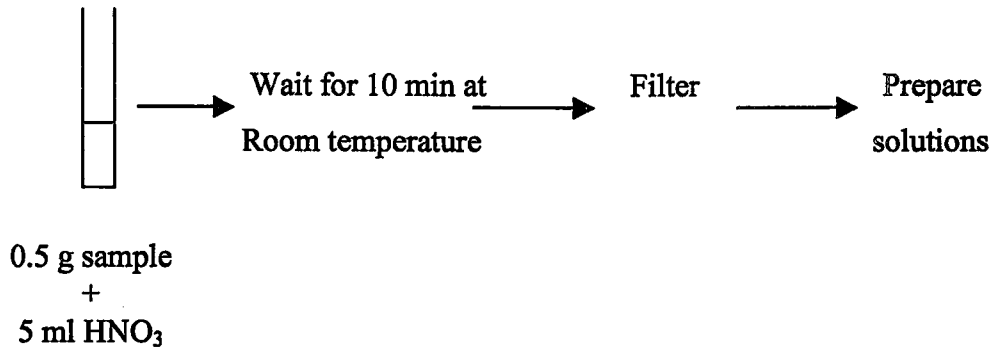



Figure 4.3. Schematic representation of the simple digestion method

## 4.4. Industrial Fermentations

In commercial baker's yeast production, fermentations are done in 100 tonnes fed-batch reactors for 17 h at 30 °C.

To determine the effect of $Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ concentrations in a commercial baker's yeast fermentation process, samples were taken from all of the different raw materials entering the fermentor, products (yeast milk and compressed yeast) and disposal stream. Sampling points are illustrated in Figure 4.4.

Samples taken during the fermentation period were acidified first and then filtered to separate yeast cells, referred to as biomass; the fermentation medium, referred to as filtrate. The coding used to label the samples are given in Table 4.4.

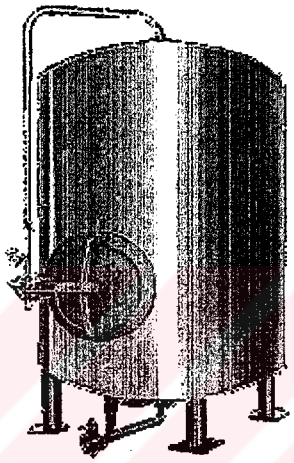
Table 4.4. Samples taken during industrial fermentations

| SAMPLE CODE | SAMPLE |
|---|---|
| (M) | **Molasses :** cane molasses or beet molasses contain 45-47 Brix fermentable sugar. |
| (SW) | **Supplemented Water:** process water contains urea, vitamins, magnesium sulfate, copper sulfate and zinc sulfate. |
| (SY) | **Seed Yeast:** cream yeast that is used to start fermentation; fed to the system from another tank. |
| (F0) | **Fermentation Medium:** obtained at $0^{th}$ hour. |
| (F0a) | **Biomass:** obtained at 0 hour after filtration of the fermentation medium. |
| (F4a) | **Biomass:** obtained at 4 hour after filtration of the fermentation medium. |
| (F8a) | **Biomass:** obtained at 8 hour after filtration of the fermentation medium. |
| (F12a) | **Biomass:** obtained at 12 hour after filtration of the fermentation medium. |
| (F17a) | **Biomass:** obtained at 17 hour after filtration of the fermentation medium. |
| (F0b) | **Filtrate:** obtained at 0 hour after filtration of the fermentation medium. |
| (F4b) | **Filtrate:** obtained at 4 hour after filtration of the fermentation medium. |
| (F8b) | **Filtrate:** obtained at 8 hour after filtration of the fermentation medium. |
| (F12b) | **Filtrate:** obtained at 12 hour after filtration of the fermentation medium. |
| (F17b) | **Filtrate:** obtained at 17 hour after filtration of the fermentation medium. |
| (D) | **Disposal**: collected from three points and mixed. |
| (YM) | **Yeast Milk:** viscous yeast cream after the separation. |
| (CY) | **Compressed Yeast :** dry matter content is 28-32 % . |

**MOLASSES (M)**
$Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$, $Zn^{2+}$

**SUPPLEMENTED WATER (SW)**
$Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$, $Zn^{2+}$

**SEED YEAST (SY)**
$Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$, $Zn^{2+}$



**FERMENTATION**
**Sampling Intervals**

| | | |
|---|---|---|
| (F0) | 0.h | F0a, F0b |
| (F4) | 4.h | F4a, F4b |
| (F8) | 8.h | F8a, F8b |
| (F12) | 12.h | F12a, F12b |
| (F17) | 17.h | F17a, F17b |

$Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$, $Zn^{2+}$

**SEPARATOR**

**DISPOSAL (D)**
$Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$, $Zn^{2+}$

**YEAST MILK (YM)**
$Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$, $Zn^{2+}$

**DISPOSAL (D)**
$Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$, $Zn^{2+}$

**COMPRESSED YEAST (CY)**
$Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$, $Zn^{2+}$

Figure 4.4. Sampling points in industrial process

The sampling was done as stated below:

**1.** Water samples were acidifed with 65 %$HNO_3$ until pH<2 (23).

**2.** Fermentation liquids collected from the fermentation tank were first acidified with 65 %$HNO_3$ until pH<2.

**3.** Samples were filtered with a vacuum pump. Whatman no:41 filter paper was used. Volumes of the filtrate and the weights of the yeast cakes were measured. Dry matter content of yeast cakes were determined with the Sartorius moisture analyzer M140.

**4.** Filtrates were stored at +4°C in the refrigerator.

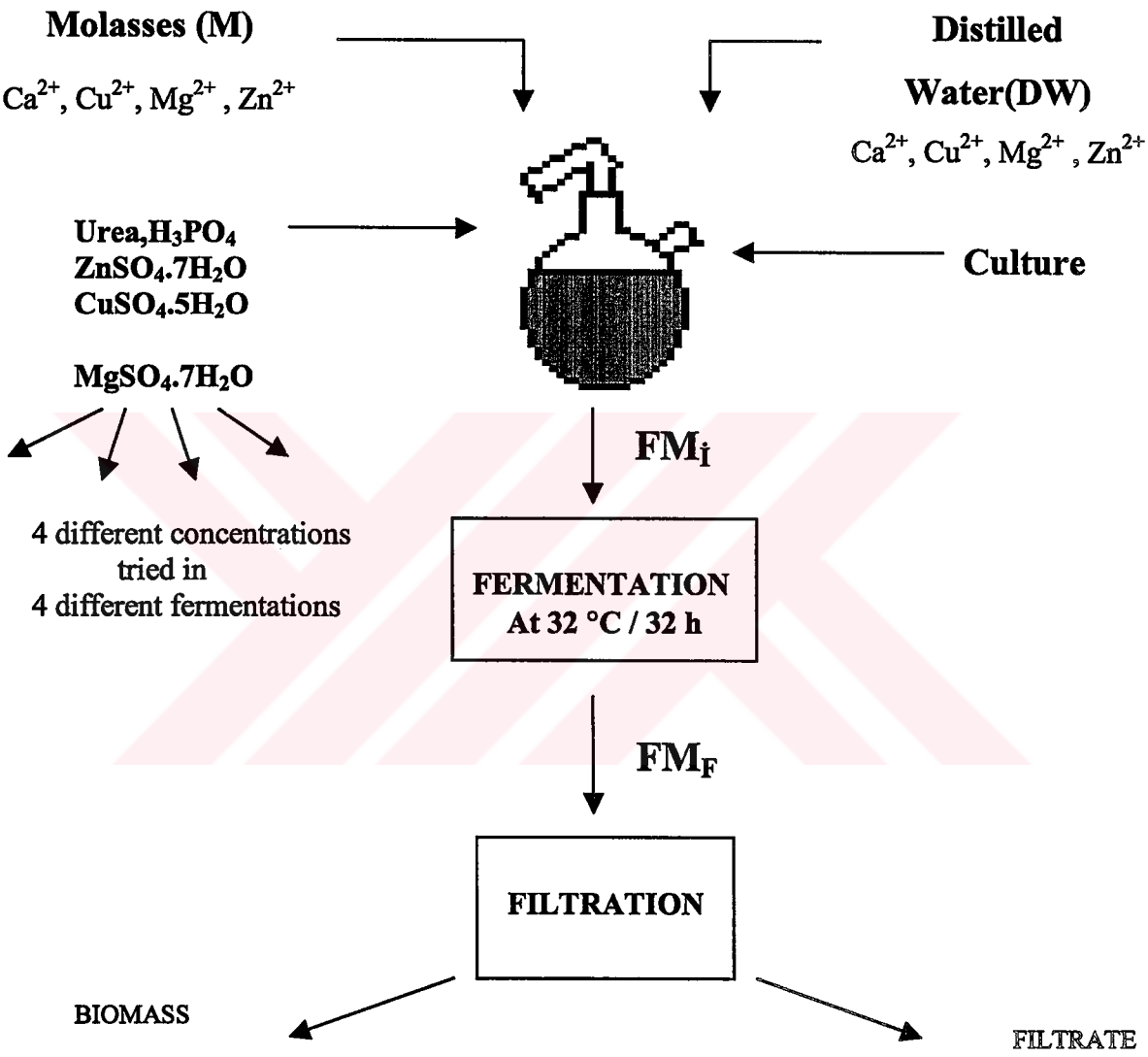
**5.** Biomasses were stored below 0 °C in the deepfreezer.

### 4.5. Lab-Scale Fermentations

A fermentor with a volume of 8.5 L was used in lab-scale fermentations. Fermentation medium contained 2 l, 45 brix molasses, 6.5 l water and urea, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$ and $MgSO_4.7H_2O$. After sterilization of the fermentor and the substrate at 121 °C / 15 min, the fermentor was inoculated with pure culture.
Four different lab-scale fermentations with different $Mg^{2+}$ concentrations were done. $Mg^{2+}$ was added to the lab-scale fermenter as $MgSO_47H_2O$. The different concentrations of $Mg^{2+}$ in each fermentation is shown in Table 4.5. Experimental procedure is shown in Figure 4.5.

Table 4.5. Magnesium concentrations in lab-scale fermentations

| | **$MgSO_4.7H_2O$ (g/l)** | **Mg (g/l)** |
|---|---|---|
| **Study 1** | 0.5 | 0.05 |
| **Study 2** | 0 | 0 |
| **Study 3** | 0.25 | 0.025 |
| **Study 4** | 1 | 0.1 |




**$FM_I$:** Fermentation medium after all the ingredients were added

**$FM_F$:** Fermentation medium at the end of the fermentation

Figure 4.5. Experimental procedure of lab-scale fermentations

The samples collected during lab-scale studies were molasses (M), distilled water (DW), fermentation medium after all the ingredients were added (FMi) and fermentation medium at the end of the fermentation ($FM_F$). All the samples except molasses were acid treated after collection for sample storage. Samples taken at the end of the fermentations were filtered and dry matter percentages of the filtered biomasses were analysed by Sartorius moisture analyzer M140.

### 4.6. Calibration Curves

Standard solutions of each element at the suitable concentration ranges were prepared in deionized water and introduced to ICP-AES for calibration. All the standard curves obtained had high correlation coefficients. These standard curves are shown in Figures 4.6, 4.7, 4.8, 4.9 for $Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2+}$, respectively.

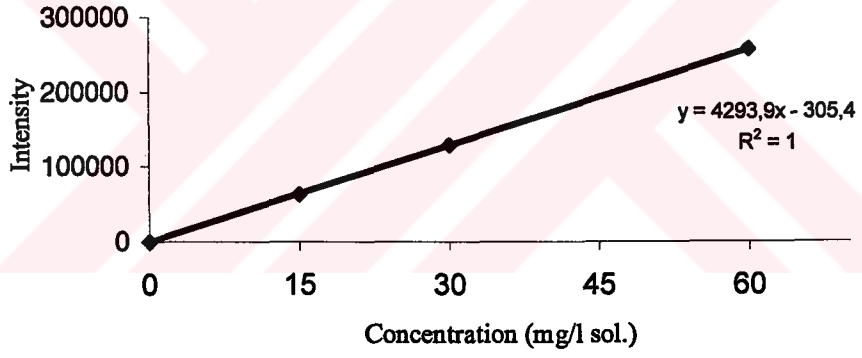



Figure 4.6. Calcium calibration curve

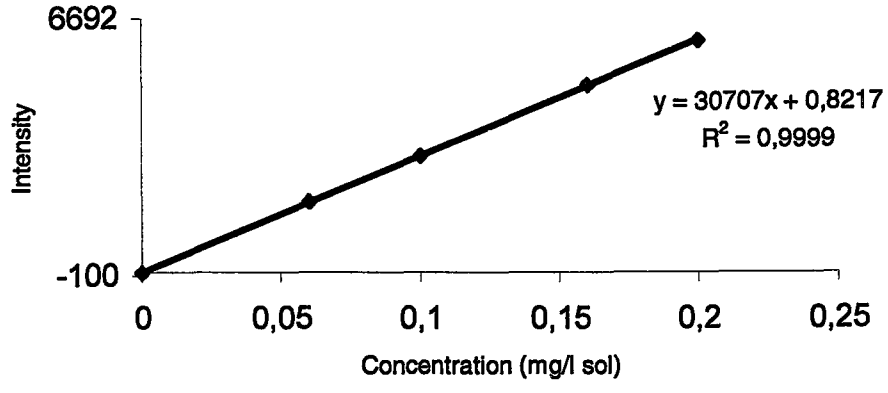



Figure 4.7. Copper calibration curve

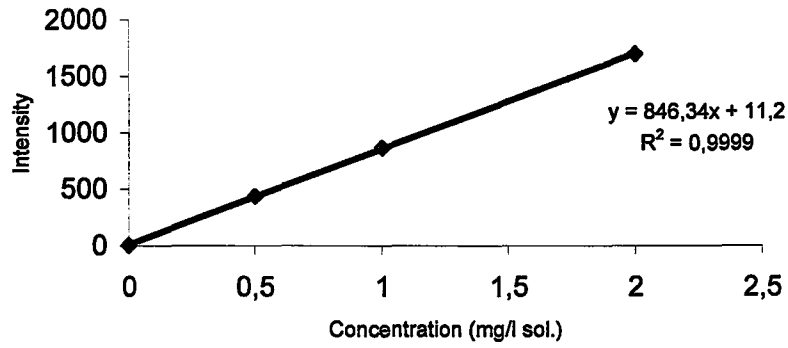



Figure 4.8. Magnesium calibration curve

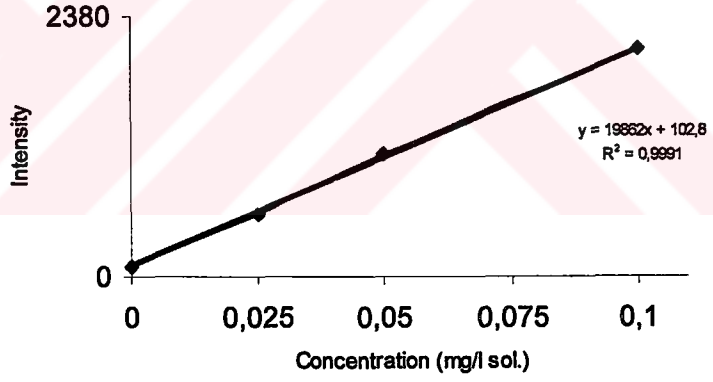



Figure 4.9. Zinc calibration curve

# CHAPTER V

# RESULTS AND DISCUSSION

## 5.1. Evaluation and Verification of the Digestion Methods for ICP-AES Measurements

In this study, the matrices of the samples were different and the concentration of $Cu^{2+}$and $Zn^{2+}$ were very low. Hence, a proper digestion method was an obligation for correct measurements by ICP-AES. The different digestion methods were evaluated on precision and accuracy. The most precise and accurate method was chosen to determine the concentrations of $Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ in molasses, yeast and fermentation samples.

### 5.1.1. Preparation of the Standard Curves

The simple digestion method was used in the preparation of standard curves. Different concentrations of standard solutions of $Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ ions were added to 45 brix molasses samples, and standard curves prepared are illustrated in Figures 5.1, 5.2, 5.3 and 5.4, respectively.

The standard curves of $Ca^{2+}$ and $Mg^{2+}$ ions were linear up to 150 ppm concentration. Since standard calcium ion solution was added to molasses sample, the intercept on the x-axis is the amount of $Ca^{2+}$ ion present in molasses sample.

The standard curve of $Cu^{2+}$ ion was linear up to 100 ppm concentration. The standard curve of $Zn^{2+}$ ion was not linear at concentrations higher than 30 ppm.

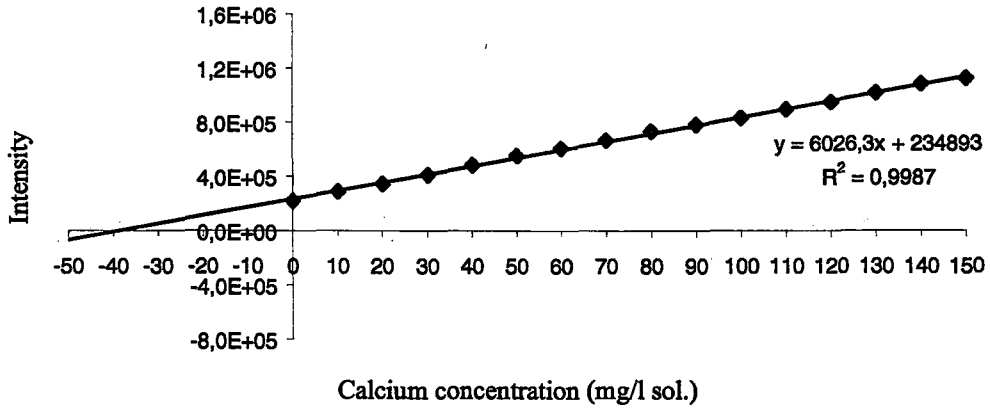



Figure 5.1. Calcium standard curve

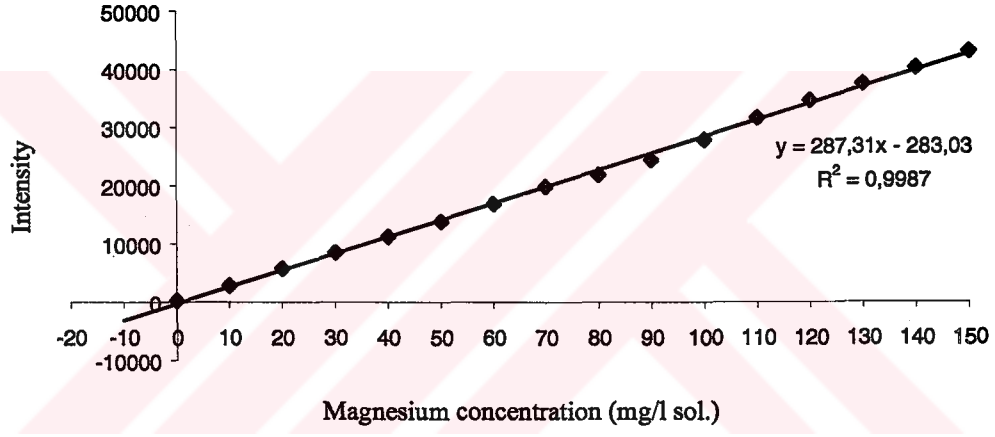



Figure 5.2. Magnesium standard curve

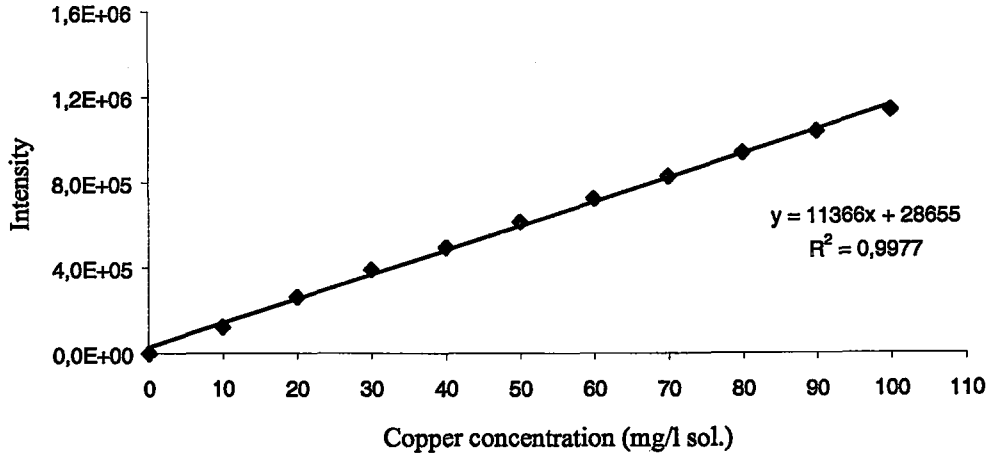



Figure 5.3. Copper standard curve

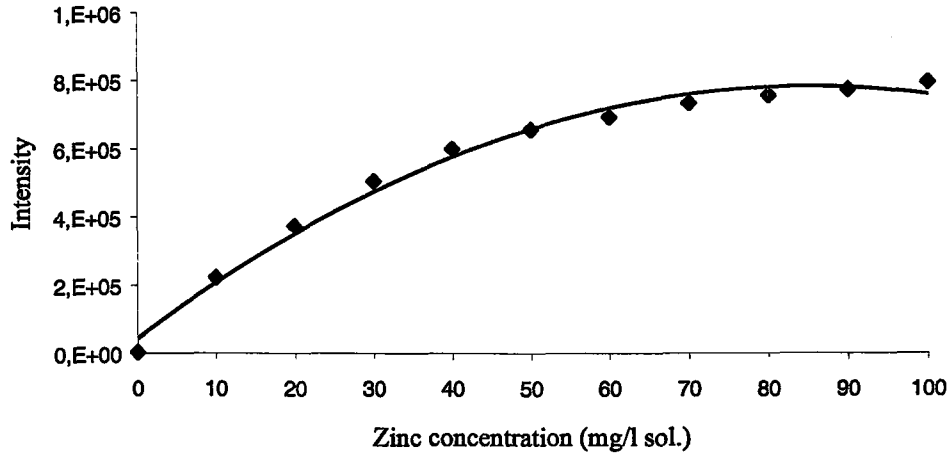



Figure 5.4. Zinc standard curve

### 5.1.2. Preparation of Standard Addition Curves

Initially 5 and 10 ppm concentrations of standard solutions of each element were added to dry yeast and 45 brix molasses samples to observe roughly the concentrations of $Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ in these samples. The data obtained was used to determine the concentrations of each element in the standard addition curves of yeast and molasses samples.

The concentrations of elements used to construct the standard addition curves of elements in dry yeast and molasses samples are given in Table 5.1 and 5.2, respectively.

Table 5.1. Standard additions for dry yeast samples

| Elements | Ca | Mg | Cu | Zn |
|---|---|---|---|---|
| Addition1 | 5 ppm | 5 ppm | 0.05 ppm | 0.25 ppm |
| Addition2 | 10 ppm | 10 ppm | 0.1 ppm | 0.5 ppm |
| Addition3 | 20 ppm | 20 ppm | 0.2 ppm | 1 ppm |

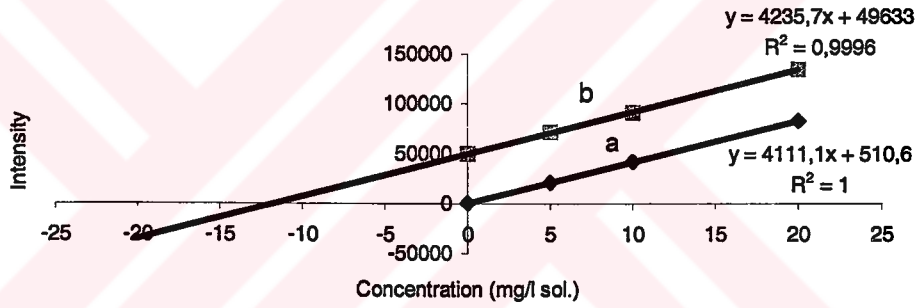
Table 5.2. Standard additions for molasses samples

| Elements | Ca | Mg | Cu | Zn |
|---|---|---|---|---|
| Addition1 | 15 ppm | 0.5 ppm | 0.05 ppm | 0.025 ppm |
| Addition2 | 30 ppm | 1 ppm | 0.1 ppm | 0.05 ppm |
| Addition3 | 60 ppm | 2 ppm | 0.2 ppm | 0.1 ppm |

### 5.1.3. Comparison of Standard Curves to Standard Addition Curves

The standard addition curves of $Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ ions prepared with dry yeast sample were compared to the standard addition curves of the same ions prepared with deionized water. These curves are illustrated in Figures 5.5, 5.6, 5.7 and 5.8.

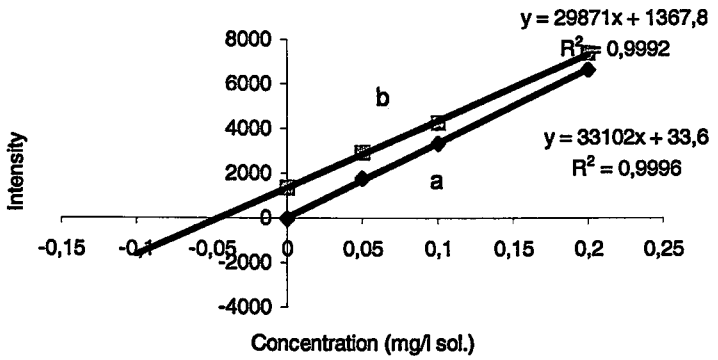
The standard addition curves prepared in dry yeast matrix and standard curves of the same elements prepared in deionized water matrix have nearly the same slope. A high correlation coefficient was observed for each curve. FCL method was used to prepare the standard addition curves of $Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ ions in dry yeast and molasses samples.




a: Standard addition curve of $Ca^{2+}$ in deionized water matrix

b: Standard addition curve of $Ca^{2+}$ in dry yeast matrix

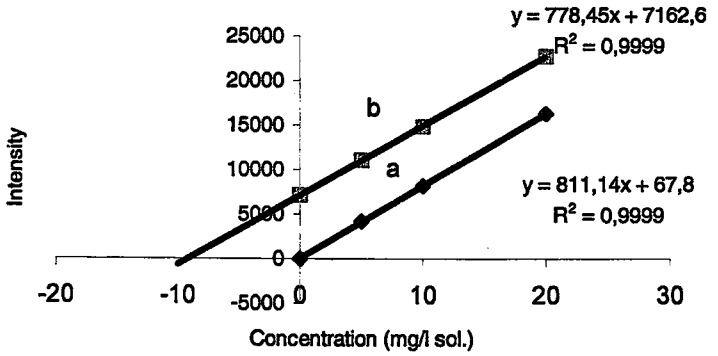
Figure 5.5. $Ca^{2+}$ standard addition curve of dry yeast




a: Standard addition curve of $Cu^{2+}$ in deionized water matrix

b: Standard addition curve of $Cu^{2+}$ in dry yeast matrix

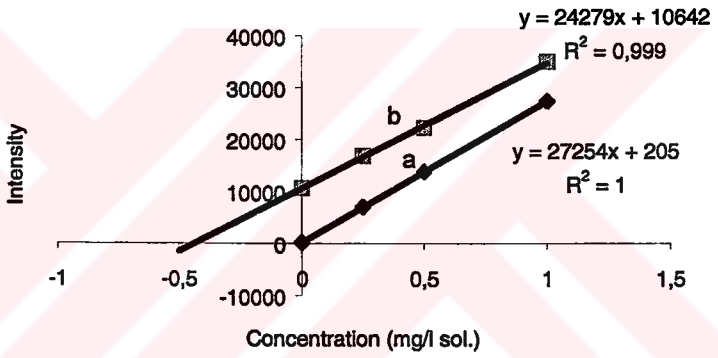
Figure 5.6. $Cu^{2+}$ standard addition curve of dry yeast




a: Standard addition curve of $Mg^{2+}$ in deionized water matrix

b: Standard addition curve of $Mg^{2+}$ in dry yeast matrix

Figure 5.7. $Mg^{2+}$ standard addition curve of dry yeast

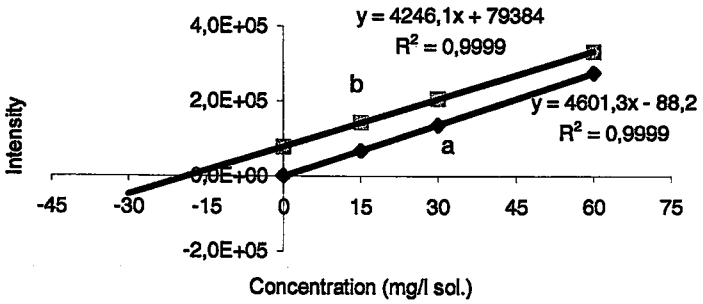



a: Standard addition curve of $Zn^{2+}$ in deionized water matrix

b: Standard addition curve of $Zn^{2+}$ in dry yeast matrix

Figure 5.8. $Zn^{2+}$ standard addition curve of dry yeast

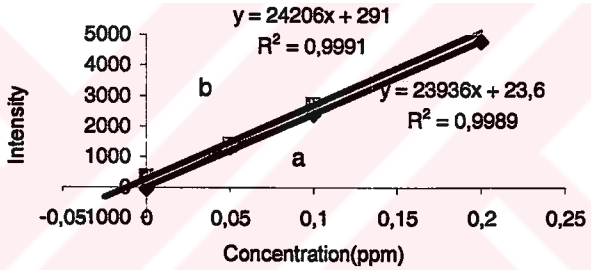
Standard addition curves of elements $Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ prepared in molasses matrix have nearly the same slope as the standard curves of the same elements in pure water matrix. These curves indicate that FCL method can be used to determine the concentration of these elements in molasses samples The standard addition curves of $Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ are shown in Figures 5.9, 5.10, 5.11 and 5.12, respectively.




a: Standard addition curve of $Ca^{2+}$ in deionized water matrix

b: Standard addition curve of $Ca^{2+}$ in molasses matrix

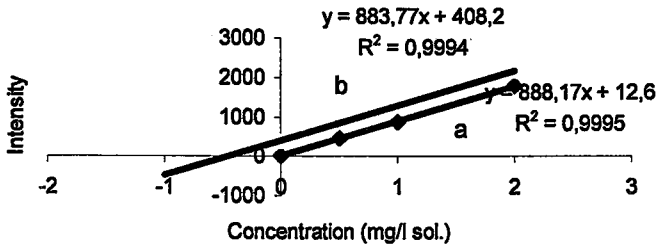
Figure 5.9. $Ca^{2+}$ standard addition curve of molasses




a: Standard addition curve of $Cu^{2+}$ in deionized water matrix

b: Standard addition curve of $Cu^{2+}$ in molasses matrix

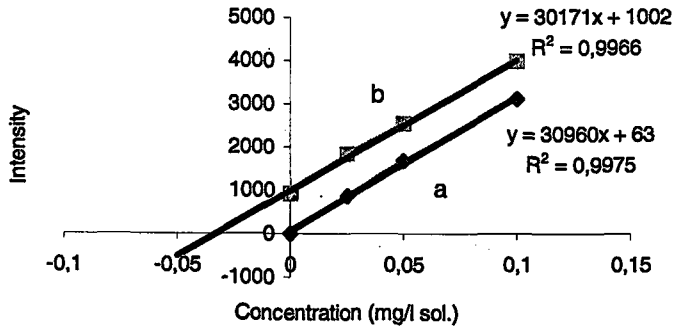
Figure 5.10. $Cu^{2+}$ standard addition curve of molasses




a: Standard addition curve of $Mg^{2+}$ in deionized water matrix

b: Standard addition curve of $Mg^{2+}$ in molasses matrix

Figure 5.11. $Mg^{2+}$ standard addition curve of molasses




a: Standard addition curve of $Zn^{2+}$ in deionized water matrix

b: Standard addition curve of $Zn^{2+}$ in molasses matrix

Figure 5.12. $Zn^{2+}$ standard addition curve of molasses

For both molasses and the dry yeast samples, FCL method gave precise results. This method can be applied to fermentation samples since fermentation samples consist of molasses and yeast cells.

### 5.1.4. Calculation of Spike Recovery and Standard Deviation Values

Recovery of the standard added should be either between 95% and 105% or within established control limits of ±2 standard deviations around the mean (28). Since there are matrix effects of yeast and molasses samples, the spike recoveries obtained in this study can not conform to the above statement. In Table 5.3, spike recovery percentages and standard deviations are shown for 3 standard additions of 5 parallel experiments.

Table 5.3. Spike recovery values of the FCL method:

| **Dry Yeast** | **Ca (spike rec.%)** | **Cu (spike rec.%)** | **Mg (spike rec.%)** | **Zn (spike rec.%)** |
|---|---|---|---|---|
| Addition 1 | 99.1 ± 2.06 | 92.9 ± 1.28 | 96.3 ± 0.80 | 92.2 ± 3.11 |
| Addition 2 | 99.4 ± 3.14 | 89.5 ± 1.89 | 96.4 ± 1.10 | 89.7 ± 2.22 |
| Addition 3 | 102.3 ± 0.62 | 93.4 ± 0.65 | 100.5 ± 1.03 | 88.8 ± 2.22 |
| **Mean** | **100.3 %** | **91.9 %** | **97.7 %** | **90.2 %** |
| **Molasses** | **Ca (spike rec.%)** | **Cu (spike rec.%)** | **Mg (spike rec.%)** | **Zn (spike rec.%)** |
| Addition 1 | 96.1 ± 1.49 | 90.5 ± 9.83 | 96.1 ± 2.77 | 104.6 ± 10.46 |
| Addition 2 | 95.9 ± 2.78 | 78.5 ± 2.02 | 94.6 ± 1.86 | 92.7 ± 7.50 |
| Addition 3 | 97.3 ± 0.58 | 92.9 ± 7.54 | 102.5 ± 2.20 | 99.9 ± 9.10 |
| **Mean** | **96.4 %** | **87.3 %** | **97.7 %** | **99.0 %** |

As seen in Table 5.3, recoveries were very high and nearly within the mentioned range above. The concentration values of zinc and copper were lower than calcium and magnesium and almost all standard deviation values were less than 10 units.

Spike recovery percentages and standard deviation values were also calculated for 3 different additions of 5 parallel molasses and dry yeast samples prepared by the simple digestion method. Although the standard deviations were low, recovery percentages of elements were also low compared to the FCL method. Especially copper recoveries in molasses samples were very low as seen in Table 5.4. It was concluded that simple method was not suitable for the samples in this study.

Table 5.4. Spike Recovery Values of the Simple Digestion Method:

| **Dry Yeast** | **Ca (spike rec.%)** | **Cu (spike rec.%)** | **Mg (spike rec.%)** | **Zn (spike rec.%)** |
|---|---|---|---|---|
| Addition 1 | 93.5 ± 1.66 | 91.5 ± 2.86 | 90.3 ± 2.45 | 91.4 ± 2.06 |
| Addition 2 | 91.1 ± 3.99 | 92.3 ± 3.64 | 86.8 ± 3.28 | 88.6 ± 2.60 |
| Addition 3 | 91.4 ± 3.11 | 91.0 ± 1.65 | 92.1 ± 6.50 | 91.4 ± 3.50 |
| **Mean** | **92.0 %** | **91.6 %** | **89.7 %** | **90.5 %** |
| **Molasses** | **Ca (spike rec.%)** | **Cu (spike rec.%)** | **Mg (spike rec.%)** | **Zn (spike rec.%)** |
| Addition 1 | 92.0 ± 1.60 | 65.9 ± 2.74 | 88.0 ± 3.01 | 92.7 ± 3.46 |
| Addition 2 | 90.8 ± 1.61 | 62.6 ± 2.52 | 84.7 ± 3.15 | 90.8 ± 2.38 |
| Addition 3 | 96.7 ± 5.97 | 70.3 ± 3.95 | 99.4 ± 8.09 | 86.4 ± 2.45 |
| **Mean** | **93.2 %** | **66.3 %** | **90.7 %** | **90.0 %** |

All the data related to the FCL method and simple digestion method are in Appendix A.

## 5.2. Evaluation of Industrial Fermentation

### 5.2.1. Production of Biomass

Commercial baker's yeast is produced in a multiple-stage process. The early stages are batch fermentations in which all ingredients are in the fermentor before yeast inoculum.

This study was performed on fed-batch fermentation stages. During 17 hours of fermentation in 2 industrial fermentors (Fer-1 and Fer-2), the biomass increases were linear (Figure 5.13 and Figure 5.14). Amount of feed molasses, the capacities of two fermentors and kg biomass produced at each time interval are shown in Table 5.5.

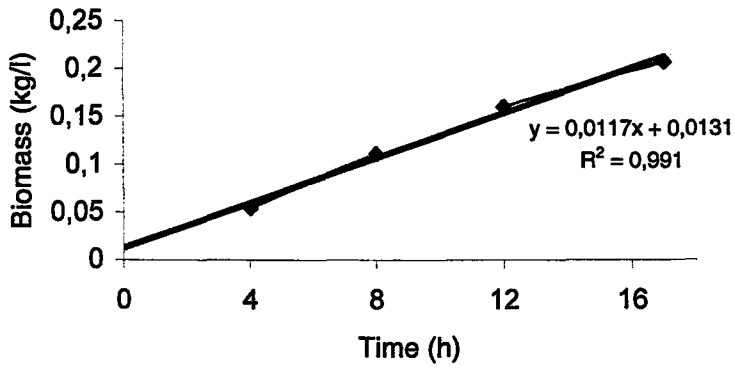



Figure 5.13. Biomass increase during Fer-1

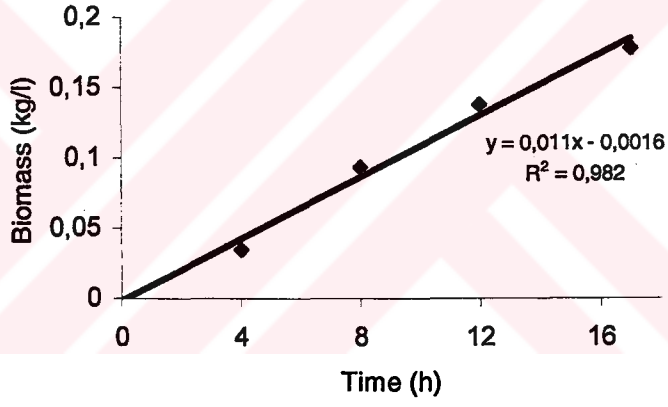



Figure 5.14 Biomass increase during Fer-2

Table 5.5. Obtained biomass and capacities of two fermentors

| Fermentors | Time (h) | Molasses (kg) | Biomass (kg) | Capacity (kg) |
|---|---|---|---|---|
| FER1 | 4 | 5,208 | 5,460 | 107,000 |
| | 8 | 16,492 | 11,132 | |
| | 12 | 30,876 | 16,030 | |
| | 17 | 43,554 | 20,738 | |
| FER2 | 4 | 6,450 | 3,408 | 114,153 |
| | 8 | 17,083 | 9,313 | |
| | 12 | 28,491 | 13,781 | |
| | 17 | 38,473 | 17,853 | |

At the end of the fermentations, 20,738 kg biomass was produced from nearly 43,554 kg of total molasses in Fer-1 and 17,853 kg biomass was produced from 38,473 kg of molasses in Fer-2. It was obtained that 47% of sucrose in molasses was converted to biomass.

### 5.2.2. $Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ Concentrations in the Industrial Fermentation

Triplicate analyses were made for each sample. Related data and standard deviations are in Appendix B and mean values are in Tables 5.6 and 5.7. In Appendix A and B, quantities of materials entering and leaving the fermentors are shown for the two industrial fermentations. The concentrations were calculated in mg element / kg sample.

Table 5.6. Element concentrations in fermentation-1

| Sample Code | Sample | $Ca^{2+}$ mg/kg sample | $Cu^{2+}$ mg/kg sample | $Mg^{2+}$ Mg/kg sample | $Zn^{2+}$ mg/kg sample |
|---|---|---|---|---|---|
| (M) | Molasses | 3480 | 2.8 | 130 | 6.0 |
| (SW) | Suppl.water | 73 | 0.1 | 159 | 0 |
| (SY) | Seed yeast | 606 | 1.8 | 236 | 20 |
| (F0) | 0th h liquid | 220 | 1.5 | 143 | 8.3 |
| (F0a) | 0th h yeast cake | 259 | 5.5 | 428 | 53.5 |
| (F4a) | 4th h yeast cake | 241 | 5.1 | 609 | 56.6 |
| (F8a) | 8th h yeast cake | 386 | 4.5 | 576 | 33 |
| (F12a) | 12th h yeast cake | 553 | 3.3 | 425 | 26.6 |
| (F17a) | 17th h yeast cake | 812 | 2.9 | 374 | 24.5 |
| (F0b) | 0th h filtrate | 131 | 0.6 | 129 | 2.7 |
| (F4b) | 4th h filtrate | 333 | 4.1 | 112 | 0.3 |
| (F8b) | 8th h filtrate | 743 | 0.2 | 83 | 0.3 |
| (F12b) | 12th h filtrate | 1120 | 0.3 | 82 | 0.3 |
| (F17b) | 17th h filtrate | 1454 | 0.4 | 90 | 0.7 |
| (CY) | Comp.yeast | 496 | 2.9 | 360 | 24.2 |
| (D) | Disposal | 1195 | 0.4 | 70 | 0.4 |
| (YM) | Yeast milk | 433 | 1.5 | 212 | 17 |

Table 5.7. Element concentrations in fermentation-2

| Sample Code | Sample | $Ca^{2+}$ mg/kg sample | $Cu^{2+}$ mg/kg sample | $Mg^{2+}$ Mg/kg sample | $Zn^{2+}$ mg/kg sample |
|---|---|---|---|---|---|
| **(M)** | **Molasses** | 311 | 2.8 | 407 | 7.5 |
| **(SW)** | **Suppl.water** | 10 | 1.2 | 144 | 0.1 |
| **(SY)** | **Seed yeast** | 38 | 1.7 | 205 | 26.8 |
| **(F0)** | **0th h liquid** | 18 | 1.5 | 136 | 7.6 |
| **(F4a)** | **4th h yeast cake** | 28 | 7.5 | 661 | 133.3 |
| **(F8a)** | **8th h yeast cake** | 40 | 5.6 | 538 | 61.6 |
| **(F12a)** | **12th h yeast cake** | 55 | 5.4 | 446 | 46.1 |
| **(F17a)** | **17th h yeast cake** | 85 | 3.9 | 424 | 45.5 |
| **(F4b)** | **4th h filtrate** | 25 | 0.4 | 105 | 0.8 |
| **(F8b)** | **8th h filtrate** | 25 | 0.5 | 107 | 0.8 |
| **(F12b)** | **12th h filtrate** | 75 | 0.3 | 126 | 0.5 |
| **(F17b)** | **17th h filtrate** | 94 | 0.4 | 150 | 0.6 |
| **(CY)** | **Comp.yeast** | 89 | 3.5 | 50 | 62.1 |
| **(D)** | **Disposal** | 98 | 0.6 | 156 | 0.4 |
| **(YM)** | **Yeast milk** | 43 | 1.7 | 246 | 25.6 |

The total concentration of the elements entering the system with the incoming materials is nearly the same as their total concentration in fermentation-1. In fermentation-2, the total concentrations of the elements entering and leaving the system have some differences which might have resulted from undetectable changes during the production period of the product.

### 5.2.3. Uptake of Elements During Industrial Fermentations

The amount of $Ca^{2+}$ initially present in the fermentation medium and $Ca^{2+}$ uptake of biomass during fermentation 1 and fermentation 2 are shown in Table 5.8.

The initial concentration of $Ca^{2+}$ in fermentation 1 was approximately 10 times higher than in fermentation 2. This concentration difference resulted from a change in

molasses composition. Therefore, the uptake values of $Ca^{2+}$ by biomass in fermentation 1 was nearly 10 times higher than fermentation 2.

Increases of $Ca^{2+}$ uptake by biomass in Fer-1 and Fer-2 by the time are illustrated in figures 5.15 and 5.16, respectively.

Available calcium concentrations and uptake amount of calcium by the cells are shown in Table 5.8.

Table 5.8. Concentrations of $Ca^{2+}$ in fermentation media and its uptake by yeast cells

| Sampling Hour | Fermentation 1 | | Fermentation 2 | |
|---|---|---|---|---|
| | Total $Ca^{2+}$ present in the medium (kg) | Total $Ca^{2+}$ uptake of yeast cells (kg) | Total $Ca^{2+}$ present in the medium (kg) | Total $Ca^{2+}$ uptake of yeast cells (kg) |
| 4 | 24 | 1.3 | 2.8 | 0.1 |
| 8 | 64 | 4.3 | 6.1 | 0.4 |
| 12 | 114 | 8.8 | 9.7 | 0.7 |
| 17 | 158 | 16.8 | 12.8 | 1.2 |

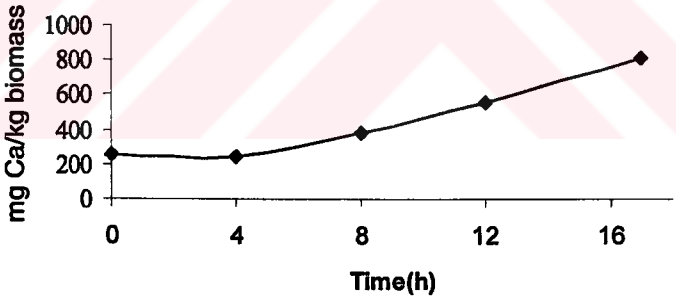



Figure 5.15. $Ca^{2+}$ uptake of biomass during fermentation 1

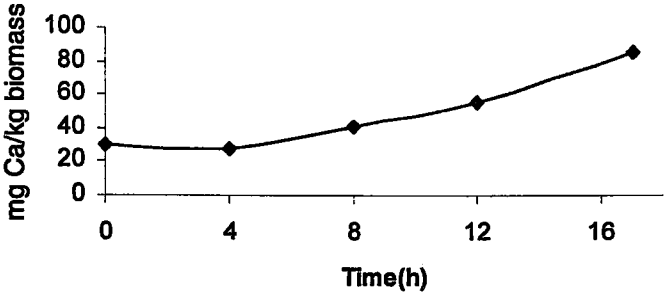



Figure 5.16. $Ca^{2+}$ uptake of biomass during fermentation 2

As for the $Mg^{2+}$ ion, it is very clear that for each time intervals, the uptake of $Mg^{2+}$ ion by yeast cake almost the same for two fermentations. It is seen that uptake of this ion by biomass is maximum in first hours of fermentation and decreases slowly during the fermentation period of two industrial productions. Changes in $Mg^{2+}$ concentrations of biomass in fermentation 1 and fermentation 2 are shown in Figure 5.17 and Figure 5.18, respectively.

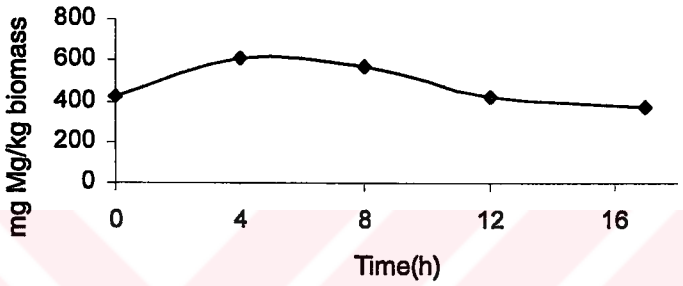



Figure 5.17. $Mg^{2+}$ uptake of biomass during fermentation 1

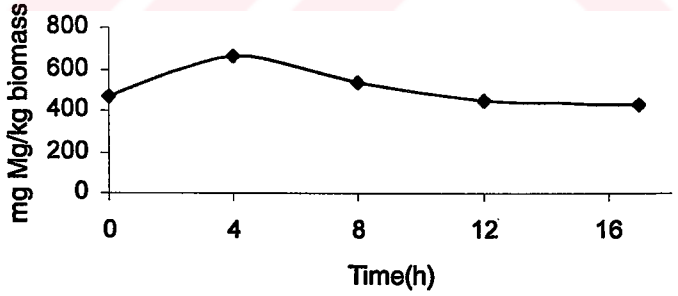



Figure 5.18. $Mg^{2+}$ uptake of biomass during fermentation 2

The concentrations of $Mg^{2+}$ in fermentation medium of fermentation 1 and fermentation 2 are different due to the differences in molasses composition. Same amount of $Mg^{2+}$ was taken up by biomass in both fermentations regardless of the $Mg^{2+}$ concentration difference in the fermentation medium. This says that although $Mg^{2+}$ concentrations vary process-to-process, yeast needs fix amount of this ion for its

metabolism. The necessity of $Mg^{2+}$ ion in yeast metabolism was further demonstrated with lab-scale studies.

Copper concentration during fermentation 1 and fermentation 2 are shown in Figure 5.19 and Figure 5.20, respectively. $Cu^{2+}$ ion uptake during the two fermentations show almost the same characteristics.

At the end of the first 4-hours, $Cu^{2+}$ uptake was 17.73 mg $Cu^{2+}$/ kg biomass in fermentation 1; 23.31 mg/kg biomass in fermentation 2.

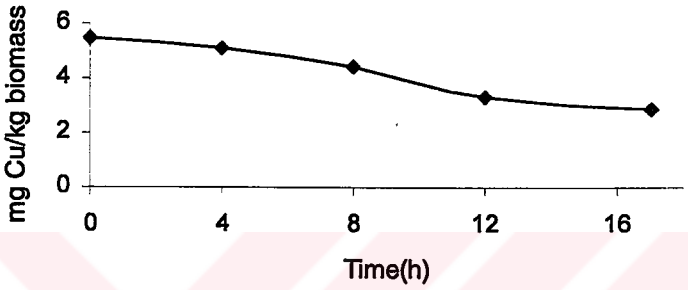



Figure 5.19. $Cu^{2+}$ uptake of biomass during fermentation 1

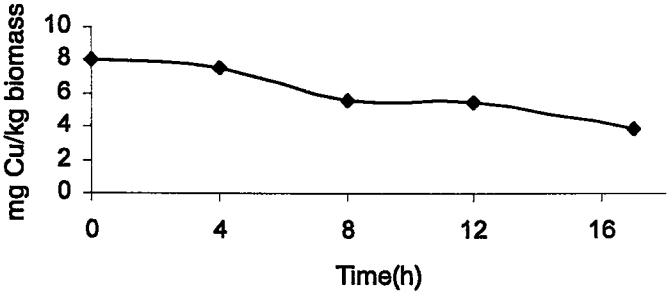



Figure 5.20. $Cu^{2+}$ uptake of biomass during fermentation 2

The first stage of copper uptake is dominant for approximately the first 6 h of growth, which also corresponds to the major period of protein synthesis during sporulation of the yeast cells. This rapid uptake stage may represent the incorporation of copper into numerous proteins and enzymes. The second stage displays a much slower rate of copper uptake, and is independent of the initial copper concentration. Uptake

rates of $Cu^{2+}$ by *S.cerevisiae* are stated as 0.018, 0.0175 and 0.015 mg $Cu^{2+}$/ (L.min) (10).

In this study, the observed copper uptake rates in terms of mg $Cu^{2+}$ / (L.min) are given in Table 5.9;

Table 5.9. $Cu^{2+}$ uptake rates during fermentations 1 and 2

| Sampling hour | Uptake rate (mg $Cu^{2+}$ / L.min) Fermentation 1 | Uptake rate (mg $Cu^{2+}$ / L.min) Fermentation 2 |
|---|---|---|
| 4 | 0.00162 | 0.00132 |
| 8 | 0.00127 | 0.00122 |
| 12 | 0.000603 | 0.000794 |
| 17 | 0.000568 | 0.000648 |

$Zn^{2+}$ concentrations in yeast cakes decrease by showing same characteristics with time during fermentation periods of two different fermentations. As if the uptake of the element stops after 8th hour of fermentation in both fermentations. Although adding extra amounts of $Zn^{2+}$ are equal in each fermentors, total $Zn^{2+}$ in the medium in Fer-1 is less than Fer-2. In addition to this, uptake values in Fer-1 are also less than Fer-2 as shown in Figure 5.21 and 5.22. It is also seen that the yeast took almost all zinc in the fermentor in two fermentations. Bromberg et.al explained that $Zn^{2+}$ results in a higher rate of fermentation but does not affect cell growth (21).

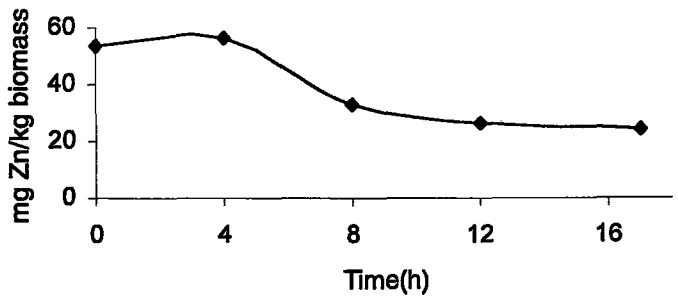



Figure 5.21. Zn uptake of biomass during fermentation 1

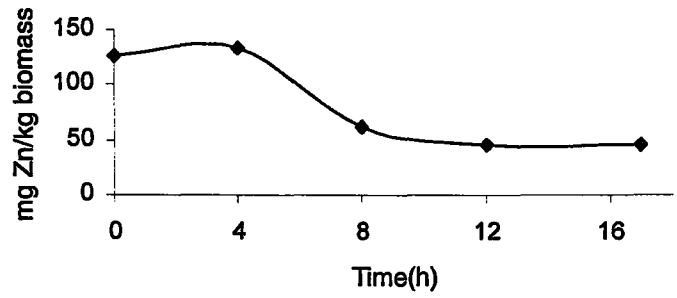



Figure 5.22. Zn uptake of biomass during fermentation 2

Zn uptake rates are shown for two fermentations in the Table 5.10.

Table 5.10. Zn uptake rates during fermentation 1 and 2

| | **Uptake rate (mg Zn / L.min) Fermentation 1** | **Uptake rate (mg Zn / L.min) Fermentation 2** |
|---|---|---|
| **4.h** | 0.0178 | 0.0234 |
| **8.h** | 0.00947 | 0.0134 |
| **12.h** | 0.00479 | 0.00672 |
| **17.h** | 0.00483 | 0.00747 |

$Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2}$ are cationic yeast nutrients. They are involved in structural and enzymatic regulatory activities during growth and metabolism (2). Their uptake depends on their concentration and bioavailability in the growth environment. Cells have the ability to select required cations and insert them into appropriate complexing sites (13).

## 5.3. Evaluation of Uptake of Ions in Lab-Scale Fermentations

Microbial cells have a high growth demand for magnesium ions that can not be met by other metals. High intracellular concentrations of magnesium (generally around 10 mM total concentration) are manifested in absolutely essential structural and metabolic functions of cells (35). Although several studies have been conducted in the past on Mg-limited growth of bacteria, very little information is available concerning yeasts.

$Mg^{2+}$ ion concentration of industrial fermentations revealed that yeast cells uptake a certain amount of $Mg^{2+}$ from the fermentation medium. In the laboratory, experiments were designed to observe the effect of different concentrations of Mg in the fermentation medium. Henceforth, *S.cerevisiae* was grown aerobically in batch culture with different Mg ion concentrations.

### 5.3.1. Uptake of Ions in Lab-Scale Fermentations

Amounts of Mg added to the 8.5 l fermentation medium and the concentration of the elements in the input and the output of the fermentation system are in Table 5.11. Mg is added to the media in lab-scale and industrial scale as $Mg.SO_4.7H_2O$. Generally, no change is observed in the uptake values of each element in 4 different fermentation studies. In each fermentation, average 165.5 mg Mg/kg biomass was consumed. Mg demand of yeast cells seemed to be constant and this average required concentration could be solely met by molasses.

Change in biomass with added concentrations of $Mg^{2+}$ were shown in Figure 5.23. Uptake of Mg ions by 1 kg filtered cells was on the average 1938.3 mg. This value was nearly the same as the uptake values of industrial fermentations at 4 and 8 h time intervals. Uptake of Mg decreased by time and it reached its minimum values at the end of large-scale fermentations. Although the yeast cell wall plays a significant role in divalent metal ion binding in yeast, it is unlikely that the timing of $Mg^{2+}$ uptake observed in yeast cells during fermentation represents a surface-binding phenomenon. Although Mg-transporters have now been characterized at the molecular level in bacteria, the mechanism of active $Mg^{2+}$ transport through the yeast plasma membrane remains to be elucidated (14).

Table 5.11. Element concentrations of inputs & outputs of lab-scale fermentations

| Added $MgSO_47H_2O$ Concentration | Inputs& Outputs | Element Concentrations (mg/kg) | | | |
|---|---|---|---|---|---|
| | | Ca | Cu | Mg | Zn |
| Study-1 (0 g/l) | M | 121.9 | 1.5 | 185.6 | 1.2 |
| | DW | 0.0291 | 0.0765 | 0.008 | 0.0078 |
| | FMi | 69.7 | 1.6 | 106.2 | 0.8 |
| | FMf | 77.1 | 1.6 | 115.3 | 0.8 |
| | Fa | 63.1 | 8.1 | 552.0 | 15.4 |
| | Fb | 71.9 | 0.2 | 93.6 | 0.1 |
| Study-2 (0.25 g/l) | Inputs& Outputs | Element Concentrations (mg/kg) | | | |
| | | Ca | Cu | Mg | Zn |
| | M | 121.9 | 1.5 | 185.6 | 1.2 |
| | DW | 0.0291 | 0.0765 | 0.008 | 0.0078 |
| | FMi | 68.0 | 1.5 | 128.8 | 0.8 |
| | FMf | 76.0 | 1.4 | 145.4 | 0.8 |
| | Fa | 86.7 | 8.8 | 575.6 | 15.9 |
| | Fb | 68.6 | 0.2 | 114 | 0.2 |
| Study-3 (0.5 g/l) | Inputs& Outputs | Element Concentrations (mg/kg) | | | |
| | | Ca | Cu | Mg | Zn |
| | M | 121.9 | 1.5 | 185.6 | 1.2 |
| | DW | 0.0291 | 0.0765 | 0.008 | 0.0078 |
| | FMi | 65.5 | 1.6 | 150.7 | 0.6 |
| | FMf | 74.4 | 1.6 | 167.8 | 0.6 |
| | Fa | 66.8 | 9.2 | 596.5 | 15.9 |
| | Fb | 70.3 | 0.2 | 142.7 | 0.2 |
| Study-4 (1 g/l) | Inputs& Outputs | Element Concentrations (mg/kg) | | | |
| | | Ca | Cu | Mg | Zn |
| | M | 121.9 | 1.5 | 185.6 | 1.2 |
| | DW | 0.0291 | 0.0765 | 0.008 | 0.0078 |
| | FMi | 69.7 | 1.5 | 197.8 | 0.7 |
| | FMf | 76.3 | 1.5 | 222.3 | 1.2 |
| | Fa | 90.4 | 9.0 | 611.5 | 16.0 |
| | Fb | 67.2 | 0.2 | 185.9 | 0.1 |

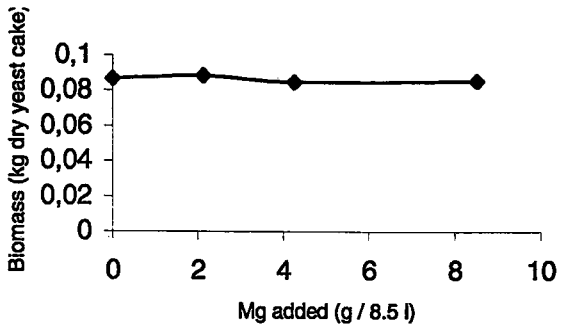



Figure 5.23. Change in biomass with added $Mg^{2+}$

### 5.3.2. Uptake of $Mg^{2+}$

Magnesium serves as an enzyme activator for many enzymes and is involved in many essential, physiological and biochemical functions in yeast cells like cell growth and division (2). Therefore, yeasts have a very high growth demand for magnesium ions, and magnesium accumulation by yeast correlates closely with the progress of fermentation (17).

For *Saccharomyces cerevisiae* under $Mg^{2+}$ limitation, the biomass yield coefficient for $Mg^{2+}$ utilized is relatively high at low dilution rates and gradually declines as the growth rate increases. One interpretation of this type of behaviour is that the limiting substrate is utilized in the production of intracellular reserves when growth is other than carbon limited. Magnesium ions decrease the proton and, especially, anion permeability of the plasmalemma by interacting with membrane phospholipids, resulting in stabilization of the membrane bilayer. Observations of a protective effect of magnesium in response to toxic levels of ethanol have suggested that magnesium plays a more important role in the cellular protection of and recovery from stress (20).

Uptake values of $Mg^{2+}$ in fermentation medium with different $Mg^{2+}$ concentrations in lab-scale fermentations are shown in Table 5.12.

Table 5.12. Uptake values in different available $Mg^{2+}$ concentrations

| Added $MgSO_47H_2O$ Concentration | $Mg^{2+}$ uptake (mg Mg/kg biomass) | $Mg^{2+}$ uptake (μM) |
|---|---|---|
| **Study-1** (0 g/l) | 552 | 784 |
| **Study-2** (0.25 g/l) | 576 | 818 |
| **Study-3** (0.5 g/l) | 596 | 786 |
| **Study-4** (1 g/l) | 611 | 839 |
| **Mean** | **583.7** | **806.7** |

$Mg^{2+}$ uptake is almost constant for all the high concentrations of $Mg^{2+}$ supplied in the medium. Figure 5.24 and 5.25 show the uptake of $Mg^{2+}$ ions versus Mg concentrations of filtrate, total Mg for each 8.5 l fermentation medium.

As a result of the 4 lab-scale fermentation studies, it may be concluded that yeast cells utilize, on the average, 806.7 μM $Mg^{2+}$ ions.

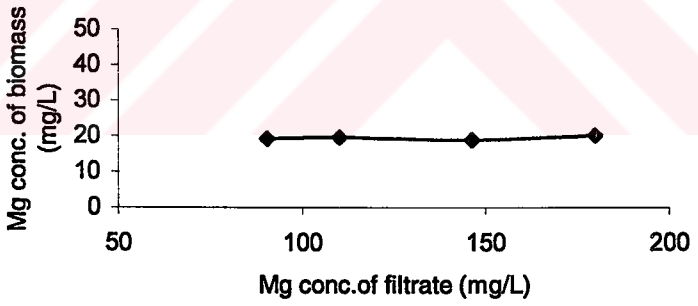



Figure 5.24. $Mg^{2+}$ concentration of biomass versus $Mg^{2+}$ in filtrate

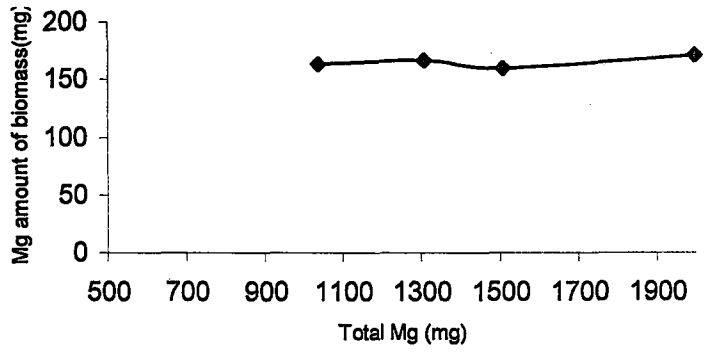



Figure 5.25. Mg concentration of biomass versus total $Mg^{2+}$ in the fermentation medium

$Mg^{2+}$ transport is concentration dependent and shows saturation with increasing external concentration.

Walker et.al found that both ethanol production and glucose consumption were highly dependent on the availability of $Mg^{2+}$ ions in the yeast growth medium. Concentrations of $Mg^{2+}$ above 500 μM did not affect fermentation when expressed on a cellular productivity basis. Yeast cells absorbed a constant amount of $Mg^{2+}$ per cell so long as the medium is sufficient in this ion (17,18).

$Mg^{2+}$ is necessary for the stabilization of the cell membrane as well as for the activation and stabilization of structures and enzymes involved in ATP synthesis. It can prevent harmful effects of Cu towards *Saccharomyces cerevisiae* under certain Mg concentration and Mg:Cu ratios. It was also claimed that Mg could repair the toxic effects of $Co^{2+}$ in *Saccharomyces cerevisiae*. Both $Ca^{2+}$ and $Mg^{2+}$ may interact with negatively charged functional groups and crosslink carboxylated and phosphorylated anionic polymers on cell surfaces and like other metals, alter electrosurface properties of yeast cell (11).

Excess amounts of $Mg^{2+}$ in fermentation medium can cause toxic effects, which include the blocking of functional groups of important biomolecules, e.g. enzymes, inhibition of transport systems for essential ions and nutrients, the displacement and substitution of essential metal ions from cellular locations and biomolecules, denaturation of enzymes, and disruption of membrane structure. Therefore, it is suggested that magnesium should not be added to the fermentor in excess amounts.

Above certain concentrations, all divalent cations, besides $Mg^{2+}$, are toxic and may cause impairment of cell metabolism.

### 5.3.3. Uptake of Other Ions

In this study, $Ca^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ uptake of dry yeast did not change with increasing concentration of Mg as seen in Table 5.13.

Table 5.13. Comparison of the uptake values of ions in lab-scale fermentations

| | Uptake (mg/kg biomass) | | | |
|---|---|---|---|---|
| **Lab-scale Fermentations** | **$Ca^{2+}$** | **$Cu^{2+}$** | **$Mg^{2+}$** | **$Zn^{2+}$** |
| Study-1 | 66.8 | 9.2 | 596.5 | 15.9 |
| Study-2 | 63.1 | 8.1 | 552.0 | 15.4 |
| Study-3 | 86.7 | 8.8 | 575.6 | 15.9 |
| Study-4 | 90.4 | 9.0 | 611.5 | 16.0 |

The cell wall of *Saccharomyces cerevisiae* contains polysaccharides, proteins and lipids. These organic molecules have many functional groups such as carboxylate, hydroxyl, phosphate and amino groups that can bind metal ions. Since biomass particles have an overall negative charge, $Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ cations can bind easily. Their dissociation is not very difficult due to low energy requirement of the reversible reactions.

For maximal uptake rate, yeast cells require $K^+$ and $H_2PO_4^-$ for the activation of their transport systems. Phosphate increases the negative charge of the membrane. After binding on live cells of *S.cerevisiae* , $Ca^{2+}$, $Cu^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ are transported into the cytoplasm via passive uptake and active transport mechanisms. $Ca^{2+}$and $Mg^{2+}$may enter *S. cerevisiae* as low affinity substrates of the monovalent cation transport system.

To increase the uptake of divalent cations, there should be appropriate temperature control, plenty of substrate and no metabolic inhibitors in the fermentation medium (9,14).

## 5.4.Comparison of Industrial and Lab-Scale Fermentations

The equilibrium distribution of $Mg^{2+}$ ion between the biomasses and filtrates was studied. The Langmuir isotherm was used to check for the data fitting. The experimental data were exposed to linear transformation to see the deviations of experimental data from the theoretical model line. The equations and their respective expressions for the linear transformation of data are summarized in Table 5.14 (35).

Table 5.14. Isotherm equations and linear transformation expressions

| | Langmuir Equation |
|---|---|
| Equation | $q / q_{max}=bC / (1+bC)$ |
| Linear Transformation Expression | $C / q_{max}=1 / (q_{max}b) + (1/q_{max}C)$ |

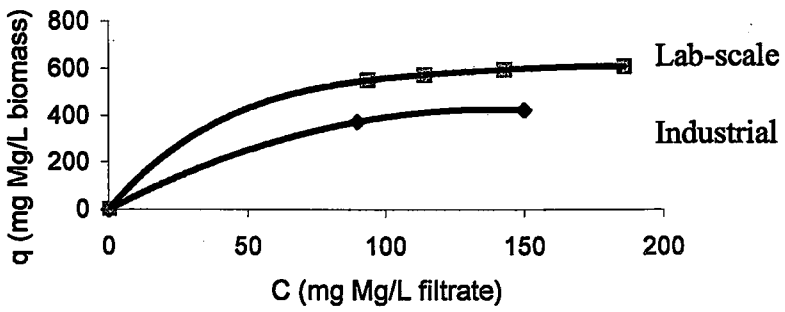
q: $Mg^{2+}$ concentration in biomass

$q_{max}$: Maximum uptake amount of $Mg^{2+}$

C: $Mg^{2+}$ concentration in filtrate

b: Affinity constant

To make a comparison between the uptake of $Mg^{2+}$ ion in industrial and lab-scale fermentations, equilibrium sorptions were performed under identical conditions. Only two industrial fermentations were observed. Although the data related to the industrial fermentations were not sufficient to compare the theoretical results with lab-scale fermentations, they were shown in the graph as predictive values. $Mg^{2+}$ equilibria for industrial and lab-scale fermentations are shown in Figure 5.26.

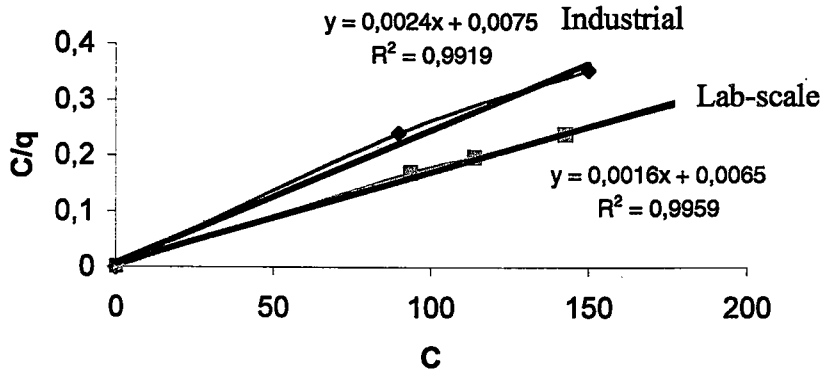



**c:** Equilibrium $Mg^{2+}$ concentration in filtrate (mg $Mg^{2+}$ uptake / l filtrate)

**q:** Equilibrium $Mg^{2+}$ concentration in biomass (mg $Mg^{2+}$ uptake / l biomass)

Figure 5.26. $Mg^{2+}$ uptake equilibria for industrial and lab-scale fermentations

The linearization of the Langmuir equation is achieved by converting q vs C data into C/q vs C. The regression coefficients were to be found bigger than 0.99. The parameters $q_{max}$ and b were calculated from the slope and intersection of the linearized plots respectively in Figure 5.27.




**C:** Equilibrium $Mg^{2+}$ concentration in filtrate (mg $Mg^{2+}$ uptake / l filtrate)

**q:** Equilibrium $Mg^{2+}$ concentration in biomass (mg $Mg^{2+}$ uptake / l biomass)

Figure 5.27. Langmuir Linear Curve

The calculated model parameters and the regression coefficients are tabulated in Table 5.15.

Table 5.15. Langmuir Parameters

| | $q_{max}$ (mg $Mg^{2+}$/L) | B | $R^2$ |
|---|---|---|---|
| Laboratory Fermentations | 625.0 | 0.25 | 0.9959 |
| Industrial Fermentations | 416.7 | 0.32 | 0.9919 |

As can be seen in Figures 5.30 and 5.31 and $q_{max}$ values, tha data are correlated quite well by the Langmuir equation. The equilibrium constants were very close to each other. $q_{max}$ value in laboratory fermentations was found as 625 mg $Mg^{2+}$/L .This is greater than $q_{max}$ for industrial fermentations. Since the pure culture was used for the

laboratory fermentations, perfect experimental conditions were provided for lab-scale fermentations. Therefore, the uptake of $Mg^{2+}$ element in lab-scale fermentations was greater than industrial fermentations.

### 5.5. Interpretations of Uptake Results for Industrial Production of Baker's Yeast

Baker's yeast production is a very old process in food industry. Hence, the process has undergone changes through many years of production experience. Since the industrial production has been standardized and become a traditional procedure, there are not any recent studies on improving biomass productivity in industrial scale.

In baker's yeast production, $Mg^{2+}$ is added as $MgSO_4.7H_2O$ to fermentors. In industrial scale, 95 kg of $MgSO_4.7H_2O$ was added to 100 tonnes of fermentation medium, which is equivalent to 9.27 kg $Mg^{2+}$/ 100 tonnes of medium. At the end of the $17^{th}$ hour, 7.7 kg $Mg^{2+}$ was taken by biomass in fermentation 1 and 7.5 kg $Mg^{2+}$ in fermentation 2. These uptake values were close to the amount of $Mg^{2+}$ added to each fermentor. In fermentation 2, 100 % of $Mg^{2+}$ demand of the yeast was met by molasses; whereas in fermentation 1, molasses could meet 73 % of the total requirement. Because of the concentration differences that exist among different batches of molasses, magnesium is added to each fermentation run with a safety margin.

$Zn^{2+}$ concentration of molasses was not enough and additional amounts were necessary for production. Zinc increases the rate of fermentation. Therefore, the medium must contain sufficient quantity of this ion.

# CONCLUSIONS

In this study, the effect of trace elements on the production of baker's yeast was investigated in industrial and laboratory scale fermentations. Molasses, supplemented water, seed yeast, fermentation media, dry yeast, compressed yeast, disposal, yeast milk samples were collected from Pakmaya Inc. All samples except water were digested with the method of FCL and analysed with ICP-AES.

Verification of FCL method was done by standard additions. Average spike recovery percentages of all standard additions were calculated for $Ca^{2+}$, $Mg^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ in dry yeast and molasses samples. The average spike recovery percentages obtained for all elements in both dry yeast and molasses with the FCL method were higher than the simple digestion method. Therefore the FCL method was used for sample digestion prior to ICP-AES analysis.

In industrial fermentations, $Mg^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ are added to the fermentors. Therefore, the effects of these elements on production have an economical impact. Since water containing high amounts of $Ca^{2+}$ enters the fermentation medium in large quantities, $Ca^{2+}$ was also investigated.

Molasses used in industrial scale fermentations had different elemental composition that affected the uptake values of these elements by yeast cells. Uptake of $Ca^{2+}$ was proportional to the available concentration of this cation. In fermentation 1, calcium concentration of molasses was nearly 10 fold of fermentation 2. Therefore, the utilization of $Ca^{2+}$ in fermentation 1 was 10 times higher than fermentation 2. An increase in time was observed on calcium uptake in both industrial scale fermentations. The constructed mass balances for both fermentations showed that total $Ca^{2+}$ concentration entering the fermentors was the same as total $Ca^{2+}$ concentration in the outputs of the system.

Mass balances of $Cu^{2+}$ for the two industrial scale fermentations revealed that the total $Cu^{2+}$ concentration entering the fermentors was not the same as total $Cu^{2+}$ concentration in the outputs of the system. This difference is due to low $Cu^{2+}$ concentrations in the samples and slightly low spike recovery percentage obtained with the FCL method. Maximum copper uptake concentrations attained were 17.23 mg/kg dry yeast in fermentation 1 and 23.31 mg/kg dry yeast in fermentation 2. A decrease in time was observed on $Cu^{2+}$ uptake in both industrial scale fermentations. It may be

concluded that yeast cells can uptake almost all their $Cu^{2+}$ demand from molasses within the first 4 hours of fermentation.

Mass balances of $Zn^{2+}$ for the two industrial scale fermentations showed that the total $Zn^{2+}$ concentration entering the fermentors was nearly the same as total $Zn^{2+}$ concentration in the outputs of the system. Maximum $Zn^{2+}$ uptake concentrations attained were 195.64 mg/kg dry yeast in fermentation 1 and 413.46 mg/kg dry yeast in fermentation 2. Almost all $Zn^{2+}$ was consumed in both fermentations and this may be the reason for adding $Zn^{2+}$ to industrial fermentation media.

Mass balances of $Mg^{2+}$ for the two industrial scale fermentations showed that the total $Mg^{2+}$ concentration entering the fermentors was the same as total $Mg^{2+}$ concentration in the outputs of the system. Maximum $Mg^{2+}$ uptake concentrations attained were 2051.73 mg/kg dry yeast in fermentation 1 and 2106.46 mg/kg dry yeast in fermentation 2. Although molasses used in these 2 different industrial scale fermentations had different $Mg^{2+}$ concentrations, similar uptake values were obtained. A decrease in time was observed on $Mg^{2+}$ uptake in both industrial scale fermentations.

$Mg^{2+}$ is essential for yeast cells. It is involved in many metabolic activities such as cellular growth, cellular division and enzyme activation (2). Therefore yeasts have a high demand for $Mg^{2+}$. On the other hand, excessive amounts of this element can have toxic affects on yeast metabolism (14,16). Since excessive or insufficient amounts of this element alters industrial baker's yeast production, determination of its concentration in the raw materials entering the fermentation is very important. The results obtained from industrial scale fermentations were backed up with four lab-scale fermentations.

The equilibrium sorption isotherms were generated to understand the $Mg^{2+}$ uptake mechanism of baker's yeast. The biosorption of $Mg^{2+}$ ions was occurred as monolayer adsorption since the equilibrium isotherms could be described by the Langmuir model. The calculated isotherm parameters were qm=625 mg $Mg^{2+}$/L (maximum biosorption capacity) and b= 0.25 (affinity constant) for lab-scale fermentations. The maximum biosorption capacity of *S. cerevisiae* was found to be 416.7 in industrial scale fermentations. It can be concluded that $Mg^{2+}$ ions binding on S. cerevisiae cells was a result of passive uptake and active transport of metal ions inside the cells were negligible. Further studies should be conducted to quantify the metabolic uptake.

$MgSO_4.7H_2O$ added to industrial fermentations cause settling problems in waste treatment due to $SO_4^{2-}$ ions. This results in high waste treatment costs.

It is evident that excessive or low amounts of required elements alter the productivity of industrial baker's yeast productions, increase the cost and present problems in waste treatment. Therefore, it is important to be able to determine the concentrations of these elements in the raw materials, especially molasses, before the fermentations.

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# APPENDIX A

Table A.1.Conformity data for dry yeast by FCL

| | **Ca conc.units ppm.** | **mg/g dry yeast** | **Cu conc.units ppm.** | **mg/g dry yeast** | **Mg conc.units ppm.** | **mg/g dry yeast** | **Zn conc.units ppm.** | **mg/g dry yeast** |
|---|---|---|---|---|---|---|---|---|
| Add0 | 11.922 | 1.1922 | 0.043102 | 0.0043102 | 8.8326 | 0.88326 | 0.38522 | 0.038522 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 17.191 | 101.6 | 0.086644 | 93.06 | 13.467 | 93.35 | 0.61146 | 96.25 |
| Add2 | 22.877 | 104.3 | 0.13175 | 92.06 | 18.453 | 97.98 | 0.82096 | 92.74 |
| Add3 | 32.860 | 102.9 | 0.22774 | 93.68 | 29.041 | 100.72 | 1.2910 | 93.19 |
| | **Ca conc.units ppm.** | **mg/g dry yeast** | **Cu conc.units ppm.** | **mg/g dry yeast** | **Mg conc.units ppm.** | **mg/g dry yeast** | **Zn conc.units ppm.** | **mg/g dry yeast** |
| Add0 | 11.335 | 1.1335 | 0.041149 | 0.0041149 | 8.5707 | 0.85707 | 0.37416 | 0.037416 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 16.361 | 100.16 | 0.08590 | 94.24 | 13.181 | 97.12 | 0.57868 | 92.713 |
| Add2 | 21.225 | 99.48 | 0.12826 | 90.86 | 18.020 | 97.03 | 0.79086 | 90.47 |
| Add3 | 32.642 | 104.17 | 0.22778 | 93.69 | 29.191 | 102.17 | 1.28810 | 81.59 |
| | **Ca conc.units ppm.** | **mg/g dry yeast** | **Cu conc.units ppm.** | **mg/g dry yeast** | **Mg conc.units ppm.** | **mg/g dry yeast** | **Zn conc.units ppm.** | **mg/g dry yeast** |
| Add0 | 11.269 | 1.1269 | 0.044286 | 0.0044286 | 8.3223 | 0.83223 | 0.37663 | 0.037663 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 15.622 | 96.02 | 0.08307 | 91.13 | 12.959 | 97.27 | 0.55157 | 88.02 |
| Add2 | 20.621 | 96.95 | 0.12646 | 87.64 | 17.572 | 95.90 | 0.76603 | 89.38 |
| Add3 | 32.269 | 103.19 | 0.22995 | 94.13 | 28.775 | 101.59 | 1.23860 | 89.97 |
| | **Ca conc.units ppm.** | **mg/g dry yeast** | **Cu conc.units ppm.** | **mg/g dry yeast** | **Mg conc.units ppm.** | **mg/g dry yeast** | **Zn conc.units ppm.** | **mg/g dry yeast** |
| Add0 | 10.865 | 1.0865 | 0.040191 | 0.0040191 | 8.3135 | 0.83135 | 0.35586 | 0.035586 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 15.698 | 98.95 | 0.083128 | 92.16 | 12.743 | 95.71 | 0.54883 | 90.58 |
| Add2 | 20.635 | 98.9 | 0.125230 | 89.32 | 17.473 | 95.41 | 0.75400 | 88.09 |
| Add3 | 31.714 | 102.75 | 0.232328 | 92.96 | 28.325 | 100.04 | 1.20640 | 88.97 |
| | **Ca conc.units ppm.** | **mg/g dry yeast** | **Cu conc.units ppm.** | **mg/g dry yeast** | **Mg conc.units ppm.** | **mg/g dry yeast** | **Zn conc.units ppm.** | **mg/g dry yeast** |
| Add0 | 11.358 | 1.1358 | 0.041877 | 0.0041877 | 8.3231 | 0.83231 | 0.37644 | 0.037644 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 16.142 | 98.68 | 0.086326 | 93.95 | 13.039 | 97.86 | 0.5865 | 93.62 |
| Add2 | 20.833 | 97.54 | 0.124720 | 87.90 | 17.511 | 95.56 | 0.7722 | 88.10 |
| Add3 | 32.612 | 103.90 | 0.223760 | 92.50 | 28.242 | 99.72 | 1.2594 | 91.50 |
| **Mean** | | 100.63 | | 91.95 | | 97.88 | | 90.21 |
| **Std.d.** | | ±2.79 | | ±2.17 | | ±2.23 | | ±3.79 |

Table A.2. Conformity data for dry yeast by simple digestion method

| | **Ca conc.units ppm.** | **Mg/g dry yeast** | **Cu conc.units ppm.** | **Mg/g dry yeast** | **Mg conc.units ppm.** | **Mg/g dry yeast** | **Zn conc.units ppm.** | **Mg/g dry yeast** |
|---|---|---|---|---|---|---|---|---|
| Add0 | 11.476 | 1.077 | 0.044010 | 0.004202 | 8.4564 | 0.7938 | 0.36193 | 0.034 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 16.304 | 94.45 | 0.09462 | 91.98 | 12.600 | 89.00 | 0.60176 | 93.25 |
| Add2 | 19.936 | 93.18 | 0.13846 | 95.14 | 16.807 | 90.97 | 0.78674 | 90.97 |
| Add3 | 30.287 | 90.84 | 0.24445 | 89.08 | 28.305 | 81.93 | 1.29670 | 89.33 |
| | **Ca conc.units ppm.** | **Mg/g dry yeast** | **Cu conc.units ppm.** | **Mg/g dry yeast** | **Mg conc.units ppm.** | **Mg/g dry yeast** | **Zn conc.units ppm.** | **Mg/g dry yeast** |
| Add0 | 10.971 | 1.070 | 0.040713 | 0.0040 | 8.0966 | 0.7900 | 0.34536 | 0.0337 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 16.067 | 91.74 | 0.09062 | 90.57 | 12.482 | 86.77 | 0.57017 | 87.11 |
| Add2 | 19.193 | 90.67 | 0.13184 | 92.29 | 15.597 | 85.22 | 0.75842 | 88.62 |
| Add3 | 29.519 | 96.06 | 0.24385 | 92.75 | 27.904 | 99.93 | 1.2760 | 95.36 |
| | **Ca conc.units ppm.** | **Mg/g dry yeast** | **Cu conc.units ppm.** | **Mg/g dry yeast** | **Mg conc.units ppm.** | **Mg/g dry yeast** | **Zn conc.units ppm.** | **Mg/g dry yeast** |
| Add0 | 10.851 | 1.035 | 0.042305 | 0.00403 | 7.8611 | 0.7496 | 0.34259 | 0.0326 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 15.150 | 95.24 | 0.08902 | 95.07 | 11.877 | 91.69 | 0.56803 | 95.03 |
| Add2 | 19.621 | 88.76 | 0.13528 | 88.73 | 15.901 | 83.64 | 0.77274 | 86.04 |
| Add3 | 30.706 | 86.00 | 0.24083 | 87.62 | 28.455 | 96.03 | 1.30180 | 91.06 |
| | **Ca conc.units ppm.** | **Mg/g dry yeast** | **Cu conc.units ppm.** | **Mg/g dry yeast** | **Mg conc.units ppm.** | **Mg/g dry yeast** | **Zn conc.units ppm.** | **Mg/g dry yeast** |
| Add0 | 11.037 | 1.057 | 0.044525 | 0.00426 | 8.0888 | 0.7752 | 0.34929 | 0.0334 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 15.313 | 92.64 | 0.08706 | 88.54 | 12.671 | 93.64 | 0.56055 | 90.35 |
| Add2 | 19.036 | 91.73 | 0.13381 | 93.00 | 15.677 | 87.57 | 0.74796 | 88.85 |
| Add3 | 30.681 | 92.78 | 0.24851 | 94.69 | 28.576 | 95.21 | 1.29460 | 89.69 |
| **Mean** | | 92.67 | | 92.23 | | 90.58 | | 90.06 |
| **Std. Dev.** | | ±3.71 | | ±2.95 | | ±6.42 | | ±3.03 |

Table A.3. Conformity data for molasses by FCL

| | **Ca conc.units ppm.** | **mg/g molasses** | **Cu conc.units ppm.** | **mg/g molasses** | **Mg conc.units ppm.** | **mg/g molasses** | **Zn conc.units ppm.** | **mg/g molasses** |
|---|---|---|---|---|---|---|---|---|
| Add0 | 32.714 | 3.2714 | 0.014017 | 0.0014017 | 0.84826 | 0.084826 | 0.063903 | 0.0063903 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 46.718 | 97.91 | | 89.17 | | 91.79 | | 116.26 |
| Add2 | 60.309 | 96.16 | | 79.67 | | 96.53 | | 100.80 |
| Add3 | 90.423 | 97.53 | | 89.17 | | 105.16 | | 98.31 |
| | **Ca conc.units ppm.** | **mg/g molasses** | **Cu conc.units ppm.** | **mg/g molasses** | **Mg conc.units ppm.** | **mg/g molasses** | **Zn conc.units ppm.** | **mg/g molasses** |
| Add0 | 32.576 | 3.2576 | 0.014053 | 0.0014053 | 0.82583 | 0.082583 | 0.070961 | 0.007096 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | | 95.45 | | 83.61 | | 97.69 | | 113.76 |
| Add2 | | 98.39 | | 75.10 | | 92.64 | | 83.45 |
| Add3 | | 97.84 | | 94.28 | | 100.36 | | 115.81 |
| | **Ca conc.units ppm.** | **mg/g molasses** | **Cu conc.units ppm.** | **mg/g molasses** | **Mg conc.units ppm.** | **mg/g molasses** | **Zn conc.units ppm.** | **mg/g molasses** |
| Add0 | 31.390 | 3.1390 | 0.012584 | 0.0012584 | 0.81924 | 0.081924 | 0.052782 | 0.0052782 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | | 94.99 | | 90.57 | | 95.65 | | 103.64 |
| Add2 | | 93.52 | | 80.03 | | 95.15 | | 99.86 |
| Add3 | | 97.12 | | 88.83 | | 104.06 | | 97.05 |
| | **Ca conc.units ppm.** | **mg/g molasses** | **Cu conc.units ppm.** | **mg/g molasses** | **Mg conc.units ppm.** | **mg/g molasses** | **Zn conc.units ppm.** | **mg/g molasses** |
| Add0 | 28.689 | 2.8689 | 0.012911 | 0.0012911 | 0.73701 | 0.073701 | 0.060598 | 0.0060598 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | | 94.59 | | 82.13 | | 96.28 | | 91.36 |
| Add2 | | 98.96 | | 78.13 | | 96.01 | | 88.05 |
| Add3 | | 97.70 | | 105.47 | | 102.60 | | 94.28 |
| | **Ca conc.units ppm.** | **mg/g molasses** | **Cu conc.units ppm.** | **mg/g molasses** | **Mg conc.units ppm.** | **mg/g molasses** | **Zn conc.units ppm.** | **mg/g molasses** |
| Add0 | 32.696 | 3.2696 | 0.0013283 | 0.0013283 | 0.85003 | 0.085003 | 0.068962 | 0.0068962 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | | 97.84 | | 106.85 | | 99.19 | | 98.14 |
| Add2 | | 92.78 | | 79.50 | | 92.58 | | 91.33 |
| Add3 | | 96.38 | | 86.77 | | 100.27 | | 93.93 |
| **Mean** | | 96.47 | | 87.28 | | 97.73 | | 99.07 |
| **Std. Dev.** | | ±1.85 | | ±9.36 | | ±4.13 | | ±9.84 |

Table A.4. Conformity data for molasses by simplified digestion method

| | **Ca conc.units ppm.** | **mg/g molasses** | **Cu conc.units ppm.** | **mg/g molasses** | **Mg conc.units ppm.** | **mg/g molasses** | **Zn conc.units ppm.** | **mg/g molasses** |
|---|---|---|---|---|---|---|---|---|
| Add0 | 29.212 | 2.9212 | 0.014324 | 0.001432 | 0.7272 | 0.07272 | 0.038251 | 0.003825 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 41.067 | 92.88 | 0.015860 | 65.22 | 1.0581 | 86.22 | 0.058906 | 93.13 |
| Add2 | 50.873 | 85.91 | 0.019641 | 57.22 | 1.3946 | 80.74 | 0.078108 | 88.50 |
| Add3 | 88.180 | 98.84 | 0.038938 | 71.67 | 2.6433 | 96.92 | 0.122990 | 88.96 |
| | **Ca conc.units ppm.** | **mg/g molasses** | **Cu conc.units ppm.** | **mg/g molasses** | **Mg conc.units ppm.** | **mg/g molasses** | **Zn conc.units ppm.** | **mg/g molasses** |
| Add0 | 28.543 | 2.8543 | 0.015387 | 0.0015387 | 0.69862 | 0.069862 | 0.038714 | 0.0038714 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 39.420 | 90.53 | 0.014250 | - | 1.0354 | 86.38 | 0.057215 | 89.80 |
| Add2 | 52.520 | 89.71 | 0.021525 | 60.82 | 1.4360 | 84.53 | 0.079385 | 89.48 |
| Add3 | 88.947 | 100.45 | 0.038998 | 70.41 | 2.6408 | 97.85 | 0.119480 | 86.13 |
| | **Ca conc.units ppm.** | **mg/g molasses** | **Cu conc.units ppm.** | **mg/g molasses** | **Mg conc.units ppm.** | **mg/g molasses** | **Zn conc.units ppm.** | **mg/g molasses** |
| Add0 | 29.094 | 2.9094 | 0.045041 | 0.0015041 | 0.7100 | 0.071 | 0.038097 | 0.0038097 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 40.527 | 91.91 | 0.017184 | 68.62 | 1.0915 | 90.20 | 0.059642 | 94.52 |
| Add2 | 57.033 | 96.51 | 0.022647 | 64.63 | 1.5132 | 88.49 | 0.082866 | 94.06 |
| Add3 | 86.449 | 97.03 | 0.039201 | 71.22 | 2.9919 | 110.41 | 0.124440 | 90.11 |
| | **Ca conc.units ppm.** | **mg/g molasses** | **Cu conc.units ppm.** | **mg/g molasses** | **Mg conc.units ppm.** | **mg/g molasses** | **Zn conc.units ppm.** | **mg/g molasses** |
| Add0 | 28.509 | 2.8509 | 0.014205 | 0.0014205 | 0.70335 | 0.070335 | 0.0378894 | 0.0037894 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 40.601 | 94.31 | 0.016458 | 67.69 | 1.0962 | 91.09 | 0.057515 | 91.44 |
| Add2 | 52.249 | 89.30 | 0.021916 | 64.07 | 1.4026 | 82.34 | 0.078177 | 88.94 |
| Add3 | 82.641 | 93.27 | 0.038449 | 70.93 | 2.5201 | 93.22 | 0.11171 | 81.01 |
| | **Ca conc.units ppm.** | **mg/g molasses** | **Cu conc.units ppm.** | **mg/g molasses** | **Mg conc.units ppm.** | **mg/g molasses** | **Zn conc.units ppm.** | **mg/g molasses** |
| Add0 | 28.350 | 2.8350 | 0.015387 | 0.0015387 | 0.70435 | 0.07043 | 0.036718 | 0.0036718 |
| | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** | | **Spike recovery %** |
| Add1 | 39.154 | 90.32 | 0.015798 | 62.22 | 1.0389 | 86.26 | 0.058379 | 94.59 |
| Add2 | 54.140 | 92.78 | 0.023516 | 66.45 | 1.4905 | 87.45 | 0.080834 | 93.21 |
| Add3 | 83.067 | 94.02 | 0.037386 | 67.49 | 2.6620 | 98.43 | 0.117660 | 86.06 |
| Mean | | 93.18 | | 66.33 | | 90.702 | | 90.00 |
| Std. Add. | | ±3.87 | | ±4.28 | | ±7.68 | | ±3.74 |

# APPENDIX B

Table B.1. ICP-AES results of Fermentation-1

| | | Ca mg/g sample | Cu mg/g sample | Mg mg/g sample | Zn mg/g sample |
|---|---|---|---|---|---|
| M | Molasses | 3.2988 | 0.00286 | 0.1336 | 0.00500 |
| | | 3.3132 | 0.00277 | 0.1288 | 0.00590 |
| | | 3.3355 | 0.00291 | 0.1345 | 0.00547 |
| S | Suppl. Water | 0.0729 | 0.000133 | 0.1571 | 0.00001 |
| | | 0.0737 | 0.000141 | 0.1575 | 0.000009 |
| | | 0.0743 | 0.000147 | 0.1633 | 0.000009 |
| SY | Seed yeast | 0.605 | 0.00174 | 0.238 | 0.0209 |
| | | 0.609 | 0.00178 | 0.240 | 0.0195 |
| | | 0.604 | 0.00184 | 0.232 | 0.0197 |
| F0 | Fer.0.h | 0.236 | 0.00142 | 0.144 | 0.00897 |
| | | 0.213 | 0.00152 | 0.142 | 0.00783 |
| | | 0.211 | 0.00146 | 0.144 | 0.00805 |
| F0a | Fer.0.h Yeast cake | 0.24928 | 0.005334 | 0.4299 | 0.05316 |
| | | 0.26850 | 0.005544 | 0.4270 | 0.05325 |
| | | 0.25876 | 0.005550 | 0.4264 | 0.05423 |
| F4a | Fer.4.h Yeast cake | 0.2200 | 0.005257 | 0.6017 | 0.05683 |
| | | 0.2959 | 0.005009 | 0.6150 | 0.5685 |
| | | 0.2069 | 0.005128 | 0.6116 | 0.0562 |
| F8a | Fer.8.h Yeast cake | 0.3262 | 0.004638 | 0.5655 | 0.0334 |
| | | 0.3616 | 0.004350 | 0.5619 | 0.0322 |
| | | 0.4173 | 0.004450 | 0.5741 | 0.0335 |
| F12a | Fer.12.h Yeast cake | 0.5532 | 0.003533 | 0.4197 | 0.0262 |
| | | 0.5537 | 0.003226 | 0.4208 | 0.0268 |
| | | 0.5521 | 0.003320 | 0.4356 | 0.0269 |
| F17a | Fer.17.h Yeast cake | 0.7990 | 0.002789 | 0.3806 | 0.0248 |
| | | 0.8206 | 0.002890 | 0.3682 | 0.0245 |
| | | 0.8155 | 0.002970 | 0.3743 | 0.0242 |
| F0b | Fer.0.h Filtrate | 0.1301 | 0.000588 | 0.129 | 0.002703 |
| | | 0.1296 | 0.000575 | 0.129 | 0.002664 |
| | | 0.1339 | 0.000602 | 0.130 | 0.002689 |
| F4b | Fer.4.h Filtrate | 0.3327 | 0.000415 | 0.11254 | 0.000425 |
| | | 0.3344 | 0.000411 | 0.11310 | 0.000314 |
| | | 0.3314 | 0.000404 | 0.11050 | 0.000278 |
| F8b | Fer.8.h filtrate | 0.7479 | 0.000244 | 0.0824 | 0.000376 |
| | | 0.7425 | 0.000240 | 0.0828 | 0.000314 |
| | | 0.7389 | 0.000245 | 0.0828 | 0.000374 |
| F12b | Fer.12.h Filtrate | 1.1271 | 0.000278 | 0.0831 | 0.000355 |
| | | 1.1165 | 0.000273 | 0.0809 | 0.000348 |
| | | 1.1171 | 0.000264 | 0.0811 | 0.000347 |
| F17b | Fer.17.h Filtrate | 1.4930 | 0.000428 | 0.0930 | 0.000712 |
| | | 1.4224 | 0.000388 | 0.0866 | 0.000589 |
| | | 1.4480 | 0.000382 | 0.0900 | 0.000720 |
| CY | Comp. Yeast | 0.4885 | 0.00274 | 0.363 | 0.0237 |
| | | 0.4880 | 0.00269 | 0.355 | 0.0244 |
| | | 0.5116 | 0.00324 | 0.364 | 0.0246 |
| D | Disposal | 1.176 | 0.000384 | 0.0690 | 0.000405 |
| | | 1.171 | 0.000369 | 0.0687 | 0.000381 |
| | | 1.240 | 0.000379 | 0.0746 | |
| YS | Yeast milk | 0.444 | 0.00154 | 0.212 | 0.018 |
| | | 0.456 | 0.00157 | 0.216 | 0.018 |
| | | 0.401 | 0.00138 | 0.208 | 0.015 |

Table B.2. ICP-AES results of Fermentation-2

| | | Ca mg/g sample | Cu mg/g sample | Mg mg/g sample | Zn mg/g sample |
|---|---|---|---|---|---|
| M | Molasses | 0.31307 | 0.002792 | 0.4085 | 0.007496 |
| | | 0.31108 | 0.002780 | 0.4045 | 0.007503 |
| | | 0.31020 | 0.002794 | 0.4071 | 0.007716 |
| S | Suppl. Water | 0.009694 | 0.001169 | 0.1355 | 0.000102 |
| | | 0.010188 | 0.001145 | 0.1432 | 0.000129 |
| | | 0.010859 | 0.001194 | 0.1529 | 0.0001477 |
| SY | Seed yeast | 0.03631 | 0.001737 | 0.2057 | 0.01905 |
| | | 0.03730 | 0.001619 | 0.2041 | 0.02764 |
| | | 0.04095 | 0.001745 | 0.2055 | 0.03380 |
| F0 | Fer.0.h | 0.01909 | 0.001737 | 0.2057 | 0.01905 |
| | | 0.01653 | 0.001619 | 0.2041 | 0.02764 |
| | | 0.01781 | 0.001745 | 0.2055 | 0.0338 |
| F4a | Fer.4.h Yeast cake | 0.02884 | 0.007562 | 0.65560 | 0.1328 |
| | | 0.03073 | 0.007641 | 0.65530 | 0.1344 |
| | | 0.02533 | 0.007348 | 0.67354 | 0.1327 |
| F8a | Fer.8.h Yeast cake | 0.04209 | 0.005748 | 0.52896 | 0.0601 |
| | | 0.04171 | 0.005737 | 0.53989 | 0.0626 |
| | | 0.03737 | 0.005392 | 0.054436 | 0.0622 |
| F12a | Fer.12.h Yeast cake | 0.05425 | 0.005414 | 0.44277 | 0.0458 |
| | | 0.05621 | 0.005397 | 0.45028 | 0.0465 |
| | | 0.05559 | 0.005547 | 0.44486 | 0.0461 |
| F17a | Fer.17.h Yeast cake | 0.08114 | 0.004045 | 0.4210 | 0.0478 |
| | | 0.08237 | 0.003912 | 0.4176 | 0.0435 |
| | | 0.09318 | 0.003883 | 0.4348 | 0.0452 |
| F4b | Fer.4.h Filtrate | 0.02612 | 0.000458 | 0.1049 | 0.000963 |
| | | 0.02536 | 0.000449 | 0.1044 | 0.000922 |
| | | 0.02487 | 0.000448 | 0.1050 | 0.000724 |
| F8b | Fer.8.h filtrate | 0.05270 | 0.000322 | 0.1121 | 0.000492 |
| | | 0.02524 | 0.000468 | 0.1046 | 0.0008445 |
| | | 0.02598 | 0.000460 | 0.1095 | 0.0007491 |
| F12b | Fer.12.h Filtrate | 0.07449 | 0.000314 | 0.1266 | 0.0004520 |
| | | 0.07524 | 0.000303 | 0.1256 | 0.0006290 |
| | | 0.07479 | 0.000339 | 0.1275 | 0.0005433 |
| F17b | Fer.17.h Filtrate | 0.09346 | 0.000405 | 0.1502 | 0.0005445 |
| | | 0.09332 | 0.000384 | 0.1486 | 0.0004916 |
| | | 0.09491 | 0.000427 | 0.1515 | 0.0006664 |
| CY | Comp. Yeast | 0.1162 | 0.003754 | 0.4995 | 0.06823 |
| | | 0.07387 | 0.003397 | 0.4835 | 0.05629 |
| | | 0.07615 | 0.003359 | 0.4918 | 0.06197 |
| D | Disposal | 0.09807 | 0.000573 | 0.1558 | 0.000498 |
| | | 0.09847 | 0.000578 | 0.1572 | 0.000333 |
| | | 0.09787 | 0.000549 | 0.1567 | 0.000291 |
| YM | Yeast milk | 0.042 | 0.001727 | 0.2403 | 0.02535 |
| | | 0.0415 | 0.001658 | 0.2440 | 0.02569 |
| | | 0.0457 | 0.001949 | 0.2550 | 0.02582 |

# APPENDIX C

**Mass balance calculations**

**$C_s$:** concentration of element in seed yeast (mg/kg)

**$C_w$:**concentration of element in water (mg/kg)

**$C_m$:**concentration of element in molasses (mg/kg)

**$Cc_4$:** concentration of element in biomass at 4.h (mg/kg)

**$Cc_8$:** concentration of element in biomass at 8.h (mg/kg)

**$Cc_{12}$:** concentration of element in biomass at 12.h (mg/kg)

**$Cc_{17}$:** concentration of element in biomass at 17.h (mg/kg)

**$Cf_4$:** concentration of element in filtrate at 4.h (mg/kg)

**$Cf_8$:** concentration of element in filtrate at 8.h (mg/kg)

**$Cf_{12}$:** concentration of element in filtrate at 12.h (mg/kg)

**$Cf_{17}$:** concentration of element in filtrate at 17.h (mg/kg)

**S :** mass of seed yeast (kg)

**W:** mass of supplemented water (kg)

**$M_4$:** mass of molasses at 4.h (kg)

**$M_8$:** mass of molasses at 8.h (kg)

**$M_{12}$:** mass of molasses at 12.h (kg)

**$M_{17}$:** mass of molasses at 17.h (kg)

**$Y_4$ :** mass of biomass (kg)

**$Y_8$ :** mass of biomass (kg)

**$Y_{12}$ :** mass of biomass (kg)

**$Y_{17}$ :** mass of biomass (kg)

**$F_4$ :** mass of filtrate (kg)

**$F_8$ :** mass of filtrate (kg)

**$F_{12}$:** mass of filtrate (kg)

**$F_{17}$:** mass of filtrate (kg)

**4.h-Mass balance:** $S*C_s + W*C_w + M_4*C_m = Y_4*Cc_4 + F_4*Cf_4$

**8.h-Mass balance:** $S*C_s + W*C_w + M_8*C_m = Y_8*Cc_8 + F_8*Cf_8$

**12.h-Mass balance:** $S*C_s + W*C_w + M_{12}*C_m = Y_{12}*Cc_{12} + F_{12}*Cf_{12}$

**17.h-Mass balance:** $S*C_s + W*C_w + M_{17}*C_m = Y_{17}*Cc_{17} + F_{17}*Cf_{17}$

# APPENDIX D

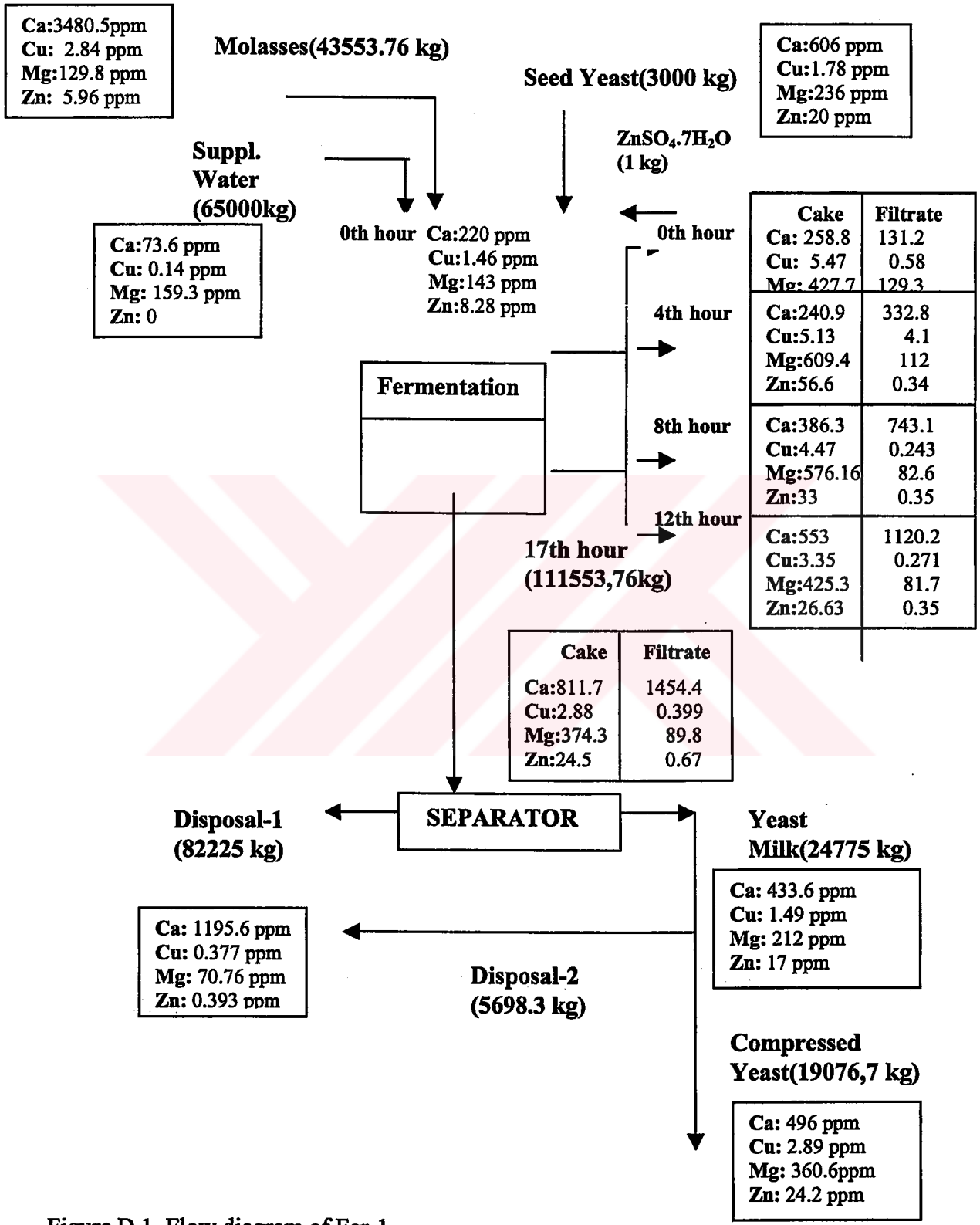



Figure D.1..Flow diagram of Fer-1

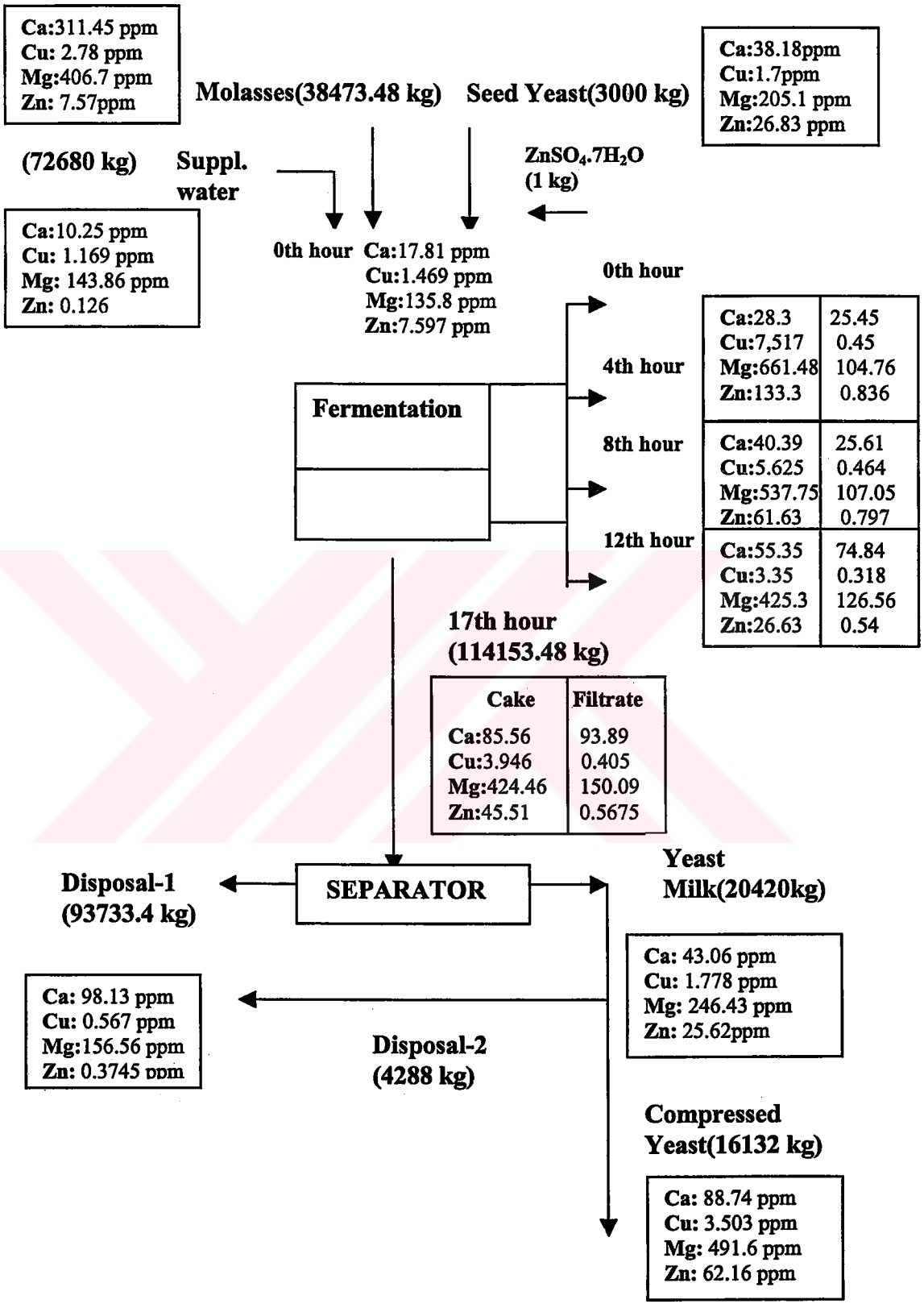
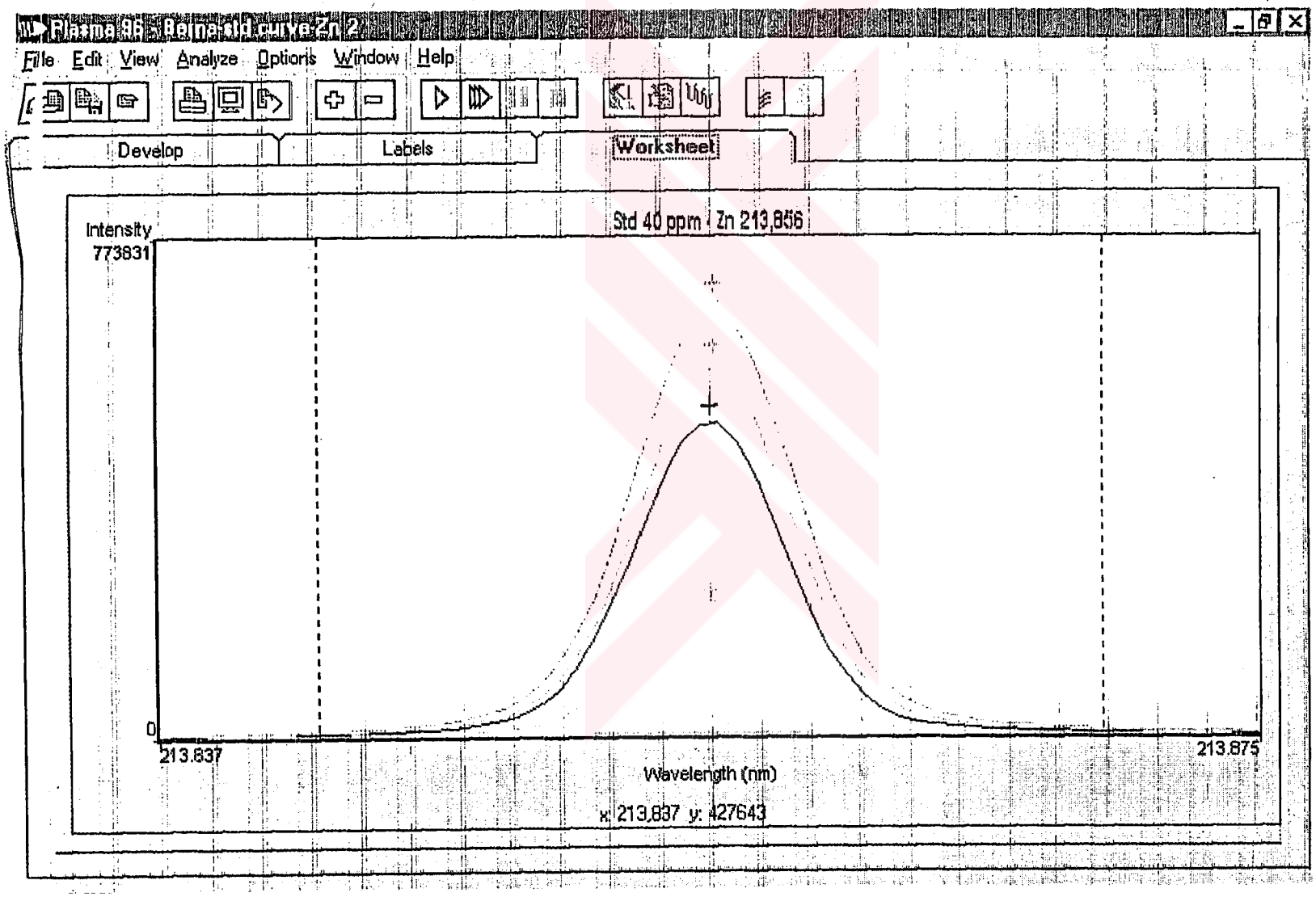



Figure D.2. Flow diagram of Fer-2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File Edit View Analyze Options Window Help
Develop
Labels
Worksheet
Std 40 ppm - Zn 213,856
Intensity
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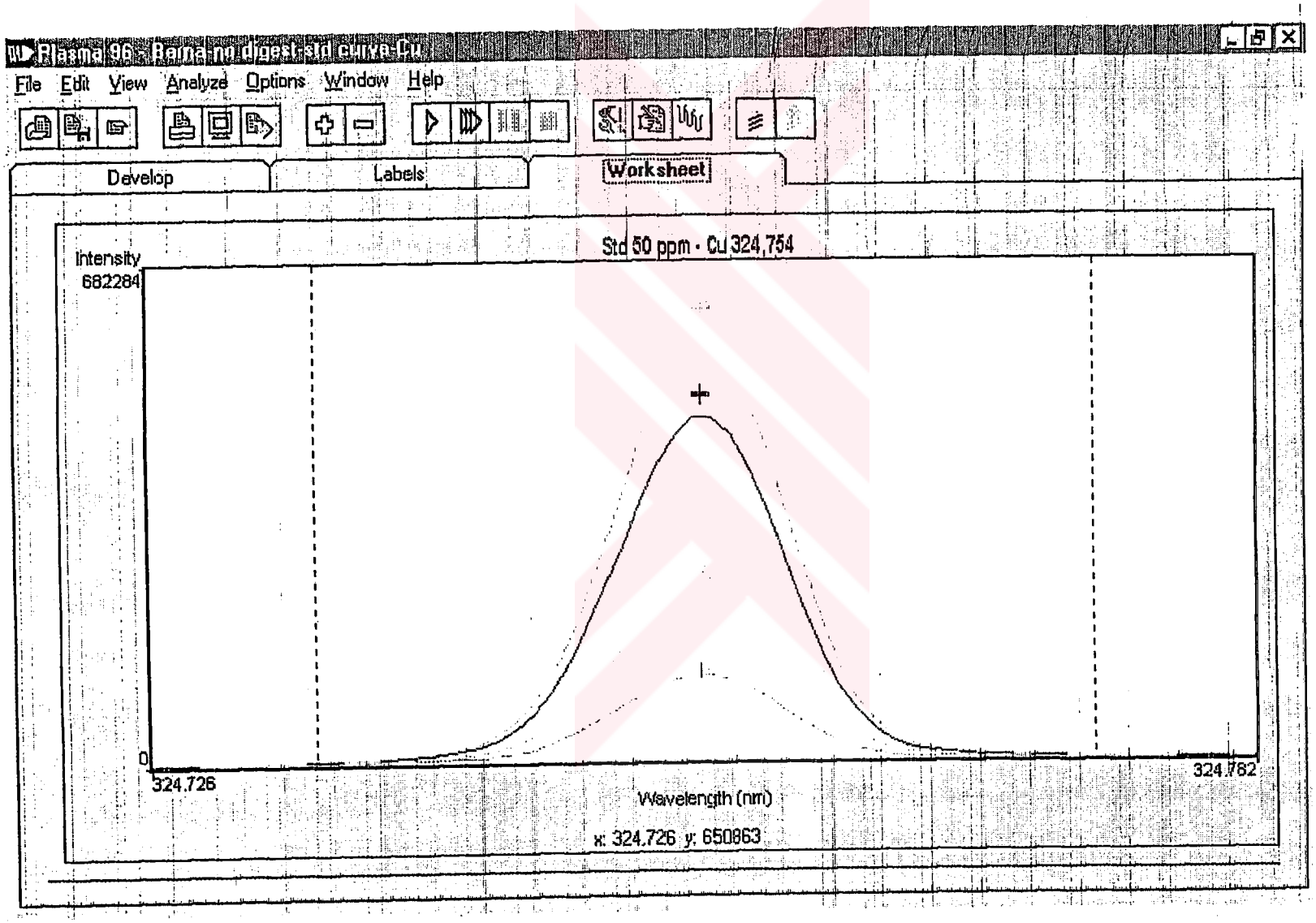
0
213.837
213.875
Wavelength (nm)
x: 213,837 y: 427643

E4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File Edit View Analyze Options Window Help
Develop
Labels
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Intensity
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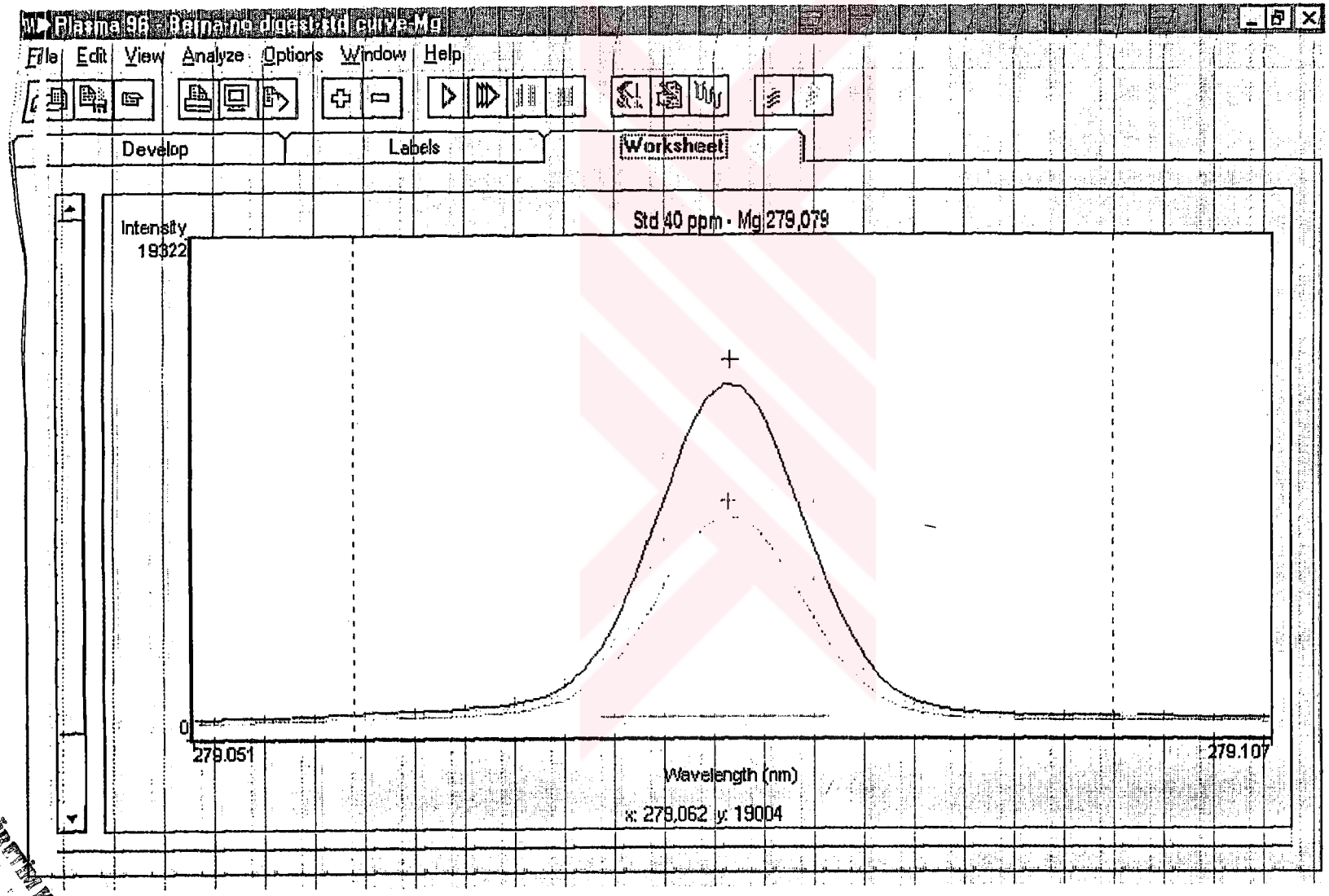
0
324.726
324.782
Wavelength (nm)
x: 324.726 y: 650863

E3



# APPENDIX E

## ICP-AES Standard Peaks at Suitable Wavelengths