Development of a sustainable method for the disposal of chromated copper arsenate(CCA) treated wood

Azita Hajizadeh Moghaddam

A Thesis

In

The Department of Building, Civil and Environmental Engineering

Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

Concordia University

Montreal, Quebec, Canada

November 2010

CONCORDIA UNIVERSITY

Department of Building, Civil and Environmental Engineering

This is to certify that the thesis prepared

By: **Azita Hajizadeh Moghaddam**

Entitled: **Development of a sustainable method for the disposal of Chromated Copper Arsenate (CCA) treated wood**

And submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy (Environmental Engineering)

Complies with the regulations of the University and meets with the accepted standards with respect to originality and quality.

Signed by the final examining committee:

Dr. W. Xie, Chair

Dr. J. Powlowski, External-to-Program, (Chemistry & Biochemistry)

Dr. F. Haghighat, BCEE Examiner

Dr. J. Hadjinicolaou, BCEE Examiner

Dr. J.F. Blais, External Examiner, (Institut National de la Recherche Scientifique Québec)

Dr. C. Mulligan, BCEE, Supervisor

Approved by:

Dr. M. Zaheeruddin, Chair, Department of Building, Civil & Environmental Engineering

Dr. R. Drew, Dean Faculty of Engineering and Computer Science

Abstract

Development of a sustainable method for the disposal of chromated copper arsenate (CCA) treated wood

Azita H. Moghaddam, Ph.D.

Concordia University, 2010

Preserved wood is commonly found in solid waste. Among the different types of preserved wood, CCA wood has received much attention due to the scale of usage and its significant role in soil and water contamination after disposal. As the ash of CCA wood is hazardous, it cannot be burned, and the best available disposal method is thus landfilling. Leaching of the metals from disposed CCA wood in landfills pollutes the environment. To reduce the contamination of CCA, treatment before landfilling is required. Nowadays, ethanol is seen as a promising source of energy. Lignocellulosic materials such as wood are resources for ethanol production. This research focuses on the possibility of producing ethanol from CCA wood. It suggests that production of ethanol will not only be a solution to the disposal but will also generate a clean fuel.

The results showed the existence of copper, chromium and arsenic did not have a negative effect on the fermentation, and producing ethanol from CCA wood is feasible. The copper removed by sulfuric acid completely precipitated during the hydrolysis and

neutralization. In addition about 50% of the chromium (VI) and also 60% of the arsenic (V) were removed from the leachate by yeast during fermentation.

TCLP tests of the hydrolyzed wood leached less than 4 ppm of arsenic while minimal amounts of chromium and copper remained in the hydrolyzed wood which makes landfilling of hydrolyzed wood acceptable.

Baker's yeast behaves selectively by uptaking arsenic (V) and chromium (VI) but not arsenic (III) and chromium (III). There is competition between copper and chromium sorption by yeast. The kinetic model for removal of copper and chromium is a zero order model while the appropriate model for uptaking arsenic by yeast is a first order model. The kinetic models confirm that there are different mechanisms of uptaking metals by yeast, a diffusion mechanism for removal arsenic and a surface adsorption mechanism for copper and chromium.

As an overall conclusion of this study, using discarded CCA wood as the feed for ethanol production is a sustainable method for disposal of CCA treated wood.

Acknowledgements

I would like to express my sincere gratitude to my supervisor Dr. Catherine N. Mulligan for her great help and comments throughout my work, unfailing patience for correction of my errors including those of grammatical nature, encouragement and support.

I am thankful to my colleagues Thejashree Nagarakere Ramalingaiah for her help on editing my thesis, Dr. Mahmood Alimahmoodi and Andre Habel for their help in conducting the laboratory work and also Joseph Hrib.

I would like to thank the members of the evaluation committee and also Concordia University faculty and staff members.

My deepest appreciations to my parents, Khojasteh and Mohammad-Ali, for their endless support for my entire life. And thanks to my sisters Rozita and Parisa, my brother Hamid-Reza and my spouse, Siamak.

Table of contents

List of Figures

List of Tables

List of Abbreviation

AFEX	Ammonia Freeze Explosion
As	Arsenic
ATP	Adenosine Triphosphate
AU	Absorbance Units
AWPA	Amrican Wood Preservers' Association
C,C_0	Concentration
CCA	Chromated Coppet Arsenate
Cr	Chromium
Cu	Copper
EDTA	Ethylene Diamine Tetra Acetic acid
EPA	United States of America Environmental Protection Agency
HPLC	High Performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
k, k_0	Reaction rate
ppm	Parts per Million
SPLP	Synthetic Precipitation Leaching Procedure
TCLP	Toxicity Characteristic Leaching Procedure
UV/Visible	Ultraviolet/Visible Spectrophotometer
XRF	X-ray fluorescence

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND INFORMATION

Wood is one of the most commonly used building materials in North America. Modern commercial forestry and sawmills mainly are providers of material for the building industry (Sloot et al., 1997). The Environmental Protection Agency (EPA) estimated that 5.8% of 236,000 million kg of generated waste in 2003 was wood (U.S. EPA, Office of Solid Waste, 2006).

Following wood removal from the forests, several types of deterioration by fungi and insects threaten the untreated wood and reduce the lifetime of the wood and the wood building materials. Chemicals are utilized to treat the wood to protect wood against bacterial, fungal, and insect attack. Chemical treatment has been practiced for centuries and is intended to enhance wood durability, and thereby increasing the life expectancy of wood in service. As an example, railroad cross ties that are used in North America would have an average life time of five years without treatment (Konasewich and Henning, 1998), whereas the lifetime of most creosote-preserved wood is estimated to be 30 years (Webb, 1990). In Florida, it has been found that up to 30% of the construction and

demolition (C&D) wood can be CCA-treated wood (Solo-Gabriele et al., 2003). The most utilized method of disposal used for treated wood is landfilling in U.S., Canada and Australia without any pretreatment. Countries such as Germany ban landfill disposal but the ash of treated incinerated wood can be hazardous since CCA concentrates in the ash (Solo-Gabriele et al., 2002) and in addition, proper air pollution equipment is required as toxic gases are produced during incineration (Iida et al., 2004). Some methods for the treatment of CCA treated wood are being developed such as thermochemical means as reviewed by Helsen and Van den Bulck (2005) and by biological treatment.

One of the most problematic types of preserved wood in the United States as well as in Canada is CCA treated wood which is used in outdoor decks, playgrounds, and fences. CCA was favored for lumber treatment because it is inexpensive, leaves a dry, paintable surface, and binds to become relatively leach-resistant. However, there is increasing concern about potential environmental contamination from leaching of Cu, Cr and As from treated wood in service and from wood removed from service and placed in landfills in North America (Shalat et al., 2006). The life cycle of treated wood is estimated to be about 25 years and then the wood is discarded as waste (Illman and Highley, 1996). By 1995, more than 90% of 67 million kg of utilized waterborne preservatives was CCA (Solo-Gabriele and Townsend, 1999). Despite this, the quantity of removed treated wood from service is estimated to increase to 16 million cubic meters by the year 2020 in the U.S. (Cooper, 1993).

Preserved wood is a common part of the solid waste (Tom, 2001). Today, municipal solid waste management has become an important part of environmental protection activities. In 2003 U.S. produced more than 236,000 million kg of municipal solid waste of which 30% was recycled and the rest was disposed of by landfilling or composting (U.S. EPA, Office of Solid Waste 2006). In Canada, even though the population is less, the same problem exists. As there are some limitations for the types of wastes that can be recycled or combusted, landfilling is an important method for municipal and construction debris waste management (Ress et al., 1998).

Since the ash of treated wood is hazardous, the best existing disposal method for used treated wood is landfilling. During rain, water penetrates into landfills and causes leaching of wood preservatives (copper, chromium and arsenic) from the disposed *Chromated Copper Arsenate* (CCA) treated wood. The leached metals can pollute ground and drinking water. As the leachate of treated wood in landfills is genotoxic and carcinogenic, the contaminated water is dangerous for human beings and animals. According to Moghaddam (2002) there is the risk of soil, water and environmental contamination by chromium, copper and arsenic, wherever the chromated copper arsenate treated wood is buried. Therefore, another method of disposal of treated wood other than landfilling and incineration could help to decrease its damage to the environment.

Nowadays, ethanol is seen as a promising new source of energy and valuable substitute for gas, oil and gasoline. Lignocellulosic materials such as wood are resources for ethanol production.

This research will focus on the possibility of producing ethanol from CCA treated wood. It suggests that production of ethanol from CCA treated wood will not only be a solution to the disposal of CCA treated wood but also will generate a clean fuel as a source of energy. Uptake of chromium, copper and arsenic by yeast during ethanol production is another advantage of the proposed method.

1.2 OBJECTIVES

In the past decade most of the research about CCA wood was focused on proving that hazardous materials (especially arsenic) would be leached from CCA treated wood more than acceptable levels to force authorities to make regulations to limit the usage of this kind of wood and there is less research about the disposal of CCA treated wood. Landfilling is the general disposal method of CCA wood and in many places CCA treated wood is disposed along with the other demolished building materials in municipal landfills without any lining which causes the contamination of soil and ground water by arsenic, copper and chromium. The main goal of this study is to develop a sustainable method for the disposal of CCA treated wood by using the ethanol production process (including concentrated sulfuric acid hydrolysis and fermentation steps) prior to landfilling of CCA treated wood. The objectives can be specified as to determine:

- The effect of weathered wood and size of wood particles on leaching of metal during hydrolysis.

- The effect of metal type and concentration on yeast and the amount and the duration of ethanol production.
- The leachability and management of the solid remains of wood
- The mechanism of arsenic, copper and chromium sorption by yeast.

1.3 THESIS ORGANIZATION

Thesis includes 8 chapters:

- Chapter One is the introduction to the problem and includes the problem definition, research objectives and the organization of the thesis
- Chapter Two or 'Literature review' contains background information and most related previous studies are discussed in this chapter
- Chapter Three includes the description of the materials, laboratory instruments and experimental procedures
- Chapter Four is devoted to the results and discussion
- Chapter Five includes metal uptake and yeast growth models based on the results
- Chapter Six is a brief economical evaluation of ethanol production from CCA treated wood
- Chapter Seven summarizes the conclusions of this study and states the contribution to knowledge
- Chapter Eight suggests the directions for future work
- References are listed at the end followed by appendices

CHAPTER TWO

LITERATURE REVIEW

2.1 WOOD

2.1.1 WOOD STRUCTURE

The three main structural components of trees are:

(http://forestry.about.com/od/treephysiology/ss/part_of_tree.htm)

- Roots, which gather water and mineral nutrients and provide a firm anchor for all the structure.
- Crown including the leaves and small branches.
- Bole or trunk.

Wood is primarily composed of hollow, elongate, spindle-shaped cells that are arranged parallel to each other along the trunk of a tree. Wood cells are formed in the very thin cambium, between the bark and wood. Cells on the outside of the cambium form the phloem, or inner bark. Cells on the inside form the xylem, or wood. Many more xylem cells than phloem cells are formed (Miller, 1999).

A cross section of a tree (Figure 2.1) shows (from the outside to the center):

- Bark, which may be divided into dead and living parts. The inner living part (B), which is thin, carries food from the leaves to the growing parts of the tree. And the outer dead part (A), whose thickness varies greatly with species and age of trees.
- Cambium(C), which forms bark and wood cells.
- Sapwood (D), which contains both living and dead tissue and carries sap from the roots to the leaves.
- Heartwood (E), which usually consists of inactive cells.
- Pith (F) at the center of tree stem, which is a small core of tissue.
- Branches and twigs.
- Wood rays (G), which are horizontally oriented tissues from pith toward bark, and connect the various layers for storage and transfer of food.

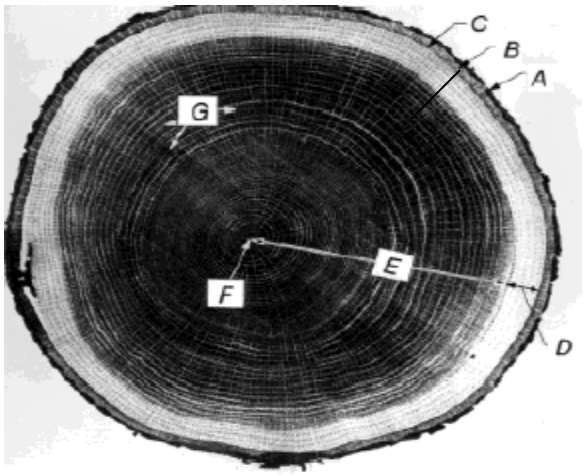

Figure 2.1: Cross section of white oak tree trunk
(A) outer bark (B) inner bark (C) cambium (D) sapwood
(E) heartwood (F) pith and(G) wood rays (Miller, 1999)

In softwood (Figure 2.2), there are two main types of cells, tracheids and parenchyma. Tracheids are the main part of wood cells, oriented longitudinally with a length of 3-8 mm. The parenchyma are the cells for storage of food (Miller, 1999).

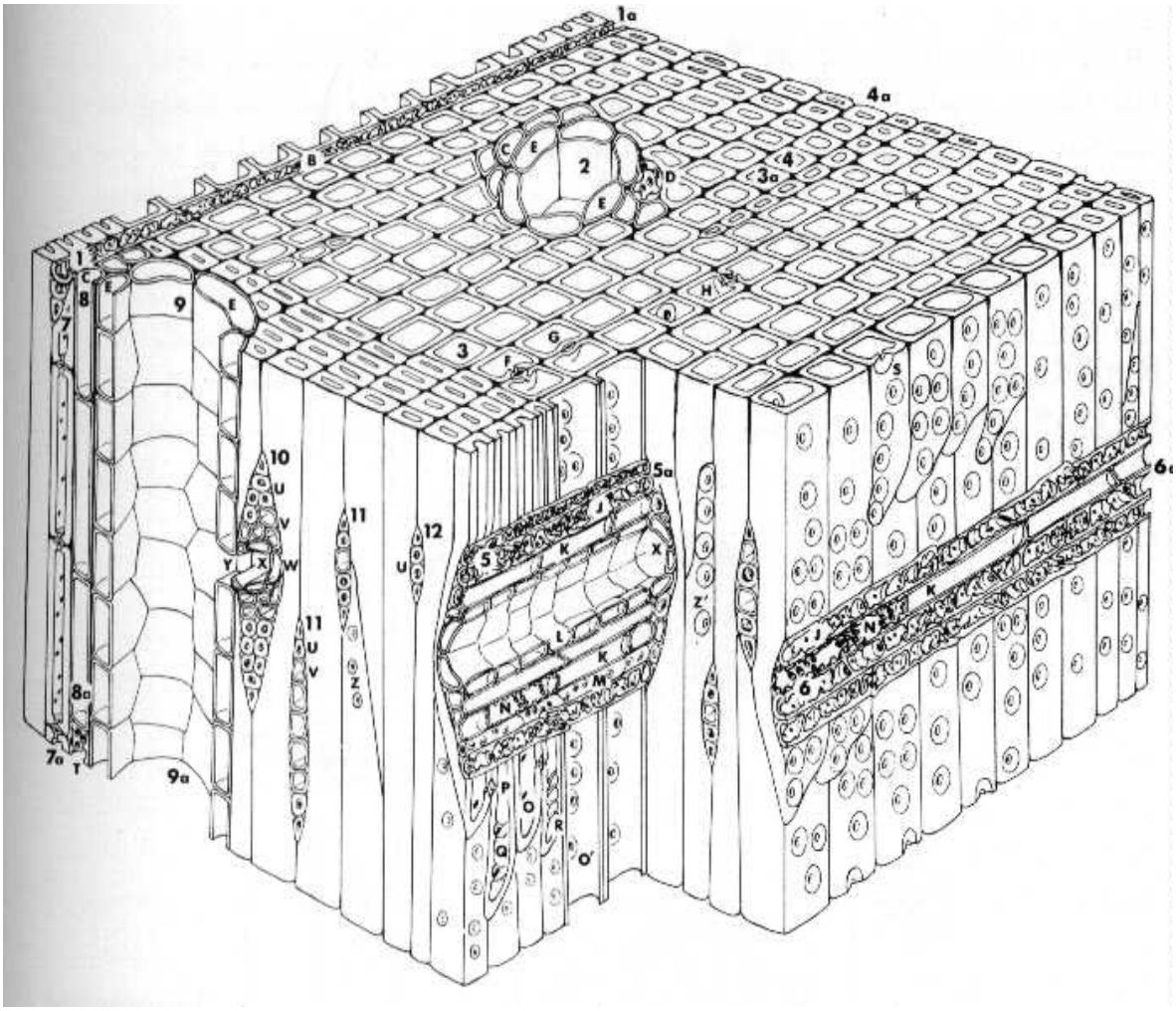

Figure 2.2: Typical softwood structure

(Howard and Manwiller, 1969. From Siau, Transport Process in Wood, 1984)

Hardwood is more complex than softwood. It consists of inactive cells that do not function in either water conduction or food storage. The main difference is that in hardwood the liquid transport through the vessels but in softwood, through the tracheids. The vessels are composed of short large diameter cells, one on top of another to make a longitudinal channel. Hardwood fibers function only as a support and do not conduct water (Miller, 1999).

The wood cell wall is composed of two walls (Figure 2.3), the primary (P layer) and the secondary layer. The secondary layer made of three layers, S1, S2 and S3 (Butterfield and Meylan, 1980). As the S2 layer is the thickest layer with the highest percentage of lignin, and with respect to physical properties, the S2 layer is the most important layer (Tsoumis, 1992).

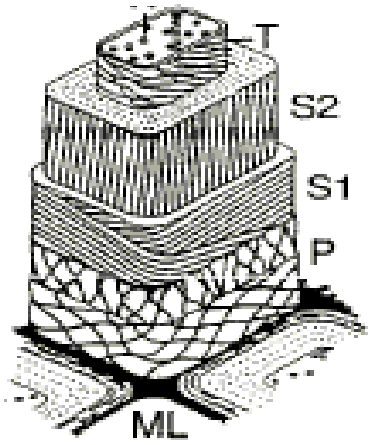

Figure 2.3: Wood cell wall structure

ML=middle lamella (mainly lignin); P= primary wall;
S1=secondary wall; S2= secondary wall (main body);
S3= tertiary wall. (**Krassig**, 1993)

2.1.2 CHEMICAL COMPOSITION OF THE WOOD CELL

Dry wood consists of cellulose, lignin, hemi-celluloses and minor amounts (5% to 10%) of extractives (Miller, 1999).

- Cellulose (Figure 2.4), the major component, is a linear crystalline polymer of glucose, which constitutes approximately 50% of wood substance by weight. It is present as microfibrils of extended cellulose chains.

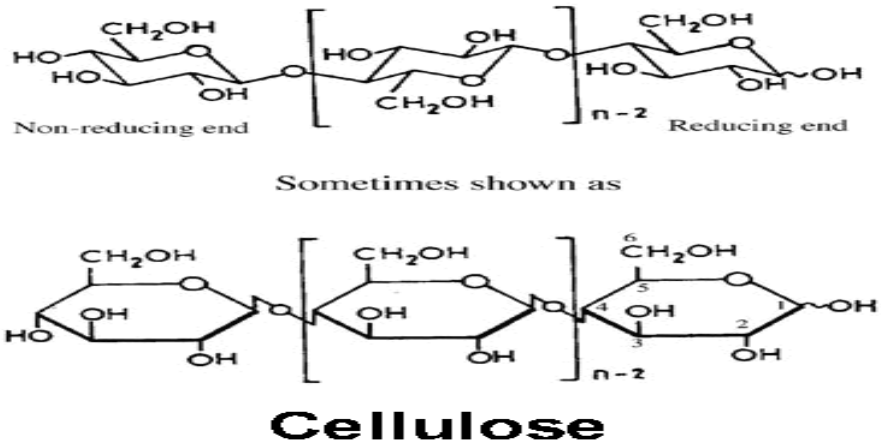

Figure 2.4: Cellulose structure

Lignin is a three-dimensional phenylpropanol polymer, which is often called the cementing agent that binds individual cells together. About 23% to 33% of the wood substance in softwoods and 16% to 25% in hardwoods is lignin. It is concentrated towards the outside of the cells and between cells.

The hemicelluloses are branched; low-molecular-weight polymers composed of several different kinds of pentose and hexose sugar monomers. The relative amounts of these sugars are related to the species. Extractives are a wide variety of small molecules in wood, which are soluble in organic solvents (Miller, 1999).

2.1.3 CCA TREATED WOOD

Chromated copper arsenate (CCA) is the major wood preservative, which is used in United States as well as in Canada. CCA is favored for lumber treatment because it is inexpensive, leaves a dry, paintable surface, and binds to become relatively leach-resistant. However, there is increasing concern about potential environmental contamination from leaching of Cu, Cr and As from treated wood in service and from wood removed from service and placed in landfills.

To preserve the wood, it is necessary for the chemicals to penetrate into wood deeply (several centimeters). A pressure treatment plant (Figure 2.5) is used to achieve such penetration followed by preservative fixation within wood. There are several factors that affect the penetration of preservatives into wood such as (Morris, 1996):

- The applied treatment process
- Wood permeability
- Heartwood /sapwood ratio
- Wood moisture content
- Wood quality

Depending on the above-mentioned factors, the preservative can either penetrate evenly into the heartwood such as Scots pine or can only be restricted to the surface like Norway spruce (Morris, 1996).

The chemical, chromated copper arsenate, is normally purchased as a premixed concentrate of 50% to 65% then stored in tanks and diluted with water to a 1.5 % to 4 % strength working solution. Diluted solution is then applied to the wood in pressure cylinders (Konasewich and Henning, 1998). The distribution of chemicals in the cell wall matrix, which affects the effectiveness of preservation, depends on wood species, cation exchange reactions and treating solution pH (Cooper, 1988).

The American Wood Preservers' Association (AWPA) specifies three formulations for CCA (Table 2.1). The differences of the A, B and C types are in the relative proportions (oxide basis) of chromium, copper, and arsenic. As CCA-C offers the best combination of performance and leach resistance, most of the produced CCA wood is treated with type C of CCA (Tom, 2001). The use of CCA–B is currently confined to field and remedial treatments, and relatively few treaters use CCA-A.

All the components of CCA have important roles in preservative properties. Fixation of CCA is a part of the complex reduction reactions of chromium from the hexavalent to

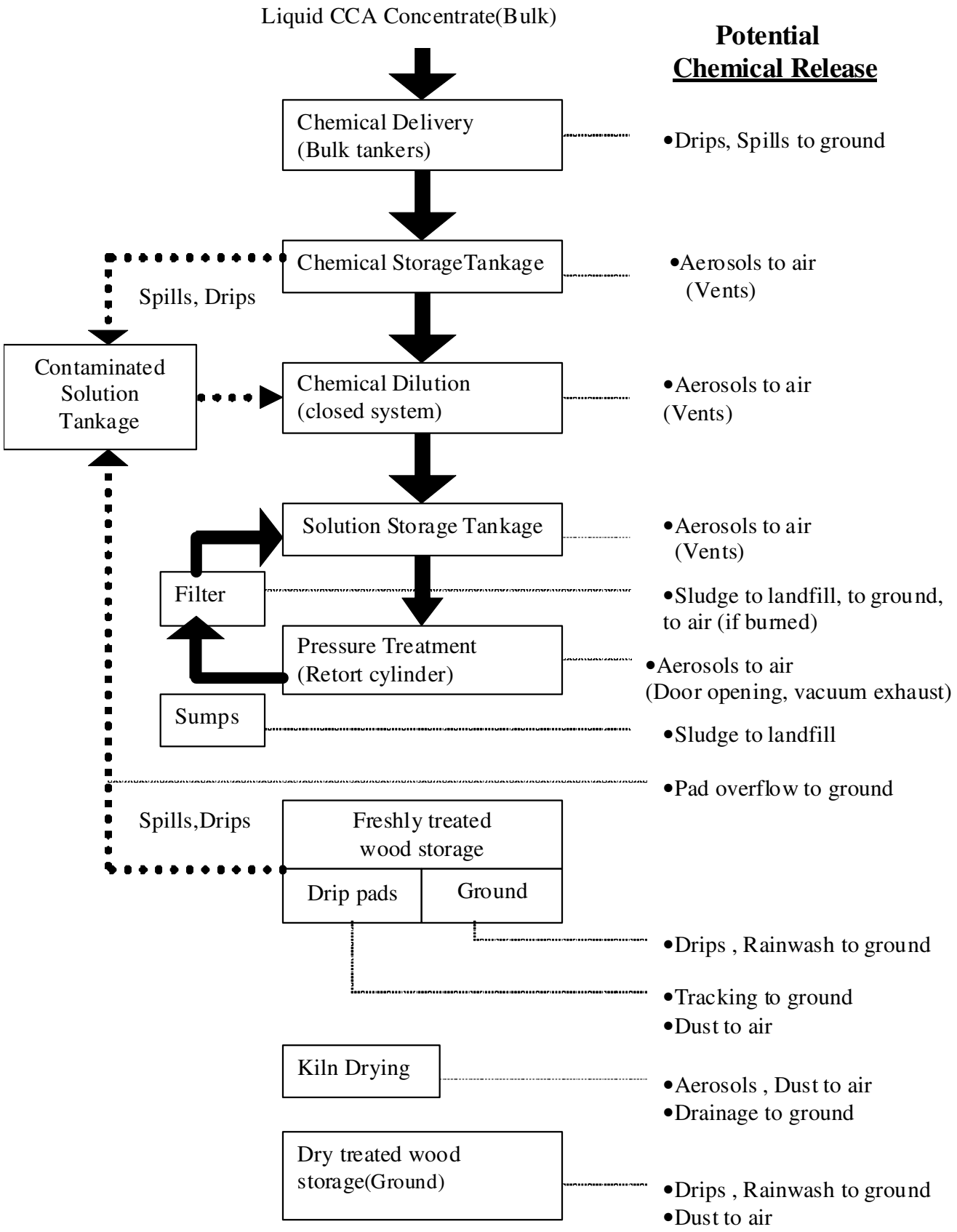

Figure 2.5: Potential chemical release from CCA pressure treating plants (Konasewich and Henning, 1998)

trivalent valence state. These reactions cause the insolubility of CCA in the wood, which resists leaching and provides lengthy service, even when the wood is in contact with ground.

Table 2.1: Composition of three CCA formulations as specified by AWPA Standards

Component	Type A (%)		Type B (%)		Type C (%)	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Cr as CrO_3	59.4	69.3	33	38	44.5	50.5
Cu as CuO	16	20.9	18	22	17	21
As as As_2O_5	14.7	19.7	42	48	30	38

From AWPA (1994)

Copper is a very strong fungicide and because of its fungicidal properties and low mammalian toxicity, other waterborne preservatives include copper. Arsenic is an insecticide and helps to protect wood against some copper – tolerant fungi.

Since the preservative composition can affect fixation and thus the leachability of chemicals, the amount of added chemicals to the wood is very important. The retention level and required amount of chemicals vary with the intended use of the product and the place of usage. AWPA standards list seven amounts of CCA for different usages (Table 2.2).

2.1.3.1 LEACHING

Leachate is the liquid that is produced when rain falls on a landfill or buildings, sinks into the wastes, and picks up chemicals as it seeps downward. In respect to environmental aspects, the leaching of compounds is an important issue for construction materials and their leaching behavior should be checked during their overall life cycle, from the production time until reuse or disposal.

Table 2.2: Retention levels of CCA active ingredients

Exposure	Retention level (kg/m³)
Above ground	4
Ground contact	6.4
Poles and foundations	9.6
Land and freshwater piling	12.8 and 16
Sea water application	24 and 40

From AWPA (1994)

In the late 1980s the treated wood became one of the environmental concerns and because of chemical leaching from disposed or in service preserved wood, the concerns have increased. After treatment there are three stages in the treated wood lifecycle that can be identified (Sloot et al., 1997):

- Storage at the treatment facility

- Actual service (Table 2.3)
- Decommissioning and waste phase.

Table 2.3: Leaching hazards for preservative treated wood

Degree of wetting	Risk of leaching	Typical application
Interior, fully protected from liquid water	No leaching	Framing lumber, joists, flooring
Interior, occasional wetting	No or slight leaching	Sillplates
Exterior, intermittent wetting	Periodic moderate leaching	Windows, fascia boards, decks, fence boards
Exterior, permanent wetting	Severe leaching	Wood foundations, utility poles, marine piling, piers, cribs, cooling towers

Ref. (Morris, 1996)

There are several factors that affect leaching of preservatives from wood. According to Sloot et al. (1997), they are classified into three main groups, which are shown in Table 2.4.

Table 2.4: Factors affecting the leaching of preserved components from wood

Other relevant factors	Physical factors	Chemical Factors
The natural properties of wood (e.g. permeability) Preservative treatment Fixation	Absorption Diffusion Dissolution Temperature	Ionic strength pH (Organic)Acids

Ref. (Sloot et al. 1997)

An important factor in both the mobility and toxicity of leached preservatives is the form in which the chemicals leave the wood. Although chromium and arsenic may exist in either of two stable valence states (Cr^{+3}, Cr^{+6}, As^{+3}, As^{+5}), their properties like solubility, mobility, formation of complex, which are mentioned later, are completely different. Copper remains less stable in the environment in any other form than the +2 valence.

2.1.3.2 SPECIATION

CHROMIUM

Chromium is the least mobile of the CCA chemicals and its mobility depends on its valency. Trivalent chromium is very reactive with organics and fixes to soil and sediments quickly. Hexavalent chromium is more soluble but less absorbed and the rate of its movement through soil and groundwater is the same (Rouse and Pyrih, 1990). The valence state of chromium is a function of the oxygen content and redox potential, pH, the presence and type of suspended inorganics and dissolved organics, when it is introduced into water and soil (Lebow, 1996).

Hexavalent chromium is the most stable form in equilibrium with atmospheric oxygen when there are no organic compounds in the media (McGrath and Smith, 1990). However, in the presence of organic compounds, Cr (VI) may be reduced to Cr (III) as the same reactions take place within treated wood. The laboratory tests have shown that

humic acids can reduce Cr^{+6} but the reaction does not proceed rapidly under most conditions (Lebow, 1996).

Iron reduces chromium and the reaction depends on the presence of the excess iron (Lebow, 1996). Sulfides can also reduce chromium and this can be an important process near the sediments and where sulfides are produced by decomposition of organic compounds (Lebow, 1996).

In soil when there are electron-donating compounds, the chromium is reduced to trivalent state and this reaction is faster in acid soils (McGrath and Smith, 1990). In water, for example in a rapidly moving stream especially when it is alkaline or hard water, the oxidation process can occur and chromium (+3) oxidize to chromium (+6). The resistance of trivalent chromium to leaching or immobility depends on its ability to form inert complexes with organic and inorganic ligands (Lebow, 1996).

As mentioned, both hexavalent and trivalent chromium are stable in the environment. According to Lebow (1996), if chromium leaches from treated wood in the trivalent form, inert complexes with organic and inorganic ligands might be formed and then chromium mobility under these conditions would be associated with the water soluble complexes or events that move through soil and sediments. If the leached chromium is in the hexavalent form, it can remain in this more soluble, mobile, biologically available state especially in alkaline water (Lebow, 1996).

According to Solo-Gabriel et al. (2004), the results of pH stat tests for new CCA wood showed the highest chromium concentrations were at the lowest and highest pH values. All of the chromium in the acidic and neutral regions was Cr(III) only at pH >9 was Cr(VI).Unlike new, for weathered wood both As(III) and As(IV) were observed.

COPPER

Unlike chromium, neither the movement and solubility of copper is highly dependent on changing the oxidation state, nor are the toxicity and solubility of Cu (I) and Cu (II) widely different from each other. Although Cu (II) is much more stable in most aerated conditions, Cu (I) can exist in saturated soils with water when there is a low concentration of copper. In the absence of organic and inorganic adsorption agents, water soluble copper is in the forms of $[Cu(H_2O)_6]^{+2}$, $[Cu(H_2O)_5]^{+2}$, $CuOH^+$ below pH 7 and species $Cu(OH)_2$, and $CuCO_3$ at pH greater than 7. Reactions with organic and inorganic compounds affect solubility and the form of copper (Baker, 1990; Parker, 1981; McBride, 1981). For example, copper deposited in sediments from pollution sources is usually complexed with organics or precipitated with inorganic oxides (Messure et al., 1991). Copper solubility is greatest at the acid and alkaline extremes and minimum at around pH 7, in most environmental exposures (Baker, 1990; Parker, 1981; McBride, 1981).

Adsorption of copper is a very important factor in determining its mobility and is influenced by pH, amount and type of adsorbents. At low pH, because of proton adsorption, the charge of adsorbents tends to be positive and at high pH they become

more negative (Lebow, 1996). Adsorption increases with increasing pH and this increases copper precipitation as copper oxides, hydroxides and carbonates at neutral and alkaline pH (Baker, 1990; McBride, 1981; James and Barrow, 1981).

To be noted that the accumulation of copper in fine sediments can be important when there is CCA treated wood in seawater (Weis and Weis, 1992; Weis et al., 1993). Copper in sediments is not bonded as strongly as chromium (Lebow, 1996) and some deposited copper in sediments may be solubilized under oxidizing conditions, possibly because of the formation of soluble hydroxides and carbonates (Lebow, 1996). But in a reducing environment, the solubility of copper in sediments decreases, possibly through the formation of sulfides (Lebow, 1996).

ARSENIC

Arsenic is more soluble in water than copper and chromium and is less likely to be adsorbed. Its mobility in the environment can thus be considerable. Like chromium, it exists significantly in two valence states, As (III) and As (V). It almost always forms oxyanions. The trivalent arsenic is much more toxic than pentavalent arsenic and methyl arsenic forms are usually less toxic than the inorganic forms (O'Neill, 1990). According to Solo-Gabriel et al. (2004), arsenic in CCA is predominantly in the +5 valence form, but As leached from CCA is primarily either As(III) or As(V).

There are many types of microorganisms that change inorganic arsenic to more soluble species. However, their capability in the presence of chromium and copper, has not been

confirmed but there is no doubt they affect the mobility and fate of arsenic when arsenic enters the environment. For example, there are some kinds of soil fungi and bacteria that convert arsenate to arsenite (Lebow, 1996) or they can methylate the oxyanions to monomethylarsonic acid, dimethylarsinic acid, trimethylarsenic oxide, trimethylarsine, and dimethylarsine. The methyl-arsines are generally volatilized (O'Neill, 1990; Braman, 1975).

In water, arsenic enters as arsenic acid (As (V)) and by aluminum, mineral clay or iron, precipitate into sediments where it is reduced to trivalent form and methylated by microorganisms. The soluble methylates then move to the water surface, react with oxygen and the produced oxyanions precipitate again by reactions with inorganic constituents (Lebow, 1996). The microorganisms and the involved arsenic species are two factors that affect the biomethylation reactions (O'Neill, 1990; Cheng and Focht, 1979). Some microorganisms can methylate arsenic compounds completely and over a wide range of pH, but some can only methylate specific species and to a lesser degree (O'Neill, 1990). In very wet soils, As (III) may be the most stable form, although complexing species and methylating organisms in soil will alter the As (V)/As(III) equilibria (Lebow, 1996). A study of soils in England showed that As (V) made up 90% of soluble arsenic in aerobic soils but only 15-45% of the soluble arsenic in anaerobic, waterlogged soils and in mineralized areas. A small amount of monomethylarsenic acid was found (O'Neill, 1990).

In oxygenated water, inorganic arsenates are the dominant species and arsenites are usually formed in sediments or deep waters (Lebow, 1996). A study on deep waters below the photic zone showed the existence of a small amount of organic arsenic. It is suggested that the organic form was produced by plankton in the photic zone. The studies noted that phytoplankton could reduce and methylate up to 50% of As (V) in the media (Lebow, 1996). The inert organic arsenics may be more toxic than inorganic forms (Riedel et al., 1989). As treated wood is usually placed in shallow water, there is the probability of reactions by photic zone microorganisms for arsenic leachate of treated wood (Lebow, 1996).

In anaerobic conditions, like seawater sediments, it is favorable for arsenic (V) to be reduced to arsenic (III) (Riedel, et al., 1989). Brannon and Patrick (1987) found that although there were organic forms of As (III) in the sediments of several harbors, which they collected, arsenite was the predominant form and even added arsenate to sediments was reduced to trivalent form. Hence the solubility and release of arsenic from sediments is related to oxygen content of water. In anaerobic conditions, more arsenic is released. Arsenic can be adsorbed and removed from solution by organic compounds as well as inorganic compounds such as iron, aluminum, calcium and clay which prevent arsenic leaching (Lebow, 1996). The studies show that the released arsenic to environment can be mobile because of the movement of sediments in high water flow, solubility of arsenic species and changing of the species type by microorganisms.

2.2 ETHANOL

Ethanol (ethyl alcohol, grain alcohol, EtOH) is a clear, colorless liquid. Alcohol molecules contain a hydroxyl group, -OH, bonded to a carbon atom (CH_3CH_2OH). Ethanol can be produced synthetically, by direct fermentation of sugars, from other biological feedstocks that contain appreciable amounts of sugar or other carbohydrates that can be converted into sugar such as starch or cellulose. Sugar beets and sugar cane are examples of feedstocks that contain sugar. Corn contains starch that can relatively easily be converted into sugar. A significant percentage of trees and grass is made up of cellulose, which can also be converted to sugar, although with more difficulty than required to convert starch.

Indirect hydration of ethylene is the oldest process between the two major ethanol production methods from ethylene, which was invented more than one hundred years ago. Ethanol is prepared from ethylene in a three-step process using sulfuric acid (John, 1969). In the first step, the hydrocarbon feedstock containing 35-95% ethylene is exposed to 95-98% sulfuric acid in a column reactor to form mono-sulfate:

$$CH_2 = CH_2 + H_2SO_4 \longrightarrow CH_3CH_2OSO_3H$$
(Monoethyl Sulfate)

$$2\,(CH_2 = CH_2) + H_2SO_4 \longrightarrow (CH_3CH_2O)_2SO_2$$
(Diethyl Sulfate)

It is subsequently hydrolyzed with enough water to give a 50-60% aqueous sulfuric acid solution:

$$CH_3CH_2OSO_3H + H_2O \longrightarrow CH_3CH_2OH + H_2SO_4$$

$$(CH_3CH_2O)_2SO_2 + H_2O \longrightarrow 2\,CH_3CH_2OH + H_2SO_4$$

The ethanol is then separated from the dilute sulfuric acid in a stripper column. The last step of this process is to concentrate the sulfuric acid and repeat the process.

In the direct hydration process, an ethylene-rich gas is combined with water and passes through a fixed-bed catalyst reactor, in which ethanol is formed according to the following reaction (Nelson and Courter, 1954) and ethanol is then recovered in a distillation system.

$$CH_2=CH_2 + H_2O \longrightarrow CH_3CH_2OH$$

According to John (1969), other processes to make ethanol synthetically are not commercially important.

The ethanol production from fermentation of sugars, starch or lignocellulosic feedstocks starts by grinding up the feedstock so it is more easily and quickly processed in the following steps. Once ground up, the sugar is either dissolved out of the material or the starch or cellulose is converted into sugar. The sugar is then fed to microbes that use it for food, producing ethanol and carbon dioxide in the process. A final step purifies the ethanol to the desired concentration (U.S. Department of Energy, 2006).

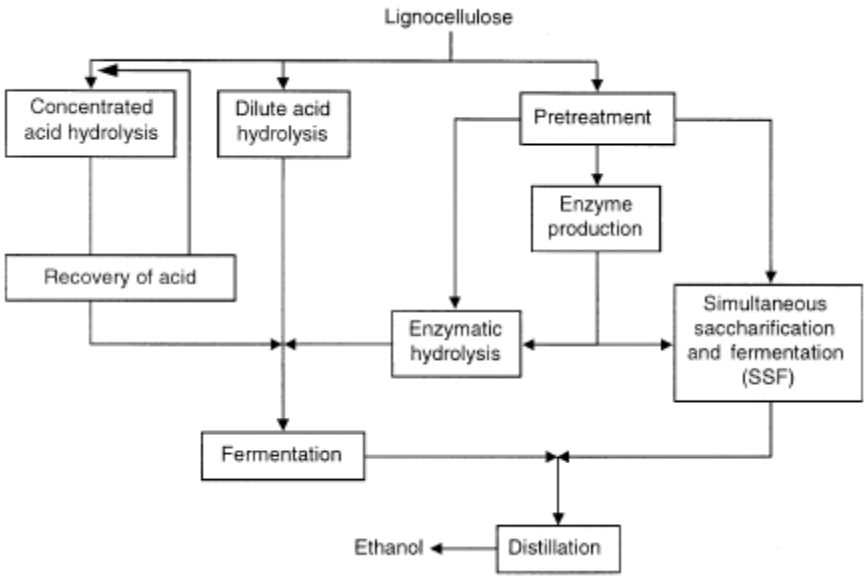

Figure 2.6. Production of ethanol from lignocellulosic materials (Galbe and Zacchi, 2002).

The United Nations Development Program "UNDP" report (2000) mentioned that world production of ethanol was estimated at 18 billion litres (equivalent to 420 petajoules) in 1998 and that also ethanol produced by the hydrolysis of lignocellulosic biomass, is a potentially low-cost and efficient option among the renewable energy technologies. According to the report, hydrolysis techniques are gaining attention, particularly in Sweden and the United States, but some fundamental issues need to be resolved. If these barriers are lowered and ethanol production is combined with efficient electricity production from unconverted wood fractions (such as lignin), ethanol costs could come close to current gasoline prices—as low as $0.12 a litre at biomass costs of about $2 per gigajoule (Lynd, 1996). Overall system conversion efficiency could increase to about 70 percent of the lower heating value (LHV) of a fuel. LHV is defined as the amount of heat released by combusting a specific quantity (initially at 25 °C or another reference state) and returning the temperature of the combustion products to 150 °C).

In the U.S. the total capacity of ethanol production was estimated 6.5 billion litres in 1994 and 88% of which was produced by fermentation. 17% of the demand was for industrial purposes and 5% was in beverage market. Therefore fuel is about 78% of ethanol market in U.S. (Taherzadeh, 1999). Hamelinck et al. (2005) stated that ethanol was produced on a fair scale of 14-26 Mtonne worldwide. Taherzadeh (1999) believed the desire to decrease U.S. dependence on foreign oil supplies, increasing the octane number of unleaded gasoline, surplus production of corn and air pollution concerns were the main reasons for the growing market of fuel-grade ethanol. Pimentel and Patzek (2005) also mentioned the desperate needs to replace liquid gasoline fuel in the future. They predicted that the use of oil supply would be extremely limited in 40-50 years.

2.3 ETHANOL PRODUCTION FROM LIGNOCELLULOSIC BIOMASS

2.3.1 CELLULOSIC CROPS

Lignocellulosic biomass contains a mixture of cellulose, hemicellulose, lignin and a small amount of other compounds (extractives). Analyses of different kinds of woods including, alder, aspen, birch, pine, and spruce are presented in Table 2.5. If we exclude the bark of the trees, the analysis shows an average of 42% cellulose, 20% hemicellulose, 21% lignin and 6% extractives in pine, spruce, alder, aspen and birch.

The efficient conversion of cellulosic biomass to ethanol requires knowledge of the chemical and physical properties of the biomass feedstock (NREL 1992). All processes comprise the same main components: hydrolysis of the hemicellulose and the cellulose to monomer sugars, fermentation of sugars to ethanol and product recovery and concentration by distillation. The hydrolysis is usually catalyzed by cellulase enzymes and the fermentation is carried out by yeast or bacteria (Figure 2.6). The main difference between the process alternatives is the hydrolysis steps, which can be performed by dilute acid, concentrated acid or enzymatically (Galbe and Zacchi, 2002).

Table 2.5:Dehydrated sugar analysis of wood samples (Based on percentage of wood, dry weight)

Material	Glucan	Mannan	Galactan	Xylan	Lignin	Extracts	Ash
Hard woods							
Alder	40.5	1.5	0.8	16.1	20.8	8.8	0.6
Aspen	43.2	2.2	0.5	15.1	16.0	4.7	1.3
Birch	40.7	1.7	0.7	20.0	19.1	4.1	0.3
Willow	33.1	1.6	1.4	10.3	23.3	7.7	2.0
Soft woods							
Pine	42.4	11.8	1.9	4.7	24.7	4.6	0.3
Spruce	41.6	11.5	2.0	4.7	25.7	5.4	0.3

Source: Taherzadeh et al.(1997)

The microorganisms traditionally used to produce ethanol cannot simultaneously convert both hexoses and pentoses to ethanol, while the lignin within the lignocellulosic matrix interferes with the hydrolysis of the carbohydrate components (Saddler 1993). Therefore, the pretreatment step must provide a high yield of both hexoses and pentoses, prevent breakdown of the carbohydrates into inhibitory components and alter the nature of the complex by reducing cellulose crystallinity, and increase the porosity of the materials (Sun and Cheng 2002), to provide effective removal of the lignin to enhance access of the hydrolytic enzymes to the carbohydrate components. Fractionation then separates the various streams to provide more effective processing of the cellulose, hemicellulose and lignin streams (Mulligan, 1994).

Lignocellulosic feedstocks provide more of a process design and operating challenge than sugar or starch feedstocks, as there is a wider variation in the type and nature of the processes and equipment needed to convert lignocellulosic feedstocks to ethanol (Hayn et al., 1993). This is primarily a consequence of the relatively early stage of development of lignocellulosic conversion to ethanol processes and the greater feedstock variability. Even sugar and starch crops can have compositional variability due to variations such as the species of feedstock used, growing site, climate, age and part of the plant that was used. However, lignocellulosic feedstocks can also have proportional variability within the mixture of its three major components, differences in the types and amounts of extractives, and natural variability in the monomeric sugars that make up the hemicellulose component.

Wood conversion to fermentable sugars and ethanol was evaluated at the pilot plant level by NREL(1992). Agricultural residues were converted to ethanol on a pilot plant scale with yields of 350-400 L / ton of feedstock.

2.3.2 HYDROLYSIS

The factors that have been identified to affect the hydrolysis of cellulose include porosity (accessible surface area) of the waste materials, cellulose fiber crystallinity, and lignin and hemicellulose content (McMillan, 1994). The presence of lignin and hemicellulose makes the access of cellulase enzymes to cellulose difficult and reduces the efficiency of the hydrolysis. Removal of lignin and hemicellulose, reduction of cellulose crystallinity, and increasing the porosity in pretreatment processes can significantly improve the hydrolysis (McMillan, 1994).

ACID HYDROLYSIS

Several types of acids, including sulphurous, sulphuric, hydrochloric, hydrofluoric, phosphoric, nitric and formic acids can be used for hydrolysis and they may be either concentrated or diluted. Processes involving concentrated acids are operated at low temperatures and give high yields (e.g. 90% of theoretical glucose yield), but the large amount of these concentrated acids are powerful agents for cellulose hydrolysis, and they are toxic, corrosive and hazardous and require reactors that are resistant to corrosion. In addition, the concentrated acid must be recovered after hydrolysis to make the process

economically feasible (Sivers and Zacchi, 1995). Furthermore, when sulphuric acid is used, the neutralization process produces large amounts of gypsum (Keller, 1996).

The main advantage of dilute acid hydrolysis is the relatively low acid consumption. However, high temperatures are required to achieve acceptable rates of conversion of cellulose to glucose, and high temperatures also increase the rates of hemicellulose sugar decomposition and equipment corrosion (Jones and Semrau, 1984). Sugar degradation products can also cause inhibition in the subsequent fermentation stage (Larsson et al., 1999). The maximum yield of glucose is obtained at high temperature and a short residence time, but even under these conditions the glucose yield is only between 50% and 60% of the theoretical value (Wyman 1996). A two-stage process has been developed to decrease sugar degradation. In the United States, BCI (Wyman, 1999), the first hydrolysis stage, the relatively easily hydrolyzed hemicellulose was released under rather mild conditions (170–190°C). This enabled the second acid hydrolysis step to proceed under harsher conditions (200–230°C) without degrading the hemicellulose sugars to furfural, hydroxymethylfurfural and other degradation products. Using a two-stage dilute acid hydrolysis process, recovery yields of as much as 70–98% of the xylose, galactose, mannose and arabinose from softwood have been reported (Nguyen et al., 1999). However, the yield of glucose was still low at 50%.

According to Ogier et al. (1999), Ackerson et al. (1981) suggested a prehydrolysis of lignocellulosic biomass by 4.4% dilute sulfuric acid (H_2SO_4) at 100 °C for 60 minutes

followed by hydrolysis of cellulose by 85% concentrated sulfuric acid (H_2SO_4) at 100°C for 10 minutes. They predicted 95% sugar recovery from cellulose and hemicellulose.

ENZYMATIC HYDROLYSIS

Enzymatic hydrolysis of cellulose is carried out by specific cellulase enzymes (Beguin and Aubert, 1994). The products of the hydrolysis are usually reducing sugars including glucose. Utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 45– 50 ºC) and does not have a corrosion problem (Duff and Murray, 1996). Both bacteria and fungi can produce cellulases for the hydrolysis of lignocellulosic materials. These microorganisms can be aerobic or anaerobic, mesophilic or thermophilic. Bacteria belonging to *Clostridium, Cellulomonas, Bacillus, Thermomonospora, Ruminococcus, Bacteriodes, Erwinia, Acetovibrio, Microbispora*, and *Streptomyces* can produce cellulases (Bisaria, 1991). Although many cellulolytic bacteria, particularly the cellulolytic anaerobes such as *Clostridium thermocellum* and *Bacteroides cellulosolvens* produce cellulases with high specific activity, they do not produce high enzyme titres (Duff and Murray, 1996). Because the anaerobes have a very low growth rate and require anaerobic growth conditions, most research for commercial cellulase production has focused on fungi (Duff and Murray, 1996). Fungi that have been reported to produce cellulases include *Sclerotium rolfsii, P. chrysosporium* and species of *Trichoderma, Aspergillus, Schizophyllum and Penicillium* (Sternberg, 1976; Fan et al., 1987; Duff and Murray, 1996). Of all these fungal genera, *Trichoderma* has been most

extensively studied for cellulase production (Sternberg, 1976). Cellulases are usually a mixture of several enzymes. At least three major groups of cellulases are involved in the hydrolysis process: (1) endoglucanase (EG, endo- 1,4-D-glucanohydrolase, or EC 3.2.1.4.) which attacks regions of low crystallinity in the cellulose fiber, creating free chain-ends; (2) exoglucanase or cellobiohydrolase (CBH, 1,4-B-D-glucan cellobiohydrolase, or EC 3.2.1.91.) which degrades the molecule further by removing cellobiose units from the free chain-ends; (3) ß-glucosidase (EC 3.2.1.21) which hydrolyzes cellobiose to produce glucose (Coughlan and Ljungdahl, 1988). In addition, to the three major groups of cellulase enzymes, there are also a number of ancillary enzymes that attack lignocellulose, such as glucuronidase, acetylesterase, xylanase, ß - xylosidase, galactomannanase and glucomannanase (Duff and Murray, 1996). During the enzymatic hydrolysis, cellulose is degraded by the cellulases to reducing sugars that can be fermented by yeasts or bacteria to ethanol. The factors that affect the enzymatic hydrolysis of cellulose include substrates, cellulase activity, and reaction conditions (temperature, pH, as well as other parameters). There are three essential types of enzymatic hydrolysis fermentation processes, separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and direct microbial conversion (DMC) (Mulligan, 1994).

2.3.3 PRETREATMENT

As the cellulose and hemicellulose, which are the aims of enzymatic hydrolysis, cannot be directly accessible by enzymes then pretreatment of lignocellulosic material before

enzymatic hydrolysis is necessary. Pretreatment methods refer to solubilization and separation of one or more of the four major components of biomass (hemicellulose, cellulose, lignin and extractives) to make the remaining solid biomass more accessible to further chemical or biological treatment. This can be accomplished through the removal of the lignin sheath, reduction of cellulose crystallinity or by increasing the surface area that is accessible to enzymes.

Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis; (2) avoid the degradation or loss of carbohydrate; (3) avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes; and (4) be cost effective (Sun and Cheng, 2002). The pretreatment methods can be physical, chemical, biological or a combination of these methods.

2.3.3.1 PHYSICAL PRETREATMENT

The applied physical pretreatment processes include mechanical milling, steam explosion, irradiation and pyrolysis. However the mechanical milling methods improve enzymatic digestibility of cellulose, but the high energy requirement and capital cost make this method uneconomical. (These methods improve enzymatic digestibility of cellulose by reduction of crystallinity index and increasing specific surface area for enzyme action with ability to produce higher slurry concentrations which reduce the reactor volume). Being ineffective on pure cellulose and high cost of energy, irradiation

fails as a pretreatment method (Mulligan, 1994). As steam explosion could be applied using sulphuric acid or SO_2, it is discussed in physico-chemical methods.

PYROLYSIS

Pyrolysis has been used for pretreatment of lignocellulosic materials. When the materials are treated at temperatures greater than 300 ºC, cellulose rapidly decomposes to produce gaseous products and residual char (Kilzer and Broido, 1965; Shafizadeh and Bradbury, 1979). The decomposition is much slower and less volatile products are formed at lower temperatures. Mild acid hydrolysis (1 N H_2SO_4, 97ºC, 2.5 h) of the residues from pyrolysis pretreatment has resulted in 80– 85% conversion of cellulose to reducing sugars with more than 50% glucose (Fan et al., 1987). The process can be enhanced with the presence of oxygen (Shafizadeh and Bradbury, 1979). When zinc chloride or sodium carbonate is added as a catalyst, the decomposition of pure cellulose can occur at a lower temperature (Shafizadeh and Lai, 1975).

2.3.3.2 PHYSICO-CHEMICAL PRETREATMENT

As a combination pretreatment method, acid prehydrolysis (dilute acid with steam) using sulfur dioxide (SO_2) has been the best of the three common dilute acids (HCl, H_2SO_4, sulfurous), and has been used in a number of pilot scale tests (Hayan et al., 1993). During the process, deciduous hemicelluloses are readily hydrolyzed into monomeric sugars, acetic acid and other components by steam at 150-200 ºC or at lower temperatures when mild acids are incorporated into the feedstock.

STEAM EXPLOSION (AUTOHYDROLYSIS)

Steam explosion is the most commonly used method for pretreatment of lignocellulosic materials (McMillan, 1994) with and without addition of an acid catalyst. According to Hayan et al. (1993) and Brownwell and Saddler (1987), it has been the most cost-effective means of pretreating agricultural and forestry residues. Of the acids, H_2SO_4 has been the most extensively investigated (Torget et al., 1990, 1991, 1996; Nguyen et al. 1998, 1999, 2000; Tengborg et al., 1998) because it is inexpensive and effective. SO_2 catalysed steam pretreatment has also been studied (Clark and Mackie, 1987; Clark et al., 1989; Schwald et al., 1989; Fein et al., 1991; Ramos et al., 1992; Stenberg et al., 1998). Pretreatment using gaseous SO_2 is not corrosive as a pretreatment with H_2SO_4 (Galbe and Zacchi, 2002), and it is also easier and faster to introduce into the material. The main problem of SO_2 is its high toxicity, which may pose safety and health risks.

Initiating at a temperature of 160-260ºC, chipped biomass is treated with high-pressure saturated steam (corresponding pressure 0.69–4.83 MPa) for several seconds to a few minutes before the material is exposed to atmospheric pressure and then the pressure is swiftly reduced, which makes the materials undergo an explosive decompression. High temperatures cause hemicellulose degradation and lignin transformation and therefore increasing the potential of cellulose hydrolysis (Sun and Cheng, 2002). Ninety percent efficiency of enzymatic hydrolysis has been achieved in 24 h for poplar chips pretreated by steam explosion, compared to only 15% hydrolysis of untreated chips (Grous et al., 1986). Residence time, temperature, chip size and moisture content are the factors that affect steam explosion pretreatment (Duff and Murray, 1996). Either a high temperature

and short residence time (270 ºC, 1 min) or lower temperature and longer residence time (190 ºC, 10 min) gives optimal hemicellulose solubilization and hydrolysis (Duff and Murray, 1996). However, according to Wright (1998), studies indicate that lower temperatures and longer residence times are more favorable.

Addition of H_2SO_4 (or SO_2) or CO_2 in steam explosion can effectively improve enzymatic hydrolysis, decrease the production of inhibitory compounds, and lead to more complete removal of hemicellulose (Morjanoff and Gray, 1987). Compared to mechanical communition, the low energy requirement and no recycling or environmental costs are the advantages of steam explosion pretreatment. The conventional mechanical methods require 70% more energy than steam explosion to achieve the same size reduction (Holtzapple et al., 1989). Steam explosion is recognized as one of the most cost effective pretreatment processes for hardwoods and agricultural residues, but it is less effective for softwoods (Clark and Mackie, 1987). Limitations of steam explosion include destruction of a portion of the xylan fraction, incomplete disruption of the lignin-carbohydrate matrix, and generation of compounds that may be inhibitory to microorganisms used in downstream processes (Mackie et al., 1985). Because of the formation of degradation products that are inhibitory to microbial growth, enzymatic hydrolysis, and fermentation, pretreated biomass needs to be washed by water to remove the inhibitory materials along with water-soluble hemicelluloses (McMillan, 1994). The water wash decreases the overall saccharification yields due to the removal of soluble sugars, such as those generated by hydrolysis of hemicelluloses. Typically, 20-25% of the initial dry matter is removed by water wash (Mes-Hartree et al., 1988). According to

Galbe and Zacchi (2002), two-step steam pretreatment of softwood has a high ethanol yield, better utilization of the raw material and lower consumption of enzymes.

2.3.3.3 CHEMICAL PRETREATMENT

Chemical pretreatments have been used to remove lignin and modify the structure of lignocellulosics. Some of the different chemical pretreatment methods are as follows:

ALKALINE AND SOLVENT PRETREATMENT

Sodium hydroxide and ammonia which, are used for cellulose alkali pretreatment on some feedstocks such as straw, could successfully break the lignocellulosic bond, partially solubilize the lignin and decrystallize the cellulose (NREL 1992). Alkaline pretreatment is more effective on agricultural residues than on wood materials (Galbe and Zacchi, 2002).

ACID

Concentrated acid hydrolysis has been used for pretreatment of lignocellulosic materials, but dilute acids have been successfully developed. Acids, such as dilute HCl, dilute H_2SO_4 and dilute sulfurous acid, have been used to break the ligno-cellulose bond and partially hydrolyse the hemicellulose, but pose a gypsum disposal problem (Mulligan 1994). The dilute sulfuric acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis (Esteghlalian et al., 1997). At moderate temperature, direct saccharification suffers from low yields because of sugar

decomposition. High temperature in dilute acid treatment is favorable for cellulose hydrolysis (McMillan, 1994). Recently developed dilute acid hydrolysis processes use less severe conditions and achieve high xylan to xylose conversion yields. Achieving high xylan to xylose conversion yields is necessary to achieve favorable overall process economics because xylan accounts for up to a third of the total carbohydrate in many lignocellulosic materials (Hinman et al., 1992). There are primarily two types of dilute acid pretreatment processes: high temperature (greater than 160 ºC), continuous-flow process for low solids loading (5– 10% [weight of substrate/weight of reaction mixture]) (Brennan et al., 1986; Converse et al., 1989), and low temperature (less than 160 ºC), batch process for high solids loading (10–40%) (Cahela et al., 1983; Esteghlalian et al., 1997). According to Ogier et al. (1999) it takes less than 10 seconds to treat the biomass at 200 ºC in a piston reactor or 30 minutes for treatment at 120-130ºC in a percolation reactor. Although dilute acid pretreatment can significantly improve the cellulose hydrolysis, its cost is usually higher than some physico-chemical pretreatment processes such as steam explosion or the Ammonia Freeze Explosion (AFEX) process. Neutralization of pH is necessary for the downstream enzymatic hydrolysis or fermentation processes (Schell et al., 1991).

AFEX

The Ammonia Freeze Explosion (AFEX) process is a treatment of lignocellulosic materials with high- pressure liquid ammonia under 1.5 MPa pressure at moderate temperature of 50-90ºC. In a typical AFEX process, the dosage of liquid ammonia is 1–2

kg ammonia/kg dry biomass, the temperature is 90 ºC, and the residence time is 30 min (Sun and Cheng, 2002).

The AFEX process is not very effective for the biomass with high lignin content such as newspaper (18–30% lignin) and aspen chips (25% lignin). It is favorable for agricultural residues (Ogier et al., 1999). The AFEX pretreatment does not significantly solubilize hemicellulose compared to acid pretreatment and acid-catalyzed steam explosion (Sun and Cheng, 2002).

ORGANOSOLV PROCESS

In the organosolv process, an organic or aqueous organic solvent mixture with inorganic acid catalysts (HCl or H_2SO_4) is used to break the internal lignin and hemicellulose bonds. The organic solvents used in the process include methanol, ethanol, acetone, ethylene glycol, triethylene glycol, and tetrahydrofurfuryl alcohol (Chum et al., 1988; Thring et al., 1990). Organic acids such as oxalic, acetylsalicylic and salicylic acids can also be used as catalysts in the organosolv process (Sarkanen, 1980). At high temperatures (above 185 ºC), the addition of catalyst was unnecessary for satisfactory delignification (Sarkanen, 1980; Aziz and Sarkanen, 1989). Usually, a high yield of xylose can be obtained with the addition of acid. Solvents used in the process need to be drained from the reactor, evaporated, condensed and recycled to reduce the cost. Removal of solvents from the system is necessary because the solvents may be inhibitory to the growth of organisms, enzymatic hydrolysis, and fermentation.

2.3.3.4 BIOLOGICAL PRETREATMENT

In biological pretreatment processes, microorganisms such as brown, white and soft-rot fungi are used to degrade lignin and hemicellulose in waste materials (Schurz, 1978). Brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. The white-rot fungus *P. chrysosporium* produces lignin-degrading enzymes, lignin peroxidases and manganese- dependent peroxidases, during secondary metabolism in response to carbon or nitrogen limitation (Boominathan and Reddy, 1992). Both enzymes have been found in the extracellular filtrates of many white-rot fungi for the degradation of wood cell walls (Kirk and Farrell, 1987; Waldner et al., 1988). Other enzymes including polyphenol oxidases, laccases, H_2O_2 producing enzymes and quinone-reducing enzymes can also degrade lignin (Blanchette, 1991). The advantages of biological pretreatment include low energy requirement and mild environmental conditions. However, the rate of hydrolysis in most biological pretreatment processes is very low.

2.3.4 FERMENTATION

Following hydrolysis, the next steps are fermentation and then ethanol recovery. During fermentation, a biological organism, generally yeast, consumes the sugar as a carbon/ energy source and produces ethanol and carbon dioxide. The produced sugars by wood hydrolysis are the pentoses (mainly xylose) and the hexoses (mannose, galactose and mainly glucose). Glucose is easily fermented to ethanol by *S. cerevisiae.* According to Beck (1993), the ease of pentose recovery from biomass sources by such means as dilute acid hydrolysis or SO_2-steam explosion and water washing, is one of the driving forces to

enhance fermentation of pentoses. Yeasts, bacteria and fungi are three xylose-fermenting organisms but both the most promising hexose-fermenting organisms; *Saccharomyces cerevisiae* (yeast) and *Zymomonas mobilis* (bacterium) are not capable of fermenting pentose sugars (Mulligan, 1994). Based on the organisms (bacteria, fungi, yeast) and process flow patterns (batch, batch recycle, continuous, continuous recycle, etc.), there are different fermentation methods.

YEAST: There are several important or at least desirable characteristics of the microorganism in an industrial "hydrolyzate-to-ethanol" process. Ideally, the microorganism should give a high ethanol yield, have a high ethanol tolerance, be resistant to hydrolysates, have no oxygen requirement, and a broad substrate utilization range. It is also desirable to have a strain with high sugar consumption rate and productivity, minimal nutrient requirement, high salt tolerance, high shear tolerance, thermotolerance, safety for humans and no spore formation (Picataggio and Zhang, 1996).

Saccharomyces cerevisiae has several essential and desirable characteristics for fermentation of hydrolyzates especially from softwoods, where glucose and mannose constitute the largest part of the sugars. It gives a high ethanol yield, has a high ethanol productivity and tolerance, and has no oxygen requirement (Schulze, 1995; Olsson and Hahn-Hägerdal, 1996). They also have the ability of fermenting a wide spectrum of substrates, which is species dependent. The main disadvantage of *S. cerevisiae* in the fermentation of the hydrolysates is its limited substrate utilization range. Yeasts can metabolize in both aerobic (via respiration) and anaerobic (fermentation) conditions.

Aerobic conditions are used to produce the biomass required for commercial fermentation. During respiration organic substrates are completely oxidized by organisms and converted to cell mass. There are two sets of requirements for the fermentation step, one for cell growth and the other for the production of ethanol (Mulligan, 1994). Fungi generally have lower fermentation rates and ethanol yields (Mulligan, 1994).

BACTERIA: Bacteria such as *Zymomonas mobilis* which has a higher fermentation rate, higher specific ethanol productivity, higher specific glucose uptake rate and is also reasonably tolerant to ethanol (7%), glucose and high temperatures, offer more advantages in comparison to yeasts. In general, the bacteria have higher yields of byproducts and lower ethanol tolerance than yeasts (Mulligan, 1994).

2.4 METAL UPTAKE

However some heavy metals are essential elements but most of them at high concentrations can be toxic to all living organisms, including microorganisms, by forming complex compounds within the cell. According to Spain (2003), heavy metals are increasingly found in natural and industrial processes and as some heavy metals are necessary for enzymatic functions and cell growth, they can be uptaken and enter the cells. There are two general uptake systems: one is quick and unspecific, driven by a chemiosmotic gradient across the cell membrane and does not need ATP, and the other is slower and more substrate-specific, which needs energy from ATP hydrolysis. The first mechanism is more energy efficient according to Nies and Silver (1995), and a variety of

heavy metals can influx into the cell and then there is a greater possibility of toxic effects in the cells. Microorganisms have evolved several mechanisms to tolerate the presence of heavy metals by efflux of metal ions out of the cells, complexation and accumulation of metal inside the cell, reduction of metal ions to a less toxic state or to use them as terminal electron acceptors in anaerobic respiration (Nies, 1999). Usually a redox reaction occurs during the uptake and efflux of metal ions by microorganisms, which can even be used for energy and growth. This resistance and growth in the presence of metals and redox reactions is an important implication of microbial heavy metal tolerance because the oxidation state of a heavy metal relates to the solubility and toxicity of the metal itself according to Spain (2003). Many scientists have been trying to use microbes that are able to oxidize or reduce heavy metals in order to remediate metal-contaminated sites.

The ability of biological materials to accumulate heavy metals from liquid through metabolically mediated or physico-chemical pathways of uptake is defined as biosorption by Ahalya et al. (2003). Algae, bacteria fungi and yeasts have proven to be potential metal biosorbents (Volesky, 1986). Kratochvil and Volesky (1998) indicated the following 6 factors as the main advantages of biosorption over conventional treatment methods.

- Low cost
- High efficiency
- Minimisation of chemical or biological sludge
- No additional nutrient requirement

- Regeneration of biosorbent
- Possibility of metal recovery

The biosorption process involves a solid biosorbent and a liquid media which contains the dissolved contaminants. There are different mechanisms for biosorption of heavy metals by microorganisms. Ahalia et al. (2003) classified mechanisms into two different categories:

A: According to dependence on cell's metabolism.

- Metabolism dependent
- Non -metabolism dependent

B: According to the location that the metal will be found in microorganism

- Extra cellular accumulation/ precipitation
- Cell surface sorption/ precipitation
- Intracellular accumulation.

The intracellular accumulation which is uptaking the metal into the cell membrane is a metabolism dependent and will take place with viable cells. However, the cell surface biosorption is a physico-chemical interaction between the metal and the functional groups of cell wall surface, according to Alhalia et al. (2003), and is not metabolism dependent. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups and can bind with metals. The surface sorption can take place with non viable

microorganisms compared to the intercellular accumulation which is related to the active defense reaction of the viable microorganism in the presence of toxic, material. According to Kuyucak and Volesky (1988), the surface biosorption is relatively rapid and can be reversible, thus allowing metal recovery. While the surface biosorption is very rapid, the intracellular accumulation is slower and takes a longer time (Volesky and Holan, 1995).

The extracellular accumulation can be metabolism dependent or metabolism independent (Ercole et al. 1994). If the microorganism produces compounds that cause precipitation, it is dependent on metabolism but if the metal precipitates after chemical reaction between the metal and cell surface then it is not dependent on metabolism.

Some other researchers such as Brady and Duncan (1994) add oxidation-reduction reactions as another metal uptake process for microorganism tolerance mechanism against metals.

As mentioned before, yeast cells are among the microorganisms which can uptake various heavy metals, preferentially accumulating those of potential toxicity and also those of value. Gupta et al. (2000) noted yeast have shown an excellent potential of metal biosorption and mentioned *Saccharomyces* specifically. Brady and Duncan (1994) found that yeast biomass could provide an effective bioaccumulation for removal and/or recovery of the metal cations from aqueous solutions with the mechanisms of accumulation in wastewaters of pH 5-9 and for copper, the quantities of accumulated

copper depended on the ratio of ambient metal concentration to biomass quantity. Copper accumulation was only slightly affected by the variations in temperature.

Table 2.6: Some biomasses tested for uptaking chromium and copper by different sources.

Metal	Biomass	Biomass class	Metal uptake (mg/g)
Cr	*Bacillus sp.*	Bacteria	118 (Cr3+)
			60 (Cr6+)
	Rhizopus arrhizus	Fungus	31
	Candida tropicalis	yeast	4.6
	Streptomyces nouresei	bacteria	1.8
	Penicillium chrysogenum	fungus	0.33
Cu	*(Bacillus subtilis)*	bacteria	152
	(Bacillus subtillis)	bacteria	146
	Candida tropicalis	yeast	80
	(fungal biomass	bacteria	76
	manganese-oxidizing bacteria	bacteria	50
	(Bacillus licheniformis)	bacteria.	32
	Cladosporium resinae	fungus	18
	Rhizopus arrhizus	fungus	16
	Saccharomyces cerevisiae	yeast	17-40
			10
			6.3
	Pichia guilliermondii	yeast	11
	Scenedesmus obliquus	freshwater algae	10
	Rhizopus arrhizus	fungus	10
	Penicillium chrysogenum	fungus	9
	Streptomyces noursei	bacteria	5
	Bacillus sp.	bacteria	5
	Penicillium spinulosum	fungus	0.4-2
	Aspergillus niger	fungus	1.7
	Trichoderma viride	fungus	1.2
	Penicillium chrysogenum	fungus	0.75

Adapted from Volesky and Holan (1995)

Volesky and Holan (1995) collected a list of metals and the microorganisms which adsorb them (Table 2.6) based on different references. They named *Saccharomyces cerevisiae* (bakers' yeast) among the microorganisms which can be used for copper biosorption.

Arsenic tolerance

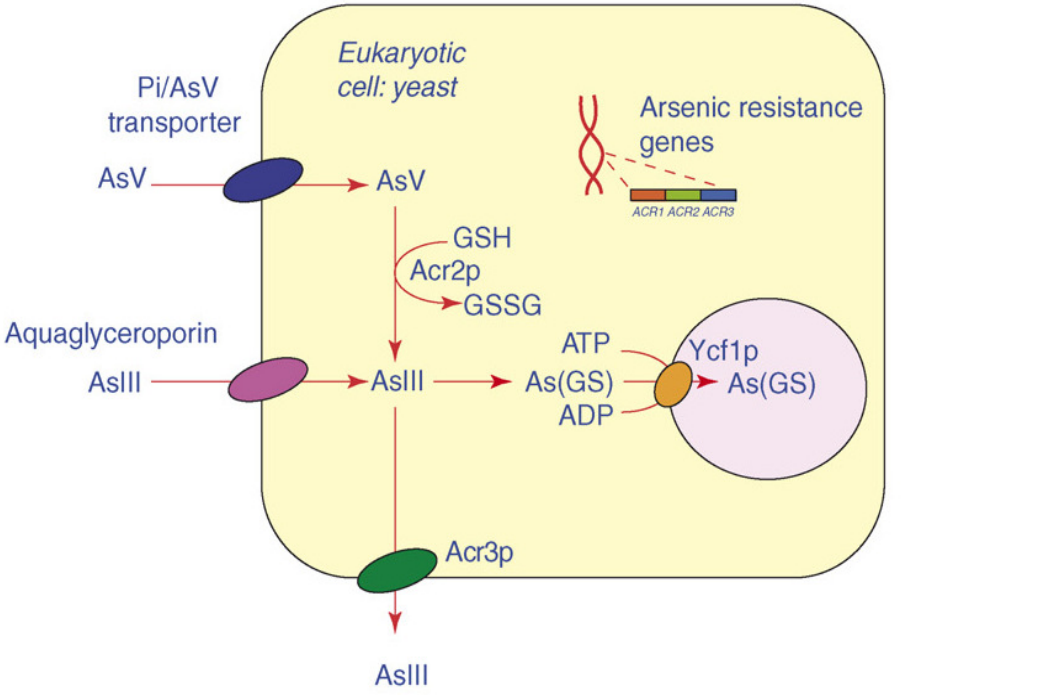

Figure 2.7: Schematic arsenic detoxification method in yeast (Tripathi et al., 2007)

Arsenic is an extremely toxic element to living organisms specially the inorganic forms of As (V) and As (III). While the toxicity of arsenate is due to its action on an analogue of phosphate and then interference with essential cellular processes such as ATP

(Adenosine Triphosphate, which supplies the energy used by an organism in its daily operations) synthesis, the toxicity of arsenite is because of its tendency to bind to sulfhydryl groups and causing damaging effects on general protein functioning (Tripathi et al., 2007). Arsenic (III) is more toxic than arsenic (V).

According to Rosen (2002), all living organisms have a detoxification system for arsenic. They uptake As (V) via phosphate transporters and As(III) by aguaglyceroporins, reduce As(V) to As(III) by arsenate reductases enzymes and then remove As(III) from the cytosol to the external medium or sequester it in interacellular compartments (Figure 2.7) as free arsenite or conjugates with GSH or other thiols in the vacuole. (Rosen, 2002; Tripathi et al., 2007).

Copper tolerance

As shown in Table 2.6, Volesky and Holan (1995) suggested *S. cerevisiae* as a microorganism capable of uptaking copper. Bakers' yeast is an inexpensive available source of biomass for removal of metals from wastewater. Cu^{+2} and Cr^{+2} are among the metals which Goksungur et al. (2003) believe the yeast which are capable of accumulating these chemicals. Crist et al. (1990) demonstrated that copper was adsorbed not only by ion exchange but also by additional covalent bonding with the carboxyl groups. Biosorbed copper was also found to bind by chelation between the *cis*-oriented hydroxyls yeast α-mannans, which form an insoluble complex and help in their isolation (Peat et al., 1961).

Goksungur et al. (2003) investigated the effects of different pretreatments on metal uptake of *S. cerevisiae* by treating with ethanol, heat and caustic soda. The highest metal uptake (21.1mg/g) was obtained by caustic treated yeast and 7.9 mg/g of metal uptake was obtained with ethanol treated yeast. They suggested that the short contact time of biosorbent with metal solution for biosorption showed the main mechanism of uptake was adsorption onto the biosorbent surface. Copper cations begin to be bound at approximately pH 4, reaching maximum sorption at the pH around 6. At lower concentrations of Cu^{+2} (10-50 mg/l), biosorption was complete in about 5 min but at higher concentrations it took 30-60 min (Goksungur et al., 2003).

Yeast cells are selective in their uptake of metal cations and more than 70% of the Cu^{+2} was removed over the total volume of 800 ml effluent passed through the immobilized yeast column used by Brady and Duncan (1994). *S. cerevisiae* is capable of accumulating considerable quantities of Cu^{+2} in the presence of excess monovalent Na^{+} (5000 μmol/L) and the level of copper accumulation is dependent on the ambient metal concentration and is inhibited by extremes of ambient pH and not inhibited by an elevated ionic strength (Brady and Duncan, 1994).

Chromium tolerance

Extremely toxic hexavalent chromium is an anion with properties that are correspondingly different from those of the usual metal cations (Volesky and Holan, 1995). It can easily be chemically or even biochemically (Paknikar and Bhide, 1993;

Reischl et al., 1993), reduced to its trivalent state, making it more removable (Siegel et al., 1986).

Immobilization

According to many researchers such as Brady and Duncan (1993) or Gupta et al. (2000) the microorganisms used for biosorption of heavy metals must be immobilized or contained and during the continuous industrial process, it is important to utilize an appropriate immobilization technique.

The free cells may provide valuable information in laboratory experimentation but immobilized biomass offers many advantages including better reusability, high biomass loading and minimal clogging in continuous flow system. The free cells generally have low mechanical strength and small particle size and excessive hydrostatic pressures are required to generate suitable flow rates. High pressures can cause disintegration of free biomass. These problems can be solved by using immobilized cell systems (Gupta et al., 2000).

Gupta et al. (2000) specifies some matrices which have been employed for immobilization of cells. One of them is the entrapment in the matrix of insoluble Ca-alginate which has been used in metal recovery by both viable and non-viable cells. Another important matrix being used for immobilization for metal removal is silica. They indicate that polyurethane and polysulfone are better than polyacrylamide and alginate matrices as immobilization supports.

Metal Recovery from Biomass

One of the important industrial applications of biosorption is recovering the metal ions from the biosorbent and regenerating the biosorbents for reuse. Effective biosorption technology needs efficient and economical regeneration of biosorbent after metal desorption, without any damage to the biomass. Physical sorption to cell wall structures should be reversible and allow for metal recovery (Kuyunak and Volesky, 1988).

Some metal ions show marked pH dependence for binding to biomass. Such metal can be desorbed easily from the biosorbent altering the pH. Dilute HCl, H_2SO_4 and HNO_3 have been successfully used for desorption of metals from the biomass. Increasing the acidity generally leads to an effective removal of metal(s) from the biomass, but it may cause permanent damage to the biomass surface structure resulting in a great reduction in metal sorption in the next cycle. Also the physico-chemically sequestered metal to the cell surface can be easily desorbed by EDTA (ethylenediamine tetraacetic acid) (Gupta et al., 2000).

For recovering bioaccumulated (Cu, Zn, Co, Cd, Ni, Cr) chlorides from immobilized *S.cerevisiae,* Wilhelmi and Duncan (1995) used 0.1M HCl. The desorption protocol utilized a minimum quantity of acid to yield concentrated, low volume metal eluents. The initial recovery of copper, cobalt and cadmium was 100%. The optimum pH for chromium adsorption was below 2. Increasing the concentration of HCl (1M) gave a 34% chromium recovery.

Other applications

Table 2.7.: Commercial Cellulosic Ethanol Plants in the U.S. (Operational or under construction)

Company	Location	Feedstock
Abengoa Bioenergy	Hugoton, KS	Wheat straw
BlueFire Ethanol	Irvine, CA	Multiple sources
Colusa Biomass Energy Corporation	Sacramento, CA	Waste rice straw
DuPont Danisco	Vonore, TN	Corn cobs
Fulcrum BioEnergy	Reno, NV	Municipal solid waste
Gulf Coast Energy	Mossy Head, FL	Wood waste
KL Energy Corp.	Upton, WY	Wood
Mascoma	Lansing, MI	Wood
POET LLC	Emmetsburg, IA	Corn cobs
Range Fuels	Treutlen County, GA	Wood waste
SunOpta	Little Falls, MN	Wood chips
US Envirofuels	Highlands County, FL	Sweet sorghum
Xethanol	Auburndale, FL	Citrus peels

(Decker, 2009) and (http://www.grainnet.com/pdf/cellulosemap.pdf)

The selective adsorption of As(V) over As(III) by *S.cerevisiae* yeast is the fundamental basis of Koh et al. (2005) and Koh and Pak (2005) research for separation and sensitive determination of arsenic species. By using an immobilized yeast column, *S.cerevisiae* was covalently bonded onto a controlled pore glass which showed selective preconcentration of As(V) over As(III), the inorganic arsenic was separated and the effluent was directly connected to hydride generation to increase sensitivity. The optimum pH and flow rate were 7 and 1.5 ml/min, respectively. The most important factor for controlling sorption of ions unto the column was pH, which was controlled

using $NaHCO_3$/ Na_2CO_3, strong acid, and acetic acid/ acetate buffer. The sorption was the most efficient at pH 7 and slowly decreased thereafter. By proper pH selection, As(V) is retained longer in the column and eluted out by nitric acid. Koh et al. (2005) claim the technique shows accurate results for determination of arsenic species.

Ethanol economics

On February 28, 2007, the U.S. Dept. of Energy announced $385 million in grant funding to six cellulosic ethanol plants for 40% of the investment costs (http://www.energy.gov/news/4827.htm). The promoters of those facilities were supposed to pay for the remaining 60%. They estimated a total of $1 billion would be invested for an approximately 530 million liter capacity which meant $1.8/annual liter production capacity in capital investment costs for pilot plants (this would work out to $.09/L over the 20-year life of a facility) and future capital costs were expected to be lower. The Department of Energy estimated that it cost about $0.58 per liter to produce cellulosic ethanol, which is twice as much as ethanol from corn.

According to the Renewables Global Status Report ,2009 (http://www.unep.fr/shared/docs/publications/RE_GSR_2009_Update.pdf), some new commercial-scale cellulosic ethanol plants were developed in 2008 (Table 2.7). In the United States, plants of capacity totaling 12 million liters (3.17 million gal) per year were operational, and an additional 80 million liters (21.13 million gal.) per year of capacity in 26 new plants were under construction. In Canada, capacity of 6 million liters per year

was operational. In Europe, several plants were operational in Germany, Spain, and Sweden and a capacity of 10 million liters per year were under construction.

2.5 CONCLUDING REMARKS

In brief, disposal of CCA treated wood is an environmental increasing concern in U.S. and Canada. Because of the leaching of chromium, copper and arsenic, landfilling cannot be a safe method for disposal of CCA treated wood. Then pretreatment to remove metals from CCA treated wood before landfilling could decrease the risk of environmental contamination after landfilling. On the other hand wood is one of the lignocellulosic sources of production of ethanol. Producing ethanol from CCA treated wood cannot only solve the disposal problem but may also make it profitable.

In this research, removal of chromium, copper and arsenic and the feasibility of producing ethanol from CCA treated wood, as a solution for disposal of CCA treated wood and the capability of Baker's yeast to uptake chromium, arsenic and copper from hydrolysate, which contains leached metal from CCA wood, are studied.

CHAPTER THREE
MATERIALS AND METHODS

To investigate the possibility of producing ethanol from CCA treated wood and the fate of existing heavy metals several experiments were performed. This chapter is devoted to the description of the materials and the methods used in this study.

3.1 TESTING PLAN

To prove the feasibility of ethanol production, fermentation tests of sugar solutions in the presence of chromium, arsenic and copper compounds were performed, followed by hydrolysis and fermentation of untreated and treated wood. In the next step, the growth of yeast and the removal of metals were monitored by applying appropriate tests and measurements (Visible absorbance and metal concentration measurements during fermentation) on fermentation of treated and untreated wood hydrolysate. At the end, TCLP and SPLP tests were performed and the amounts of metals in hydrolysed, treated, weathered and ground wood were measured. Figure 3.1 shows the protocol and testing plan used during this research.

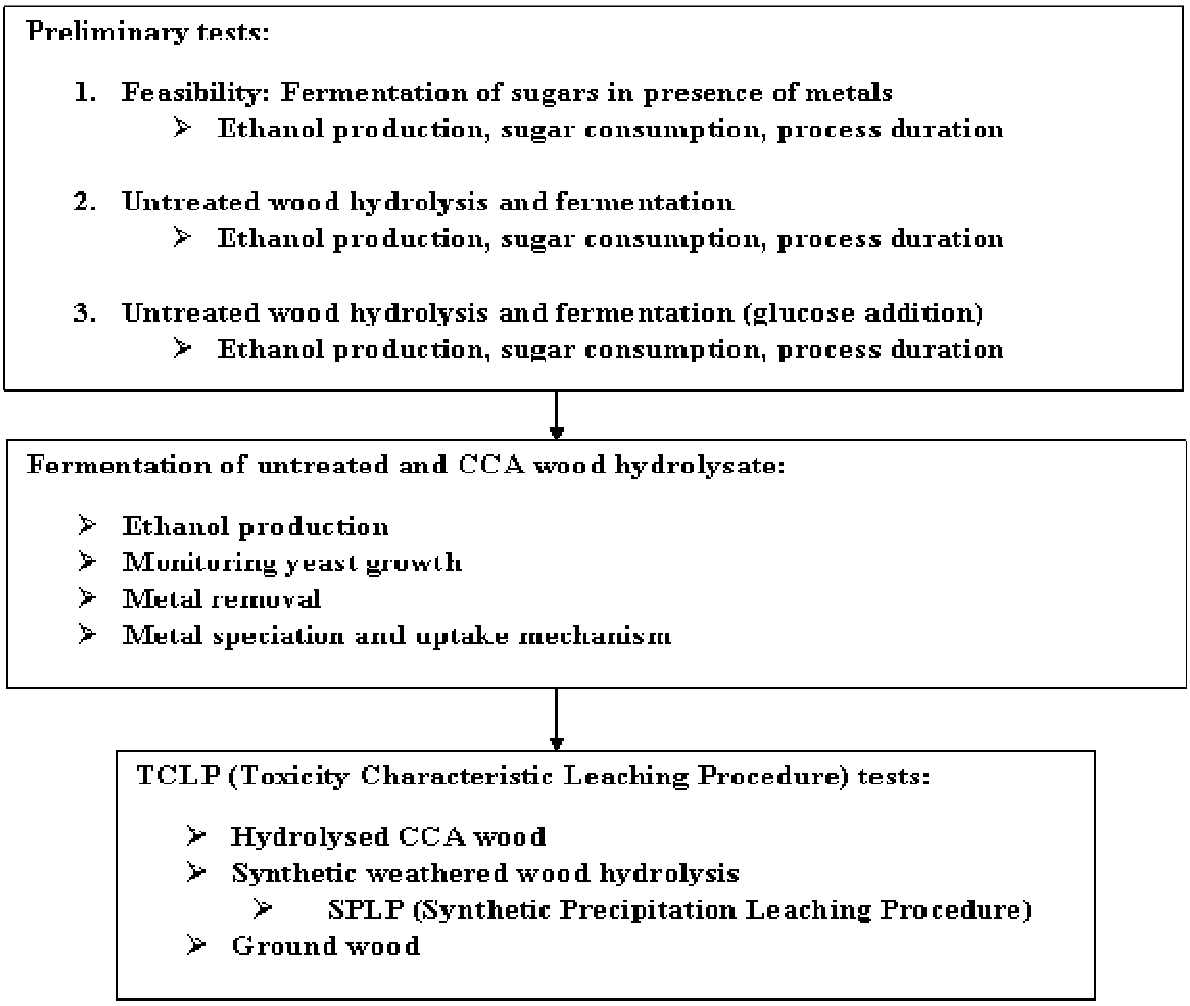

Figure 3.1: Protocol and testing plan

3.2 MATERIALS

- **Treated Wood**

CCA treated wood is the most frequently used wood, for exterior applications, in Canada. This greenish wood was sold in different renovation stores in different sizes. According to Forintek Canada Corp., the most frequently used Canadian softwood species for exterior applications are:

- Western Red Cedar
- Eastern White Cedar
- Pine (white, Murray, red)
- Douglas fir
- Spruce

The wood samples for these experiments were 2 pieces of 2.5 cm x 15 cm x 12.5 cm treated wood (were bought in 2002). According to the seller's information (RONA), for this size of wood, they usually used Gray Pine species. Most disposed wood is old preserved wood and the amounts of their remaining preservatives are less than new wood. Thus by experimenting on new treated wood the maximum content of metals and the worst condition is tested. An experiment for hydrolysis and fermentation of weathered wood was also performed.

- **Wood (not treated):**

Chips of gray pine wood, produced during cutting and rasping, were prepared by RONA.

- **Yeast:**

Active dry Fleischmann's Baker's yeast (*Saccharomyces cerevisiae*) was purchased from a local supermarket.

Other chemicals include:

- **Acids:**

Nitric acid: provided by Fisher Scientific, trace metal grade, 67-71% purified.

Sulfuric acid: provided by Fisher Scientific, reagent A.C.S, 95-98% purified.

Hydrochloric acid: provided by Fisher Scientific, trace metal grade, 67-71% purified.

Glacial acetic acid :provided by Fisher Scientific, reagent A.C.S.

- **Nutrients:**

Sodium phosphate monobasic (NaH_2PO_4), magnesium sulfate ($MgSO_4$), ammonium phosphate dibasic $(NH_4)_2HPO_4$, yeast extract.

- **Sugars:**

Dextrose certified anhydrous, D-galactose, D- (+)xylose, D(+)-mannose(99%).

- **Heavy Metals:**

Sodium arsenate dibasic (Na_2HAsO_4 .$7H_2O$), chromium chloride ($CrCl_3$. $6H_2O$), cupric chloride ($CuCl_2$. $2H_2O$), arsenic standard for atomic adsorption (1000 ppm), chromium standard for atomic adsorption (1000ppm), copper standard for atomic adsorption (1000 ppm).

- **pH adjustment chemicals :**

KOH(1N), Sulfuric acid (85%, 4.4% and 1N), NaOH(50% and 0.2N).

3.3 ANALYTICAL PROCEDURES

HPLC

A Beckman Coulter HPLC (High Performance Liquid Chromatography), System Gold with Model 508 Auto sampler was used to measure the amount of produced ethanol, consumed sugars and metal species in liquid phase. Supelco C-610H and Shodex SP0810 columns, which are ideal for separating the fermentation products according to company recommendation and a Shodex KC811 column for arsenic speciation and a Hamilton PRPX100 column for chromium speciation and copper concentration, were used. A Jasco Refractive Index (RI) detector, model RI1530 was employed.

Table 3.1 shows the specifications of the HPLC columns used for this research and the retention times of sugars and ethanol according to the Supelco Bulletin 887B, and Showdenko Company information. Table 3.2 gives the same information for arsenic (III and V) based on Shodex Company recommendations and copper based on the Hamilton

website which was modified by not adding EDTA and using 192 nm wavelength instead of 254 nm. The conditions for chromium (VI and III) were obtained based on experiments.

Table 3.1: Retention times on HPLC column

Cat.No.	Supelco C-610H 59320-U	Shodex[a] SP0810
Dimension (mm)	**300 x 7.8**	**300 x 8**
Temperature	**30° C**	**50° C**
Mobile phase	**0.1% H_3PO_4 in HPLC grade water**	**HPLC grade water**
Flow rate (ml/min)	**0.5**	**1**
Detector	**RI[b]**	**RI[b]**
Compound	**Retention time (min)**	**Retention time (min)**
Glucose	12.1	7.2
Galactose	12.9	8.8
Mannose	12.8	10.5
Xylose	12.8	11.8
Ethanol	25.6	10.4

[a] http://www.sdk.co.jp

[b]RI: Refractive index detector

Inductively Coupled Plasma Mass Spectrometry

Copper, chromium and arsenic in the liquid were measured by an Agilent 7500 Inductively Coupled Plasma Mass Spectrometry (ICP-MS) which is based on coupling an inductively coupled plasma as a method of producing ions (ionization) with a mass spectrometer as a method of separating and detecting the ions.

Table 3.2. Retention times on HPLC column for metals.

Cat.No.	**Hamilton PRPX100**	**Shodex[a] KC811**	
Dimension (mm)	**150 x 4.6**	**300 x 8**	**300 x 8**
Temperature	**25° C**	**40° C**	**50° C**
Mobile phase	**3mM sulfuric acid**	**12mM phosphoric acid**	**HPLC grade water**
Flow rate (ml/min)	**2**	**1**	**1**
Detector	**UV (192nm)**	**UV (192 nm)**	**UV (192 nm)**
Compound	**Retention time (min)**	**Retention time (min)**	**Retention time (min)**
Copper	12.1		
Chromium(VI)	1		
Chromium(III)	2.5		
Arsenic(V)		6.5	
Arsenic (III)		8.2	
Acetic acid			5

[a] http://www.sdk.co.jp

Digestion

To measure the amount of heavy metals by ICP or AA, in sulfuric acid solutions during the first and the second steps of hydrolysis and also in the solution after fermentation, the

solutions were digested. According to the Acid digestion method, *EPA 3005A* 2 ml of concentrated nitric acid (70%) and 5 ml of concentrated HCl (67%) were added to the 100 ml of sample leachates in flasks. The solutions were heated on hot plates at 90-95 °C until the volume was reduced to 15-20 ml. Then they were left to cool and adjusted to 100 ml by distilled water, before measuring by ICP or AA.

UV/VIS spectrophotometer

Microorganism cell density was determined based on optical absorbance. Measurements were made using a Perkin Elmer Model Lambda 40 spectrometer at a 600 nm wavelength. The samples were diluted to an absorbance of less than 1. For the samples after a few minutes of yeast addition, the dilution of 1/20 was acceptable while for samples after 6 hours a dilution of 1/100 was necessary.

XRF

The amount of arsenic, chromium and copper in the solid CCA treated wood was measured with the X-ray fluorescence (XRF) analyzer (Niton XLp 700 series Environmental Analyzer) according to the EPA Method 6200 (Field Portable XRF Spectrometry for the Determination of Elemental Concentrations in Soils and Sediments). The in situ mode was used and ground, air dried treated, untreated, weathered, and hydrolyzed wood were analyzed to determine the amount of heavy metals.

3.4 EXPERIMENTAL PROCEDURES

Procedure of fermentation of sugars to ethanol as preliminary tests

To investigate the possibility of the fermentation of sugar by Baker's yeast in the presence of chromium, copper and arsenic, some experiments were carried out. The fermentation was performed using 250 ml flasks placed on a Thermolyne adjustable reciprocating orbital shaker adjusted on 100 rpm with a Fisher Scientific incubator model 304, at 30 ºC under aerobic conditions. The amount of nutrients were calculated for 200 ml hydrolysate, according to the Palmqvist et al. (1996) fermentation procedure including: 2.39g NaH_2PO_4, 0.005g $MgSO_4$, 0.05g $(NH_4)_2HPO_4$, 0.5g yeast extract, 3.5g glucose, 0.95g mannose, 0.15g galactose and 0.39g xylose which were added to every flask in all experiments (unsterilized). For controls, nothing else was added. In flasks to evaluate the presence of heavy metals, 11 g ($CrCl_3$. $6H_2O$) or equivalent liquid chromium standard, 4.74 g (Na_2HAsO_4 .$7H_2O$) or equivalent liquid arsenic standard, 3.69 g ($CuCl_2$. $2H_2O$ or equivalent liquid copper standard) and a mixture of all was added (Appendix A). By adding 170 ml distilled water and adjusting pH to 5.5 using sulfuric acid and NaOH, the volumes of all flasks were brought to 200 ml. Finally, 1 g of active dry Fleischmann's Baker's yeast (purchased from a local super market) was added to all flasks except for the controls.

Using 3.5 g of glucose and assuming that all the glucan converts to glucose and that according to Table 2.5, 42.4% of pine wood is glucan, then the amount of wood fermented to 3.5 g glucose could be a maximum of 8.25 g. Using 50.5% chromium, 21%

copper and 38% arsenic according to the Table 2.1, 11 g of $CrCl_3$, 4.74 g of Na_2HAsO_4 and 3.69 g of $CuCl_2$ were measured and added to the relevant flasks (A.2, A.3, A.4, A.5).

Figure 3.2:Experimental set up for sugar fermentation in presence of Cr, Cu and As

To investigate the required time for the fermentation, the experiments using the samples without the presence of metals were performed. Several samples (including controls) were left in the incubator for 1 hour and 1 to 5 days. The experiments were set in triplicate. Results of the first set of experiments showed that no more than 32 hours were needed for fermentation.

To investigate the effect of heavy metals, the samples contained copper, chromium, arsenic separately and a mixture of all together (according to above-mentioned calculations) in addition to controls and samples without heavy metals were left in the incubator for 24 hours. The amounts of produced ethanol and consumed sugars were measured by HPLC. By using equation 3.1 and calculating the theoretical amount of produced ethanol and then the ratio of measured ethanol to the theoretical ethanol, the yields of ethanol were calculated (Appendix A).

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2 \qquad 3.1$$

Concentrated acid hydrolysis

To produce the sugars for the fermentation, the untreated and treated wood samples were chopped and hydrolyzed using the sulfuric acid hydrolysis method suggested by Ackerson et al. (1981) which is a prehydrolysis of lignocellulosic biomass by 4.4% dilute sulfuric acid (H_2SO_4) at 100°C for 60 minutes (Figure 3.3). The subsequent liquid acid and sugar stream was separated from the solids by using a buchner funnel, neutralized by sodium hydroxide and fermented. The solids, mostly cellulose and lignin, entered the second stage hydrolyzer and were mixed with 85% concentrated sulfuric acid (H_2SO_4) at 100 °C for 10 minutes. Cellulose was converted into C6 glucose sugars. Again the subsequent liquid acid and sugar stream was separated from the solids by Buchner funnel, neutralized by sodium hydroxide and were fermented. Samples of liquids from both hydrolysis stages were taken for digestion and heavy metal measurements.

No filter was used because the wood pieces were bigger than the funnel's holes.

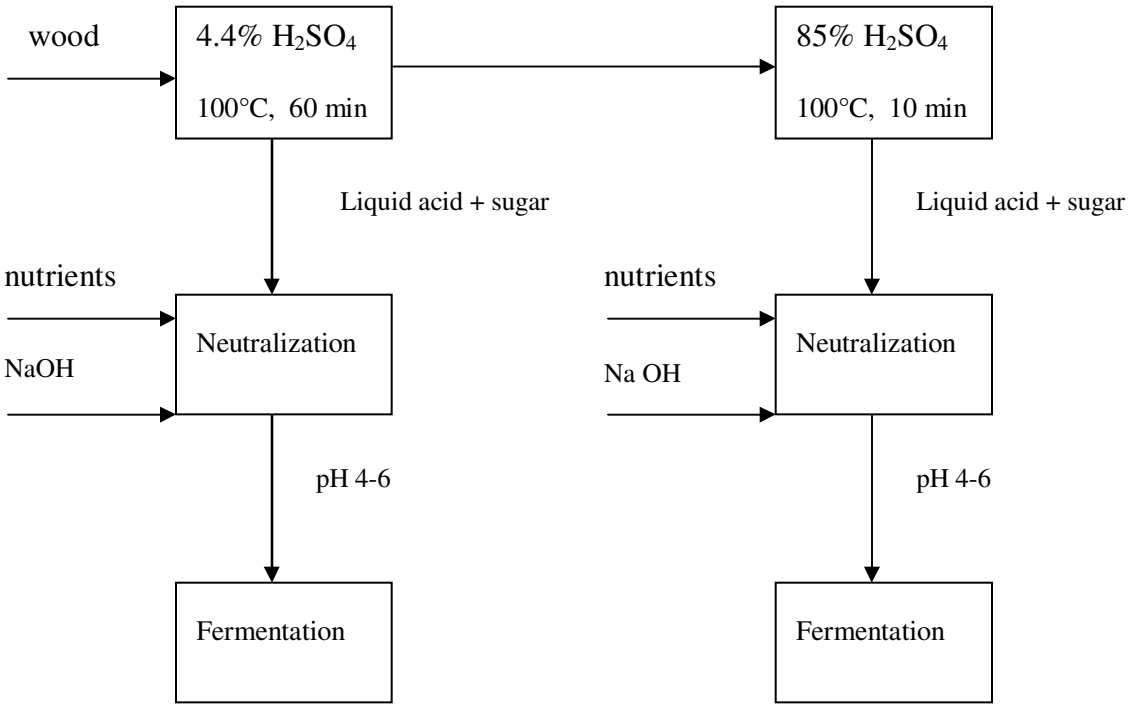

Figure 3.3: Acid hydrolysis flow sheet

Procedure of fermentation of the hydrolysate

Following the acid hydrolysis, hydrolysates were fermented using 250 ml flasks placed on a Thermolyne adjustable reciprocating orbital shaker adjusted on 170 rpm with a Fisher Scientific incubator model 304, at 30 ºC under aerobic conditions. The amount of nutrients was calculated for 100 ml hydrolysate, according to the Palmqvist et al. (1996) fermentation procedure including: 2.39 g of NaH_2PO_4, 0.005 g of $MgSO_4$, 0.05 g of $(NH_4)_2HPO_4$, and 0.5 g of yeast extract were added to every flask in all experiments (unsterilized). By adding distilled water and adjusting the pH to 5.5 using sulfuric acid

(85%, 4.4%) and NaOH (50% and 0.2N), the volume of all flasks were brought to 100 ml. Finally, 2g active dry Fleischmann's Baker's yeast (purchased from a local super market) were added to all flasks except for the controls. To compare the effects of different amounts of yeast, the samples containing 4 g of yeast were prepared also. Before starting the fermentation, after 4 hours of fermentation and at the end of fermentation (24 hours), samples were centrifuged (3500 rpm, 10 min), filtered (using a 0.45 μm syringe filter) and analyzed by HPLC for ethanol production and sugar consumption. For measuring the total amount of heavy metals, the centrifuged samples of hydrolysates before and after fermentation were digested, filtered (using Whatman No.41 filter papers) and analyzed by ICP. To measure the amounts of chromium and arsenic species and also copper, different samples were analyzed by HPLC, using appropriate columns.

Toxicity characteristic leaching procedure (TCLP)

The TCLP test (EPA Method 1311) was performed for the remaining solids of wood (Figure 4.13) after the hydrolysis to determine if the values were less than 5 ppm (the maximum acceptable limit) for chromium and arsenic. A volume of 5.7 ml of glacial acetic acid, followed by 64.3 ml of 1N sodium hydroxide was added to 500 ml of distilled water and then was diluted to a volume of 1 liter (pH of 4.93 $\pm$ 0.05) as the TCLP extraction fluid. The wood remains were extracted with an amount of extraction fluid equal to 20 times the weight of solid phase for 18 hours on a Thermolyne adjustable reciprocating orbital shaker adjusted on 30 rpm. Leached chromium and arsenic were measured by HPLC.

Synthetic precipitation leaching procedure (SPLP)

The SPLP leaching fluid is a simulated acid rain, which was prepared by adding 0.4 ml of a dilute sulfuric acid and nitric acid solution (60/40 mix) to a 2L volumetric flask and bringing the volume with reagent water to achieve a final pH of 4.20±0.05. The ratio of solid to liquid was 1:20 (US EPA method 1312). The SPLP procedure was used to simulate weathered wood from new CCA treated wood.

Biomass concentration and growth rate

The dry cell weight method was used to calibrate the measured Visible adsorption of the growth of biomass during fermentations using CCA treated and untreated wood and also for glucose fermentation in the absence and presence of copper, arsenic and chromium (each one separately). In this method, 5 ml samples of the fermentation broth were centrifuged (3800 rpm) at room temperature for 10 min. The biomass yeasts were harvested by filtration through a pre-weighed membrane filter (cellulose nitrate filter, 1.2 µm pore size, Millipore), dried at 105°C for 24 hours to a constant weight. The difference of membrane weight and the final weight (after filtration and drying) divided by the volume gives the concentration. The growth rate was determined from the graph of concentration (UV/Visible absorbance) versus time. The growth rate is L = log(concentration) of phase B(log phase) versus time (Figure 3.4).

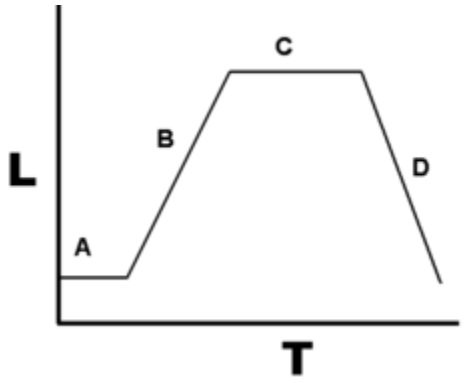

Figure 3.4: Yeast growth as a function of time

$$\frac{dL}{dT} = kL$$

The biomass weight was plotted versus time, for the yeasts which were grown without metals, harvested and exposed to the metals to compare their uptake with the yeasts that have not been previously exposed to metals and also the production of ethanol versus time were compared to the metal uptake graphs versus time to determine the mechanism of metal uptake of the yeasts.

CHAPTER FOUR
RESULTS AND DISCUSSION

As mentioned earlier, there are three main steps to produce ethanol from wood including hydrolysis, fermentation and ethanol recovery by distillation. Among them fermentation is the only step in which living organisms (baker's yeast in our case) are used. Therefore the existence of heavy metals along with wood could have negative effects on fermentation process and prevent the whole process. To investigate the feasibility of the process, fermentation of sugars in the presence of arsenic, copper and chromium was tested as the first preliminary step. Then several experiments using CCA treated wood and untreated wood were performed to investigate the effect of heavy metals on ethanol production as well as the effect of the process on heavy metal removal.

4.1 FERMENTATION

4.1.1 PRELIMINARY EXPERIMENTS

In order to find the duration of fermentation process, different batches for 1 hour, 1 day, 2 days, 4 days and 5 days were set up. The results are as shown in the Figure 4.1. It took approximately 32 hours to produce 8.3g/l of ethanol (the maximum amount obtained).

When the experiment was conducted over a longer duration, no more ethanol was produced.

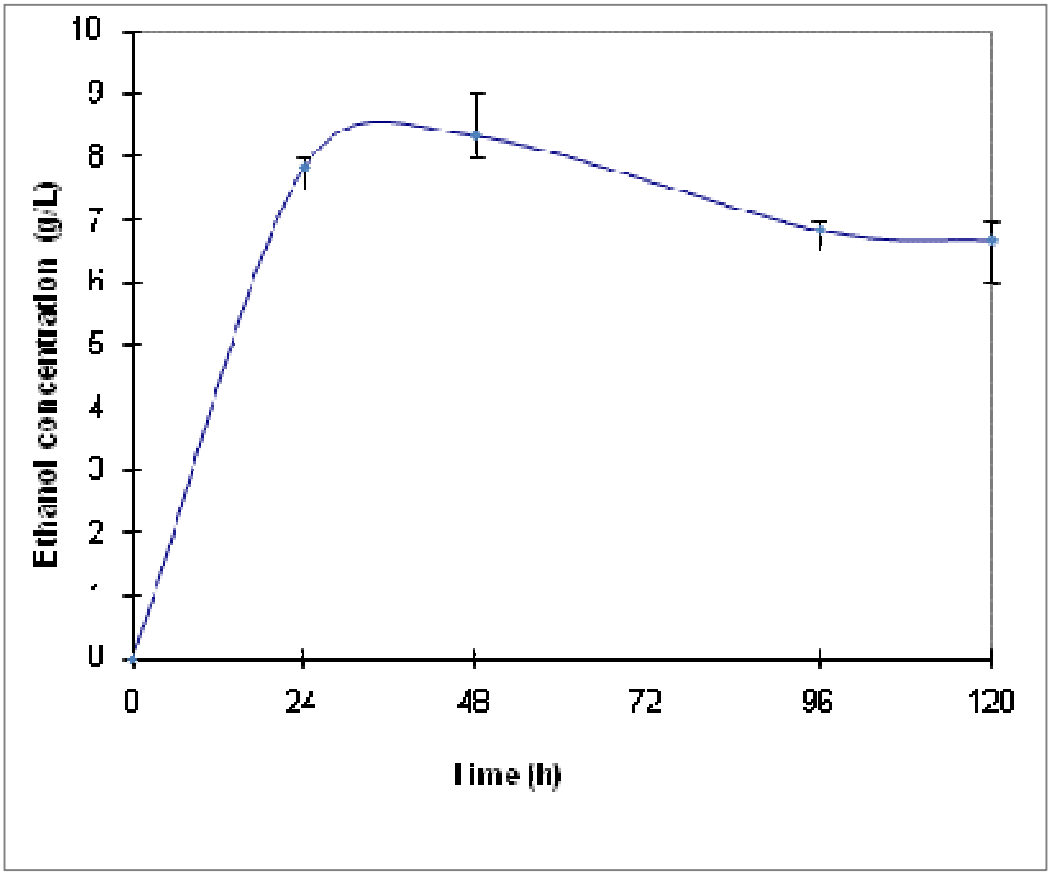

Figure 4.1: Ethanol production from sugars (3.5% glucose solution containing mannose, xylose and galactose according to AppendixA) without the presence of heavy metals over a period of 5 days

The theoretical amount of ethanol production was calculated using the formula A.1(Appendix A) and a maximum yield of 93% was obtained for the entire process. The yield as a function of time is shown in Figure 4.2.

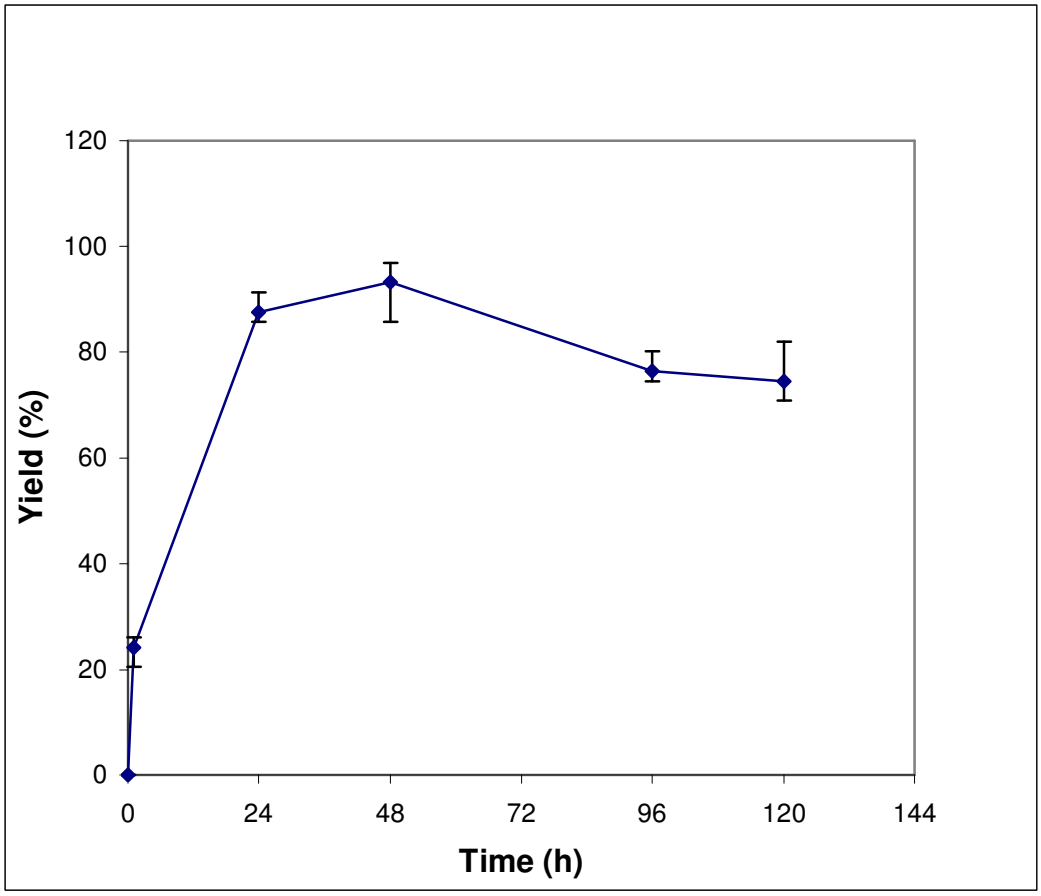

Figure 4.2: Ethanol production yield from sugars (3.5% glucose solution containing mannose, xylose and galactose according to AppendixA) without the presence of heavy metals over the period of 5 days

According to Taherzadeh et al. (1997), some by-products of hydrolysis process such as furfural (HMF) act as inhibitors for the fermentation process and then the yield of the fermentation process will be less than 100%. Lower yields require less time (Figure 4.2) and then the fermentation time can be decreased to less than one day.

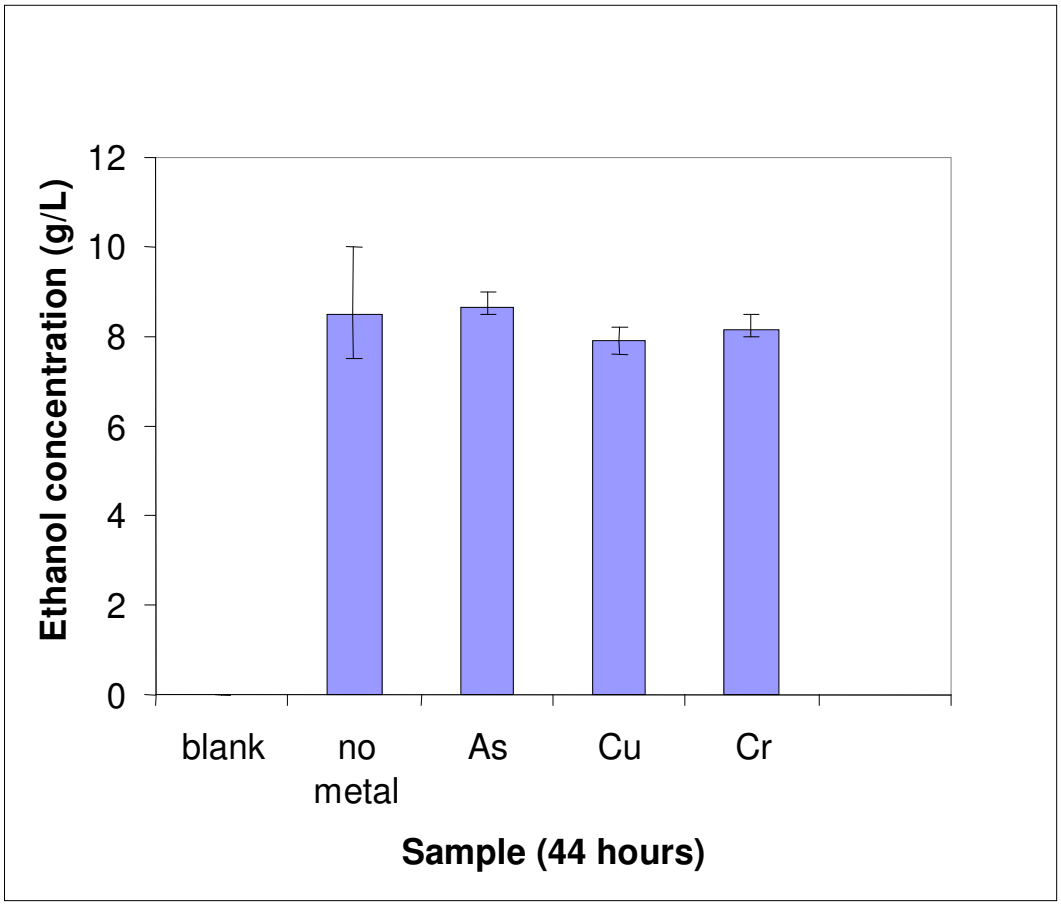

Figure 4.3: Ethanol production after 44 hours of fermentation of sugars (3.5% glucose solution containing mannose, xylose and galactose) in the presence of metals according to Appendix A

Experiments that were performed to investigate the effect of chromium, copper and arsenic on the fermentation of sugar and the results are shown in Figure 4.3. The amounts of chromium and arsenic added to the flasks were calculated according to Tables 2.1 and 2.5 and based on 3.5 g glucose per every flask. For copper, the liquid standard copper was used. In Figures 4.3 and 4.4, chromium and arsenic and copper did not have any negative effect on fermentation and almost the same amount of ethanol was produced in flasks with no metals and flasks containing chromium, copper and arsenic.

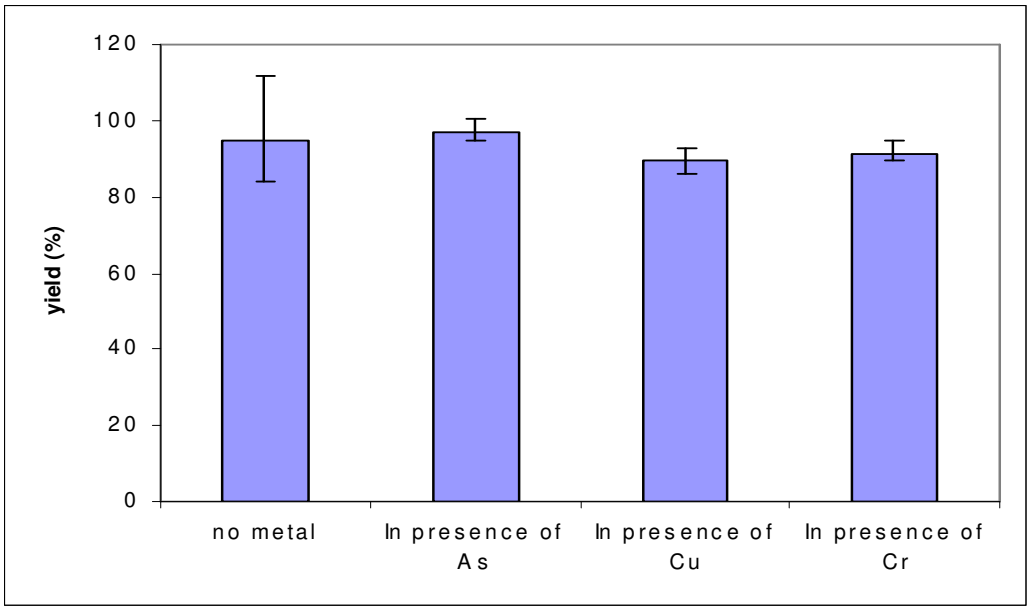

Figure 4.4: Ethanol yield after 44 hours of fermentation of sugars (3.5% glucose solution containing mannose, xylose and galactose) in the presence of metals according to Appendix A

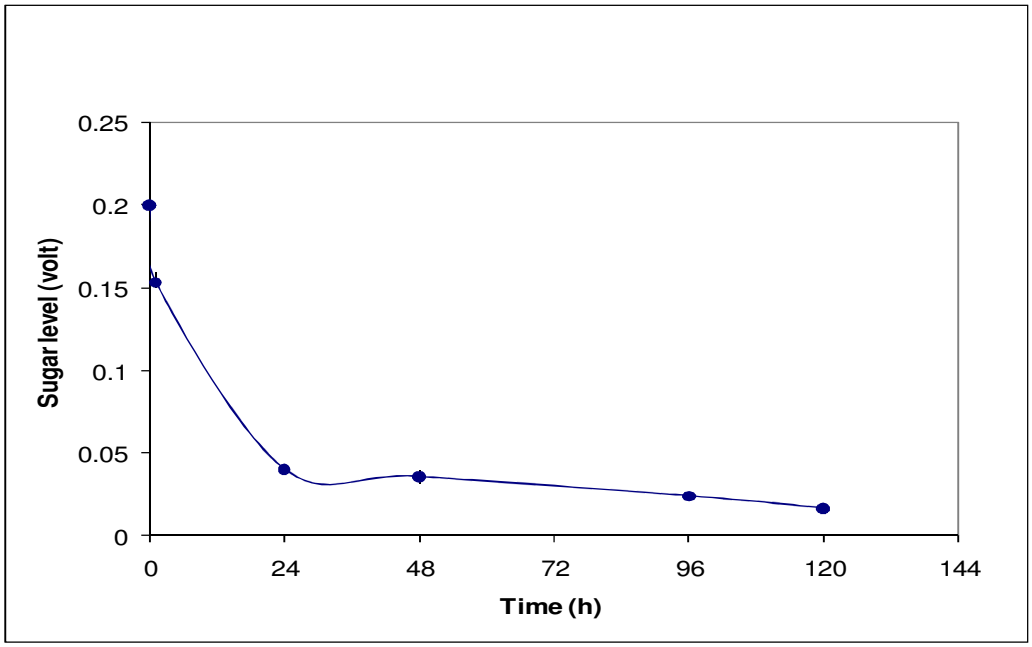

Figure 4.5: Monitoring of the amounts of mannose, xylose and galactose by HPLC during fermentation without the presence of heavy metals (3.5% glucose solution containing mannose, xylose and galactose according to AppendixA)

According to the SUPELCO bulletin 887B, the retention times of mannose, xylose and galactose are almost the same for the column used and therefore there was one common peak for all these sugars. As seen in Figure 4.5, during the fermentation the peak height reduced to the minimum of 0.04 volts after about 30 hours and then there was no noticeable reduction. It seems that some of these sugars were not consumed during fermentation. According to Ogier et al. (1999), Baker's yeast can only consume hexoses and not pentoses and then xylose, which is a pentose, would not be consumed during fermentation. More tests were performed to investigate the effect of xylose on yeast and the results will be discussed later in this chapter. Because the amount of mannose added to the flasks is almost 2.5 times of xylose and 6 times of galactose, the related peak to mannose covers the other peaks but later when the mannose and galactose were consumed by yeasts the remaining is probably xylose (Olsson and Hahn-Hagerdal, 1996). As seen in Figure 4.5 the minimum height of peak occurred around 32 hours, which was also the maximum ethanol production (Figure 4.1). According to Figure 4.6, all glucose was consumed by the yeast during fermentation and it seems that glucose was consumed and finished more quickly than the other sugars.

To have a better understanding of the behavior of the yeast and the mechanism of metal uptake by the baker's yeast, the growth rate of the microorganisms was monitored during the fermentation in all the experiments by measuring the absorption of samples, using a Visible spectrophotometer at a 600 nm wavelength.

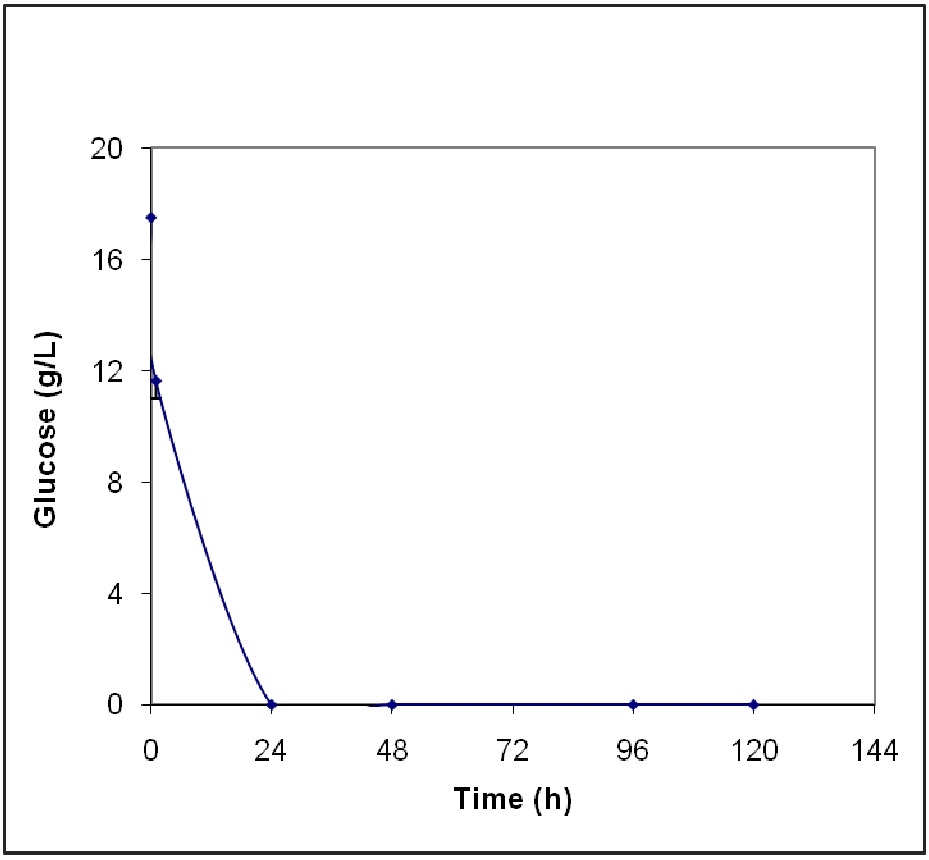

Figure 4.6: Glucose consumption during fermentation of sugars without the presence of heavy metals (3.5% glucose solution containing mannose, xylose and galactose according to AppendixA)

There is a linear relationship between absorbance and concentration of yeast (Appendix B). Therefore in all of the experiments the absorbance of samples during the fermentation was measured and the concentrations were calculated based on the linearity and the measurement of concentration and absorbance of 2 points in every sample. Monitoring of cell densities indirectly by optical methods has been mentioned by many researchers such as Taherzadeh (1999).

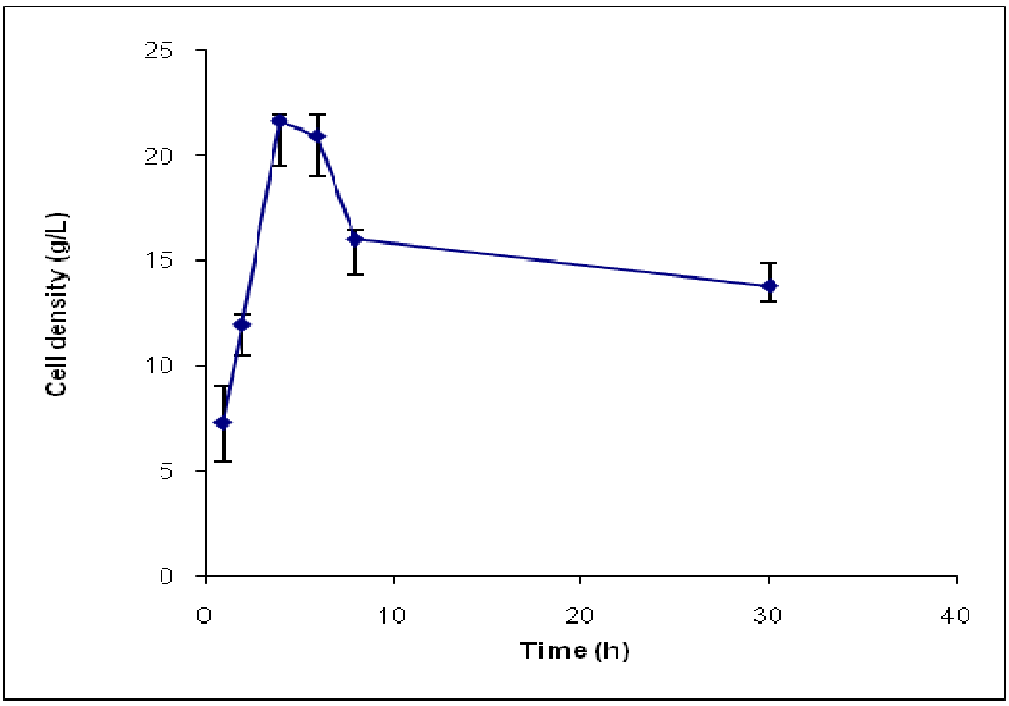

Figure 4.7: Yeast cell density during fermentation of 2% glucose solution

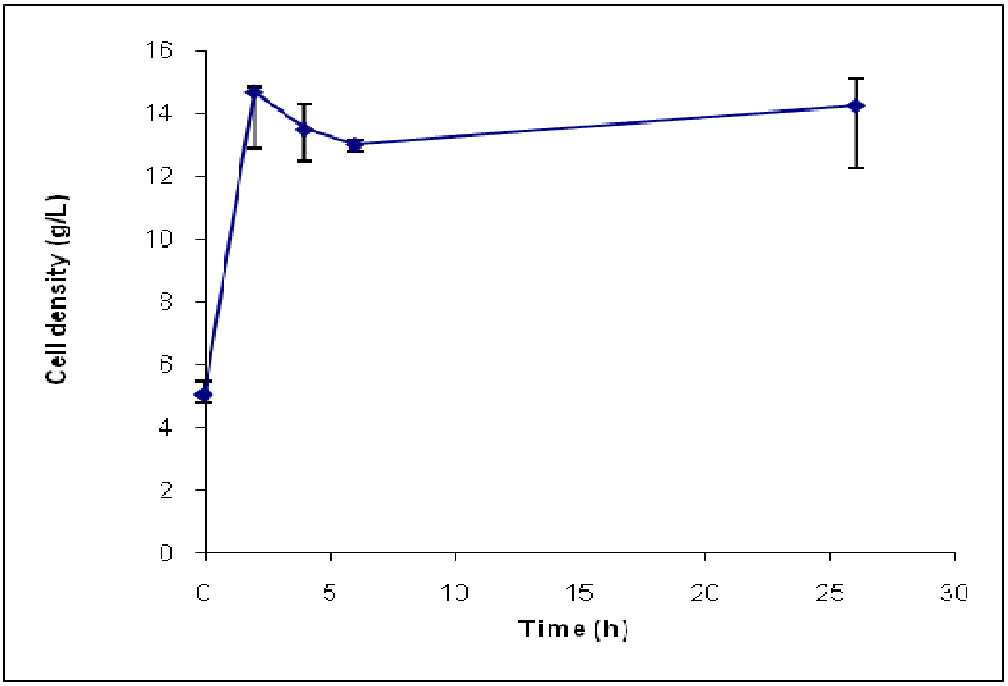

Figure 4.8: Yeast cell density during fermentation of 2% xylose solution

Figures 4.7 and 4.8 show the cell density of the yeast during the 30 hour period of fermentation using glucose and xylose as sources of carbon, respectively. During the fermentation of a glucose solution, yeast can grow almost 30 % more than fermentation of a xylose solution. Also there is no ethanol production in the flasks containing xylose. These results show that baker's yeast cannot consume xylose (which is a pentose) as easily as glucose or it may be yeast extract which is consumed as the only source of carbon during fermentation of xylose solution. As there was no sterilization in all tests then there is also the possibility of bacterial growth in the solutions.

The acclimation time for yeast when there are nutrients and glucose, pH 5.5 and temperature of 30˚C is very short and in less than 4 hours, the cell concentration reaches a maximum (Figure 4.7). Comparison of Figures 4.7 and 4.9 shows that when the maximum cell density is reached after 4 hours of fermentation, ethanol production continues and after almost 6 hours, the maximum amount of ethanol is produced.

To investigate the effect of heavy metals, existing in the CCA, on the ethanol production and the whole process, 10 g of small pieces of CCA treated wood (1.6 cm x 0.6 cm x 1cm) were hydrolyzed using 4.4% sulfuric acid followed by 85% sulfuric acid and then fermentation for 24 hours under a temperature of 30ºC and volume of 100 ml per flask. Following the first fermentation, the same amounts of yeast were added to the samples and were left in the incubator on the shaker for another 24 hours. Figures 4.10 and 4.11 show the production of ethanol and consumption of glucose, galactose and xylose in hydrolysates of 4.4% and 85% during the 1st and 2nd fermentations. Both figures show

that at some point the fermentation process stopped. However the pH was around 5 and the new amounts of yeast were added for the second fermentation but there was not much ethanol production. This may be because of the by- products during fermentation, which are toxic to the yeast. Calculating about 58.9% ethanol production yield (according to the maximum production of 6 g/l ethanol and 20 g/l glucose in the system (Figure 4.10) and comparing with the amount of 93% ethanol production yield when there is no hydrolysate in system (Figure 4.2) confirms the inhibitory effects of hydrolysis by- products. Azhar et al. (1982), Taherzadeh et al. (1997) and many other researchers confirm the inhibitory effects of by products on the fermentation process.

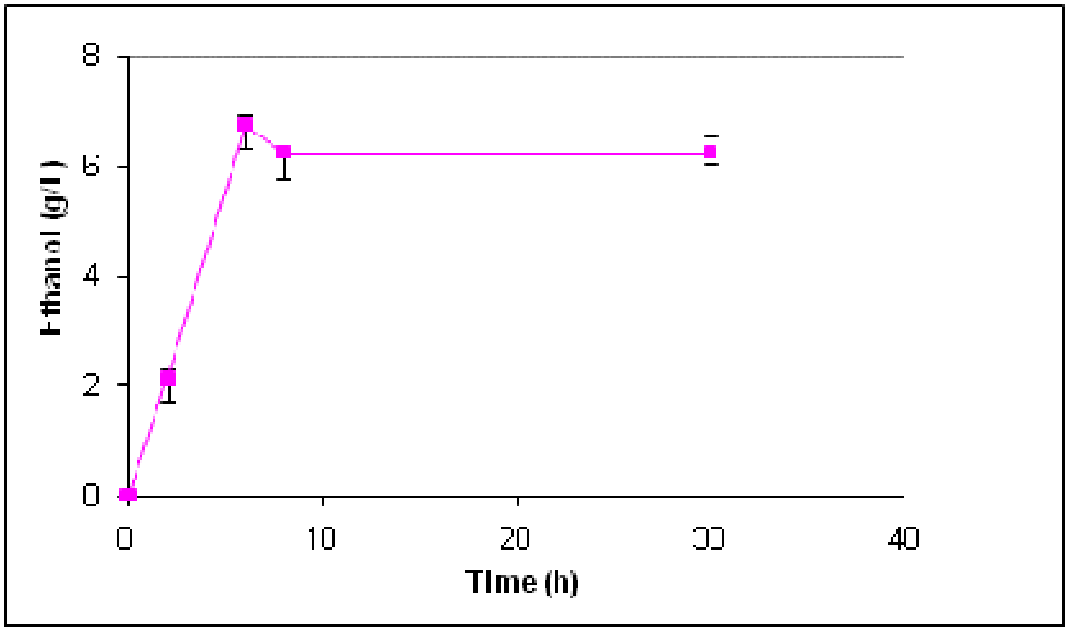

Figure 4.9: Ethanol production during fermentation of a 2% glucose solution

The most important by products are furans (furfural and 5-hydroxymethyl furfural), carboxylic acids (e.g., acetic acid) and phenolic compounds (e.g. phenol) (Taherzadeh,

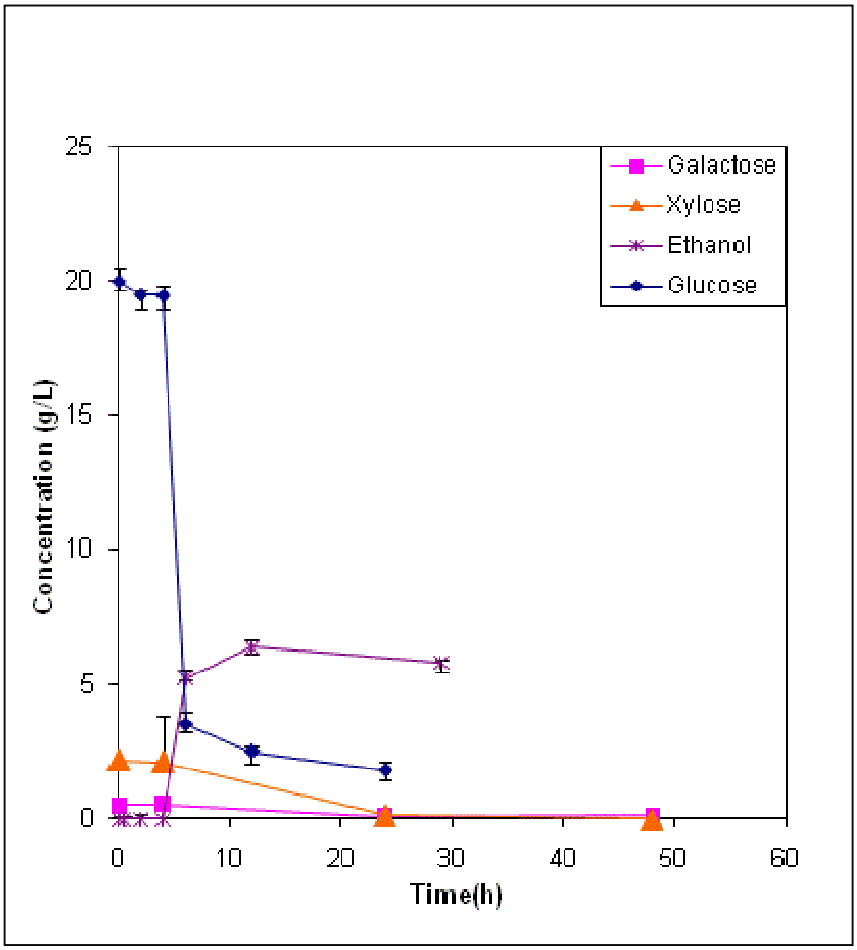

Figure 4.10: Sugar consumption and ethanol production during the fermentation of CCA treated wood hydrolysate (4.4% sulfuric acid)

1999). It seems that the acclimation time for fermentation of treated wood hydrolysate (Figure 4.12) is about 2 hours which is a little bit longer than the glucose fermentation (Figure 4.7). One of the probable reasons is because the conditions in wood hydrolysate are not as favorable as glucose media and some materials such as furans are produced during wood hydrolysis which can be inhibitory to the microorganisms. Also the maximum cell density is less than when there was a glucose solution. However, in both fermentations, after almost 4 hours, the cell density reaches its maximum.

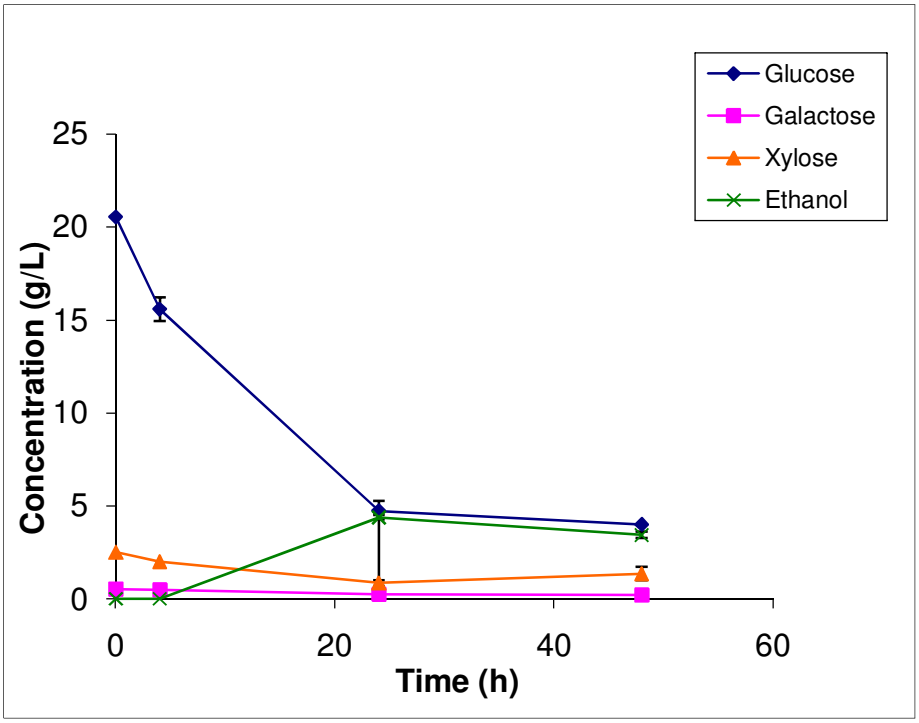

Figure 4.11: Sugar consumption and ethanol production during the fermentation of CCA treated wood hydrolysate (85% sulfuric acid)

4.1.2 COMPLEMENTARY AND CONTROL EXPERIMENTS

Figure 4.13 is the amount of ethanol production during the fermentation of CCA treated wood hydrolysate. Yeast starts to produce ethanol 4 hours after starting fermentation, which is almost at the end of log phase or start of the stationary phase (Figure 4.12). But for the glucose sample, there is no delay in the start of the ethanol production (Figure 4.9), as there is no acclimation phase (Figure 4.8). Thus, the shorter acclimation time leads to a faster ethanol production.

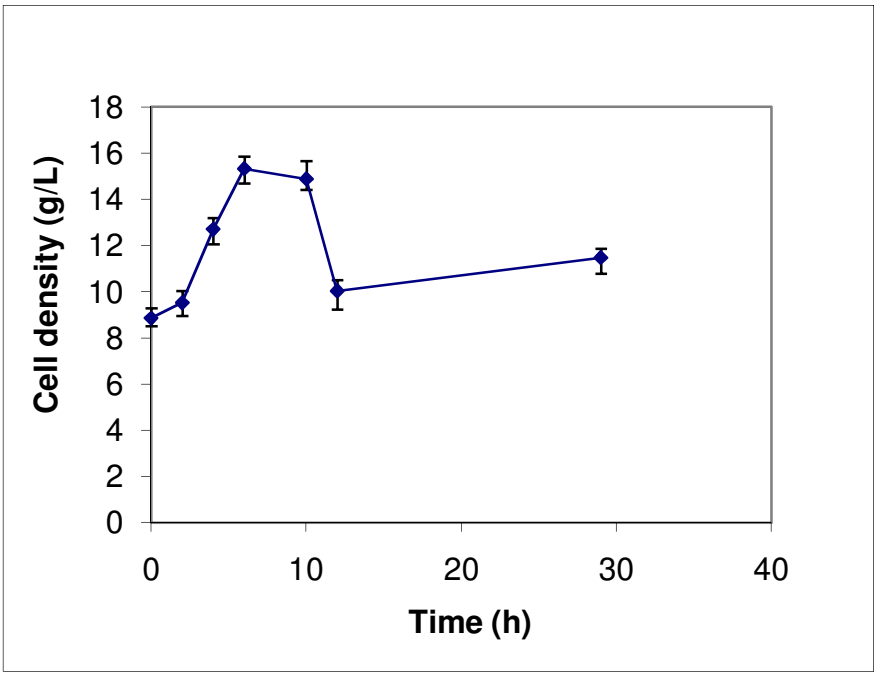

Figure 4.12: Yeast cell density during fermentation of CCA treated wood hydrolysate (4.4%)

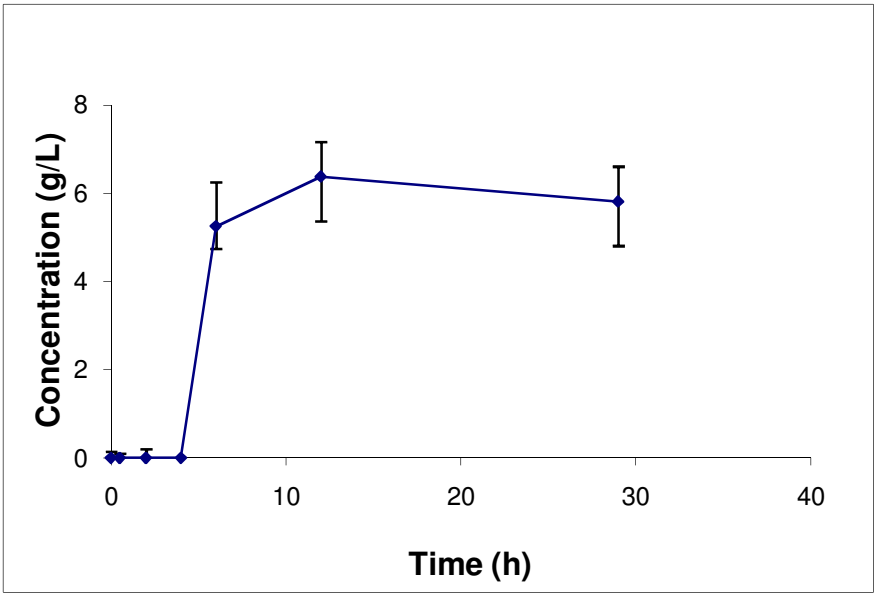

Figure 4.13: Ethanol production during fermentation of CCA treated wood hydrolysate (4.4%)

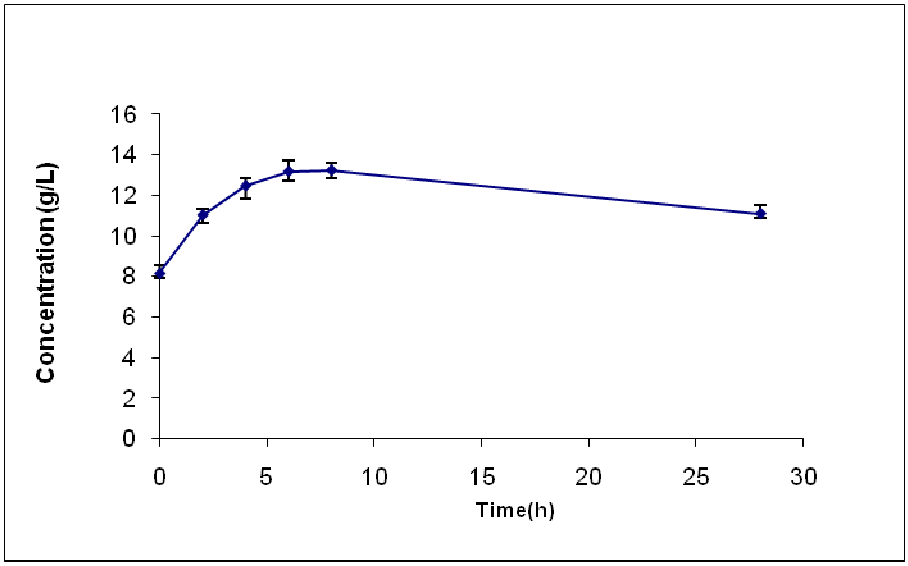

Figure 4.14: Yeast cell density during fermentation of untreated wood hydrolysate (4.4%)

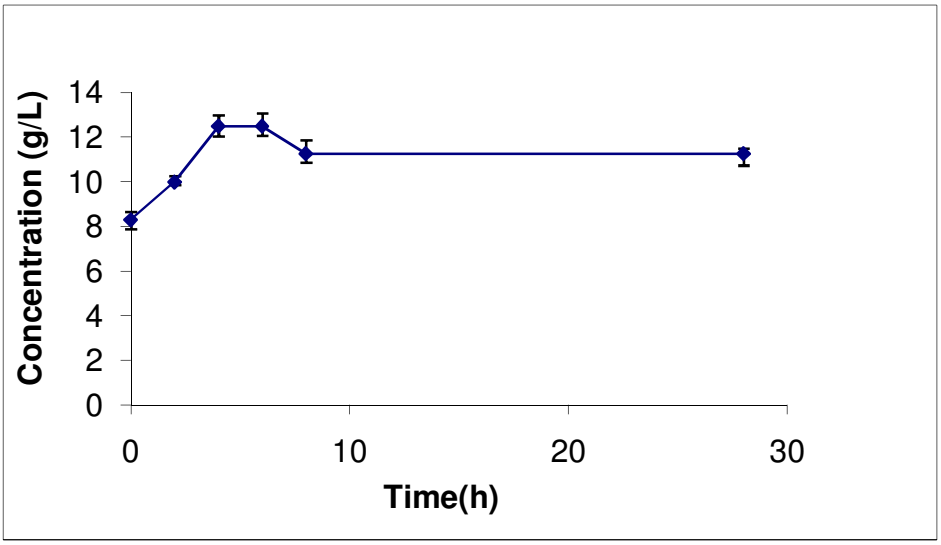

Figure 4.15: Yeast cell density during fermentation of untreated wood hydrolysate (4.4%) (2% sugar was added)

To compare the fermentation of treated and untreated wood, 10 g of untreated wood were hydrolysed and fermented by the same procedure as treated wood. At the same time to

make more comparisons, 2% sugar was added to some samples and the results of cell density and ethanol production are shown in Figures 4.14- 4.16.

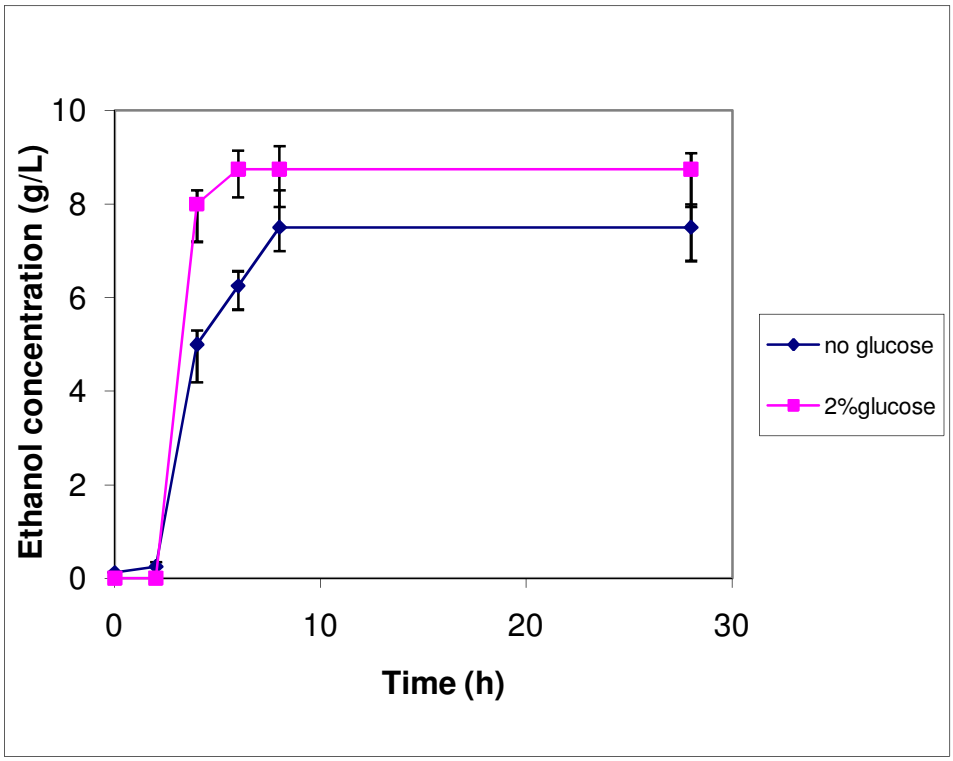

Figure 4.16:Ethanol production during fermentation of untreated wood hydrolysate (4.4%)

As shown in Figures 4.14 and 4.15, the maximum cell density is not affected by adding more glucose. However there may be a little more ethanol production when glucose was added (Figure 4.16).

As almost the same amount of ethanol was produced by treated and untreated wood thus the amount of arsenic, chromium and copper doesn't affect ethanol production (Figures

4.13 and 4.16). However there are differences between the maximum cell densities (Figures 4.12 and 4.14).

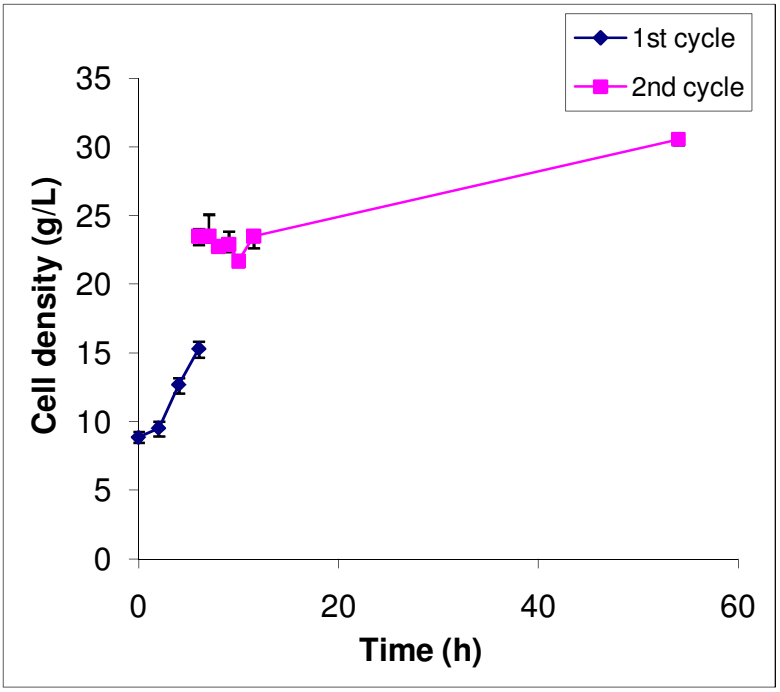

Figure 4.17:Yeast density during 1st and 2nd cycle fermentation of CCA wood hydrolysate (4.4%).
The 2nd fermentation cycle refers to fermentation of hydrolysate by adding 2g yeast at the beginning and then 2g more after 6 hours of fermentation

To understand the yeast activity better, other tests were performed and after 8 hours of fermentation of hydrolysates, the pH was measured. The pH values were about 4.5 to 5 which are still tolerable for the yeast. Again 2g of yeast were added to the samples which were left for fermentation. These samples are referred to as the 2nd cycle containing the amount of (2g + 2g) of yeast (Figure 4.17). The cell density of samples containing 4g yeast are shown in Figure 4.18.

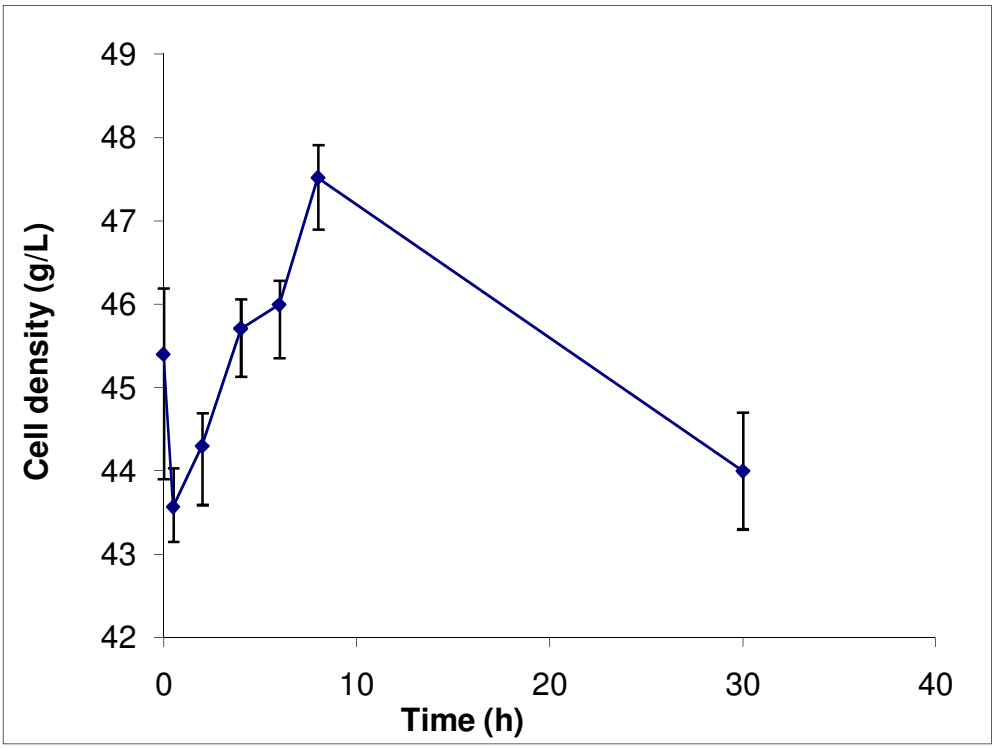

Figure 4.18:Yeast density using 4 g yeast per 100 ml CCA wood hydrolysate (4.4%)

Cell density for different samples, were summarized in Figure 4.19. However the maximum cell density in the second cycle is much higher than samples containing 2 g yeast but it is not as high as the samples containing 4g yeast. It means adding yeast in two steps (6 hour delay between yeast addition times) leads to less yeast growth, compared to adding all yeast at once. The reason could be the production of by products and inhibitors before the second yeast addition and less acclimation of newly added yeast to the media.

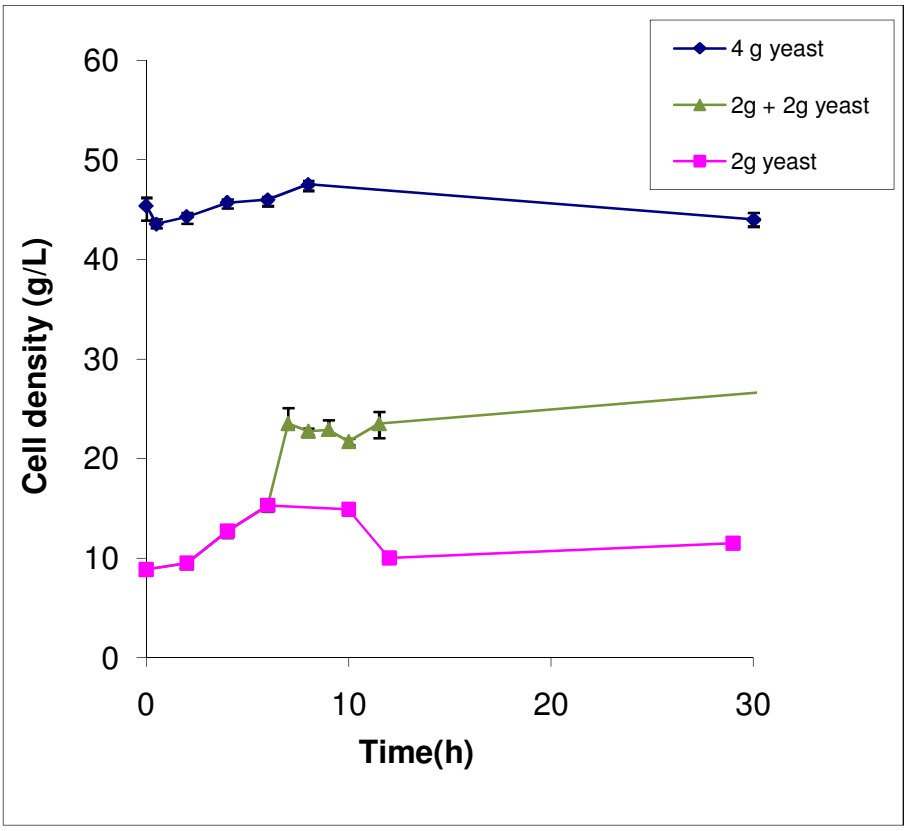

Figure 4.19:Yeast density per 100ml CCA wood hydrolysate (4.4%) using different amounts of yeast for fermentation

Figure 4.20 shows the results of production of ethanol during fermentation of hydrolysates (4.4%) using different amounts of yeast per 100 ml of hydrolysate. The experiments were performed to have a basic idea on how the increase of yeast could affect the ethanol production and when is the starting time of ethanol production. The figures show that even a higher amount of yeast does not increase the amount of total ethanol production but changes the pathway of ethanol production and the yeast start to produce ethanol faster. Comparison of Figures 4.19 and 4.20, the maximum ethanol production is almost 6 hours after the start of the fermentation when the stationary phase of the yeast starts or the growth stops and it could mean that there is a toleration limit for yeast and higher amount of ethanol could inhibit or kill them.

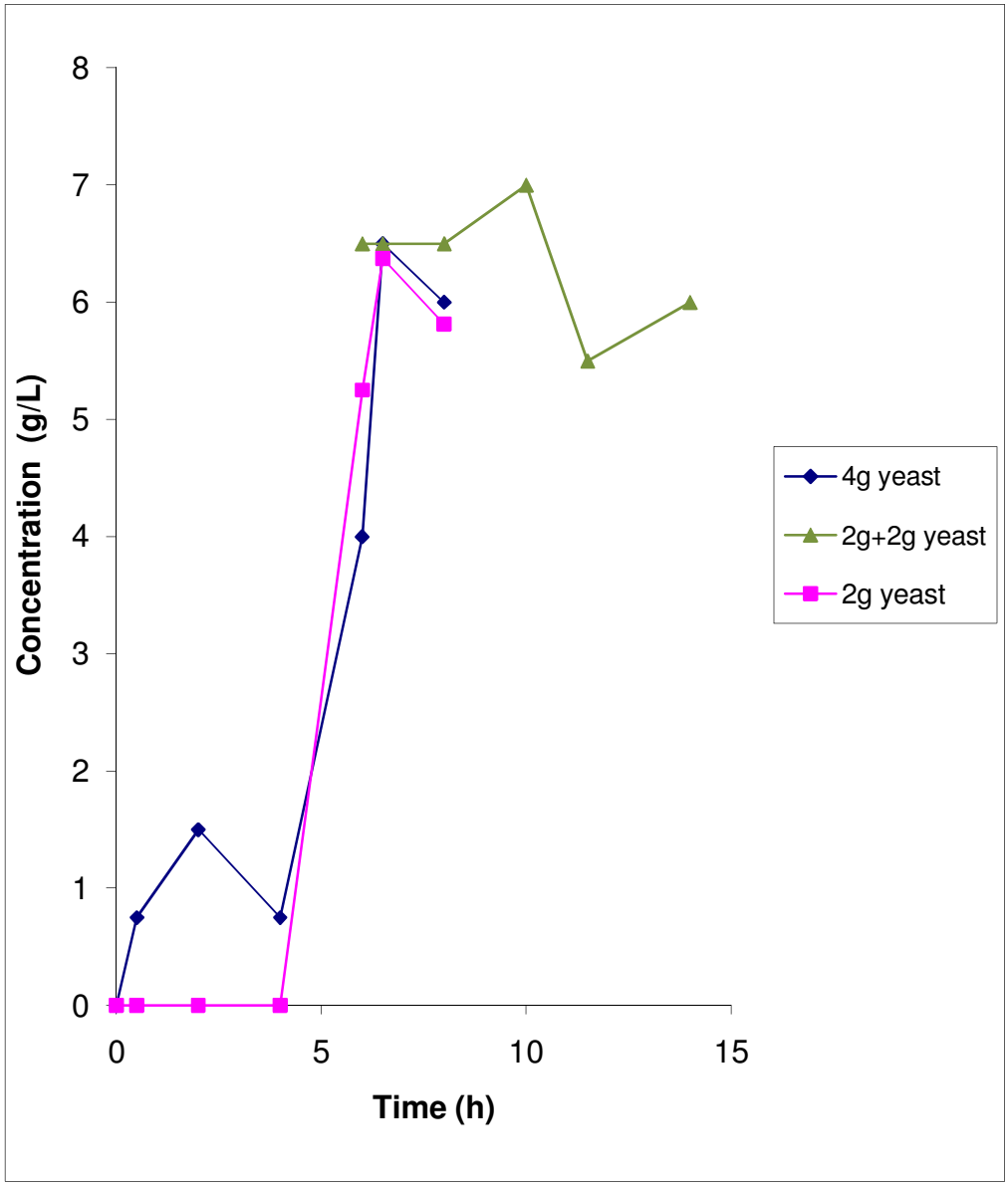

Figure 4.20: Ethanol production from CCA treated wood hydrolysate (4.4%) using different amounts of yeast

4.2 HYDROLYSATE METAL REMOVAL

Figures 4.21 to 4.23 are the results of monitoring the amount of chromium (VI), (III), and arsenic (V) and(III) in the hydrolysate liquid phase (using sulfuric acid, 4.4%) during fermentation by 2 g, 4g and (2g+2g) yeast. The solutions were filtered to be measured by HPLC using appropriate columns. During wood treatment, CrO_3, As_2O_5 and CuO are used to treat wood. Although the Cr(VI), As(V) and Cu(II) are the main species of metals which are used, the different steps of treatment could change the speciation. In particular, during the fixation step chromium (VI) will change to chromium (III) to fix the preservative on wood (Lebow, 1996). As chromium (VI) and arsenic (V) are the more soluble forms then the high levels of chromium (VI) and arsenic (V) in comparison to chromium (III) and arsenic (III) in the leached hydrolysate is logical (Figures 4.21 to 4.23). In all samples the amounts of arsenic (III) and also chromium (III) does not change which means that yeast do not uptake arsenic (III) and chromium (III) but uptake chromium (VI) and arsenic (V).

Adding yeast in 2 steps (Figures 4.21 and 4.23) contributes for maximum removal of almost 50% chromium (VI) and 60% arsenic (V) in comparison with using 2g yeast and 4g yeast. Comparing Figures 4.21 and 4.23 with Figure 4.19, chromium (VI) and arsenic(V) are taken up when the yeast are in their log phase or during their growth phase and by stopping the growth there is no uptake. Figure 4.19 shows that adding yeast in two steps requires a longer growth time which enables more chromium (VI) and arsenic(V) to be taken up.

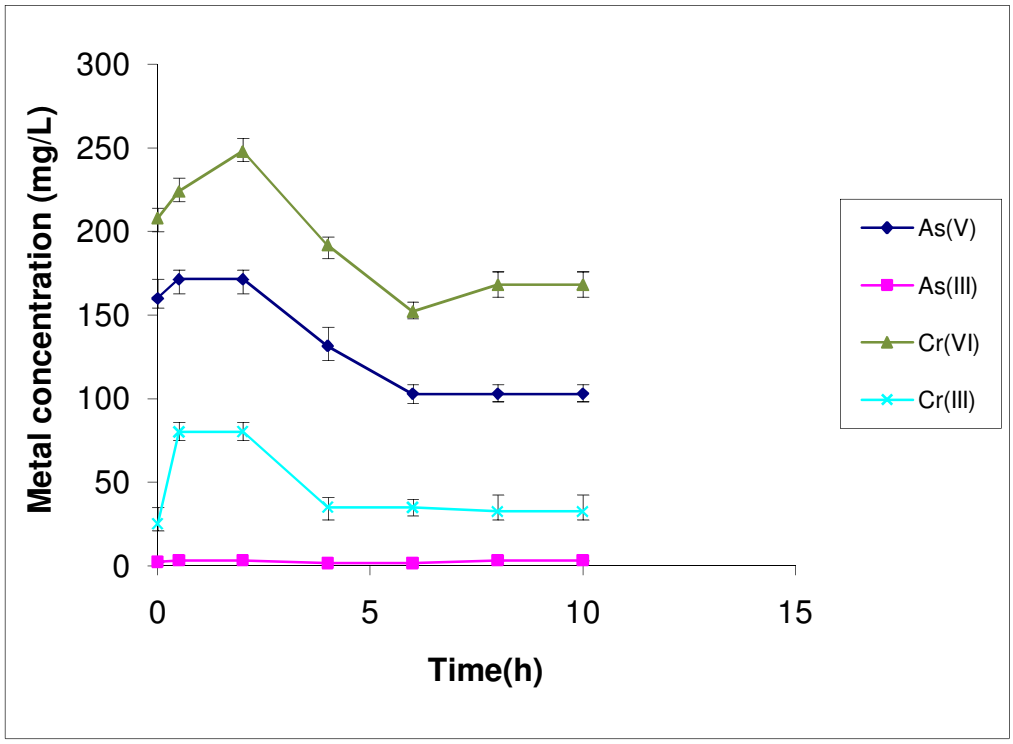

Figure 4.21: Metal concentrations during fermentation of CCA wood hydrolysate (4.4%) with 2g yeast per 100ml

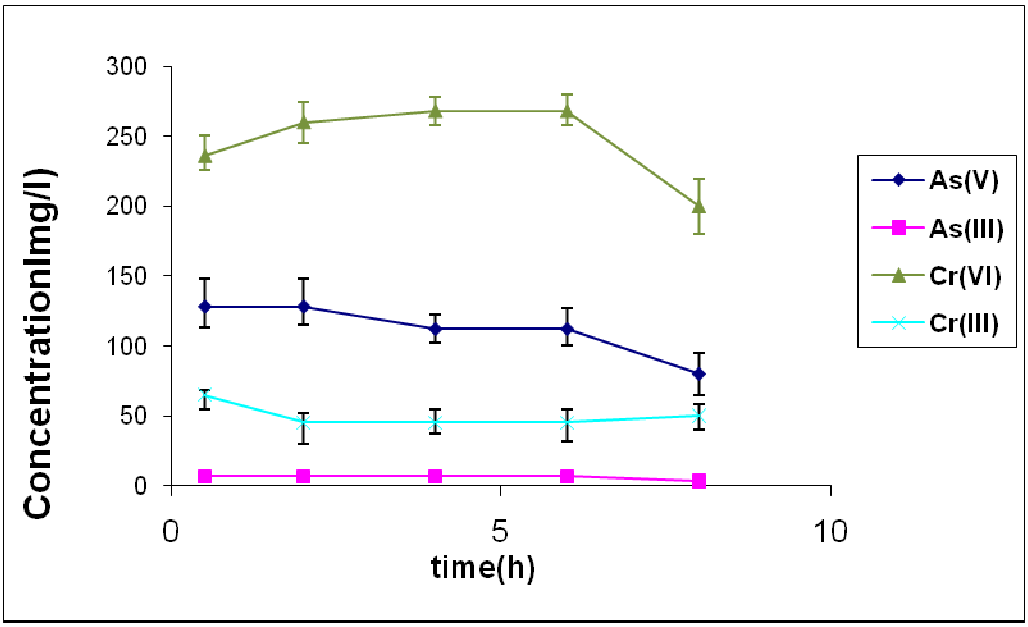

Figure 4.22: Metal concentration during fermentation of CCA wood hydrolysate (4.4%) with 4g yeast per 100ml

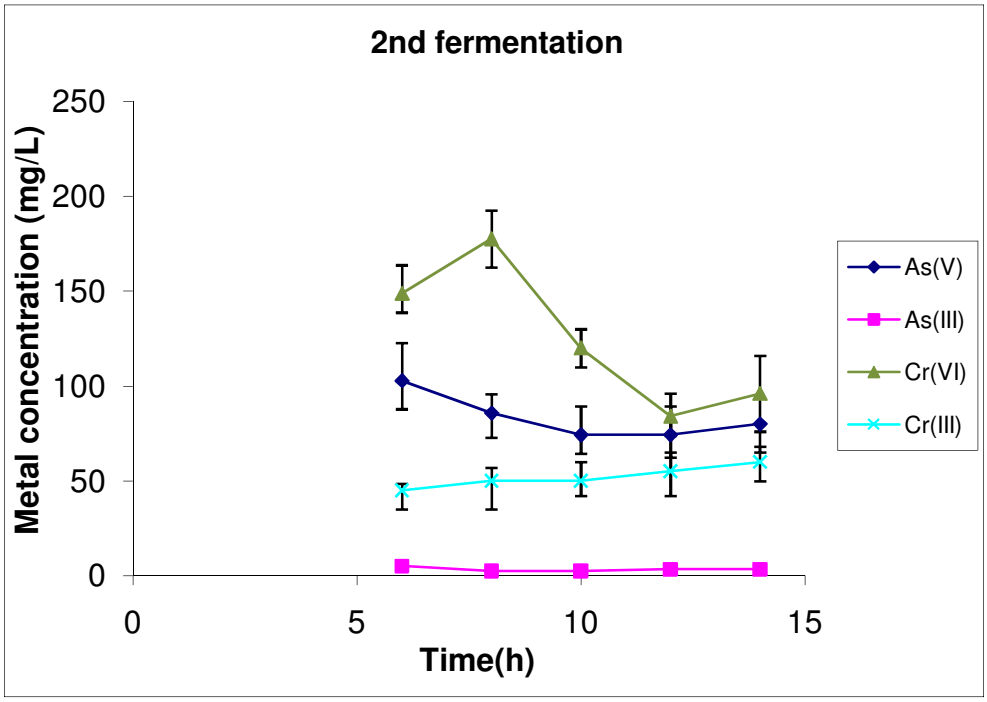

Figure 4.23: Metal concentration during fermentation of CCA wood hydrolysate (4.4%) , 2nd cycle

Copper was not detected by HPLC probably because of precipitation of copper compounds resulted from reactions of sulfuric acid and sodium hydroxide with copper during hydrolysis and pH adjustments, then separate experiments for investigation of copper removal were performed.

Figures 4.24 to 4.27 are the results of separate experiments on the removal of metals by yeast during the fermentation of 2% glucose solution containing mixture of chromium, arsenic and copper. Except for arsenic (V), there was not any significant removal for the others in Figure 4.24. For arsenic(III) and chromium (III), it was predictable but low levels of removal of chromium (VI) and copper could not be explained. Other

experiments were then performed to test copper and chromium removal separately and the results are shown in Figures 4.26 and 4.27 respectively.

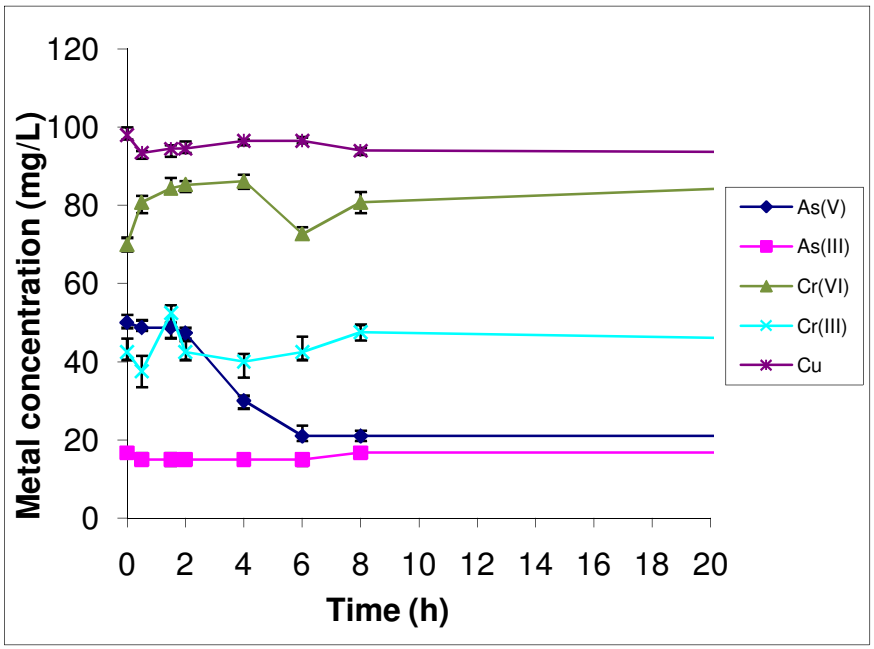

Figure 4.24: Metal concentration during fermentation of 2% glucose solution (containing metal mixture)

Figure 4.26 shows that the concentration of copper could decrease about 50% during the first 2 hours of fermentation and then increase during the next 4 hours and finally decreases after 7-8 hours of fermentation. Figure 4.25 which is the magnification of the same results of copper concentration from Figure 4.24 shows the same behavior for removal of copper however the removal was not more than 2%. Comparison of these figures shows that the yeast will adsorb copper during the acclimation time and later yeast will take copper when it is not active. Then for copper, yeast acts as an adsorbent and the maximum removal will be less than 2 hours after the start of the fermentation.

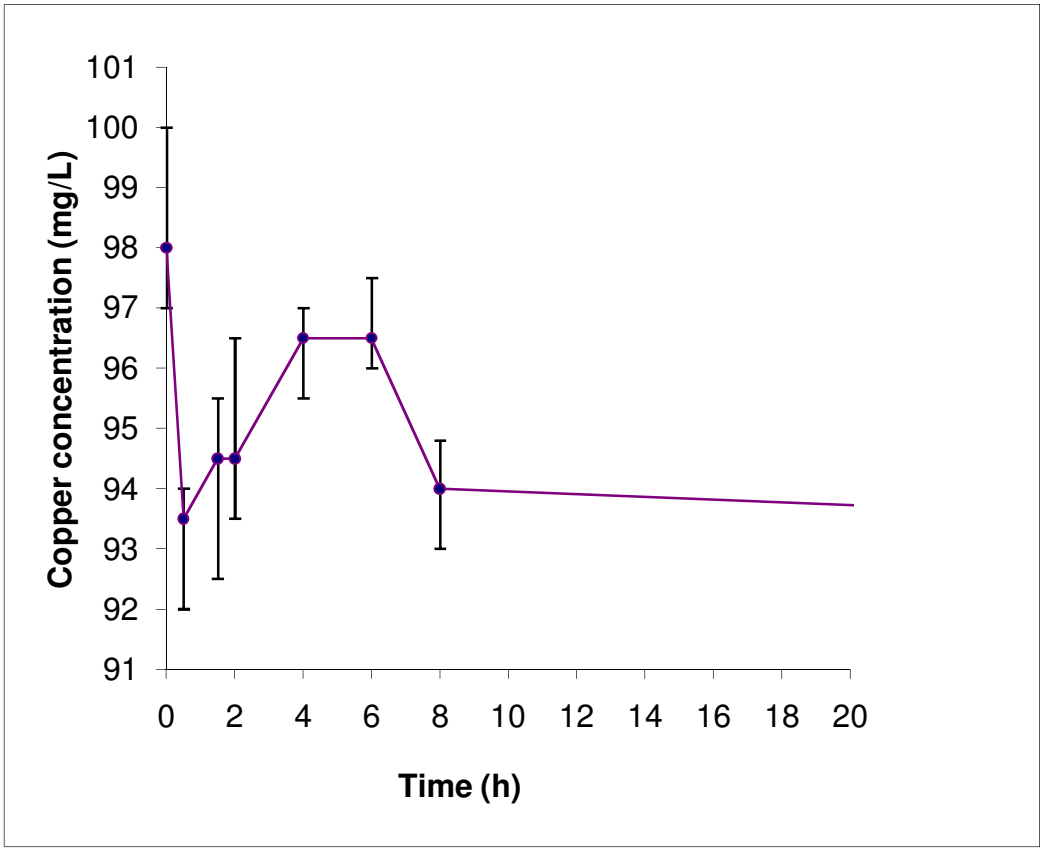

Figure 4.25: Copper concentration during fermentation of the metal mixture

More fermentation experiments were performed using a mixture of copper and chromium(III,VI) but no removal was found. As there was chromium (VI) removal in samples containing CCA wood hydrolysate which did not contain copper, it seems there is a competition between these metals to be removed by yeast. More experiments using different concentrations of chromium and copper are essential as future work.

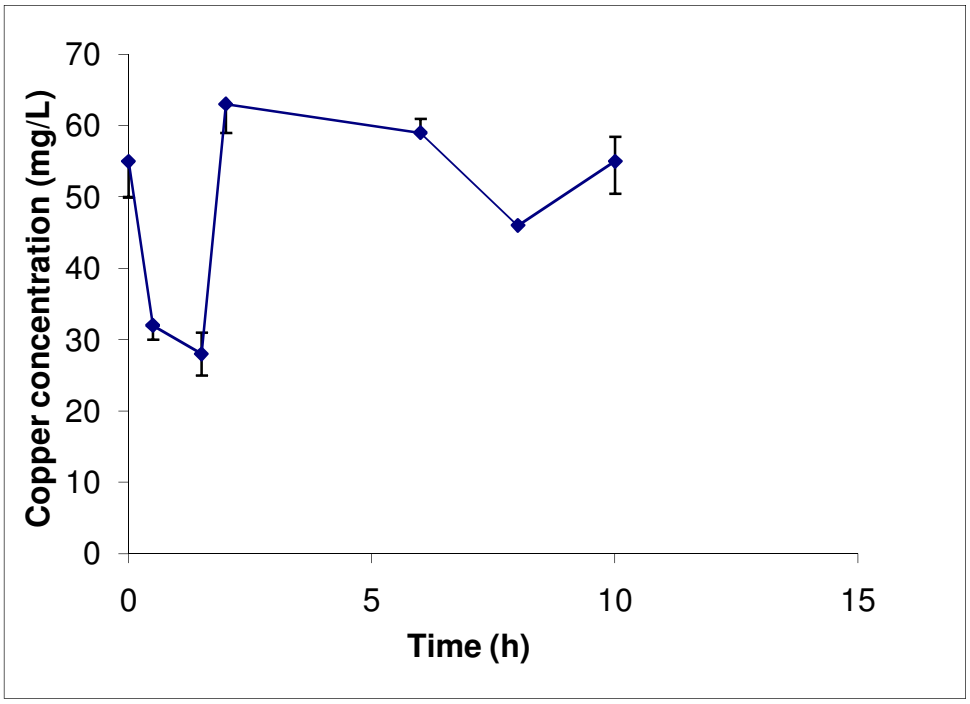

Figure 4.26: Copper concentration during fermentation of 2% sugar solution containing only copper

The behavior of yeast for removal of arsenic is completely different (Figures 4.21 and 4.24). There was no arsenic removal during the first 2 hours. All removal occurs when the yeast are active, then it seems that yeast do not adsorb arsenic but accumulate it inside. This is bioaccumulation and not biosorption. Thripathi et al.(2007) also suggests that the yeast acts as a pump to take As(V) inside the cell and to reduce to As(III) as a part of its detoxification mechanism.

The results obtained for chromium (Figure 4.21 and 4.27) were similar to the results obtained for arsenic removal, which was mostly during the 2 to 6 hours of the fermentation or during the yeast growing period (growth or log phase) (Figures 4.21 and 4.24). This was in spite of the copper (Figure 4.26) adsorbed before starting the log phase. On the other hand, there was a competition for uptake by yeast for copper and

chromium, as there is almost no chromium uptake when copper exist in solution. Then it can be another process in addition to surface adsorption of chromium, such as reduction which needs electron exchange during cell activity.

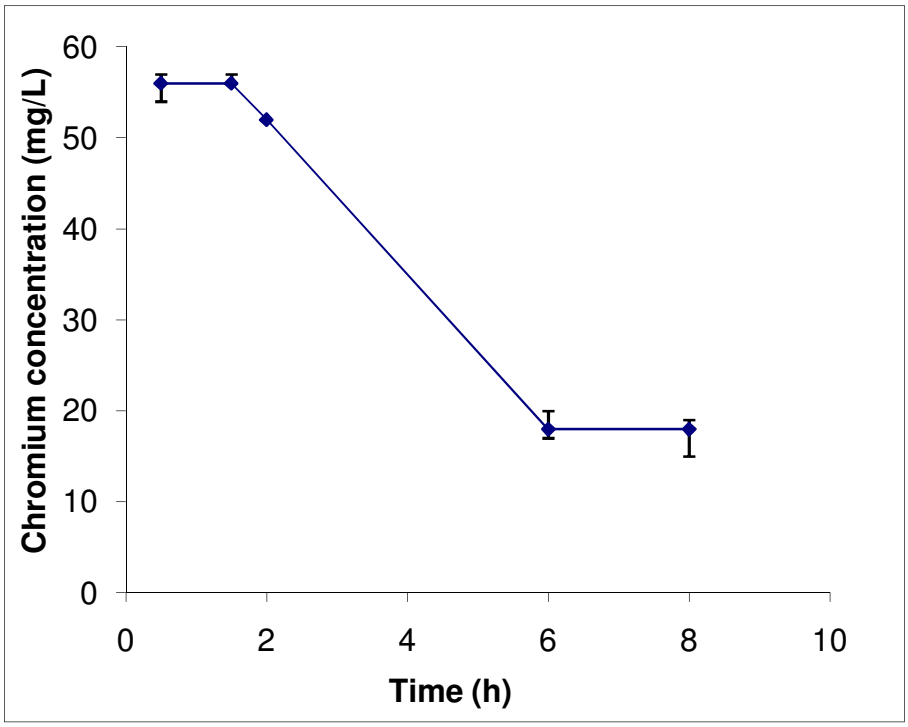

Figure 4.27: Chromium (VI) concentration during fermentation of 2% sugar solution containing only chromium

Figures 4.28 and 4.29 are the pictures of yeast cells during the fermentation of glucose solution without and with metals respectively. The photos were taken using a microscope and a digital camera with a total magnification of 2000 times (yeast cell diameter is about 5-10 micrometer). Some black dots can be seen inside the yeast which was fermenting the solution containing heavy metals. The black dots could be metals which are up taken by yeast and stored inside the cell, but no similar photos from other references were found to confirm the idea.

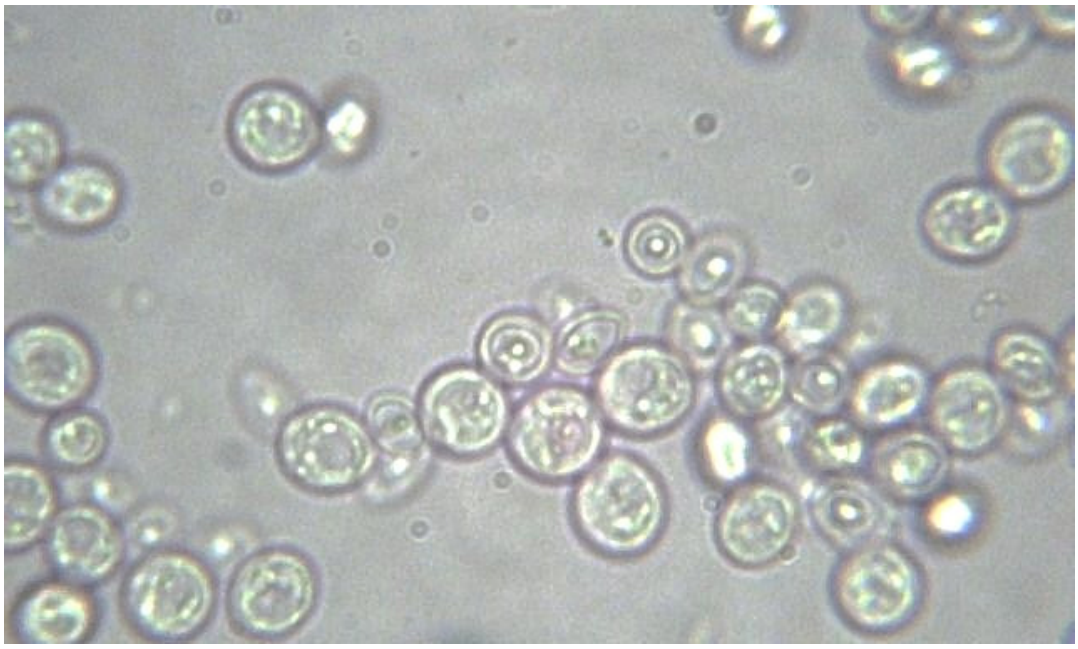

Figure 4.28: Microscopic picture of yeast in glucose solution (contains no metal)

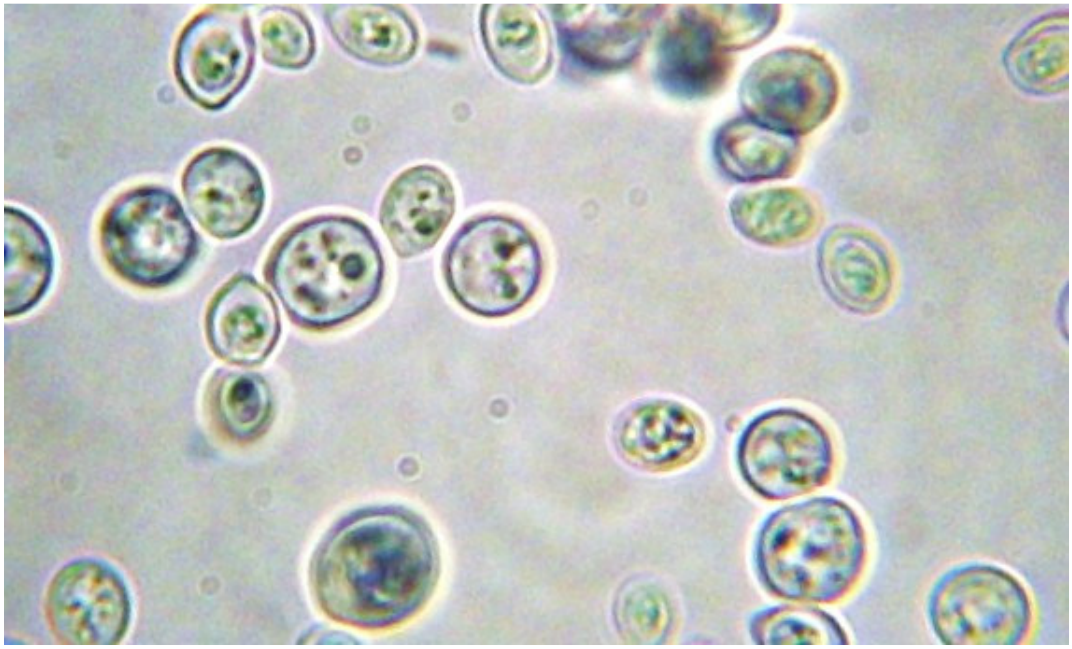

Figure 4.29: Microscopic picture of yeast in glucose solution (contains metal)

4.3 HYDROLYSED WOOD METAL REMOVAL

Figure 4.30 is the photo of hydrolyzed and non- hydrolyzed treated wood and Figure 4.31 shows their metal contents.

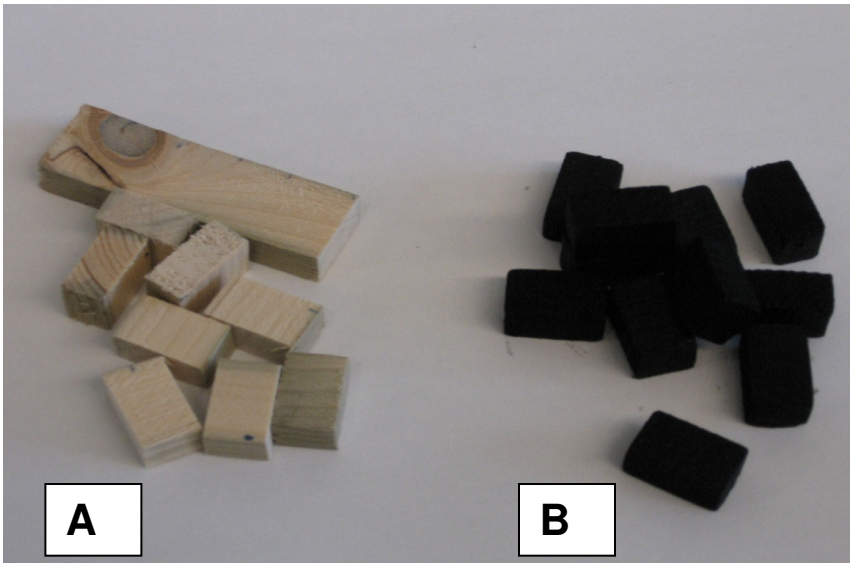

Figure 4.30: CCA treated wood particle samples
A) before hydrolysis B)after hydrolysis

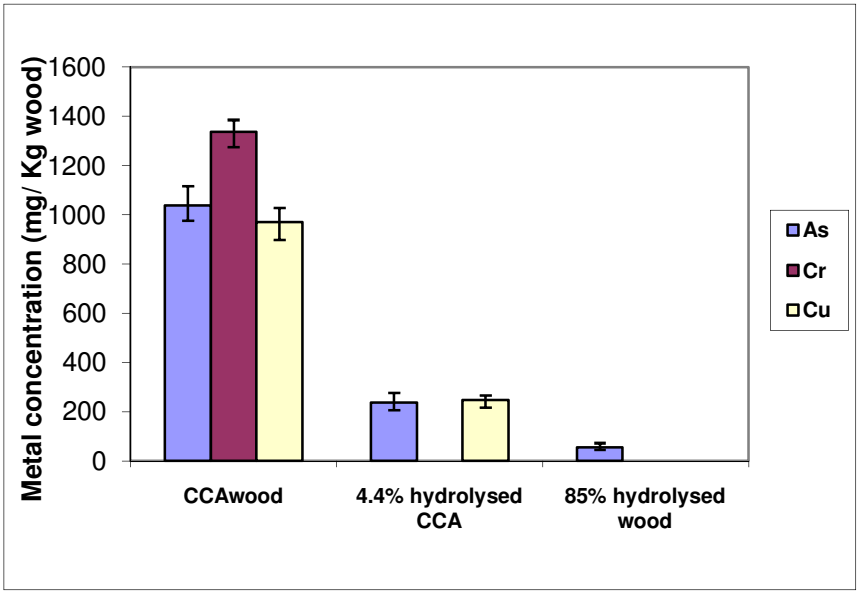

Figure 4.31: Metal contents of treated and hydrolyzed treated wood

During the 1-hour hydrolysis of treated wood by sulfuric acid (4.4%), almost all the chromium and 80% of copper and arsenic were removed from wood. The rest of the copper and more than 75% of the remaining arsenic were removed in the second step of hydrolysis by sulfuric acid (85%) after 10 minutes.

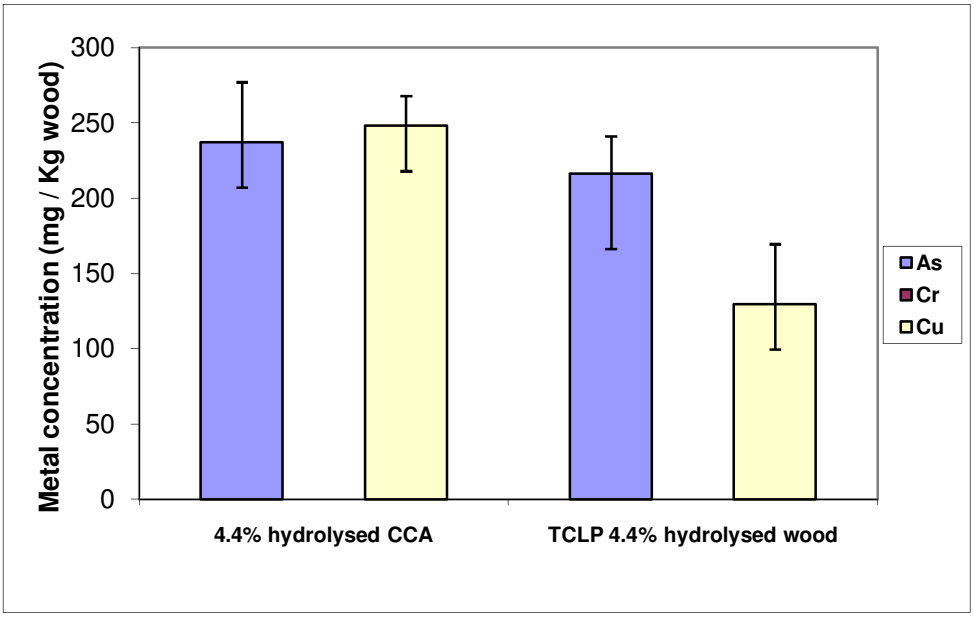

Figure 4.32: Metal contents of 4.4% hydrolyzed CCA wood leached by TCLP

The strong ability of sulfuric acid to leach almost all the chromium from the CCA wood (Figures 4.31,4.32) was also observed during the leaching tests using different types of acids by Moghaddam and Mulligan (2008).

To determine if the hydrolyzed wood can be landfilled, TCLP tests for both 4.4% and 85% hydrolyzed wood were performed (Figures 4.32 and 4.33). During the TCLP test, the arsenic leached from 4.4% hydrolyzed wood, was about 4% and for 85% hydrolyzed wood, it was less than 4 ppm. This is acceptable as the maximum arsenic of 5 ppm in

drinking water is allowed. Then two-step hydrolysis of CCA wood is an effective pretreatment method prior to landfilling and the CCA treated wood is not hazardous.

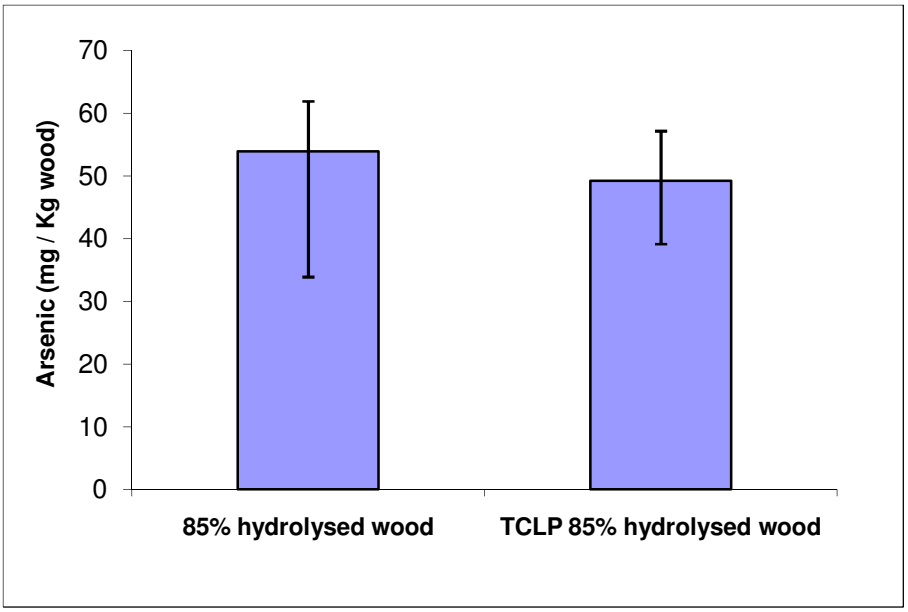

Figure 4.33: Metal contents of 85% hydrolyzed CCA wood leached by TCLP

As most disposed wood particles are weathered and have been in service for several years (usually they were installed outdoors), some experiments were performed to compare the leaching of weathered CCA wood and new CCA wood. Because the type and the retention time of wood during preservation, the time of service and the surrounding condition of wood affects the weathered wood, the CCA wood was synthetically weathered using the SPLP procedure. The metal contents of weathered CCA wood are much lower than new wood especially for chromium (Figure 4.34).

During hydrolysis of weathered wood, the total chromium was removed (Figure 4.35) as for new wood but it seems weathering makes arsenic and copper, more fixed in the wood

and more resistant to leaching in comparison with unweathered CCA wood even during hydrolysis by sulfuric acid (Figures 4.35 and 4.31), which means hydrolyzed weathered wood can be landfilled.

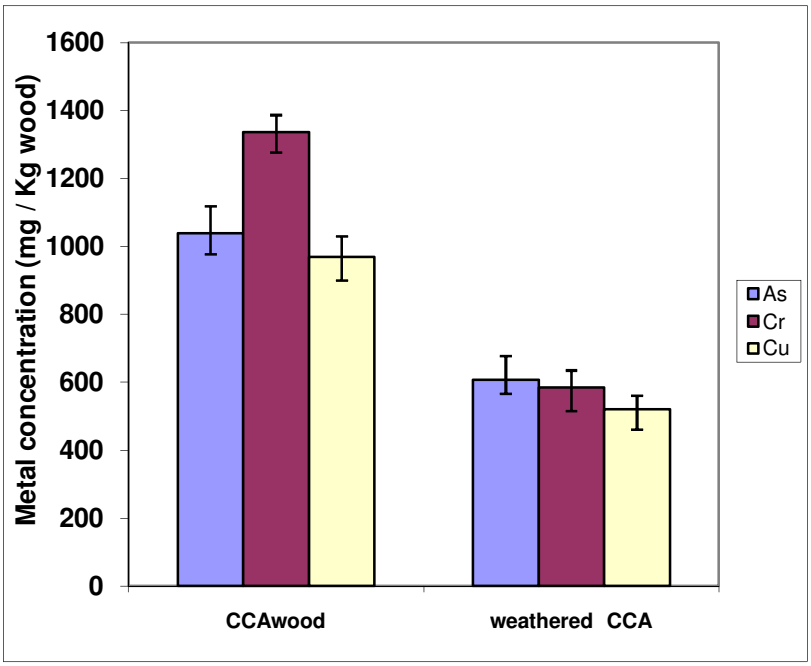

Figure 4.34: Metal contents of weathered CCA wood

Figure 4.36 gives a comparison between the leachability of weathered, unweathered and ground (passes through sieve no.10 and not passing through sieve no.60) CCA wood during the hydrolysis. The smaller size of the wood leads to more metal removal during hydrolysis.

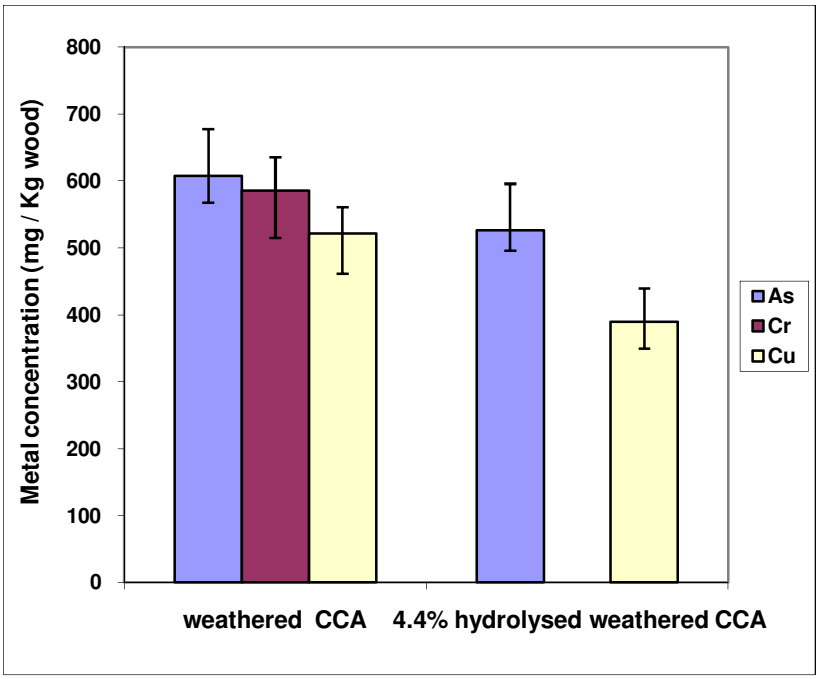

Figure 4.35: Metal contents of weathered and hydrolyzed weathered CCA wood

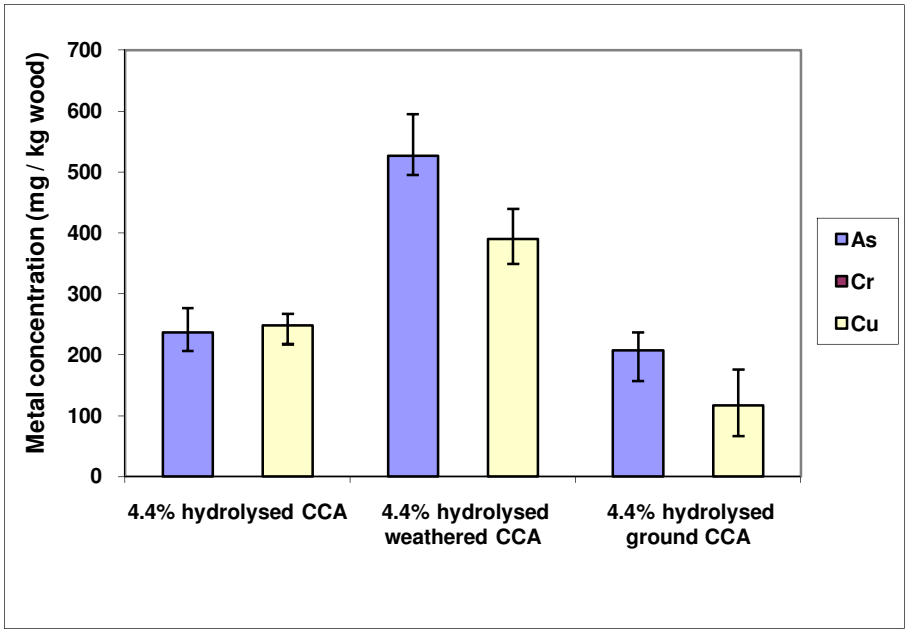

Figure 4.36: Metal contents of hydrolyzed weathered and ground CCA wood

4.4 BY PRODUCTS

During ethanol production some by-products are generated. According to Taherzadeh et al. (1997), the most important by-products are carboxylic acids, furans and phenolic compounds. These compounds could act as inhibitors for continuation of yeast activity. Among several byproducts, acetic acid has taken some attentions. Almost 0.9 g/L acetic acid produced during fermentation of CCA treated wood. According to Taherzadeh et al. (1997), acetic acid can be a friend or a foe in the process of conversion glucose to ethanol and "the permissible region of growth of *Saccharomyces cerevisiae* on glucose was determined as a function of both pH and the concentration". They believed the addition of acetic acid would affect growth energetics and then ethanol yield.

4.5 ETHANOL PRODUCTION

The maximum yield of ethanol was almost 0.2 g of ethanol per g of wood in these experiments. According to Taherzadeh (1999), the maximum theoretical yield of ethanol production is 0.32 g of ethanol per g wood based on an average of 42% cellulose and 21% hemicellulose in wood. The yield could be higher if a better and more economical hydrolysis was applied. Hydrolysis under high temperature and pressure for a very short time will give a higher sugar production and less harmful by-products such as furfural and 5-hydroxymetyl furfural (HMF), which are produced from sugar decomposition. Also ethanol itself is toxic for the microorganisms, and then removing the produced

ethanol from the fermentor will help to increase the yield of ethanol production. Mulligan (1994) mentioned there had been research directed towards enhancing the conditions for the ethanol tolerance of the yeast.

4.6 MECHANISM AND FATE OF METALS

The results show that there are different pathways for copper, chromium and arsenic in the whole process. Copper was removed before adding yeast, as no copper was detected by HPLC in the liquid. During acid and then later sodium hydroxide addition, copper precipitated and was removed from the liquid phase. In the next step chromium (VI) and arsenic (V) were taken up by the yeast when the yeast starts to produce ethanol. Scientists such as Rosen (2002) believe that yeast uptake and accumulate arsenate and convert it to arsenite as a method of detoxification. Our results also are consistent with this mechanism for arsenic because there is no removal during the first 2 hours when the yeast is acclimating. If there was adsorption on to the cell wall similar to the mechanism suggested by Wilhelmi and Duncan (1995) for copper then the removal and concentration changes would have occurred very fast and at the first moments of yeast addition.

Copper competes with chromium to be sorbed by yeast and also the chromium uptake model (Chapter 5) is similar to the copper model, but despite the presence of copper and arsenic, chromium is uptaken during the log growth period and when cell is active, then it may be a surface sorption (same as copper) followed by a reduction process (same as

arsenic) for chromium. The suggested mechanisms for chromium, copper and arsenic are summarized in Table 4.1.

In brief almost all of the chromium and copper were removed from CCA wood and less than 4 ppm of arsenic would leach out of 50 ppm arsenic remained in hydrolyzed wood during the TCLP test which makes landfilling of the residue acceptable and safe as the maximum acceptable arsenic leaching for TCLP is 5 ppm.

All the removed copper by sulfuric acid is precipitated during the hydrolysis and neutralization and before the fermentation step. However if copper wasn't precipitated it would be adsorbed by yeast.

Table 4.1: Proposed metal removal mechanisms during neutralization and fermentation of CCA treated wood hydrolysate

Metal	Removal mechanism
Copper	Precipitation
Chromium	Sorption on the cell wall + Reduction
Arsenic	Diffusion inside the cell + Reduction

About 50% of chromium VI (the more soluble form) and 60% of arsenic were removed by yeast from the hydrolysate. The change of speciation (inside the yeast) from more soluble forms to less soluble forms is one of the good aspects of the process. By adding

some fresh yeast (like what was done as second fermentation), the removal yield by yeast can be developed. As ethanol is one of the toxic materials for yeast, removing ethanol during fermentation and making it a semi-batch process could also improve the removal process as the yeast would live for a longer time.

4.7 DISPOSAL

The metals remaining in the effluent from the fermentation reactor can be treated by "Osmose water purification system to remove CCA contaminants from water" manufactured by Zenon Environmental Systems, which is suggested by EPA for CCA treatment industry. The typical system would be composed of three components: 1-prefilter 2-primary method of membrane separation 3- ion exchanger polishing unit (EPA, 1992). In general a prefilter and an ion exchanger can be used to remove chromium and arsenic from the fermentation reactor effluent. According to Wilhelmi and Duncan (1995), the accumulated metals (including chromium and copper) can be recovered from the yeast by up to 100%. Figure 4.37 shows an overall scheme of the proposed CCA wood waste management process.

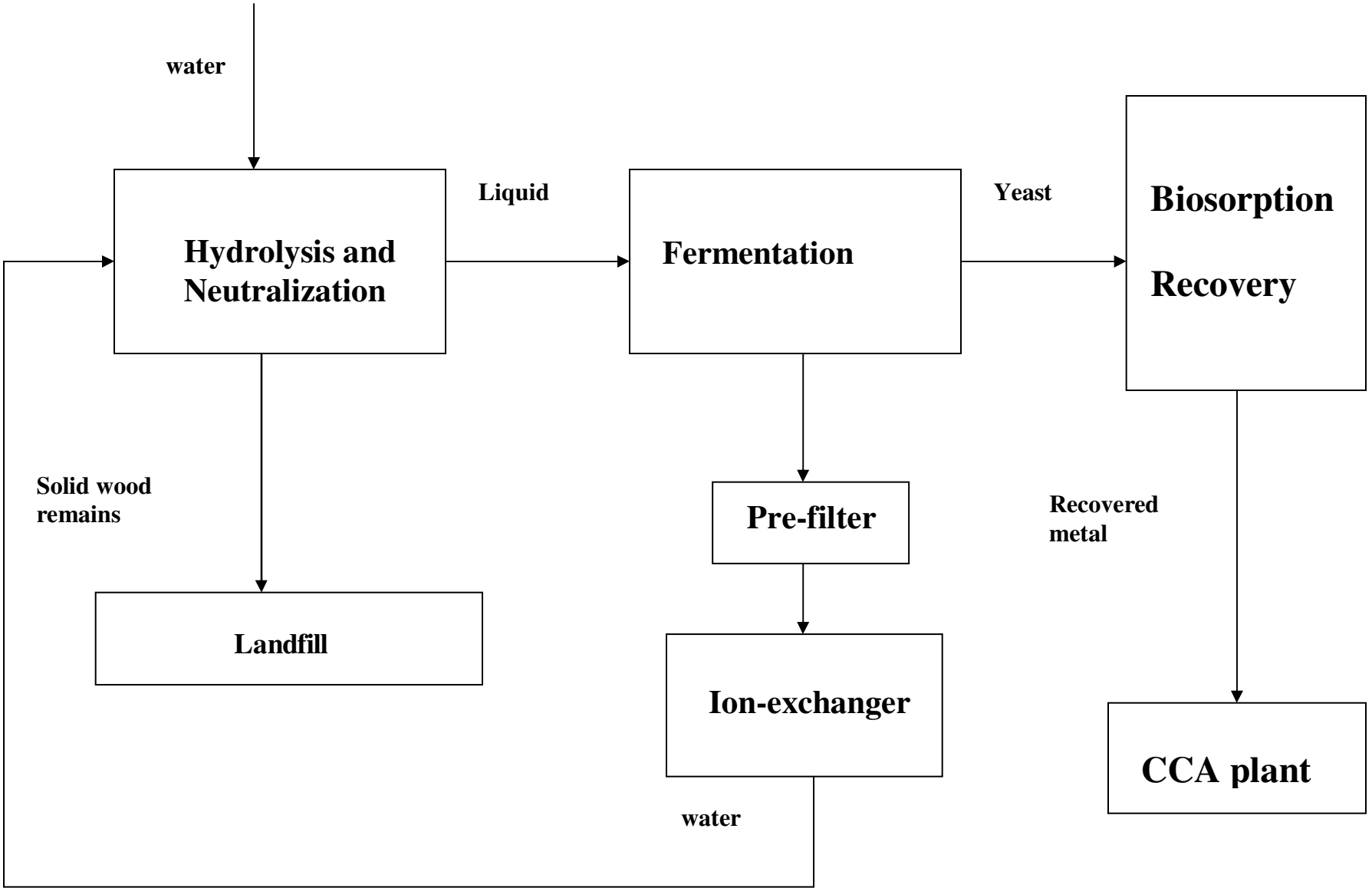

Figure 4.37 Overall proposed scheme of the CCA wood waste management process.

CHAPTER FIVE

MODELING AND KINETICS

In this chapter, the kinetics of uptake of arsenic, chromium and copper and also the growth of microorganism (baker's yeast) are discussed to simulate the removal of metals by yeast.

Table 5.1: Reaction kinetic models

Reaction	Kinetic model	Order
A ⟶ P	$\frac{\partial C_A}{\partial t} = -k$	Zero
	$\frac{\partial C_A}{\partial t} = -kC_A$	First
A + B ⟶ P	$\frac{\partial C_A}{\partial t} = -kC_AC_B$	Second

Three different kinetic models (zero, first and second order reaction) are usually applied to the experimental data (Table 5.1). A and B represent reactants while C_A and C_B are the concentrations of A and B at time t of reaction, P represents the product and k is the reaction rate.

5.1 ARSENIC UPTAKE

The kinetic model that provided the best fit for As(V) was the first-order depletion kinetics, which is described as:

$$\frac{\partial C}{\partial t} = -kC$$

$$\int_{C_0}^{C} \frac{\partial C}{C} = -k\int_{t_0}^{t} \partial t$$

$\ln C - \ln C_0 = -kt + \text{const.}$

$$\ln \frac{C}{C_0} = -kt + const$$

Where, C is the concentration at time t, C*o* is the initial concentration, k is the kinetics rate constant and t is time. The data fit to the first-order depletion model is presented in Figure 5.1. The calculated process kinetic rate and regression are 0.0843 h^{-1} and 0.85, respectively. The regression of R^2= 0.04 for a zero-order model makes the first order model the best model for arsenic (V) removal by yeast.

The experimental data used for this modeling were the results of fermentation of CCA treated wood hydrolysates and fermentation of glucose solution containing a mixture of

chromium, copper and arsenic compounds. Having a regression of R^2= 0.85 for a suggested model which fit on the experimental data despite different initial concentrations and different initial solutions, could make the model more general for uptaking arsenic(V) by baker's yeast. Also as the existence of chromium and copper doesn't change the model for arsenic, it seems that uptake of arsenic(V) by baker's yeast is a unique independent process, not affected by the existence of other metals (chromium and copper).

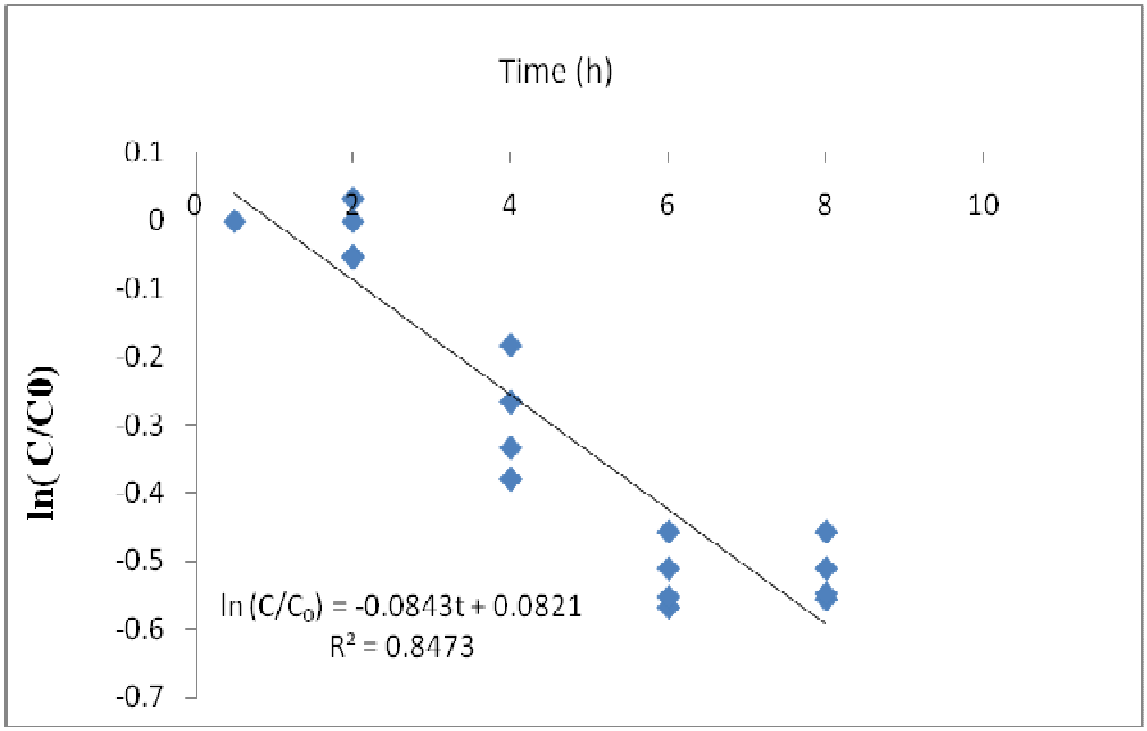

Figure 5.1: First-order kinetics model for arsenic(V)uptake by baker's yeast

5.2 CHROMIUM UPTAKE

The kinetic model that provided the best fit for Cr(VI) was the zero-order kinetics, which is described as:

$$\frac{\partial C}{\partial t} = -k_0$$

$$\int_{C_0}^{C} \partial C = -k_0 \int_{t_0}^{t} \partial t$$

$C - C_0 = -k_0 t + \text{const.}$

$C/C_0 = -kt + \text{const.}$

Where, C is the concentration at time t, C*o* is the initial concentrations, k is the kinetics rate constant and t is time. The data fit to the zero-order depletion model is presented in Figure 5.2. The calculated process kinetic rate and regression are 0.0811 h^{-1} and 0.73, respectively. The regression of R^2=0.0002 for a first order model eliminates it as a kinetic model for chromium uptake process.

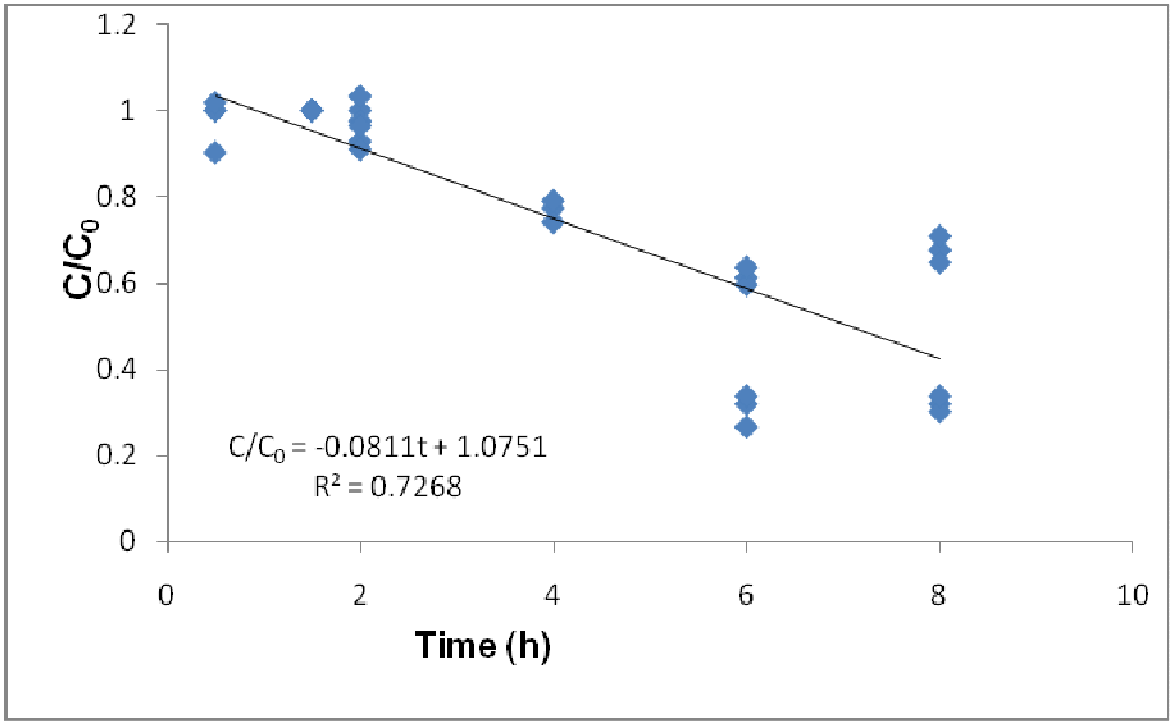

Figure 5.2: Zero-order kinetics model for chromium(VI) uptake by baker's yeast

The experimental data used for this modeling were the results of fermentation of CCA treated wood hydrolysates and fermentation of glucose solution containing chromium compound. The results of glucose solution containing a mixture of chromium, copper and arsenic compounds did not fit on zero and first order models. The difference of fitting and not fitting data on the model is the existence of copper in the mixture solution. It seems uptake of chromium and copper are not independent processes and the model should contain both processes at the same time and probably it is a second order kinetic model. Because of neutralization of wood hydrolysate and precipitation of copper before commencing fermentation process, there is no copper in liquid phase and the zero-order kinetic model with regression of R^2= 0.73 can be suggested for chromium removal process by baker's yeast during fermentation of wood hydrolysate.

5.3 COPPER UPTAKE

Some experiments of fermentation of solutions containing mixture of chromium and copper and fermentation of copper solution were performed separately. The results of mixture of chromium and copper did not fit to any zero and first order kinetic models (as was explained before) but a zero order model was fit on the solution containing only copper.

The data fit to the first-order depletion model is presented in Figure 5.3. The calculated process kinetic rate and regression are 0.159 h^{-1} and 0.7, respectively. The model for copper is similar to chromium uptake but the difference is the start and end time of removal. Removal of copper starts when yeast is added to the prepared solution and ends

earlier than 2 hours but for chromium it starts 2 hours after adding the yeast and ends in 6 to 8 hours. It means copper is absorbed by yeast when it is not active but chromium is absorbed when yeast starts to grow and is active similar to arsenic.

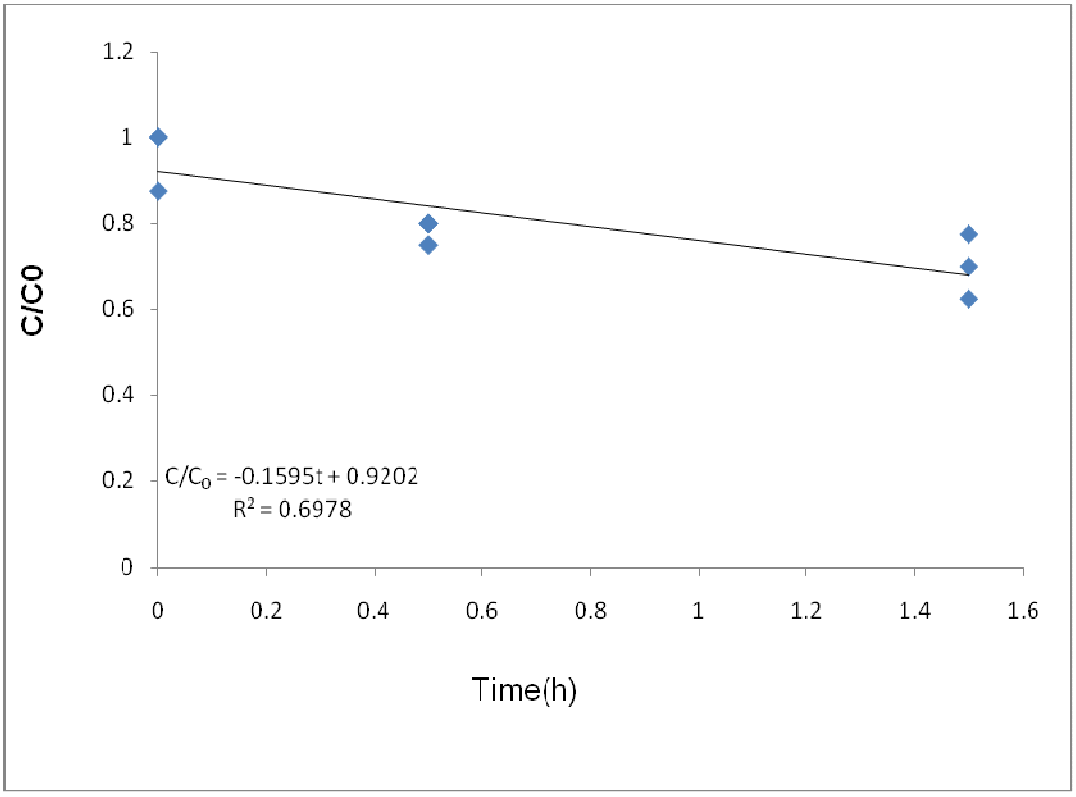

Figure 5.3: Zero-order kinetics model for copper uptake by baker's yeast

5.4 YEAST GROWTH

The growth of microorganisms (including yeasts) is presented by 4 phases (Figure 5.4). There is usually a lag phase, then the exponential growth commences. As essential nutrients are depleted or toxic products build up, growth ceases, and the population enters the stationary phase. If incubation continues, cells may begin to die (the death phase).

Microbial populations show a characteristic type of growth pattern called exponential growth, which is best seen by plotting the number or concentration of cells over time on a semi-logarithmic graph (numbers of cells is plotted on a logarithmic scale and time is plotted arithmetically).

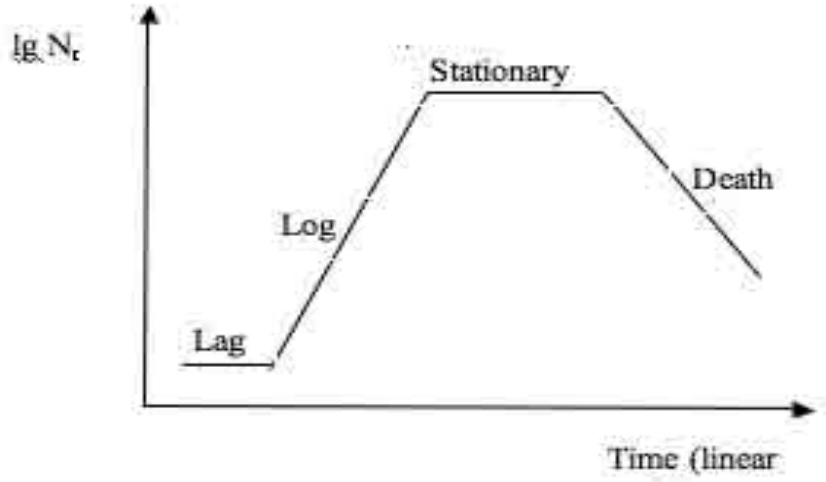

Figure 5.4:Typical microorganism growth curve

Figures 5.5, 5.6 and 5.7 present the growth curves of yeast which were used for fermentation of CCA treated and untreated wood hydrolysates.

The results presented by Figures 5.5-5.7 show a first order kinetic model or an exponential growth of yeast for both treated and untreated wood hydrolysates, using 2g or 4g initial values of yeast. For growth of yeast in different conditions, the rates of growth (k) are different.

$$\ln \frac{C}{C_0} = kt + a$$

$$C = C_0 e^{kt} + b$$

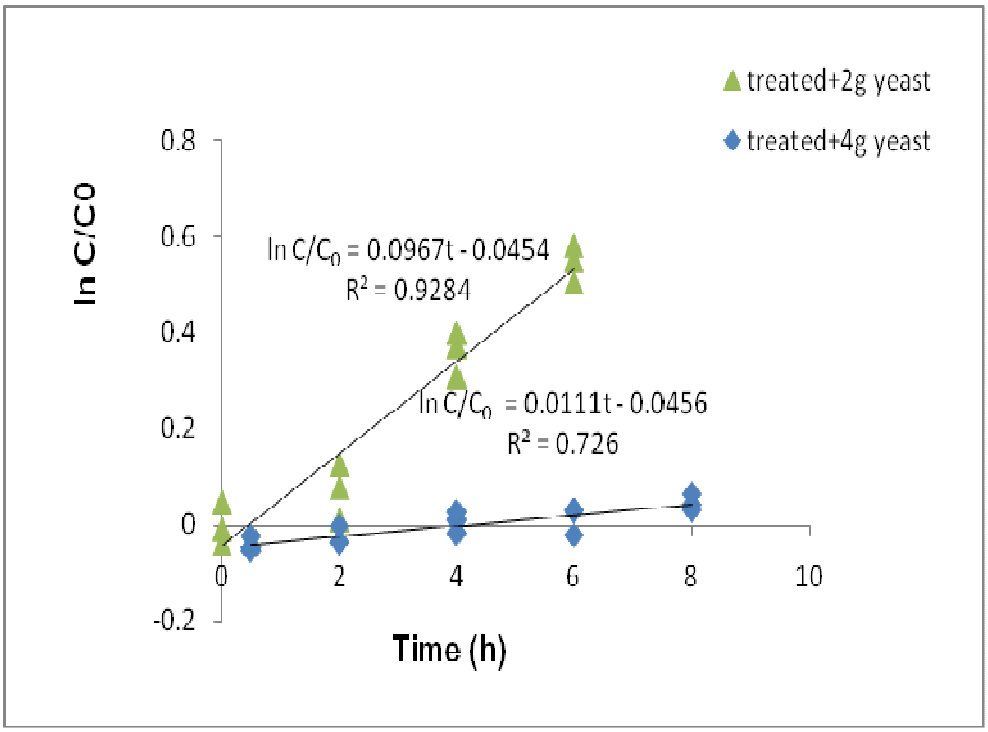

Figure 5.5: Log growth density versus time for treated wood hydrolysate

Figure 5.5 shows the logarithmic growth curves for fermentation of treated wood hydrolysate by two different inocula of 2g and 4g yeast per 100 ml of hydrolysate. It shows a much higher growth rate when using less yeast (k_{2g}=0.097>>k_{4g}=0.011) and also a much greater regression factor of 0.93 which means a better fit to the model.

Figure 5.6 shows that a lack of glucose is not the limiting factor for yeast growth as the growth rates are the same with almost the same regression factors.

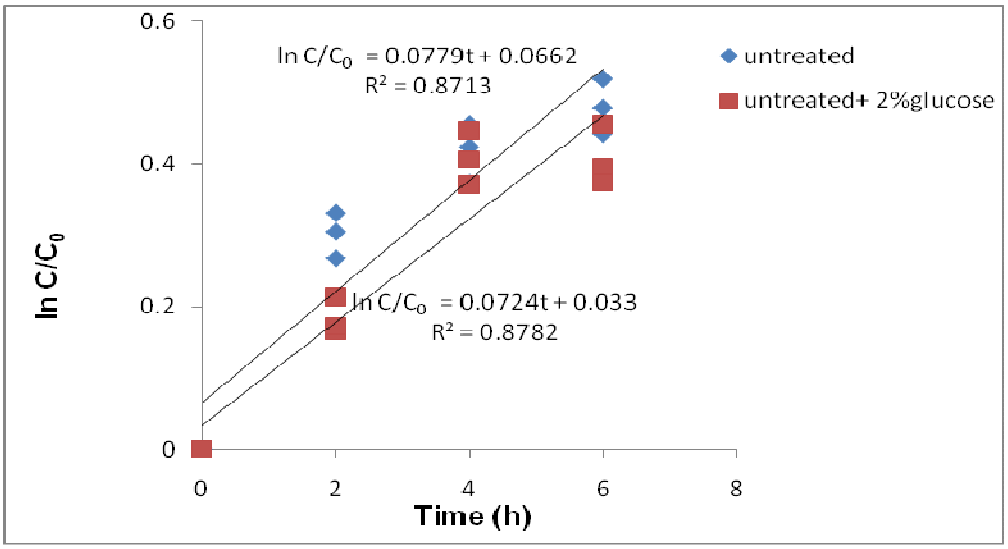

Figure 5.6: Logarithm of growth density versus time for untreated wood hydrolysate

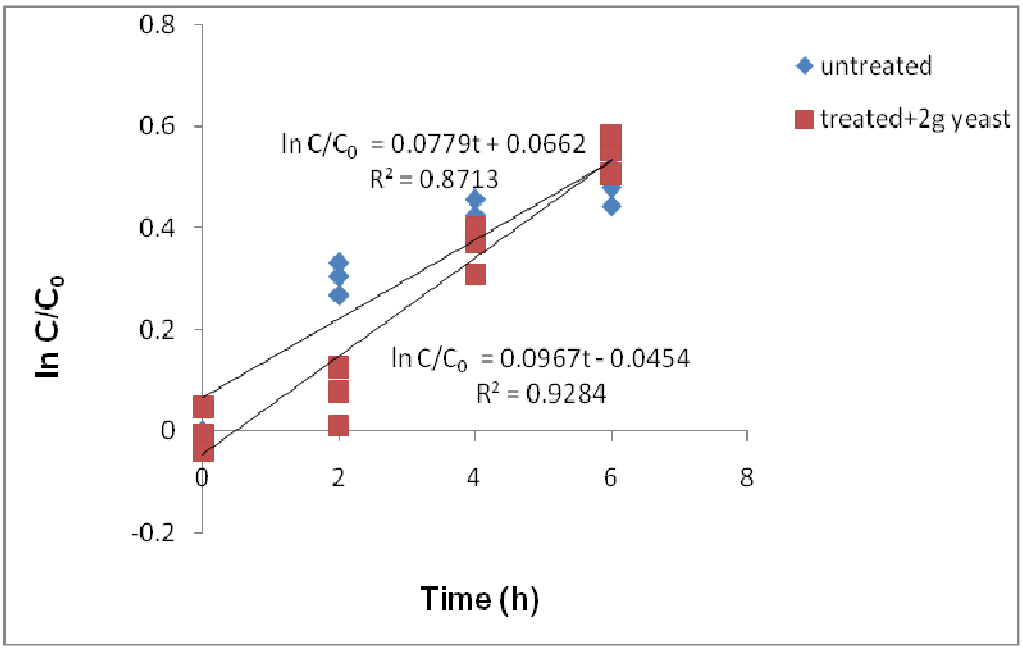

Figure 5.7: Log growth density versus time for untreated and treated wood hydrolysates

It seems that the growth of yeast in treated wood hydrolysate is much faster and the result fits on kinetic model a little better than untreated wood.

5.5 CONCLUDING REMARKS

The suggested models and regression factors for arsenic, chromium and copper uptake by yeast are summarized on table 5.2. Table 5.3 contains the yeast growth rates for fermentation of untreated and treated wood hydrolysates using different amounts of yeast.

Table 5.2: Proposed kinetic models for metal uptake by Bakers' yeast

Uptaken metal	Model	Order	R^2
Arsenic	$\ln C/C_0 = -0.0843\ t + 0.0821$	First	0.85
Chromium (VI)	$C/C_0 = -0.0811\ t + 1.0751$	Zero	0.73
Copper	$C/C_0 = -0.1595\ t + 0.9202$	Zero	0.7

Table 5.3: Yeast growth rates for different conditions

Feed	**Yeast per 100 cm^3 hydrolysate**	**Growth rate**	**R^2**
Untreated wood + 2% glucose	2g	0.074 h^{-1}	0.88
Untreated wood	2g	0.0779 h^{-1}	0.87
Treated wood	2g	0.096 h^{-1}	0.93
Treated wood	4g	0.011 h^{-1}	0.73

CHAPTER SIX

ETHANOL ECONOMICS

Global warming and the role of greenhouse gases, the oil crises and fossil sources depletion make countries to find other replacements for energy sources. Ethanol from biomass as one of the new sources of energy has attracted significant attention especially ethanol from cellulosic sources. Ethanol production economics have been evaluated around the world. This chapter will compare some cost evaluations using different references and also estimates the cost of the proposed system .

6.1 Different economical evaluations

In an assessment of alcohol process technologies, Mulligan (1994) reported that Arkenol which produced ethanol from cellulosic feedstocks (specifically wood) and used concentrated sulfuric acid hydrolysis, could generate 0.43 kWh electricity per liter of ethanol. The steam (150 psi) was required to give a total energy input to output ratio of 1.2-2.0. She reported a cost of $US 0.4-0.85 / US gal ($Cdn 0.14-0.3 / L) depending on feedstock cost and capital requirements of $US 2.25-3.50 / US gal ($Cdn 0.8-1.24 / L). However the report was prepared 15 years ago and the system was not proven at large

scale at that time, but as the feedstock was wood and the hydrolysis method was the same as the hydrolysis used in this research, it was included in this chapter.

Table 6.1: Inputs per 1000 l of 99.5% Ethanol Produced from U.S. wood cellulose Pimentel and Patzek (2005)

	Quantities	KJ x 1000	Costs ($)
Wood	2500 kg	1674	250
Machinery	5 kg	418	10
Replace nitrogen	50 kg	3347	28
Transport wood	2500 kg	1255	15
Water	125000 kg	293	20
Stainless steel	3kg	188	11
Steel	4kg	192	11
Cement	8kg	63	11
Grind wood	2500 kg	418	8
Sulfuric acid	118 kg	0	83
Steam production	8.1 kg	18426	36
Electricity	666 kWh	7125	46
Ethanol conversion to 99.5%			
	9 kcal/ L	38	40
Sewage effluent	20 kg (BOD)	289	6
Total		33727	575

1000 l of ethanol = 5.13 million kcal

Table 6.2: Parameters for the economic evaluation (Hamelinck et al., 2005)

Interest rate		10%
Economical lifetime		15 years
Technical lifetime		25 years
Investment path		20% in first year
		30% in second year
		50% in last year
Operational costs		
Fixed variable		
	Maintenance	3% of TCI
	Labour	0.5% of TCI at 400 MW
	Insurance	0.1 % TCI
Consumed materials		
	Dilute acid	22 €/ tone biomass
	Lime	0.87 €/ tone biomass
	Cellulase	0.13 €/ l ethanol
Biomass		2-3 $€_{2002}/GJ_{HHV}$
Electricity		0.03 €/kWh
Annual load		8000 h (91% of time)

TCI: Total capital investment

According to Pimentel and Patzek (2005), the ethanol cost per liter for wood produced ethanol is about 0.58$ (Table 6.1). Some European researchers (Hamelinck et al., 2005) estimated an investment cost of 2.1 k€ / kW_{HHV}[1] (at 400 MW_{HHV} input, i.e a nominal 2000 $tonne_{dry}$/day input) which could have increased to 900 k€/ kW_{HHV} for a 5 times larger plant ($2GW_{HHV}$). They said "A combined effect of higher hydrolysis-fermentation efficiency, lower specific capital investments, increase of the scale and cheaper biomass feedstock costs (from 3 to 2 €/GJ_{HHV}) could bring the ethanol production costs from 22 €/GJ_{HHV} in the next 5 years, to 13 €/GJ over the 10-15 year time scale, and down to 8.7 €/GJ in 20 or more years".

Hamelinck et al. (2005) calculated ethanol production costs by dividing the total annual costs of each system by the produced amount of ethanol. The total annual costs consist of annual capital requirements, operating and maintenance, feedstock and electricity which are given by Table 6.2. The total cost investment of each system component was calculated by multiplying the scaled base cost by an installation factor (Table 6.3).

According to Hamelink et al.(2005): Ethanol from sugar cane in Brazil cost 10-12 €/GJ (Moreira, (2000) and Larson et al.(2001)) and projected cellulosic ethanol production in Europe would cost 34-45 €/GJ (Reith et al., (2002), de Boer and den Uil (1997)). Future costs were projected 4.5-10 €/GJ by Lynd (1996), 6-8 €/GJ by de Boer and den Uil

---

[1] Higher Heating Values for a fuel include the full energy content as defined by bringing all products of combustion to 77°F (25° C).

Table 6.3:Costs of system components in M€$_{2003}$(1€$_{2003}$= 1 U.S$_{2003}$)

Component	Base cost	Scale factor	Base scale	Installation factor	Maximum size*
Pretreatment					
Mechanical	4.44	0.67	83.3 tonne $_{dry}$/h	2	83.3
Mill	0.37	0.7	50 tonne wet /h	1	
Acid	14.1	0.78	83.3 tonne $_{dry}$/h	2.36	
Steam explosion	1.41	0.78	83.3 tonne $_{dry}$/h	2.36	
Ion exchange	2.39	0.33	83.3 tonne $_{dry}$ /h	1.88	
Overliming	0.77	0.46	83.3 tonne $_{dry}$ /h	2.04	
Hydrolysis+fermentation					
Cellulase production (SSF)	1.28	0.8	50 kg/h	2.03	50
Seed fermentors (SSF+SSCF)	0.26	0.6	3.53 tonne/h ethanol	2.2	3.53
Hydrolyse-fermentation (SSF)	0.67	0.8	1.04 tonne/h ethanol	1.88	1.04
Distillation and purification	2.96	0.7	18466 kg/h ethanol	2.75	18466
Molecular sieve	2.92	0.7	18466 kg/h ethanol		18466
Residuals					
Solid separation	1.05	0.65	10.1 tonne dry/h	2.2	10.1
Aerobic digestion	1.54	0.6	43 tonne/h waste water	1.95	43
Drier	7.98	0.8	33.5tonne $_{wet}$/h	1.86	110
Power					
Boiler	27.1	0.73	173 MW steam raised	2.2	
Gasifier	40	0.7	68.8 tonne $_{dry}$ /h	1.69	75
Gas turbine	16.9	0.7	26.3 MW	1.86	
Steam system +turbine	5.36	0.7	10.3 MW	1.86	

* all units are same as relevant base scale
(Hamelinck et al., 2005)

(1997) and 10 to 11 €/GJ (within 10 years) by Wooley et al. (1999), approaching the costs of methanol production via biomass gasification and fossil bulk fuels according to Lynd (1996), Novem (1999), Hamerlink and Faiij (2001).

6.2 Cost estimation

Our suggested ethanol production system is similar to the system evaluated in Table 6.1, which, contains acid hydrolysis of wood. The inflation ratios for the years 2005, 2006, 2007, 2008, 2009 are 3.39%, 3.24%, 2.85%, 3.85% and -0.4% respectively (http://www.inflationdata.com/inflation/inflation_rate/historicalinflation.aspx). Based on Table 6.1 and the inflation ratio, the cost of production of 1000 L ethanol in the year 2010 is estimated about US$653 (C.1, Appendix C).

Because of the existence of chromium, copper and arsenic in the effluent from the fermentation unit, additional treatment (compared to the ethanol production unit) should be performed. The same treatment unit suggested by the EPA for CCA treatment plant or in brief a prefilter, and ion exchanger could be used. Hamelinck et al. (2005) suggests prices for ion exchanger and solid separation units (Table 6.3) which can be used as basic estimations for calculation of our additional system prices.

Based on Table 6.3, the total investment cost for a 83.3 tonne ion exchanger , 2.5 tonne ion exchanger and an ion exchanger for a plant of production of 1000 litre ethanol per hour would be MU.S$$_{2003}$$4.49, MU.S$$_{2003}$$0.135 and U.S$$_{2003}$$ 135000 respectively

(C.2,C.3,Appendix C). The total investment cost for the ion exchanger for a plant of production of 1000 litre ethanol per hour in the year 2010 can be U.S$153,296 (C.4,Appendix C).

Also the total investment cost for 83.3 tonne solid separation and then for a 2.5 tonne solid separation would be U.S$$_{2003}$ 2.31 and U.S$$_{2003}$ 0.0693 respectively (C.5, C.6, Appendix C). Total investment cost for solid separation for a plant of production of 1000 litre ethanol per hour would be U.S$$_{2003}$ 69300 for the year 2003 and US$78,692 for the year 2010 (C.7 Appendix C).

The total investment costs for the main equipment which could be added to an ethanol production plant (ethanol from wood feedstock), to use CCA treated wood as their feedstock are summarized in Table 6.4.

Figure 6.4: Basic equipment cost estimation for year 2010 (1000 litre ethanol per hour)

Investment cost	US$
Ion exchanger	153,296
solid separation	78,693
Total	231,989

Nowadays with more ethanol production plants under construction, U.S$ 250,000 additional investment for an ethanol production plant to be able to produce ethanol from wasted CCA wood will be more profitable and a cheaper way to dispose of CCA treated

wastes than building pretreatment plants containing acid treatment units (similar to hydrolysis units) to remove hazardous metals from wood before landfilling. The profit of ethanol production could somehow compensate the transportation expenses. Also special equipment for burning CCA treated wood will not be cheaper and better as the remaining ashes contain high concentration levels of metals which leads to another disposal and environmental problem.

CHAPTER SEVEN
CONCLUSIONS AND CONTRIBUTION

7.1 CONCLUSIONS OF THE STUDY

Overall the process conclusions include:

- The existence of copper, chromium and arsenic did not have a negative effect on fermentation process and producing ethanol from CCA treated wood is feasible.

- Using the disposed CCA wood as a source of ethanol production will be even more sustainable as the source is a troublesome waste.

- The produced ethanol could be used as a fuel for transportation of wood to the factory.

- Additional investment for an ethanol production plant to produce ethanol from waste CCA wood will be a more profitable and cheaper way to dispose of CCA treated wastes than building pretreatment plants containing acid treatment units

(similar to hydrolysis units) to remove hazardous metals from wood before landfilling.

Conclusions concerning the solid wood phase:

- TCLP tests of the hydrolyzed wood leached less than 4 ppm of arsenic while minimal amounts of chromium and copper remained in the hydrolyzed wood which makes landfilling of hydrolyzed wood acceptable and safe.

- Weathering makes arsenic and copper, more fixed in the wood and more resistant to leaching in comparison with unweathered CCA wood even during hydrolysis by sulfuric acid. Hydrolyzed weathered wood can thus be landfilled.

- The smaller size (ground) wood leads to more metal removal during hydrolysis.

- Good leachability of sulfuric acid for removal of chromium, copper and arsenic from CCA treated wood makes it the main part of pretreatment method. As the hydrolysis of wood by sulfuric acid is the first step of ethanol production from wood, disposed wood could be the feed of ethanol production factory. During the two step hydrolysation most of the metals leach out of wood and as it was mentioned earlier the landfilling of residue would be acceptable according to environmental rules.

Conclusions regarding the liquid phase:

- Bakers' yeast can remove metals from solution, especially copper, arsenic and chromium and using more fresh yeast increases the removal percentages. About 50% chromium(VI) and 60% arsenic (VI) were removed by adding 4 g yeast in two steps. In separate tests, up to 50% of the copper was removed by yeast during the first 2 hours of yeast addition for initial value of 60 ppm, however in our wood hydrolysate, copper was removed by precipitation before yeast addition.

- Baker's yeast behaves selectively by uptaking arsenic (V) and chromium (VI) and not arsenic (III) and chromium (III). The selective behavior of yeast can be used as a method of speciation separation where it is needed.

- The change of speciation (inside the yeast) from more soluble forms to less soluble forms is one of the positive aspects of the process.

- Copper competes with chromium to be sorbed by yeast and the chromium uptake model is a zero order kinetic model similar to the copper kinetic model, while the kinetic model for arsenic removal by yeast is a first order model.

- It seems that during glucose solution fermentation, yeast can grow almost 30 % more than when they are in xylose solution. Also there is no ethanol production in the flasks containing xylose. These results can be consistent with previous

studies showing that baker's yeast cannot consume xylose which is a pentose but can consume hexoses like glucose. The low level of growth in xylose solution can be because of consumption of yeast extract as the only source of carbon. As there was no sterilization in all tests then there possibly was also bacterial growth in solutions.

Figure 7.1 shows a schematic of the proposed process and metal mass balance.

7.2 CONTRIBUTION

Although, producing ethanol from wood is not a new research and has been researched for a long time, using this method as a pretreatment for disposal of CCA wood is a new proposal and the main goal of this study. Producing ethanol at the same time of removing chromium, arsenic and copper is a sustainable method for disposal of hazardous waste wood to such an extent that makes landfilling of disposed wood acceptable and safe.

The mechanism of metal removal and fate of chromium, copper and arsenic in the whole process was investigated and kinetic models for uptaking arsenic, chromium and copper by bakers' yeast were suggested. The models helped to explain the difference between uptaking arsenic and uptaking copper or chromium.

A diffusion mechanism for removal of arsenic was determined and a different mechanism for copper and chromium removal was obtained (surface adsorption).

This was the first determination of the selective behavior of Baker's yeast for chromium.

It was shown that the yield of metal removal would increase by adding yeast in two continuous steps (6 hour interval).

It was determined that arsenic removal by yeast is an independent process and the existence of copper and chromium do not affect the capacity of yeast for uptaking arsenic while chromium and copper removal are dependent processes and each of them decreases the removal of the other one by yeast.

During this study an HPLC method including the column, eluent and wavelength for chromium speciation (Cr^{+6}/Cr^{+3}) was determined and used that has not been previously suggested. A Hamilton HPLC column model PRPX100 (150 x 4.6 mm) and UV detector was used to separate Cr^{+6} and $Cr^{+3}$and to detect at 192 nm wavelength. 3mM sulfuric acid (flow rate 2 ml/min) was the mobile phase. The retention times of Cr^{+6} and Cr^{+3} were 1 and 2.5 minutes respectively.

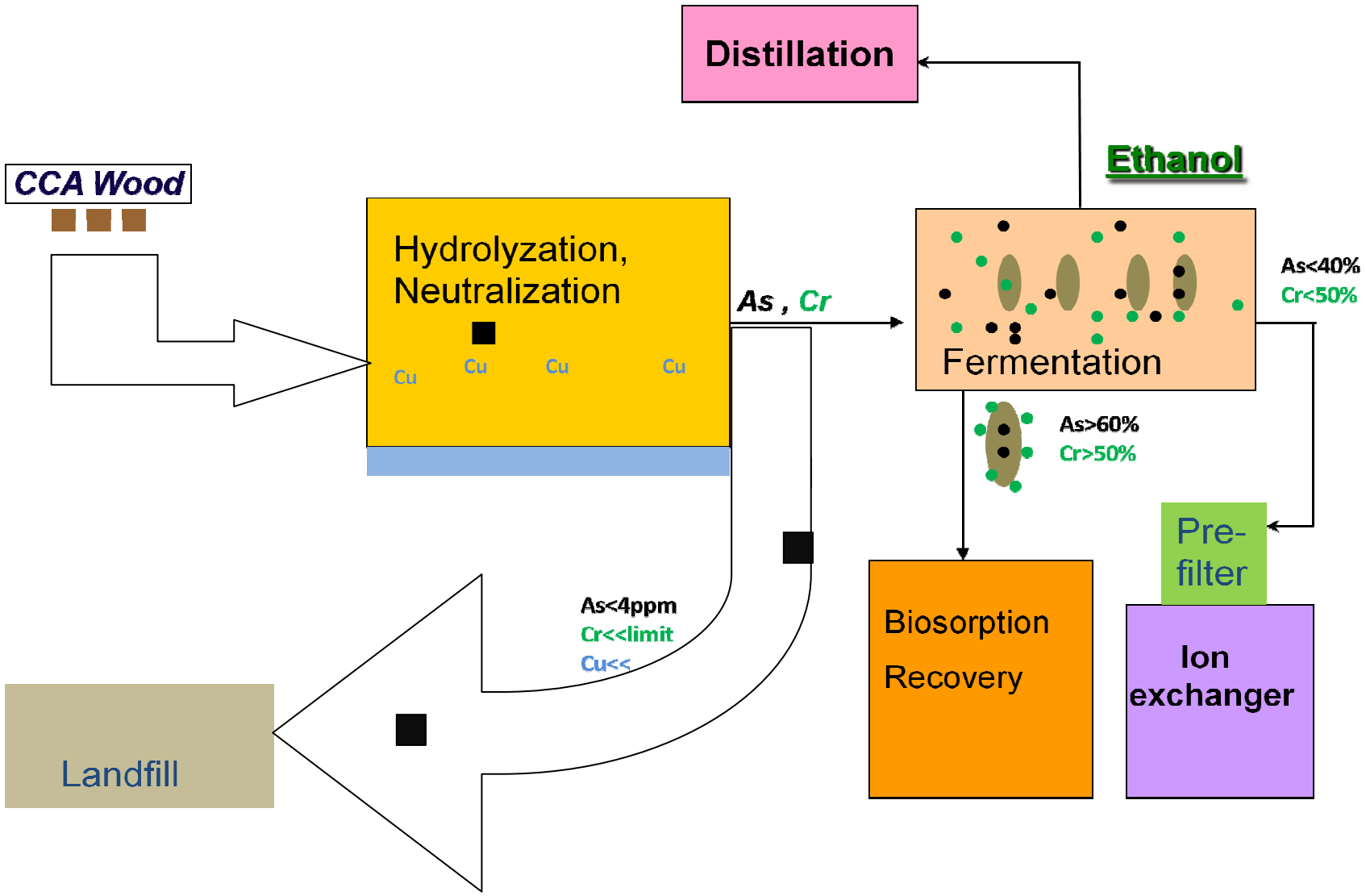

Figure 7.1: Schematic proposed process and metal mass balance

CHAPTER EIGHT
FUTURE WORK

The hydrolysis method applied in this research was not an economical and optimal method. The industries usually used dilute acid hydrolysis or enzymatic method (which gets more attention these days). The effect of other applied hydrolysis methods on leaching of chromium, copper and arsenic should be investigated and tested.

The detoxification and removal of the products which kill yeast will help to improve the yield of ethanol production and probably metal uptake. Ethanol itself will kill yeast, then removing ethanol and employing a semi-batch fermentation process will improve the yield. The effect of detoxification methods and removing ethanol from fermentation batches, on metal uptake yield should be tested to find the maximum capacity of metal uptake by yeast.

Finding some alternative usage for hydrolyzed wood remains instead of landfilling can be another subject for research to lead to more economical methods. Recycling and burning could be better methods compared to landfilling but more tests are necessary to make

sure that the air emissions of burning wood is not hazardous and also the leachability of metals from ashes should be tested.

The speciation of metals like arsenic and chromium is an important factor for their toxicity and mobility. It is suggested that yeast changes the speciation of arsenic and probably chromium and also it may store the uptaken metal inside the cell (arsenic) or only adsorb on the cell wall (copper). Performing some tests to identify the speciation of chromium and arsenic inside the yeast and also the adsorption points of chromium, copper and arsenic inside or on cell wall of the yeast will help us for recovery processes and even separation methods.

There are some other microorganisms such as *Zymomonas mobilis* bacteria which may be used in the fermentation process in some ethanol industries, the same tests can be performed to determine if they also uptake metals or not.

As it was mentioned earlier the metal can be stored inside the vacuole or be adsorbed on the cell wall, therefore depending on the point of adsorption, the type and the speciation of metal, different recovery methods might be considered. More investigation on recovery processes might enable the reuse of yeast and metals.

REFERENCES

Ackerson, M., Ziobro, M., Gaddy, G.L. 1981. Two-Stage Acid Hydrolysis of Biomass. Biotechnol. Bioeng. Symp. 11**:** 103-112.

Ahalya, N., Ramachandra, T.V., Kanamadi, R.D. 2003. Biosorption of Heavy Metals. Research Journal of Chemistry and Environment,7(4): 71-79.

AWPA, 1994. Book of standards. Woodstock, MD: American Wood Preservers' Association.

Azhar, AF., Bery, MK., Colcord AR., Roberts RS. 1982. Development of a yeast strain for the efficient ethanol fermentation of wood acid hydrolyzate. Dev. Ind. Microbiol. 23:351-60.

Aziz, S., Sarkanen, K. 1989. Organosolv pulping – a review. Tappi. J. 72: 169–175.

Baker, D.E. 1990. Copper. In: Alloway, B.J., ed. Heavy metals in soils. New York: John Wiley and Sons.

Beck, M.J. 1993. Fermentation of pentoses from wood hydrolysates, In: Saddler, J.N. Bioconversion of forest and agricultural residues (ed) C.A.B. International Wallingford. pp.211-229.

Beguin, P., Aubert, J.P. 1994. The biological degradation of cellulose. FEMS Microbiol. Rev. 13: 25–58.

Bisaria, V.S. 1991. Bioprocessing of Agro-residues to glucose and chemicals. In: Martin, A.M. (Ed.), Bioconversion of Waste Materials to Industrial Products. Elsevier, London, pp. 210–213.

Blanchette, R.A. 1991. Delignification by wood-decay fungi. Annu. Rev. Phytopathol. 29: 381–398.

Boominathan, K., Reddy, C.A. 1992. cAMP-mediated differential regulation of lignin peroxidase and manganese-dependent peroxidase production in the white-rot basidiomycete *Phanerochaete chrysosporium*. Proc. Natl. Acad. Sci. (USA) 89 (12): 5586–5590.

Brady, D., Duncan, J.R. 1994. Bioaccumulation of metal cations by Saccharomyces cerevisiae. Applied Microbiology and Biotechnology 41: 149-154.

Braman, R.S. 1975. Arsenic in the environment. In: Woolson, E.A., ed. Arsenical pesticides, ACS symposium series 7. Washington, DC: American Chemical Society: 108-123.

Brennan, A.H., Hoagland, W., Schell, D.J. 1986. High temperature acid hydrolysis of biomass using an engineering-scale plug flow reactor: result of low solids testing. Biotechnol. Bioeng. Symp. 17: 53–70.

Brannon, J.M., Patrick, W.H., Jr.1987. Fixation, transformation, and mobilization of arsenic in sediments. Environmental Science and Technology. 21:450-459.

Brownell, H.H., Saddler, J.N. 1987. Steam Pretreatment of Lignocellulosic Materials for Enhanced Enzymatic Hydrolysis, Biotechnology and Bioengineering, 29: 228-235.

Butterfield, B.G., Meylan, B.A. 1980. Three dimensional structure of wood. An ultrastructural approach. London, New York; Chapman and Hall, 99 pp.

Cahela, D.R., Lee, Y.Y. Chambers, R.P. 1983. Modeling of percolation process in hemicellulose hydrolysis. Biotechnol. Bioeng. 25: 3–17.

Campbell, C.J., Laherrere, J.H., 1998. The end of cheap oil. Sci. Am. 3: 78–83.

Cheng, C.N., Focht, D.D. 1979. Production of arsine and methylarsines in soil and culture. Applied and Environmental Microbiology. 9:494-498.

Christ RH., Martin, J.R., Guptill, P.W., Eslinger, J.M., Crist, D.R. Interactions of metals and protons with algea 2. Ion exchange in adsorption and metal displacement by protons. Environ. Sci Technol. 24:337-342.

Chum, H.L., Johnson, D.K., Black, S. 1988. Organosolv pretreatment for enzymatic hydrolysis of poplars: 1. enzyme hydrolysis of cellulosic residues. Biotechnol. Bioeng. 31: 643–649.

Clark, T.A., Mackie, K.L. 1987. Steam explosion of the softwood *Pinus radiata* with sulphur dioxide addition. I. Process optimisation. J. Wood Chem. Technol. 7:373–403.

Clark, T.A., Mackie, K.L., Dare, P.H. 1989. McDonald, A.G. Steam explosion of the softwood *Pinus radiata* with sulphur dioxide addition. II. Process characterization. J. Wood Chem. Technol. 9:135–166.

Cooper, P.A. 1988. Diffusion and interaction of components of water-born preservatives in the wood cell wall. IRG/WP/3474.

Cooper, P.A. 1993. Disposal of treated wood removed from service: the issues, In: Proceedings, Environmental Considerations in Manufacture, Use and Disposal of Preservative Treated Wood. Madison,WI, USA Carolinas-Chesapeake Section. Forest Products Society, pp. 85-90.

Converse, A.O., Kwarteng, I.K., Grethlein, H.E., Ooshima, H. 1989. Kinetics of thermochemical pretreatment of lignocellulosic materials. Appl. Biochem. Biotechnol. 20/21,:63–78.

Coughlan, M.P., Ljungdahl, L.G. 1988. Comparative biochemistry of fungal and bacterial cellulolytic enzyme system. In: Aubert, J.-P., Beguin, P., Millet, J. (Eds.), Biochemistry and Genetics of Cellulose Degradation, FEMS Sym- posium no. 43, pp. 11–30.

de Boer, AJ., den Uil, H. 1997. An evaluation of three routes for the production of liquid fuels from biomass. 1997. ECN-R- 97-001. Energy Research Centre of the Netherlands. Petten the Netherlands.

Decker, J. 2009. Going Against the Grain: Ethanol from Lignocellulosics, Renewable Energy World, January 22, 2009. Retrieved February 1, 2009

Duff, S.J.B., Murray, W.D. 1996. Bioconversion of forest products industry waste cellulosics to fuel ethanol: a review. Bioresour. Technol. 55: 1–33.

EPA (U.S. Environmental Protection Agency). 1992. Mercury and arsenic wastes, removal, recovery, treatment, and disposal. Pollution technology review No.214. pp. 89-90.

Ercole, C., Veglio, F., Toro, L., Ficara, G. and Lepidi, A.1994. Immobilisation of microbial cells for metal adsorption and desorption. In: Mineral Bioprocessing II. Snowboard. Utah

Esteghlalian, A., Hashimoto, A.G., Fenske, J.J., Penner, M.H. 1997. Modeling and optimization of the dilute-sulfuric-acid pretreatment of corn stover, poplar and switchgrass. Bioresour. Technol. 59: 129–136.

Fan, L.T., Gharpuray, M.M., Lee, Y.H. 1987. In: Cellulose Hydrolysis Biotechnology Monographs. Springer, Berlin, p. 57.

Fein, J.E., Potts, D., Good, D., O'Boyle, A., Dahlgren, D., Beck, M.J., Griffith, R.L. 1991. Development of an optimal wood-to-fuel ethanol process utilizing best available technology. In: Klass, D.L. (ed.) Proceedings of the ASAE 1991. International Winter Meeting. Energy from biomass and wastes, vol.15. Institute of Gas Technology, Chicago, Ill. pp 745–765.

Galbe M., Zacchi, G. 2002. A review of the production of ethanol from softwood. Applied Microbiology and Biotechnology 59:618–628.

Goksungur, Y., Uren, S., Guvenc, U. 2003. Biosorption of copper ions by caustic treated waste Baker's yeast biomass. Turk Journal of Biology. 27: 23-29.

Grous, W.R., Converse, A.O., Grethlein, H.E. 1986. Effect of steam explosion pretreatment on pore size and enzymatic hydrolysis of poplar. Enzyme Microb. Technol. 8: 274–280.

Gupta, R., Ahuja, P. Khan, Saxena, S. R. K. and Mohapatra, H. 2000. Microbial biosorbents: Meeting challenges of heavy metal pollution in aqueous solutions. Current Science. 78(8): 967-973.

Hamelinck, C.N., Hooijdonk, G., Faaij, A.PC. 2005. Ethanol from lignocellulosic biomass: techno-economic performance in short, middle and long term. Biomass & Bioenergy Journal, 28: 384-410.

Hamelinck CN. and Faaij APC. Future prospects for production of methanol and hydrogen from biomass. 2001. Report NWS-E-2001-49. Utrecht University, department of Science Technology and Society. Utrecht the Netherlands. 32pp+annexes.

Hayn, M., Steiner, S., Klinger, R., Steinmuller, H., Sinner, M. and Esterbauer, H. 1993.Basic Research and Pilot Studies on the Enzymatic Conversion of Lignocellulosics, in: Bioconversion of Forest and Agricultural Plant Residues, J.N.Saddler(ed), C.A.B. International, Wallingford, 1993.pp.33-72.

Helsen, L., Van den Bulck, E. 2005. Annual Meeting, Boston, Review of disposal technologies for chromated copper arsenate (CCA) treated wood waste, with detailed analyses of thermochemical conversion processes, Environmental Pollution, 134: 301-314.

Hinman, N.D., Schell, D.J., Riley, C.J., Bergeron, P.W., Walter, P.J. 1992. Preliminary estimate of the cost of ethanol production for SSF technology. Appl. Biochem. Biotechnol. 34/35: 639–649.

Holtzapple, M.T., Humphrey, A.E., Taylor, J.D. 1989. Energy requirements for the size reduction of poplar and aspen wood. Biotechnol. Bioeng. 33: 207–210.

John, JA. Hydration in Miller SA (ed.), 1969. Ethylene and industrial derivatives. Ernest Benn, London. pp. 690-801.

Howard, E. T., Manwiller, F.G. 1969. Anatomical characteristics of southern pine sternwood. Wood Sci. 2: 77-86.

Iida, K., Pierman, J., Tolaymat, T., Townsend, T., Wu, C.Y. 2004. Control of chromated copper arsenate wood incineration air emissions and ash leaching using sorbent technology. Journal of Environmental Engineering, February, 1302: 184-192.

Illman, B. L., Highley, T. L. 1996. Fungal degradation of wood treated with metal-based preservatives, USDA Forest Service, Forest Products Laboratory, Madison, Wisconsin, U.S.A.

James, R.O., Barrow, N.J. 1981. In: Lonegran, J.F. et al., eds. Copper in soil and plants. New York: Academic Press, 47-68.

John, J.A. 1969. "Hydration" in Miller SA (ed.), *Ethylene and industrial derivatives*. Ernest Benn. London. 690-801.

Jones, J.L., Semrau, K.T. 1984. Wood hydrolysis for ethanol production – previous experience and the economics of selected processes. Biomass 5:109–135.

Keller, F.A. 1996. Integrated bioprocess development for bioethanol production. In: Wyman CE (ed) Handbook on bioethanol: production and utilization. Taylor & Francis, Bristol, Pa. pp 351–379.

Kilzer, F.J., Broido, A. 1965. Speculations on the nature of cellulos pyrolysis. Pyrodynamics 2: 151–163.

Kirk, T.K., Farrell, R.L. 1987. Enzymatic combustion: the microbial degradation of lignin. Annu. Rev. Microbiol. 41, 465–505.

Koh,J., Kwon, Y., Pak, Y.N. 2005. Separation and sensitive determination of arsenic species (As^{+3}/As^{+5}) using the yeast immobilized column and hydride generation in ICP-AES. Microchemical Journal 80 195-199.

Koh,J., and Pak, Y.N. 2005. Speciation of Arsenic ions (As^{+3}/ As^{+5}) with the yeast-immobilized column. Bull. Korean Chem. Soc. , 26(2: 217-218.

Konasewich, D.E., Henning, F.A. April 1998. Chromated Copper Arsenate Wood Preservation facilities, Environment Canada, Report EPS 2 /WP/3.

Krassig, H.A. 1993. Polymer Monographs, Vol 2, Elsevier Press, New York, NY.

Kratochvil, D., Volesky, B. 1998.Advances in biosorption of heavy metals. Trends in Biotechnology, 1998, vol. 16, p. 291-300.

Kuyucak, N., Vole sky, B. 1988.Biosorbents for recovery of metals from industrial solutions. *Biotechnol Left.,* 10 (2): 137-142.

Larson, E.D., Williams, R.H., Regis, M., Leal LV. 2001. A review of biomass integrated-gasifier/gas turbine combined cycle technology and its application in sugarcane industries, with an analysis for Cuba. Energy for Sustainable Development;V(1):54–76.

Larsson, S., Palmqvist, E., Hahn-Hägerdal, B., Tengborg, C., Stenberg, K., Zacchi, G., Nilvebrant, N.O. 1999. The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. Enzyme Microb Technol 24:151–159.

Lebow, S. 1996. Leaching of wood preservative components and their mobility in the environment, United States Department of Agriculture, General Technical Report FPL-GTR-93.

Lynd LR. 1996. Overview and evaluation of fuel ethanol from cellulosic biomass: technology, economics, the environment, and policy. Annual Reviews, Energy Environment;21:403–65.

Moreira JR. 2000.Sugarcane for energy—recent results and progress in Brazil. Energy for Sustainable Development;IV(3):43–54.

Mackie, K.L., Brownell, H.H., West, K.L., Saddler, J.N. 1985. Effect of sulphur dioxide and sulphuric acid on steam explosion of aspen wood. J. Wood Chem. Technol. 5: 405–425.

McBride, M.B. 1981. Forms and distribution of copper in solid and solution phases of soil. In: Loneragan, J.F. ed. Academic Press, New York: pp. 25-45.

McGrath, S.P., Smith, S. 1990. Chromium and Nickel, In: Alloway, B.J., ed. Heavy metals in soils. New York: John Wiley and Sons: pp. 125-150.

McMillan, J.D. 1994. Pretreatment of lignocellulosic biomass. In Himmel, M.E., Baker, J.O., Overend, R.P. (Eds.). Enzymatic conversion of Biomass for fuels production. American Chemical Society. Washington, DC, pp292-324.

Mes-Hartree, M., Dale, B.E., Craig, W.K. 1988. Comparison of steam and ammonia pretreatment for enzymatic hydrolysis of cellulose. Appl. Microbiol. Biotechnol. 29: 462–468.

Messure, K., Martin, R.E.; Fish, W. 1991. Identification of copper contamination in sediments by a microscale partial extraction technique. Journal of Environmental Quality. 20:114-118.

Miller, R.B. 1999. Structure of Wood (Ch2), Forest Products Laboratory. Wood handbook-Wood as an engineering material. Gen. Tech. Rep. FPL-GTR-113. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory.

Moghaddam, A.H. 2002. Leaching of heavy metals from chromated copper arsenate treated wood, M.A.Sc. thesis , Concordia University.

Moghaddam, A., Mulligan, C. 2008. Leaching of heavy metals from chromated copper arsenate treated wood after disposal. Waste Management, 28: 628- 637.

Morjanoff, P.J., Gray, P.P. 1987. Optimization of steam explosion as method for increasing susceptibility of sugarcane bagasse to enzymatic saccharification. Biotechnol. Bioeng. 29: 733–741.

Morris, P.I., 1996. Towards a unified international hazard class system. 26 Annual Meeting - International Research Group on Wood Preservation. IRG / WP / 96-20081.

Mulligan, C. 1994. Assessment of alcohol process technology. Prepared for agriculture and agri-food Canada – green plan ethanol program. SNC Research Corporation. Reference SNC: 6231.

National Research Energy Lab. 1992. Conversion Technologies: Biomass to Ethanol - Alcohol Fuels Reference Work #3.

Nelson, C. R., Courter, M. L. 1954. Ethanol by hydration of ethylene. Chem. Eng. Prog. 50: 526-532.

Nguyen, Q.A., Tucker, M.P., Boynton, B.L., Keller, F.A., Schell, D.J. 1998. Dilute acid pretreatment of softwoods. Appl. Biochem. Biotechnol. 70–72:77–87.

Nguyen, Q.A., Tucker, M.P., Keller, F.A., Beaty, D.A., Connors, K.M., Eddy, F.P. 1999. Dilute acid hydrolysis of softwoods. Appl. Biochem. Biotechnol. 77–79:133–142.

Nguyen, Q.A., Tucker, M.P., Keller, F.A., Eddy, F.P. 2000. Two-stage dilute acid pretreatment of softwoods. Appl. Biochem. Biotechnol. 84–86:561–576.

Nies, D.H. (1999). Microbial heavy metal resistance. Appl Microbiol Biotechnol *51*: 730-750.

Nies, D.H., Silver, S. (1995). Ion efflux systems involved in bacterial metal resistances. Journal of Industrial Microbiology 14: 186-199.

Novem. 1999. Analysis and evaluation of GAVE chains, Management Summary. GAVE report 9921. Utrecht the Netherlands.

Ogier, J.C., Ballerini, D., Leygue, J.P., Rigal, L., Pourquie, J. 1999. Production d'ethanol a partir de biomasse lignocellulosique. Oil and Gas Science and Technology – Revue de l'IFP.54(1):67-94.

Olsson, L. and Hahn-Hagerdal, B. 1996. Fermentation of lignocellulosic hydrolysates for ethanol production. Enzyme Microb. Technol. 18:312-331.

O'Neill, P. 1990. Arsenic. In: Alloway, B.J., ed. Heavy metals in soils. New York: John Wiley and Sons: pp.83-99.

Paknikar, K. M.; Bhide, J. V. 1993. Aerobic reduction and biosorption of chromium by a chromate resistant *Aspergillus* sp. In Biohydrometallurgical Technologies; Torma, A. E., Apel, M. L., Brierley, C. L., Eds.; The Minerals, Metals & Materials Society: Warrendale, PA; 2: 237-244.

Palmqvist, E., Hahn-Hegerdal, B., Galbe, M., Larsson, M., Stenberg, K., Szengyel, Z., Tengborg, C., Zacchi, G. 1996. Design and operation of bench-scale process development unit for the production of ethanol from lignocellulosics. Biosource Technology. vol. 58:171-179.

Parker, A.J. 1981. In: Lonegran, J.F. and others, eds. Copper in soils and plants. New York: Academic Press: 1-22.

Peat, S., Whelan, W.J., Edwards, T.E. 1961. Polysaccharides of bakers' yeast. Part IV. Mannan. J. Chem. Soc, 29-34.

Picataggio, S.K., Zhang, M. 1996. Biocatalyst development for bioethanol production from hydrolysates, In Wyman CH(ed.), Handbook on bioethanol: production and utilization. Tylor & Francis, Washington DC, pp. 163-178.

Pimentel, D., Patzek, T.W. 2005. Ethanol production using corn, switchgrass and wood; Biodiesel production using soybean and sunflower. Natural Resources Research, vol.14, No. 1

Ramos, L.P., Breuil, C., Saddler, J.N. 1992. Comparison of steam pretreatment of eucalyptus, aspen and spruce wood chips and their enzymatic hydrolysis. Appl. Biochem. Biotechnol. 34–35:37–48.

Reischl, G., Wittorf, F., Deckwer, W. D. 1993. Biological treatment of chromium and COD-polluted wastewater from the leather industry. Leder (Ger.) 44 (81), 172-9.

Reith, JH., den Uil, H., van Veen, H., de Laat, WTAM., Niessen, JJ., de Jong, E., Elbersen, HW., Weusthuis, R., van Dijken, JP. and Raamsdonk L. 2002. Co-production of bioethanol, electricity and heat from biomass residues. In: Palz, W., Spitzer, J., Maniatis, K., Kwant, K., Helm, P. and Grassi A., editors. Proceedings of Twelfth European Biomass Conference. Florence Italy. ETA-Florence. pp. 1118–1123.

Ress, B.B., Calvert, P.P., Pettigrew, C.A., Barlaz, M.A. 1998. Testing Anaerobic Biodegradability of Polymers in a Laboratory-Scale Simulated Landfill, Environ. Sci. Technol., 32 (6): 821- 827.

Riedel, G.F., Sanders, J.G. Osman, R.W. 1989. The role of three species of benthic invertebrates in the transport of arsenic from contaminated estuarine sediment. Journal of Experimental Marine Biology and Ecology. 134 : 143-155.

Rosen, BP. 2002. Biochemistry of arsenic detoxification. FEBS Lett. 529(1):86-92.

Rouse, J.V., Pyrih, R.Z. 1990. In place clean –up of heavy metal contamination of soil and ground water at wood preservation sites. In: Proceedings, American Wood Preservers' Association;86:215-220.

Saddler, J.N. 1993. Bioconversion of forest and agricultural residues (ed) C.A.B. International Wallingford. pp. 211-229.

Sarkanen, K.V. 1980. Acid-catalyzed delignification of lignocellulosics in organic solvents. Prog. Biomass Convers. 2, 127–144.

Schell, D.J., Torget, R., Power, A., Walter, P.J., Grohmann, K., Hinmann, N.D. 1991. A technical and Economic Analysis of Acid-Catalysed Steam Explosion and Dilute Sulfuric Acid Pretreatment Using Wheat Straw or Aspen Wood Chips. *Appl. Biochem. and Biotechnol.,* 28/29, 87-97.

Schulze, U. 1995. Anaerobic physiology of Saccharomyces cerevisiae. Ph.D. thesis, Technical University of Denmark, Denmark.

Schurz, J. 1978. In: Ghose, T.K. (Ed.), Bioconversion of Cellulosic Substances into Energy Chemicals and Microbial Protein Symposium Proceedings, IIT, New Delhi, pp. 37.

Schwald, W., Smarridge, T., Chan, M., Breuil, C., Saddler, J.N. 1989. The influence of SO_2 impregnation and fractionation on the product recovery and enzymatic hydrolysis of steam-treated sprucewood. In: Coughlan, M.P. (ed.) Enzyme systems for lignocellulose degradation. Elsevier, London, pp 231–242.

Shafizadeh, F., Lai, Y. Z. 1975. Thermal degradation of 2-deoxy-Darabino- hexonic acid and 3-deoxy-D-ribo-hexono-1,4-lactone. Carbohyd. Res. 42: 39–53.

Shafizadeh, F., Bradbury, A.G.W. 1979. Thermal degradation of cellulose in air and nitrogen at low temperatures. J. Appl. Poly. Sci. 23: 1431–1442.

Shalat, S.L., Solo-Gabriele, H.M., Fleming, L.E., Buckley, B.T., Black, K., Jimenez, M., Shibatea, T., Durbin, M., Graygo, J., Stephan, W., Van DeBogart, G. 2006. A pilot study of children's exposure to CCA-treated wood from playground equipment, Science Total Environ. 367: 80-88.

Siegel, S., Keller, P., Galam, M., Zehr, H., Siegel, B., Galum, E. 1986. Biosorption of lead and chromium by Penicillium preparation. Water, Air, Soil Pollut. 27: 69-75.

Sivers, M., Zacchi, G. 1995. A techno-economical comparison of three processes for the production of ethanol from wood. Bioresour Technol 51:43–52.

Sloot, H.A.,van der Heasman, L., Quevauviller, P.H., 1997. Harmonization of leaching extraction tests. Elsevier Science. Studies in Environmental Science,Vol.70.

Solo-Gabriele, H., Townsend, T. 1999. Disposal – end management of CCA – treated wood 95th Annual Meeting of the American Wood Preservers' Association, May 16-19 Ft.Lauderdale, Florida , 95, pp. 65-73.

Solo-Gabriele, H., Townsend, T., Messick, B., Calitu, V. 2002. Characteristics of chromated copper arsenate-treated wood ash. Journal of Hazardous Materials. B89, 213-232.

Solo-Gabriele, H., Townsend, T., Schert, J. 2003. Environmental impacts of CCA treated wood: a summary from seven years of study focusing on the US Florida environment. Presented at the 34th Annual IRG Meeting, Brisbane, Australia, IRG/WP 03-50205.

Solo-Gabriele, H., Townsend, T., Yong, C. 2004. Arsenic and chromium speciation of leachates from CCA treated wood. Florida center for solid and hazardous waste management, report No.03-07.

Spain, A. 2003. Implications of microbial heavy metal tolerance in the environment. Reviews in Undergraduate Research, 2: 1-6.

Sternberg, D. 1976. Production of cellulase by Trichoderma. Biotechnol. Bioeng. Symp., (6) : 35–53.

Stenberg, K., Tengborg, C., Galbe, M., Zacchi, G. 1998. Optimization of steam pretreatment of SO_2-impregnated mixed softwoods for ethanol production. J. Chem. Technol. Biotechnol. 71:299–308.

Sun, Y., Cheng, J. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource Technology 83: 1-11.

Taherzadeh, M.J. 1999. Ethanol from lignocellulose: Physiological effects of inhibitors and fermentation strategies(Ph.D. thesis). Chalmers University of Technology. Sweden.

Taherzadeh, M.J., Eklund, R., Gastafsson, L., Niklasson, C., Liden, G. 1997. Characterization and fermentation of dilute-acid hydrolyzates from wood. Industrial & Engineering Chemistry Research.36:4659-4665.

Tengborg, C., Stenberg, K., Galbe, M., Zacchi, G., Larsson, S., Palmqvist, E., Hahn-Hägerdal, B. 1998. Comparison of SO_2 and H_2SO_4 impregnation of softwood prior to steam pretreatment on ethanol production. Appl. Biochem. Biotechnol. 70–72:3–15.

Thring, R.W., Chorent, E., Overend, R. 1990. Recovery of a solvolytic lignin: effects of spent liquor/acid volume ration, acid concentration and temperature. Biomass 23: 289–305.

Tom, P. 2001. Good wood gone bad, Waste Age 32(8), 36-51.

Torget, R., Werdene, P., Himmel, M., Grohmann, K. 1990. Dilute acid pretreatment of short rotation woody and herbaceous crops. Appl. Biochem. Biotechnol. 24–25:115–126.

Torget, R., Werdene, P., Himmel, M., Grohmann, K. 1991. Dilute acid pretreatment of corn residues and short-rotation woody crops. Appl. Biochem. Biotechnol. 28–29:75–86.

Torget, R., Hatzis, C., Hayward, T.K., Hsu, T-A., Philippidis, G.P. 1996. Optimization of reverse-flow, two-temperature dilute-acid pretreatment to enhance biomass conversion to ethanol. Appl. Biochem. Biotechnol. 57–58:85–101.

Tripathi, R.D., Srivastava, S., Mishra, S., Singh, N., Tuli, R., Gupta, D.K. and J.M. Maathuis, F.2007**.** Arsenic hazards: strategies for tolerance and remediation by plants. Trends in Biotechnology 25(4): 158-165.

Tsoumis, G. 1992. Science and technology of wood; structure, properties, utilisation. New York: Van Nostrand Reinhold: London; Chapman and Hall, 494 pp.

Volesky, B. 1986. Biosorbent Materials. Biotechnol. Bioeng Symp., 16: 121-126.

Volesky, B. Holan, Z.R. 1995. Biosorption of heavy metals. Biotechnol. Prog. 11:235-250.

Waldner, R., Leisola, M.S.A., Fiechter, A. 1988. Comparison of ligninolytic activities of selected fungi. Appl. Microbiol. Biotechnol. 29: 400–407.

Webb, David A. 1990. Wood preservative treatments for crossties and potential future treatments. The Railway Tie Association Annual Meeting ,Birmingham, Alabama, October 10-12.

Weis, J.S., Weis P., 1992. Transfer of contaminants from CCA-treated lumber to aquatic biota. Journal of Experimental Marine Biology and Ecology.161: 189-199.

Weis, P., Weis J.S., Procter. T. 1993. Copper, chromium and arsenic in esturarine sediments adjacent to wood treated with chromated copper arsenate. Estuarine, Coastal and Shelf Science. 36: 71-79.

Wilhelmi, B.S., Duncan J.R., 1995. Metal recovery from *Saccharomyces cerevisiae* biosorption columns. Biotech. Letters 17(9):1007-10012.

Wooley, R., Ruth, M., Sheehan, J., Ibsen, K., Majdeski, H. and Galvez, A. 1999. Lignocellulosic biomass to ethanol—Process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hyrolysis—Current and futuristic scenarios. Report No. TP-580-26157. National Reneawable Energy Laboratory. Golden Colorade USA. 130pp.

Wright, J.D. 1998. Ethanol from biomass by enzymatic hydrolysis. Chem. Eng. Prog. 84 (8): 62–74.

Wyman, C.E. 1996. Ethanol production from lignocellulosic biomass: overview. In: Wyman CE (ed) Handbook on bioethanol: production and utilization. Taylor & Francis, Bristol, Pa. pp 1–18.

Wyman, C.E. 1999. Production of low cost sugars from biomass: progress, opportunities, and challenges. In: Overend RP, Cornet E (eds) Biomass – a growth opportunity in green energy and value added products. Proceedings of the 4th Biomass Conference of the Americas, vol 1. Pergamon, Oxford, pp 867–872.

United Nations Development Program report. 2000. World Energy Assessment. Washington D.C. ISBN: 92-1-126126-0.

U.S. Department of Energy.2006.
http://www.eere.energy.gov/afdc/altfuel/eth_made.html

U.S. EPA.2006.
http://www.epa.gov/msw/facts.htm

APPENDICES

APPENDIX A

Calculations:

$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2$ A.1

Glucose	Ethanol
180 g	2 x 46 g
17.5	?=8.95

(42.4/100) x (pine wood amount) = 3.5 g glucose A.2

wood amount =8.25 g

8.25 g pine wood x (50.5/100) =4.16 g CrO_3 A.3

CrO_3	$CrCl_3 + 6\ H_2O$
100	158.3 + 6 x 18
4.16	x=11.1g

8.25 g pine wood x (21/100) =1.73 g CuO A.4

CuO	$CuCl_2 + 2\ H_2O$
80	170.48
1.73	x=3.7g

8.25 g pine wood x (38/100) =3.31 g As_2O_5 A.5

As_2O_5	$Na_2HasO_4 + 7\ H_2O$
230	312
3.5	x=4.74 g

APPENDIX B

There is a linear relationship between absorbance and concentration of yeast. Figure B.1 is a typical graph which shows the linear relation of concentration and absorbance for one of the fermentation batches. In all of the experiments the absorbance of all samples during the fermentation and the concentration of 2 samples were measured. Based on 2 measured concentrations and their relevant absorbances (for every fermentation batch), the line of cell density versus absorbance was sketched. Using the line formula and the measured absorbance , the concentration of the other samples were calculated.

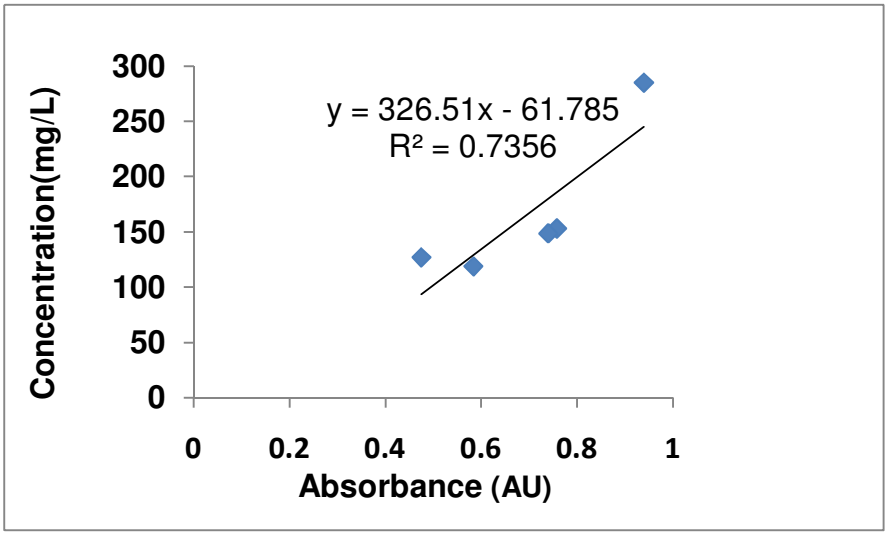

Figure B.1: A typical sample of yeast cell density versus optical absorbance

APPENDIX C

US$575 x (1.0339 x 1.0324 x 1.0285 x 1.0385 x 0.996) = US$653 C.1

Total investment for Ion exchanger (83.3 tonne) : 2.39 x 1.88 = MU.S$$_{2003}$$4.49 C.2
(Based on Table 6.3)

Total investment for Ion exchanger (2.5 tonne) : 4.49 x 2.5 / 83.3 = MU.S$$_{2003}$$0.135 C.3

U$135000 x (1.0339 x 1.0324 x 1.0285 x 1.0385 x 0.996) = U.S$153,296 C.4

Total investment for 83.3 tonne solid separation: 1.05 x 2.20 = M U.S$$_{2003}$$ 2.31 C.5

Total investment for 2.5 tonne solid separation : 2.31 x 2.5 / 83.3 = U.S$$_{2003}$$ 0.0693 C.6

U$69300 x (1.0339 x 1.0324 x 1.0285 x 1.0385 x 0.996) = US$78,692 C.7