**ADDIS ABABA UNIVERSITY**

**ADDIS ABABA INSTITUTE OF TECHNOLOGY**

**SCHOOL OF CHEMICAL AND BIO ENGINEERING**


# Bioaccumulation of Cr(VI) from Tannery Wastewater Using *Saccharomyces cerevisiae*

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*By*

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*A Thesis Submitted to the School of Chemical and Bio-Engineering, Addis Ababa Institute of Technology, Addis Ababa University*

*Presented in Partial Fulfillment of the Requirements for Degree of Master of Science in Chemical Engineering (Biochemical Engineering Stream)*

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*June 2018*

The undersigned have examined the thesis entitled '**Bioaccumulation of Cr(VI) from Tannery Wastewater using *S.cerevisiae*'** presented by **Tessafa Abrham**, a candidate for the degree of **Master of Science in Chemical Engineering under Biochemical Engineering Stream** and hereby certify that it is worthy of acceptance.

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# Abstract

*Chrome tanning is the most and widely used types of tanning process and the wastewater generated by these industries is a major source of chromium pollution in the form of Cr(III)&Cr(VI) which are released into the environment either after treated inefficiently or with no treatment and become a cause for many human health problem. Several researchers have published papers to find the solution for removal of Cr(VI) but at its low concentration and this research work investigates at high concentration of Cr(VI). Cell suspensions of S.cerevisiae at its exponential growth phase were added to 250ml plastic flasks of containing 50ml of YPD media at pH value of (3,5&8), initial Cr(VI) concentration of (5, 50&100mg/ml) and incubation time of(3,5&7 days) and shaken at 200rpm and 27℃. The result of bioaccumulation of Cr(VI) from tannery wastewater using S.cerevisiae shows good result after Cr(VI)-contaminated wastewater has been treated at different treatment variables of pH, initial Cr(VI) concentration. Cr(VI) uptake efficiency of S.cerevisiae increases at low values of pH, Cr(VI) and incubation time and decreases at high values of these variables. And it can be conclude that Cr(VI)uptake efficiency is better at low values of the variables and the optimum conditions obtained by using the full factorial experimental design are pH value of 4.5, Cr(VI)concentration of 5mg/ml and incubation time of 5 days resulting 84.25 % theoretical Cr(VI) uptake efficiency . Cell viability test assay of this work shows the possibility of Cr (VI) removal at its high concentration using living cells that are tolerant to high concentration though the efficiency decreases as concentration increases due to inhibition. Bioaccumulation of Cr(VI) by the cell S.cerevisiae is analyzed by FTIR by comparing their IR spectrum of functional groups of the Cr(VI) free and Cr(VI) loaded S.cerevisiae cell wall that showed change of wavenumbers of biocomponents of cell wall of the two cells which confirms the binding of chromium ion with the functional groups of cell wall of S.cerevisiae and produces Cr complex compounds /precipitates. Freundlic equilibrium and pseudo second order kinetic model are used for expressing the bioaccumulation dynamics of Cr(VI) based on their higher correlation coefficient ($R^2$)value.*


**Key words:** Tannery Wastewater, Hexavalent Chromium, Bioaccumulation, FTIR analysis, *S.cerevisiae yeast cell.*

## Acknowledgment

I express my sincere thanks to my supervisor, **Dr. Shegaw Ahmed**, postgraduate programs leader and Assistance prof at School of Chemical and Bio Engineering, Addis Ababa Institute of Technology, Addis Ababa University for his esteemed supervision, incessant support and advices throughout my project work.

I accord my thanks to **Dr. Anurada Jabasingha and Prof.Eduardo Ojito,** at School of Chemical and Bio Engineering, Addis Ababa Institute of Technology, Addis Ababa University for providing me with all necessary fruitful advices, motivations and willingness during the project work.

I would like to take the opportunity to thank all friends and lab mates Miss, Hana, Mr. Alene and Mr. for their help, advice, co-operation and motivation during my work at lab and outside who treated me as a friend.

I would also thank to all my faculty member, office staff and Technical staff of School of Chemical and Bio Engineering, Addis Ababa Institute of Technology, Addis Ababa University, for their co-operation.

I wish to extend my sincere thanks to all my class mates and friends for their help and support.

Finally, I would also express my deep sense of gratitude to **God** for giving me health and strength throughout my life and to my father, **Abrham Ashagrie,** for his encouragement and support throughout, which always inspired me.

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# Acronyms

Cr(VI)- hexavalent chromium

$BOD_5$- biological oxygen demand at day five

COD-chemical oxygen demand

TSS- total suspended solid

TDS-total dissolved solid

EPA- environmental protection agency

WHO-world health organization

FTIR-Fourier transformed infrared radiation

UV-ultra violet

DPC-Di-Phenyl Carbazide

YPD-Yeast extracts Peptone Dextrose

RPM-revolution per minute

APHA- American public health association

ANOVA- analysis of variance

Hr- hour

$C_{i\ -}$ intial Cr(VI) concentration

$C_{f-}$ final Cr(VI) concentration

# CHAPTER ONE

# 1. Introduction

## 1.1. Background of the study

Tanning is a chemical process that converts animal hides and skin into leather and related products. Tanning industry is one of the fast-growing traditional sectors with high environmental challenges. The transformation of hides into leather is usually done by means of tanning agents and the process generates highly turbid, colored and foul smelling wastewater. The major components of the effluent include sulfide, chromium, volatile organic compounds, large quantities of solid waste, and suspended solids like animal hair and trimmings. The various components present in the effluent affect human beings, agriculture, and livestock besides causing severe ailments to the tannery workers (Dargo & Ayalew, 2014).

Chromium (Cr) is one of the major environmental pollutants which enters the air, water and soil in the form of chromium(III) and chromium(VI) through natural processes and human activities such as leather tanning, electrolytic plating, metal finishing, petroleum refining, coal composition and etc. The tanning activity is vital for the leather industry and most tanneries in the world (about 90%) use chromium salts to produce leather, because these salts provide better leather flexibility, better water resistance and a high shrinkage temperature(Technologien, 2002). Now a day, the release of high chromium containing tannery waste water from tanning industries to the environment is the life threatening action of human activities. Chromium exists in the oxidation states (II–VI) but the more stable forms in the environment are trivalent Cr(III)) and hexavalent Cr(VI)) chromium. Cr(VI) is more toxic than Cr(III) due to high solubility and mobility in soil and aquatic environments, and high permeability through biological membranes (Bahafid et al, 2013) while Cr(III) in trace amount is necessary for growth of plants, animals and microorganisms but when the concentration of Cr(III) is high it becomes toxic as of Cr(VI) for life due to its adverse effect upon organisms. Generally, the permissible limit for total chromium in drinking water is 0.05 mg/L (WHO,2004;Verma et al.,2015).The permissible limits of total

chromium in tannery effluents is between 1 and 2 mg/L according to USA, UK and Ethiopian Standards. Thus, above this limit it is considered as a risk pollutant by the United States Environmental Protection Agency (EPA: www.epa.gov) and Ethiopian Environmental Protection Authority (EPA: www.epa.gov.et).

Tanning industry worldwide generates approximately 40 million L of wastewater containing Cr every year (Sujita, 2014). In most of the countries, the tanning wastewater is discharged without proper treatment into the sewerage system causing serious environmental impact. Therefore here are some of the methods used to treat the tannery waste water before discharged into the sewerage system such as mechanical, physico-chemical treatment, biological treatment and combined chemical-biological treatment.

Treatment of Cr polluted wastewater is dependent on conventional, mostly physico-chemical, methods such as chemical precipitation, reverse osmosis, membrane processes and adsorption (Bhateria & Dhaka, 2017;Saha & Orvig, 2010; Technologien, 2002). However, Conventional methods used for the removal of hexavalent Cr use chemical procedures, which are expensive and lack specificity. As an alternative, biological approaches utilizing microorganisms offer the potential for a highly selective removal of toxic metals coupled with considerable operational flexibility. Biological approaches may reduce Cr (VI) to Cr (III) intracellularly or by making the extra cellular environment more reducing or lowering pH to favor Cr (VI) reduction. Many microbial species such as bacteria, fungi, yeast and algae are known to be capable of adsorbing heavy metal on their surface and/or accumulating within the structure. Therefore, the biological treatment using these various microbial species is one of the successful approaches for Cr(VI) bio-removal from industrial wastewater(Sen & Dastidar, 2010).

Many biological materials can bind heavy metals, but only those with sufficiently high binding capacity and selectivity for heavy metals are suitable for use in a full-scale bioaccumulation processes. Bioaccumulation not only offers an innovative alternative to other remediation approaches, but it also allows metals recovery. Among the different types of biomass proposed for heavy metals bioaccumulation, yeast cells of *Saccharomyces cerevisiae*, seems to be a promising alternative.

Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site. According to the environmental protection agency (EPA), bioremediation is a treatment that uses naturally occurring organisms to break down hazardous substances into less/nontoxic substances (Adeniji, 2004). Bioaccumulation, a bioremediation process, is a biological method of environmental control using living biomass by ways of which can be classified as: extra cellular accumulation/ precipitation, cell surface sorption/ precipitation and intracellular accumulation (Hlihor, 2009).

*Saccharomyces cerevisiae* is a species of yeast that has been instrumental to baking, wine making and brewing since ancient times. Now a day because of its biochemistry, it is applicable for environmental bioaccumulation through removal of heavy metals from the contaminated environment. But most of the works reported are removal of heavy metal of low concentration and there must be an investigation of removal of heavy metals of high concentration using *S.cerevisiae.*

## 1.2. Statement of the problem

Tannery effluent is a major pollution problem not only in Ethiopia but also in the world due to lack of efficient treatment. Many tanneries have been faced to be closed because of their wastes are hazardous for the environment and the case is true in our country. Most tanneries in the world use chromium salts to produce leather and release high chromium containing tannery waste water. And the presence of chromium over its permissible limit in the environment has become a great concern due to its adverse effect on the plants, animals and microorganisms. It affects human, crop growth, marine organisms (kills fish by damaging the gill) and microorganisms by being toxic. In general chromium has an impact on human health as a mutagen, carcinogen by concentrating in bone, blood and organs. Due to its high solubility and mobility in soil and aquatic environments, and high penetration into biological membranes, Cr (VI) is the most dangerous form of chromium which causes health problems of allergic reactions, skin rash, kidney and liver damage, and may even death of the individual (DOSH directive, 2011).

Environmental pollution is caused by no or use of less efficient treatment method of the effluents. Treatment of Cr polluted wastewater is mostly dependent on physico-chemical methods such as chemical precipitation, adsorption, reverse osmosis and membrane process (Bhateria & Dhaka, 2017) which are expensive, inefficient and creates sludge which is a secondary pollutant . Therefore, there is a need for the development of a low cost process to remove chromium economically and efficiently such as biological treatment (Saha & Orvig, 2010) using *S.cerevisiae* as a biosorbent.

Bioaccumulation of Cr(VI) from tannery effluents using *S.cerevisiae* is expected to give potentially cost effective, efficient and environmental friendly treatment due to low operational cost, availability and cell wall materials of yeast cell that can bind with the heavy metals and reduce it to less or no toxic compound.

Even though few researches were done by different scholars regarding biological treatments of industrial effluents for the removal of chromium, this study will investigate bioaccumulation of Cr(VI) from tannery wastewater using *saccharomyces cerevisiae yeast cell* .

## 1.3. Objectives of the study

### 1.3.1. General objective

The objective of this work is to bioaccumulate hexavalent chromium from tannery waste water using *saccharomyces cerevisiae*.

### 1.3.2. Specific objectives

The specific objectives of this research work are to:

- Characterize tannery wastewater viz., pH, COD, BOD, TDS, sulphate, chloride, Cr(VI), conductivity and salinity
- Determine the optimum pH, initial Cr concentration, and incubation time values for the bioaccumulation of Cr(VI) from tannery wastewater

- Asses plate colony count assay for Cr(VI)tolerance test
- Model bioaccumulation of Cr(VI) by *Saccharomyces cerevisiae* using established models

## 1.4. Significance of the study

This work has huge significance in:

I. Showing the way of keeping the environment free from toxic chromium
II. Finding cheap and efficient method of Cr contaminated tannery wastewater treatment.
III. Serving the leather processing industries to treat their chromium contaminated wastes with cheap and efficient technology before discharging it to the environment
IV. Reducing the heavy metal contamination in different industries in addition to leather industry such as electroplating, textile, mining etc.

## 1.5. Scope of the study

This thesis work includes the characterization of the tannery waste water, determining the optimum conditions (pH, initial Cr concentration, and incubation time) for uptake (bioaccumulation) of chromium (VI), Cr (VI) plate colony count assay and modeling of the bioaccumulation process.

# CHAPTER TWO

## 2. Literature Review

The transformation of hides into leather is usually done by means of tanning agents and the process generates highly turbid, colored and foul smelling wastewater. In Ethiopia, though the number of tannery industries are less; their impact in terms of pollution is large, the annual volume of liquid waste discharge from over 20 tanneries based on their annual production capacities is estimated to vary between 2,000,000 and 2,500,000 cubic meter (Dargo & Ayalew, 2014). The tanning industry discharges different types of wastes into the environment, primarily in the form of liquid effluents containing volatile organic matter, chromium, sulfide, ammonia and other salts. In Ethiopia, 90% of the existing leather industries discharge the effluent directly into the environment without any form of treatment (Hunegnaw A, 2015).

Based on the tanning agents, tanning operations are further divided into vegetable tanning and chrome tanning. Vegetable tanning is usually done in series of vats by using natural organic substances. Chrome tanning is done at a higher pH using chromium salts. It is the most common type of tanning in the world and in Ethiopia. Chrome tanned leathers are considered of top handling quality, high hydro-thermal stability and excellent user properties. However, chrome waste from leather processing poses a significant disposal problem primarily because it is non-biodegradable and environmentally persistent metal(Saurav & Czech, 2011). Chromium salts used during the tanning process generate two forms of chrome; hexavalent chromium and trivalent chromium. Hexavalent chromium is highly toxic to living organisms even at low concentration causing carcinogenic effect. Trivalent chromium may be present in the waste or can be produced from the hexavalent chromium by chemical treatment. Soluble trivalent chromium causes toxicity in anaerobic digestion due to the accumulation of the metal in the intracellular fraction of biomass (Seyoum L, 2004). Therefore, Chrome discharge into environment is one of the components that have to be strictly controlled.

### 2.1. Tanning process and its waste generation

The technique of skin tanning is a complex process that consists of multiple processes and technological operations, including: beam house operation, tanning operation, retanning and finishing(Ozgunay et al, 2007).

Beam house includes the following processes: soaking, liming, chemical or mechanical removal of hair, degreasing and pickling. Then in the tanning operation the raw materials are tanned to make the actual leather. Two methods are adopted for tanning of raw hide, vegetable tanning and chrome tanning. The main difference of these two tanning methods is the tanning solution. The tanning solution used for chrome tanning is a solution of (trivalent) chrome salt while the tanning solution for vegetable tanning is prepared from bark, wood, fruits and roots that contain tannin.

Retanning and wet finishing processes give the tanned hide special or desired characteristics. Finishing includes all operations performed on the hide after fat liquoring and includes the enhancement of color resistance to stains and abrasions, smoothing and stretching the skin, drying, conditioning, staking, dry milling, buffing and plating(Ozgunay et al, 2007).

## 2.2. Characteristics of tannery effluents

The chemical composition of untreated hides or skin processing waste depends mainly on a kind and quality of raw material, treatment type and process conditions and overall wastes generated in each unit operations of a tanning process are shown in **Figure1**. The effluent includes the tanned leather wastes (such as splits, shaving and trimming), wastewater and sludge. Wastewater contains pollutants such as unused chemical, leached proteins and products, hides and skins degradation. Tannery wastewater is characterized mainly by measurements of Biochemical Oxygen Demand (BOD), Chemical oxygen demand,(COD), total dissolved solid(TDS), chromium and sulphide etc.

According UNIDO (2011),the three main categories of tannery wastewater, each one having very distinctive characteristics, are:

- Effluents emanating from the beam-house – liming, deliming/bating, water from fleshing and splitting machines; they contain sulphides, their pH is high, but they are chrome-free.

- Effluents emanating from the tanyard (tanning and re-tanning, sammying) – high Cr content, acidic.
- Soaking and other general effluents, mainly from post-tanning operations (fat-liquoring, dyeing) – low Cr content.

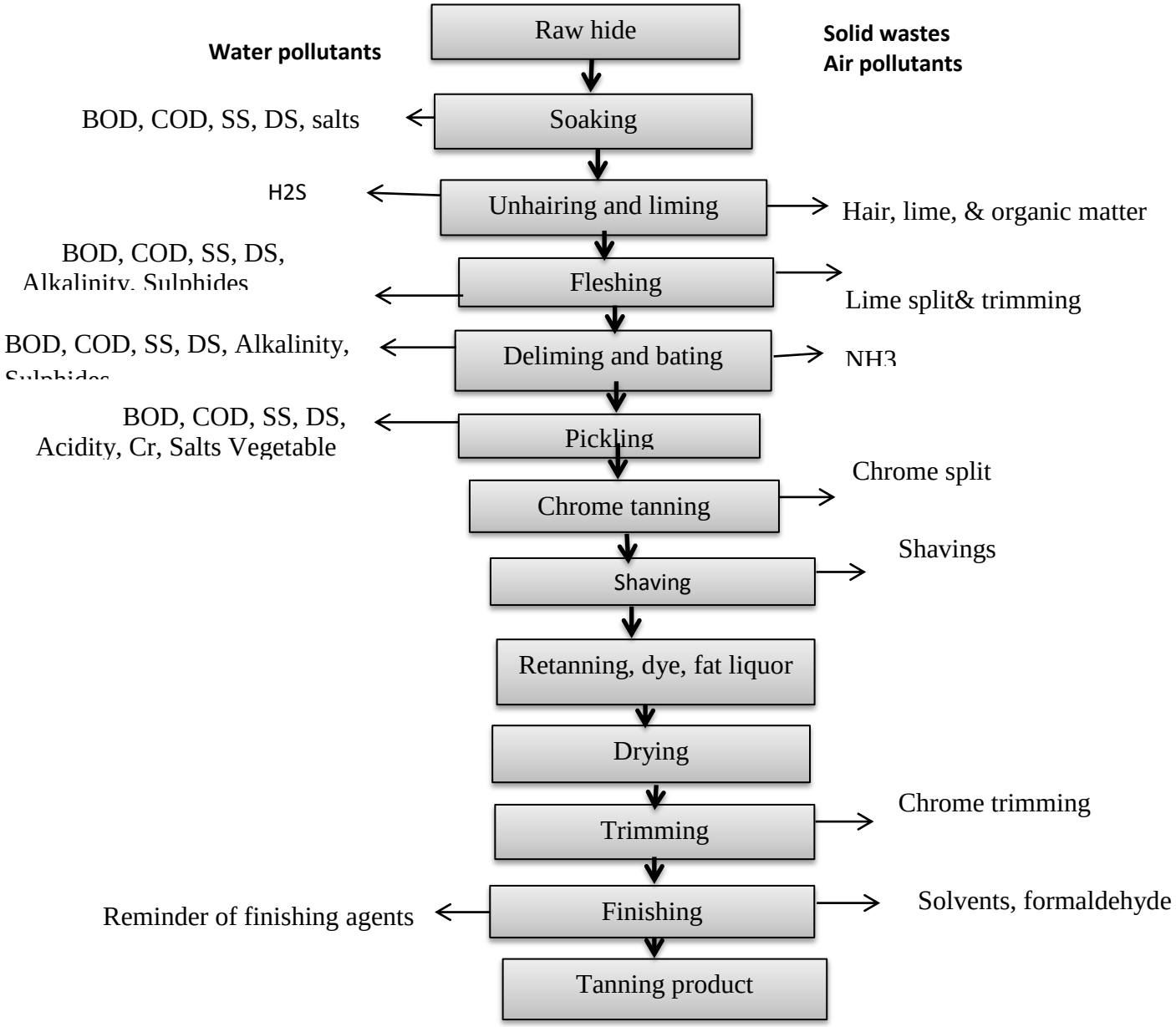



Figure 1: Waste generated from each unit operation of a tanning process

Therefore to prevent the environmental pollution due to tannery wastes generated from over 22 tanning industries in Ethiopia, emission limits values on discharge of the wastes of tannery to water has been established according to Ethiopian Environmental Impact Assessment guidelines for tanneries.

Table 1: Emission limit values for discharge to water

| Constituent Group or Parameter | Emission Limit Value (mg/L) |
|---|---|
| Temperature | 40 °C |
| pH | 6 – 9 pH units |
| BOD5 at 20˚C | >90% Removal or 200 mg/L |
| COD | 500 |
| Suspended Solids | 50 |
| Total Ammonia (as N) | 30 |
| Total Nitrogen (as N) | >80% Removal or 60 mg/L |
| Total Phosphorus (as P) | >80% Removal or 10 mg/L |
| Oils, Fats, and Grease | 15 |
| Mineral Oil (Interceptor) | 20 |
| Chromium (as total Cr) | 2 |
| Chromium (as Cr VI) | 0.1 |
| Chloride (as Cl) | 1000 |
| Sulphide (as S) | 1 |
| Phenols | 1 |

Source: EIA guidelines for tanneries, April 2005

## 2.3. Hexavalent chromium property and toxicity

The wastewater generated by tanneries is the major source of Chromium pollution. In the developing as well as in the developed countries, the industrial effluents are released directly or indirectly into the natural water resources, mostly without proper treatment, posing a major threat to the environment. The discharge of Cr (VI) into the ecosystem is a great challenge in the world, as well as in Ethiopia and it is a matter of concern in all tanning industries in the country. This pollutant enters into the environment mainly due to the use of chrome tanning process in almost all leather industries. Hexavalent chromium (Cr (VI)) is the most toxic and carcinogenic due to its high solubility in water, rapid permeability through biological membranes, and subsequent interaction with the intracellular proteins and nucleic acid. Thus, reduction of potentially toxic Cr (VI) to less toxic Cr (III) is a useful process for remediation of Cr (VI) affected environments(Ahluwalia, 2014).

The hexavalent species exists primarily as chromic acid ($H_2CrO_4$), and its salt; hydrogen chromate ($HCrO_4^-$) and chromate ion ($CrO_4^{2-}$) depending on the pH. The predominant species present as a function of pH are$H_2CrO_4$ at pH <1, $HCrO_4$- at1<pH<6 and $CrO_4^-$ at pH>6(Adeniji, 2004).

## 2.4. Tannery Wastewater Treatment Technology

Tanning industry worldwide generates approximately 40 million L of wastewater containing Cr (VI) every year(Sujita, 2014). In most of the countries, the tanning wastewater is discharged without proper treatment into the sewerage system causing serious environmental impact. Therefore here are the two basic technologies applied to treatment of Cr(VI) contaminated tannery wastewater such as conventional and biosorbent decontamination technology according to Amirnia (2015).

### 2.4.1. Conventional treatment technology

Conventional wastewater treatment technologies are the widely used, effective at high concentration of contaminant, expensive (cost and operation), environmental unfriendly and less efficient for low concentration of the contaminant. The conventional techniques available for the bio reduction of hexavalent chromium include chemical precipitation, chemical oxidation or

reduction, electrochemical treatment, evaporation, filtration, reverse osmosis, ion exchange, and membrane technologies(Bhateria & Dhaka, 2017). A summary of advantages and disadvantages of some metal treatment technologies is presented in **Table2**.

### 2.4.2. Biosorption technique

Various physiochemical techniques used for wastewater treatment can be applied to tannery wastewater (to the entire process or to individual step in the process) but these processes are expensive.

Biosorption is defined as the property of certain biomaterials to bind and concentrate selected ions or other molecules from aqueous solutions, and it can occur in both living and dead biomass. Metal uptake is a combination of a rapid metabolism independent process, followed by a slower one, associated to metabolism dependent processes known as bioaccumulation. Therefore, biosorption of heavy metals can be defined as metal removal by active and passive linkage in live and dead biomasses from aqueous solutions in a mechanism that is controlled and or not by metabolic steps. Biosorption process is known as an attractive biotechnological process which employs naturally abundant or waste biomass for removing most types of heavy metals from aqueous solutions. Biosorption is a bioremediation's emerging tool for wastewater treatment that has gained attention in the scientific community over the past three decades (Amirnia, 2015).

Table 2: Technology comparison for heavy metal treatment

| Technology | Strengths | Weaknesses |
|---|---|---|
| Ion Exchange | Commercially available,<br>Effective on co-occurring contaminants,<br>Well- understood,<br>Well-accepted by metal industry | Resin regeneration and replacement is costly,<br>Not effective on all metals,<br>Produces metal-laden waste brine,<br>Overall, high capital and operational/maintenance costs |
| Reverse | Effective removal | Capital intensive |

| | | |
|---|---|---|
| Osmosis | method<br>Accepted benchmark technology | Low through Produces Metal-laden waste<br>Membranes expensive<br>Easily foul up<br>Elevated pressure<br>sensitivity to suspended solids, organics |
| Chemical Precipitation | Effective<br>Low capital cost<br>Simple operation | Inadequate<br>Requires tight operational controls<br>Post treatment needs<br>Secondary sludge generation |
| Biosorption | Inexpensive<br>Metal selectivity<br>Easy operation<br>High Efficiency<br>High versatility<br>Tolerance to contaminants<br>Results in low volumes of high-concentration suitable for subsequent metal recovery | Not very well understood<br>difficulty in developing generic technologies |

**Source**:(Amirnia, 2015).

This above table shows that alternative methods such as biosorption could be a promising substitute to physico-chemical methods for removal of heavy metals.

## 2.5. Microbial Cr (VI) Biosorption Mechanisms

The mechanisms by which microorganisms accumulate/remove metals from solutions are: (i) extracellular accumulation/precipitation; cell-surface sorption or complexation; and (ii) intracellular accumulation.

Metal ion uptake by fungal biomass (both living and dead) is as follows. The first uptake mode involves the surface binding of Cr (VI) ions to the cell wall and extra-cellular material, which is metabolism independent. The second mode of Cr (VI) uptake into the cell across the cell membrane is dependent on the metabolic activity and is referred to as intra cellular accumulation. The first mode of metal uptake is common both in dead and living cells. Cr (VI) uptake by the second process, which is metabolism dependent, occurs only in the living cells(Gavrilescu, 2009) ,(S. Papirio et al, 2017)& (Mustapha, & Halimoon, 2015).

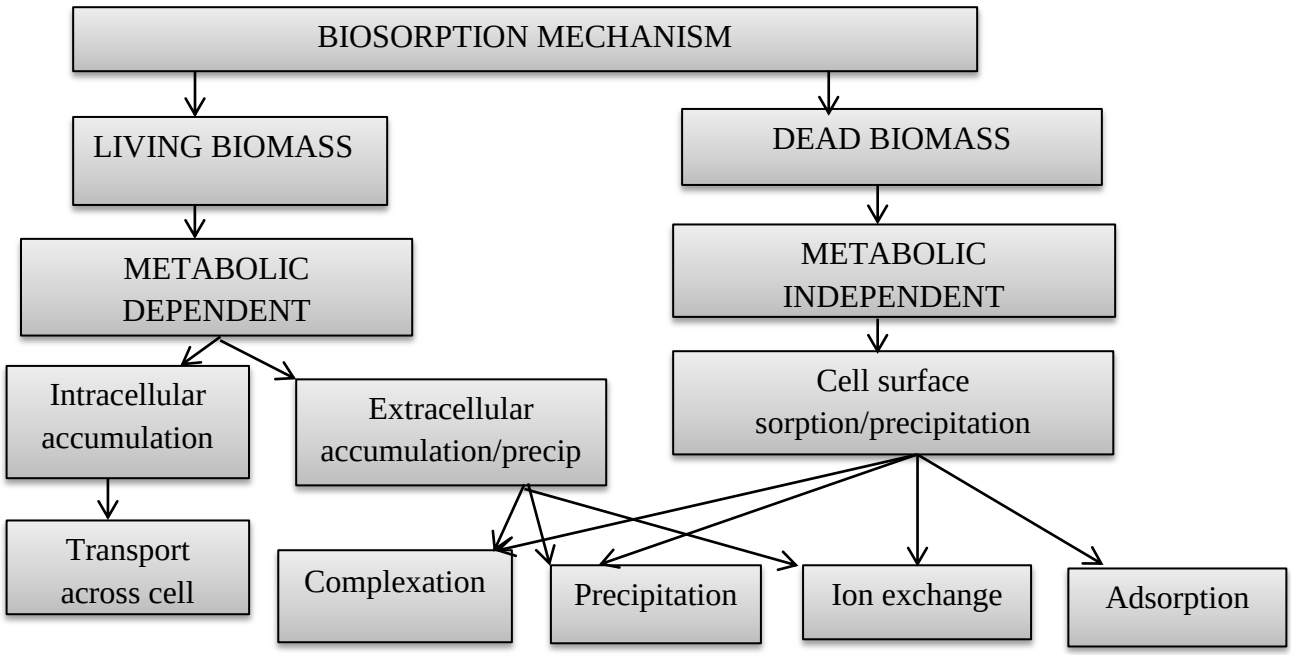



Figure 2: Flow diagram of biosorption mechanisms

The use of growing cells for metal removal has the advantage that the simultaneous removal of metal is obtained during growth of the organism and separate biomass production processes e.g., cultivation, harvesting, drying, processing. Also, the actively growing systems sequester metal through a combination of surface reactions, intracellular and extracellular precipitation and extracellular complexation reactions. However, major limitation of using growing systems for removal of metals is that cell growth is inhibited when the metal concentration is high, resulting in poor metal removal (Donmez &Aksu, 1999). This problem can be overcome by the use of metal tolerant organism. The tolerance and removal capacities are the essential characteristics of

growing biomass used in a metal ion removal process. The relation between the hexavalent and the trivalent states of chromium can be described by the following equation:

$Cr_2O_7^{2-} + 14\ H^+ + 6e \rightarrow 2\ Cr(III) + 7\ H_2O + 1.33\ V$

The study of Szpyrkowicz L and Kaul N. (2004) indicates that heavy metal removal process by *S. cerevisiae* biomass doesn't imply the formation of stable covalent bonds, but is predominantly realized by chemical interactions (ion-exchange). Chromium ions have strong interactions with functional groups hydroxyl (-OH),carboxyl(-COOH),sulfhydryl(-SH),(C=O (carbonyl) and N-H(amide) and (P=O)(Sujita, 2014)

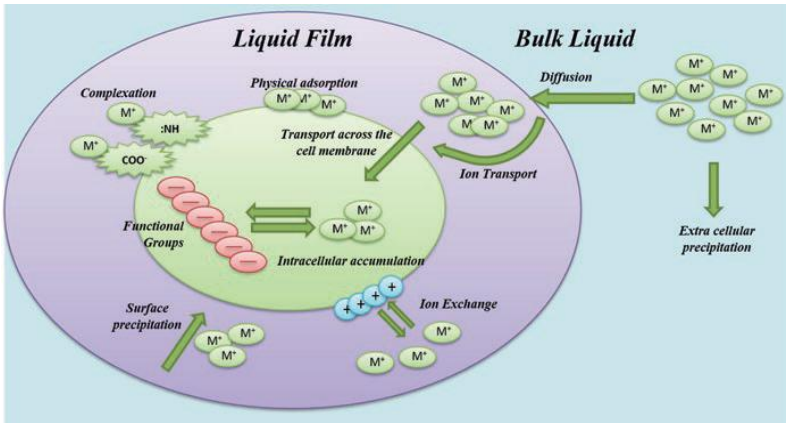



Figure 3: Illustration of the main mechanism involved in bioremoval of heavy metals

The above **Figures 2** and **3** reports a flow diagram and a schematic illustration, respectively, of the bioaccumulation mechanisms depending on the presence/absence of metabolism and the location of the metals sorbed. In Schematic illustration of the main mechanisms involved in bioaccumulation from **Figure 3**, metabolism-dependent mechanisms include the transport across the cell membrane and intracellular accumulation by reacting with active functional groups. In contrast, ion exchange, physical adsorption and complexation also occur in the absence of cellular metabolism(S. Papirio et al, 2017).

## 2.6. Bioaccumulation of Hexavalent chromium

Bioaccumulation is a biosorption technique that uses living cells to accumulate the hexavalent chromium from the Cr- contaminated environment (wastewater). Bioaccumulation of Cr(VI) using growing bacteria, fungi, yeast or algae cell biomass has been recognized as a potential

alternative to the existing conventional methods for detoxification of industrial wastewaters due to their heavy metal uptake capacities and suitability to be used as biosorbents(Sen & Dastidar, 2010 ). Biomass cell walls, consisting mainly of polysaccharides, proteins and lipids, offer many functional groups that can bind and accumulate metal ions such as carboxylate, hydroxyl, sulphate and amino groups(Islam et al., 2014).

The major advantages of the bioaccumulation process over the conventional treatment methods include(Farhan & Khadom, 2015):

- Low cost of the raw materials
- Increased metal removal
- Possibility of metal recovery

A variety of microorganisms have been identified as having the capacity to remove/accumulate Cr (VI) contamination such as:

**Bacteria**: Microbial Cr (VI) reduction was first reported in the late 1970s, when Romanenko and Koren'Kov (1977) observed a Cr (VI) reduction capability in Pseudomonas species grown under anaerobic conditions according to Ozgunay *et al.*,(2007). The active bacterial strain, isolated from sewage sludge, was classified as Pseudomonas dechromaticans. Since then, several researchers have isolated several microorganisms that catalyze the reduction of Cr(VI) to Cr(III) under varying conditions. Bacteria isolated from industrial effluents, especially those from tanneries, textile, electroplating and contaminated soil are endowed with the capacity to reduce Cr(VI) levels due to the nature of the cellular components, several functional groups are present on the bacterial cell wall, including carboxyl, phosphate, amine and hydroxyl groups(Abdi & Kazemi, 2015). However, biological treatment of Cr(VI)-contaminated waste water may be difficult because of toxicity of metal can kill the bacteria.

**Yeast**: Recently, it has been discovered that Cr(VI) detoxification occurs via extracellular reducing substances that are secreted by the yeast cells such as sulfate and riboflavin(Joutey *et al*, 2015). Indeed, many yeast strains are known to bio-transform Cr (VI) to the less toxic Cr(III including *S. cerevisiae*. Ksheminska *et al*. (2005) and Ksheminska *et al*. (2008) identified yeasts as convenient organisms to study bioaccumulation, because some strains are capable of growing

in matrices that have high concentrations of chromium compounds and adsorb or accumulate significant quantities into cells and transform them via chelation to less toxic forms.

**Fungi:** Fungal cell walls and their components have a major role in bioaccumulation and also take up suspended metal particulates and colloids. Fungi are ubiquitous in natural environments and important industrial processes. Most studies on fungi have claimed that Cr(VI) was removed from aqueous solution through an "adsorption mechanism", and that anionic chromate ions bind to positively charged groups (e.g., amines) of the living and dead fungal biomass. The chromium binding sites on fungal cell surfaces were most likely carboxyl and amine groups(Sen & Dastidar, 2010).

**Algae**: Algae are photosynthetic organisms. Both growing and non-living algal cells are capable of removing Cr(VI). The first step involved in the binding of Cr(VI) ions to algal species is binding to the cell surface. This process occurs rapidly and is independent of cellular metabolism. The second step of intracellular accumulation of a metal results from a simultaneous growth and surface bioaccumulation effects. This step requires cell metabolic energy and is a much slower process.

**Yeast**:

Ksheminska et al. (2008) identified yeasts as convenient organisms to study bioaccumulation, because some strains are capable of growing in matrices that have high concentrations of chromium compounds and adsorb and or accumulate significant quantities into cells and transform them via chelation to less toxic forms.

The uptake of chromium by yeast has also been shown to be a consequence of both, bioaccumulation and active accumulation mechanisms(Amirnia, 2015). Yeast's response to chromium stress involves various cellular processes such as oxidation–reduction reactions, interaction with cellular organelles, binding by cytosolic molecule. Yeast is able to accumulate high levels of both forms of chromium in relation to the background Cr concentration in the medium. Both living and dead cell biomass are used for removal of chromium even though there are advantages in using living cells over the dead cells as biosorbents. Living cells work the same way as dead cells at lower metal concentrations and are able to generate new cells through

growth, which allow more space for bioaccumulation mechanisms to occur(Ahluwalia, 2014). Dushenkov *et al.* reported that living cells could adsorb metal ions rapidly and provide a higher degree of separation. Indeed, many yeast strains are known to bio-transform Cr(VI) to the less toxic Cr(III) and S. cerevisiae is a typical example(Gavrilescu, 2009).

According to Abbas, S. H. *et al.*, (2014) yeast gives good, efficient and economical for sequestering heavy toxic metals from dilute aqueous solutions by bioaccumulation because: (i) It offers the advantage of having a high percentage of cell wall materials; (ii) It shows excellent metal binding properties; (iii) It is available in large quantities from the antibiotic and food industries; (iv) it provides an eco-friendly environment.

Therefore, yeasts are one of the most commonly used organisms in bioprocess engineering. Of these, *S. cerevisiae* strain is more recognized due to its high fermentation rates easy cultivation using inexpensive media and available in large quantities as industrial fermentation waste(Amirnia, 2015).

Van Wyk(2011) indicated in his study that there is an application of waste yeast for bioaccumulation of tin and chrome from metal containing industrial effluent and according to Wang and Chen(2006), *Saccharomyces cerevisiae* has received increasing attention due to its unique nature and capacity for metal sorption and is one of the most promising biosorbents capable of removing Cr (VI) from aqueous solutions.

## 2.7. Parameters that affect removal of Cr (VI)

Yeast metal accumulation is affected by many factors such as presence of anions and cations and soluble organic compounds in the solution and physicochemical parameters of the solution such as temperature, pH, initial metal ion concentration, biomass concentration and contact time of solution with yeast and to a limited extent on temperature. Particularly, pH, heavy metal concentration and incubation time on bioaccumulation experiments were investigated by optimization process(Farhan & Khadom, 2015).

PH of the solution affects the percentage of ionized groups of yeast cell wall; at low pH, the increase of protonation of yeast cell wall ligands decrease metals adsorption. On the other hand, the increase of pH can result in the precipitation of metals hydroxides, reducing metal

accumulation by the biomass. The presences of anions (carbonates, chlorides, fluorides, phosphates and sulphate) in solution can complex metal ions and thus reduce or inhibit their adsorption to S. cerevisiae. Chromium ion can also be complexed by organic compounds (ligands present in the effluents or/and cellular compounds); these soluble ligands can compete with the cells for the metals, reducing the efficiency of heavy metals removal. The following are typical factors such as pH, temperature, cell concentration, Cr (VI) concentration and contact time(Fomina & Michael, 2014).

### 2.7.1. Effect of pH

At low pH, the increase of protonation of yeast cell wall ligands decrease metals adsorption. On the other hand, the increase of pH can result in the precipitation of metals hydroxides, reducing metal accumulation by the biomass and it is best between 3-5 pH values (Juan, 2010). Bioaccumulation of anionic metal species (e.g. $CrO4^{2-}$ and $SeO4^{2-}$) is often enhanced under acidic conditions(S. Papirio et al, 2017).

### 2.7.2. Effect of temperature

Temperature has an influence on the bioaccumulation of metal ions, but to a limited extent under a certain range of temperature on the bioaccumulation of Cr(VI) and the microorganism is mesophilic growing at the temperature range of 20-35°C. According to (Farhan & Khadom, 2015) the increase of temperature indicates a decrease of sorption capacity and the maximum equilibrium uptake occurred at 27°C.

### 2.7.3. Effect of cell concentrations effect of initial concentration of Cr (VI)

Bioaccumulation has been observed to increase as initial concentration increases; this may be attributed to the active binding sites available for available sorbate ions. As initial pollutant concentration increases so as to the quantity of biosorbed pollutant per unit weight of biosorbent but decreases after all the active sites are occupied (Abdi & Kazemi, 2015).

### 2.7.4. Effect of contact time

The bioaccumulation capacity increases with an increase in time at constant temperature but decreases as the sorption site of the yeast gets occupied by the heavy metal(Farhan & Khadom, 2015)

### 2.7.5. Effect of shaking speed for bioaccumulation

When the speed of shaker increases so that biosorption removal rate of adsorptive pollutant by reducing its resistance of the mass transfer (Abdi & Kazemi, 2015).

## 2.8. Instrumentation for bioaccumulation research

Many analytical techniques can be used to study hexavalent chromium binding to biomaterials(Saha & Orvig, 2010). The functional groups on the sorbent surface that may involve metal ion sorption are usually investigated by FTIR spectroscopy. FTIR is used to characterize the functional groups present in the adsorbent and sheds very important light for chemical modification of adsorbent for better performance. UV-Vis spectrophotometer is used for the detection of hexavalent chromium. The absorbance of pink colored 1,5-diphenyl carbazide complex of hexavalent chromium is measured at 540 nm(Abdi & Kazemi, 2015) and this instrument is functional in determining the quantity of Cr(VI) in the solution.

## 2.9. Saccharomyces cerevisiae

*S.cerevisiae* is a well-known species of yeast used for baking and brewing industry as well as a bioaccumulation of mostly heavy metals to prevent the contaminated environment. It is an inexpensive, readily available source of biomass for bioaccumulation of waste water. It has been shown to accumulate heavy metals, with two distinct processes such as bioaccumulation, and intracellular accumulation. Survival of yeasts in toxic metal contaminated site is due to the presence of cell walls material within that shows excellent metal binding property and are known in detoxifying the toxics metals by the mechanisms such as intra and extracellular precipitation, active uptake and valence transformation.

Yeast cell wall consists mainly of polysaccharides, proteins and lipids. The functional groups that help in surface accumulation of heavy metal in yeast mainly are: carboxyl, amino, amide, hydroxyl, sulfhydryl and phosphate groups(S. Papirio et al, 2017).

Study of (Benazir et al., 2010) proved that *S.cerevisiae* had an efficiency of 95.6% in remedying chromium with a reduction rate of 1.829mg/L per hour and (Gavrilescu, 2009) showed that yeast *S. cerevisiae* has 99.5 % uptake efficiency of Cr (VI) bioaccumulation at the pH range of 4.5-5.5 and 25°C temperature with 120 mg/g uptake capacity. Another study by (Sujita, 2014) indicated that in a medium containing dichromate 20mg/ml more than 87% of dichromate ions was achieved within 72hours using yeast *saccharomyces cerevisiae*.

**Advantage of *S.cerevisiae***

As seen previously microorganisms including bacteria, algae, fungi, and yeasts are found to be capable of efficiently accumulating heavy metals. *Saccharomyces cerevisiae* was the first eukaryote to have its complete genome sequenced and this will undoubtedly lead to a new application. *Saccharomyces cerevisiae* is easy to cultivate at large scale. The yeast can be easily grown using unsophisticated fermentation techniques and inexpensive growth media. The biomass of *S. cerevisiae* can be obtained from various food and beverage industries. *S. cerevisiae* as a by-product is easier to get from fermentation industry, in comparison with other types of waste microbial biomass(Farhan & Khadom, 2015).

When choosing biosorbent/bioaccumulant, for large-scale industrial uses, the main factor to be taken into account is its availability and cheapness(Abbas, S, H et al.,2014) which is characteristics of *S.cerevisiae* and this is why we choose *S.cerevisiae* as a biosorbent of Cr(VI)for bioaccumulation of tannery waste water.

# CHAPTER THREE

## 3. Materials and Methods

### 3.1. Chemicals and reagents

Chemicals needed were1,5-diphenylcarbazide(DPC, 98% AR), $H_2SO_4$ (98%), Acetone (50% acetone), yeast extract (cas no 8013-01-2), peptone, dextrose (cas no 50-99-7), Potassium dichromate($K_2Cr_2O_7$), $HgSO_4$,NaOH, $HNO_3$, Thiosulphate, HCl, $H_2O_2$, ferrion, FAS (standard ferrous ammonium sulphate). The chemicals used during the course of this work were all of analytical grade.

### 3.2. Equipments used

Most of the Equipments used were available in the laboratory of school of Chemical and Bio Engineering Department, Addis Ababa Institute of Technology, Addis Ababa University.

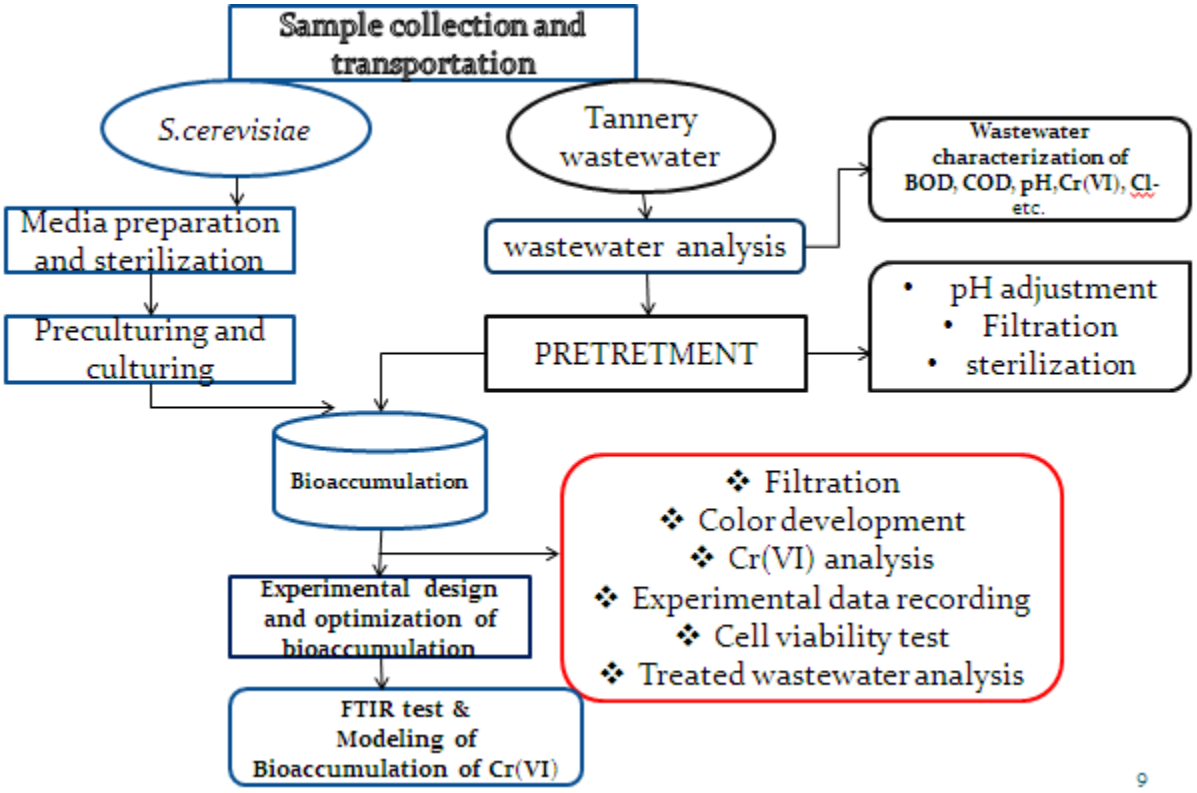
Equipments required to conduct this research work were Refrigerator (model: RMN 410), pH-meter (model: HANNA edge), UV-Vis spectrophotometer (Labomed. Inc.U.S.A), tannery wastewater, *Saccharomyces cerevisiae*, flasks, polyethylene bottles, test tubes, incubator shaker and Whatman 1filter paper, autoclave and microbiological hood.

### 3.3. Sample collection and preparation

Tannery wastewater was collected in clean polyethylene bottles from the outlet point of chromium tanning stage of Dessie Tannery Industry, Dessie, Amhara, located 387.4Km far from the city of Addis Ababa and *S.*cerevisiae was obtained from Ethiopian Biodiversity Institute found in Addis Ababa, Ethiopia. Those plastic collecting bottles were first cleaned by 8M nitric acid ($HNO_3$) solution, washed with distilled water three times and dried overnight. Because Cr (VI) is an oxidizer, care was taken during sampling and sample preparation of Cr-contaminated wastewater. Sampling and sample preparation procedures often involve changing the sample pH and in this work the pH was adjusted to 7, which may result in changes in the relative concentration of the oxidation states. In addition refrigeration of the sample and immediate

analysis are the main protocols for maintaining the integrity of sample. Therefore after collecting the sample it was stored at 4°C to minimize the chemical reactivity until analysis. For efficient removal of hexavalent chromium by the microbe, the tannery effluent sample was first filtered with whatman#1 filter paper to remove settleable solids and then the pH was adjusted to 7 using NaOH before addition of the yeast.

## 3.4. Experimental framework




## 3.5. Characterization of tannery wastewater

The characteristics of the waste water sample such as pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), TDS, chlorides, sulphide, sulphate, conductivity, salinity and Cr(VI) were determined according to the standard methods for the examination of water and waste water.

### 3.5.1. Determination of pH, conductivity and salinity

The pH was determined by measuring by digital pH meter (model: HANNA edge).Both conductivity and salinity were measure by using conductivity meter (APHA, 1999).

### 3.5.2. Determination of BOD

The sample was incubated at 20°C for five days in air tight bottles and dissolved oxygen (DO) was measured initially and after incubation period, BOD was calculated from the difference between these two values. Biochemical oxygen demand of an effluent is the milligram of oxygen required to biologically stabilize one litter of that effluent (by biodegradation of organic organic compounds with the help of microorganism) in 5 days at 20°C. If the $BOD_5$ value of an effluent is high, then that effluent contains too much of biodegradable organic compounds and so will pollute the receiving water highly.

**Procedure**

5 liters of distilled water were taken, aerated for 3.5 hours, added nutrients 1 ml nutrient for 1 liter aerated distilled water (FeCl, $CaCl_2$, $PO_4$, $MgSO_4$, domestic water), aeration for 30 minutes. And in the BOD bottle (300 ml), sample was added and filled with aerated water, covered by lid (avoid air bubbles), keeping BOD incubator at 20°C for 5 days. After 5 days the bottle was taken out and 2 ml $MnSO_4$, 2 ml alkaliazide iodide and 2 ml conc. $H_2SO_4$ were added. Shake the bottle well (yellow color) take 200 ml sample add starch solution as indicator (purple color) titrated with 0.025 N Sodium Thiosulphate end point color change from purple to colorless. Blank bottle was filled with aerated water without the sample and the procedure was followed.

**Calculation**

$$BOD_5 = (blank\ value - titerted\ value) * \frac{300}{volume\ of\ sample}$$

### 3.5.3. Determination of COD

The chemical oxygen demand of an effluent means the quantity of oxygen, in milligram, required to oxidize or stabilize the oxidizable chemicals present in one litter of effluent under specific condition. 2.5 ml of the sample was taken in tube, 1.5 ml of 0.25 N $K_2Cr_2O_7$(potassium dichromate), spatula of Mercuric sulphate $HgSO_4$ and 3.5 ml of COD acid were added and kept

in COD reactor for 2hrs at 150$^{o}$C. After cooling the sample titrated against FAS (standard ferrous ammonium sulfate 0.1N) and used ferrion as indicator. The end point is reddish brown color. In the blank tube 2.5 ml of distilled water was taken and then follow the same procedure in the sample(Islam et al., 2014).

**Calculation**

$$COD\left(\frac{mg}{L}\right) = (blank\ value - titerated\ value) * N\ of\ FAS * \frac{8000}{volume\ of\ sample}$$

8000=mill equivalent wt of $O_2$ *1000ml

### 3.5.4. Determination of chloride

Chloride is determined in a natural or slightly alkaline solution by titration with standard silver nitrate, using potassium chromate as an indicator. Silver chloride is quantitatively precipitated before red silver chromate is formed.

**Procedure**

Take sample (10 ml to 50 ml), add 2 ml of hydrogen peroxide ($H_2O_2$), add 2 ml $K_2CrO_4$ (potassium chromate indicator), titrate with silver nitrate (0.0141 N), end point formation of reddish yellow color (yellow to orange). In blank trial take distilled water instead of sample and follow the same procedure above (Hassen & Woldeamanuale, 2017).

**Calculation**

$$Chloride\left(\frac{mg}{L}\right) = (A - B) * N.of\ silver\ nitrate * 33.45 * \frac{1000}{volume\ of\ sample}$$

A = ml titration for sample, B = ml titration for blank, N = normality of $AgNO_3$

### 3.5.5. Determination of sulfide

The sulfides in the solution are oxidized with and excess of a standard iodine solution and the excess back titrated with a standard thiosulfate solution.

**Procedure**

10ml of sample was taken in conical flask, 5 ml zinc acetate (5%) was added, filtered through filter paper and the filter paper was removed and putted in the same conical flask by adding 100 ml distilled water. Then 20 ml iodine solution and 4 ml 6N HCl, and 2 drops of starch were added as an indicator (purple color will form), titrated against sodium thiosulphate (0.025N),

end point for the color change from blue color to colorless. In the blank test 100 ml distilled water was taken instead of sample and followed the same procedure as above for the sample.

**Calculation**

$$Sulfide\left(\frac{mg}{L}\right) = (BV - TV) * N.thio * \frac{400}{volume\ of\ sample} * N.iodin$$

BV= blank value, TV= titrated value

### 3.5.6. Determination of TDS

Dissolved solids are minerals, salts, cations and anions. Therefore total dissolved solids are comprised of inorganic salts (basically calcium, magnesium, potassium, sodium, bicarbonates, chlorides and sulphates) and some of small amounts of organic matter that dissolves in water (www.water-research.net).

The sample was filtered through a Whatman filter paper and a known volume of the sample was evaporated on a weighed china dish, dried and from the weight of the residue TDS were calculated.

**Procedure**

A well –mixed sample was filtered and 50ml volume of the sample was pipetted after washing into a known weight of evaporating dish. Then the sample was evaporated and dried at 105°C oven, cooled in a desiccator and finally weighed.

- **Calculation**
  - Total dissolved solid in mg/L= $\frac{A-B}{ml\ of\ sample} * 1000$. Where, A=weight of solid +weight of paper, B= weight of paper

### 3.5.7. Determination of Cr (VI)

The concentration of Cr (VI) was determined spectrophotometricaly using 1,5-diphenylcarbazide as a complexion agent. Diphenylcarbazide (DPC) stock solution (0.25%w/v in 50% acetone) was

prepared. A volume of 15ml of sample were pipetted out into 25mL standard flasks. After addition of 1mL of DPC the volume of solution was made up to 25mL using distilled water. The absorbance of the purple-colored solution was measured using UV-Vis spectrophotometer (Labomed. Inc. U.S.A) at 540nm and was allowed to stand for 10min. Cr (VI) concentrations were extrapolated from the standard curve prepared from the standard solutions of potassium dichromate.

- Cr(VI)reacts with the color reagent DPC in the following reaction;
  - $2CrO_4+3H_4L+8H^+$……Cr (iii)$(HL)_2^+$+$Cr^{3+}$+$H_2L$+$8H_2O$,

Where, $H_4L$=DiPhenylCarbazide, $H_2L$= DiPhenylCarbazone.

**Preparation of hexavalent standard calibration curve**

100ml of five flasks were prepared and to each flasks 0, 6, 10, 15, 20, and 30 ml of chromium working standard solution were added. These flasks respectively contain 0, 60, 100, 150, 200 and 300ug$Cr^{+6}$. 6N $H_2SO_4$ was used to adjust the pH of the solution to 2±0.5 and 2ml of Diphenylcarbazide (DPC) solution were added to the acidified solution, mixed well and the solution were stand for 10±5 minute until the red violet/purple color is developed. Then the solution is transferred to cuvette and read the absorbance of the sample at 540nm using cuvette after setting the UV-spectrophotometer to zero absorbance with a reagent blank. Finally, read the absorbance and draw a standard curve.

## 3.5. Bioaccumulation of Cr (VI) assay in liquid media

*Saccharomyces cerevisiae* (bakery yeast) were grown at different pH-values, chromium concentration and incubation time to investigate the effect of these factors for bioaccumulation of Cr (VI) from the effluent. The process involved steps such as sterilization of apparatus, culture media (YPD) preparation, pre-culturing and culturing and bioaccumulation study.

### 3.5.1. Sterilization of apparatus

Cleaning of glassware used in the experiments such as the Petri-plates, media bottles, deionized water, syringes and sample collecting bottles was done as follows: first, it was washed with detergent solution, rinsed with tap water, rinsed with 10 % nitric acid, rinsed with tap water, rinsed with distilled water and finally sterilized in autoclave at 121°C for 15minutes to prevent metal binding to glasses and microbial contamination.

### 3.5.2. Preparation of culture media (YPD)

According to many literatures the best media for the growth of *S.cerevisiae* is YPD sometimes called YEPD made from yeast extract, peptone and dextrose in a ratio of 1:2:2 dissolved in distilled water.

### 3.5.3. Strain and culture conditions

In this work, *S. cerevisiae* collected from Ethiopian biodiversity institute were used. The strain was routinely maintained at 4 °C, on YPD agar slants.

Pre-cultures were prepared in 40 ml of YPD broth in 100 ml Erlenmeyer flasks and cells were incubated at 27 °C on an orbital shaker at 200 rpm, during 24 h. Cultures in YEPD broth were prepared by inoculating 1 l of culture medium in 2 l Erlenmeyer flasks with 4% (v/v) from pre-cultures. Cells were incubated in the same conditions of the pre-culture for 72 h.

### 3.5.4. Batch Bioaccumulation study

A bioaccumulation process can be performed via several modes; of which, batch and continuous modes of operation are frequently employed to conduct laboratory scale bioaccumulation processes. Although most industrial applications prefer a continuous mode of operation, batch experiments have to be used to evaluate the required fundamental information, such as bioaccumulation efficiency, optimum experimental conditions, and bioaccumulation rate.

The cell suspensions of the flask from the late exponential growth phase (72 h) were added to 250 ml plastic flasks of containing 50ml of YPD media, different initial Cr (VI) concentration

and pH value for different contact time and shaken at 200 rpm, at 27◦C. *Saccharomyces cerevisiae* was used in this study. Bioaccumulation is largely dependent on pH, chromium concentration and contact (incubation)time and to optimize the experimental conditions, parameters like pH(3,5, 8),initial chromium concentration(5,50,100mg/ml) and incubation time(3,5,7 days) were investigated. At the end of selected incubation period, tannery industry waste water treated with *S.cerevisiae* were taken from the incubator and filtered by Whatman 1 filter paper. For 20ml of filtrate a 1ml of DPC were added and stand for 10minutes until the pink color develops. Then the colored filtrate absorbance were determined using UV spectrophotometer and by referring to the calibration curve of the absorbance, obtained Cr (VI) concentration in treated tannery waste water for different waste process parameters using *S.cerevisiae* were calculated. The percentage removal of Cr (VI) in tannery industry waste water is calculated respectively as follows:

Percentage uptake of Cr (VI) $=\frac{CO-C1}{CO} * 100$

Percentage of removal of Cr(VI)= where $C_0$ and $C_1$ are the initial (before treatment) and after treatment concentration of Cr(VI) solution( in mg/L), respectively.

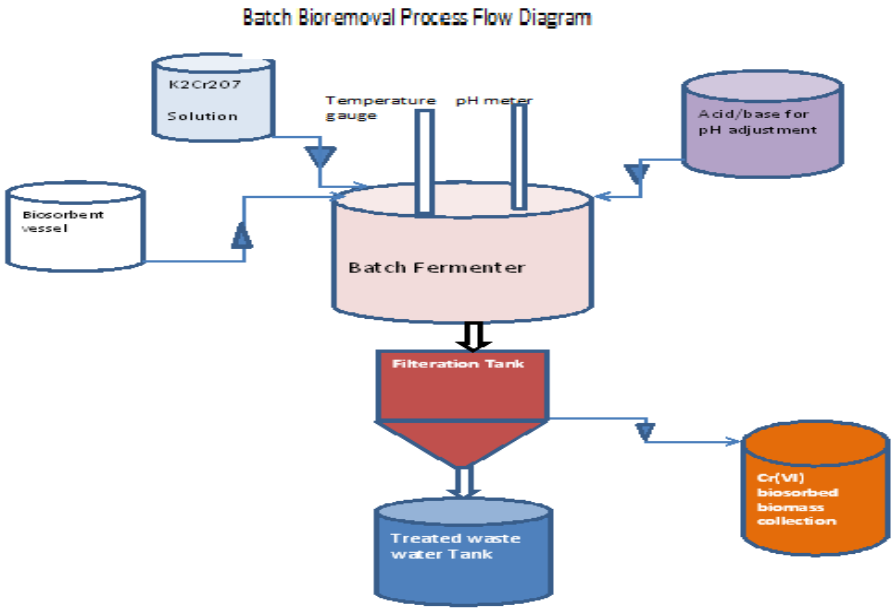



Figure 4: Process flow diagram of batch bioaccumulation process of Cr(VI)

## 3.6. Optimization of Cr (VI) bioaccumulation conditions and experimental design

Batch bioaccumulation system is used to remove Cr (VI) from contaminated sample solution and optimize various process parameters influencing the bioaccumulation. The effects of pH (3,5 &8), initial Cr(VI) concentration(5,50 &100 mg/ml) and incubation time(3,5 &7) were examined to maximize the removal of Cr(VI) from the solution. Laboratory experiment was done based on full factorial design with two replications. Bioaccumulation process parameters were analyzed for their single effects and their interaction effects on optimum bioaccumulation of Cr(VI).

| Factors | Level | | |
|---|---|---|---|
| | Minimum | Medium $(\frac{min+max}{2})$ | Maximum |
| Incubation time | 3 | 5 | 7 |
| PH | 3 | 5.5 | 8 |
| Initial Cr(VI)conc. | 5 | 52.5 | 100 |

**number of run** $= \mathbf{r} * \mathbf{L^k}$, where, $r =$ replication, $L =$ number of level, $k =$ number of factor. number of run $= 2 * 3^3 = 54$

## 3.7. Statistical analysis on factors affecting Bioaccumulation of Cr (VI)

Bioaccumulation of Cr(VI) from tannery wastewater is affected by different factors such as incubation time, pH, initial concentration of contaminant. The experimental design selected for this study was general factorial method and the response measured was the percentage uptake of Cr(VI) by the yeast cell. The three bioaccumulation process variables studied were incubation time, pH and initial Cr(VI) concentration. Design Expert 7.0.0 software was used in analysis of variance.

The response of the bioaccumulation process was used to develop a mathematical model that correlates the percentage of uptake of Cr(VI) to the bioaccumulation parameters. The model fit

summary for the response Cr(VI) uptake efficiency and statistical analysis of the ANOVA are given in **Appendix C** and **Appendix D** respectively. The multiple regression coefficients were obtained by employing a least square technique to predict a quadratic polynomial model for the Cr(VI) uptake efficiency.

## 3.8. Colony forming unit assay for cell viability assessment

Cell viability test is a test quantifying the viable cell. For the determination of tolerance to hexavalent chromium, cell viability test using a serial dilution colony forming unit count method was chosen. In order to count the colony forming units a 1ml of the diluted (1/10) cell suspensions from control and treated solution of,5mg/ml, 50mg/ml and 100mg/ml Cr(VI) containing solutions were serially diluted from $10^{-1}$-$10^{-4}$ with sterile water to a final volume of 10 ml in test tubes containing 9 ml of sterile distilled water and 1ml of the serial diluted solution was inoculated aseptically in a fresh prepared YPD agar at 37°C for 48hr and colony forming unit was counted using microscope after 48hrs. Total colony forming unit per unit volume is calculated as follows:

- The total colony count (CFU/mL)=No. of colonies*DF/volume of culture plate

## 3.9. Modeling of batch bioaccumulation system

Bioaccumulation of Cr (VI) from tannery wastewater using living *S.cerevisiae* is a two steps process as discussed previously (**section 2.5)** which are extracellular (surface bioaccumulation) and intracellular assimilation. Extracellular bioaccumulation is the first and fast process step of Cr(VI) bioaccumulation while intracellular accumulation is the final and slow process step of Cr(VI) bioaccumulation by living cells. Therefore extracellular bioaccumulation of Cr(VI) can be mathematically modeled using both batch equilibrium and kinetic models and the intracellular accumulation of Cr(VI) by living yeast cell can be analyzed by results of FT-IR.

Parallel to the extensive experimental activity carried out during the last decades on heavy metal biosorption, several mathematical models, most in the form of empirical correlations, have been developed to elucidate and represent the heavy metal adsorption on biomass binding sites (Volesky, 2003). Mathematical modeling represents a useful tool to describe the complex

mechanisms characterizing the biosorbent and the solute interactions and assists in the optimization and design of biosorption processes. Because of flow and other continuous systems are more complex and many column studies use 'breakthrough curves' to assess sorbent efficiency a batch bioaccumulation modeling system is preferred. Batch equilibrium bioaccumulation studies can provide useful information on relative bioaccumulation efficiencies and important physico-chemical factors that affect bioaccumulation; but they have drawbacks such as they usually provide no information on mechanisms and equilibrium uptake values not being attained (e.g. if insufficient incubation time is allowed or where sorbate concentrations are low and biomass concentrations high(Gadd, 2009). Modeling is very useful for scale up studies or process optimization and a number of models with varying degrees of complexity have been developed to describe the metal bioaccumulation systems. These are of two types: equilibrium models and kinetic models.

### 3.9.1. Equilibrium models

An accumulation isotherm is used to characterize the interaction of metal ions with the biosorbents/bioaccumulants. This provides a relationship between the concentration of metal ions in the solution and the amount of metal ions accumulated to the biosorbent when the two phases are at equilibrium and like any other adsorbate-adsorbent systems; isotherms are regularly used to describe the metal bioaccumulation data. Among them, Langmuir model (Langmuir, 1918) and Freundlich model (Freundlich, 1906) are commonly used by many researchers, and the adsorption equilibrium of metal ions is usually very well-described by these models.

The mathematical expressions of Langmuir and Freundlich equation are:

$$\frac{Ceq}{Q} = \frac{1}{b * Qmax} + \frac{Ceq}{Qmax} \dots\dots\dots \text{langmuir equation}$$

$$\ln Qe = \ln k + \frac{1}{n} * \ln Ce \dots\dots\dots \text{freundlich equation}$$

Where, Ceq is the liquid phase concentration of Cr(VI), b is Langmuir constant, K is constant, 1/n is the intensity of adsorption and Q is specific metal-uptake; Qmax is the maximum chromium uptake.

### 3.9.2. Kinetic model

The study of accumulation dynamics describes the metal ion uptake rate, and this rate controls the habitation time of metal ion uptake at the sorbent-sorbate interface. Chemical kinetics gives information about reaction pathways and times to reach equilibrium. Accumulation/sorption kinetics is largely dependent on the physical and/or chemical characteristics of the sorbent material. Different models have been used to investigate the mechanism of sorption and pseudo first order (Lagergren, 1898) and pseudo second order (Ho and McKay, 1999) kinetics models are used for this work since many scholars are used them in most of their work.

**Pseudo first order**: Lagergren´s pseudo-first-order model (1898) is based on the assumption that the rate of bioaccumulation is proportional to the number of free active sites on the biosorbent´s surface. The pseudo-first-order kinetic model is expressed as follows:

$$\frac{dqt}{dt} = k1(qe - qt) \qquad \text{(a)}$$

Where k1 is rate constant of first order bioaccumulation, qe metal accumulated at equilibrium (mg/g) is amount of heavy, qt (mg / g) is the amount of metals accumulated at any given time *t* (min)

By integrating Eq. (a) over the boundary conditions of *qt* =0 at *t* = 0, and *qt* = *qt* at *t* =*t* , Eq. (a) can be written as follows;

$$\ln(qe - q_t) = -k1 * t + \ln(q_e)$$

**Pseudo second order:** HO's pseudo-second-order kinetic model (Ho and McKay, 1999) is given as follows:

$$\frac{dq_t}{dt} = k2(q_e - q_t)^2 \qquad \text{(b)}$$

Where *k2* is the rate constant of pseudo-second-order adsorption (*g* /*mg*.min). By integrating Eq.(b) over the boundary conditions of *qt* =0 at *t* = 0, and *qt* = *qt* at *t* =*t* , and rearranging the following linear form of the pseudo-second-order kinetic model is obtained;

$$\frac{t}{q_t} = \frac{1}{q_e}t + \frac{1}{k2q_e{}^2}$$

# CHAPTER FOUR

## 4. Results and Discussion

### 4.1. Characterization of collected Tannery wastewater

The physico-chemical analysis of untreated tannery wastewater has shown alkaline pH, high COD, color, TDS, , chloride, sulphate and Cr (VI) as mentioned in **Table3**. The values of all the parameters measured are above the discharge limit value as seen in **Table1** and **Table3**. The electrical conductivity was also high due to the presence of inorganic substances and salts whereas elevated amount of COD may be due to high amount of organic compounds which are not affected by the microbial decomposition.

Table 3: Characteristics of tannery wastewater

| Parameter | Value | |
|---|---|---|
| | Before treatment | After treatment |
| pH | 4 | 4 |
| Temperature | 30°C | 25 |
| Color | Dark green | Turbid white |
| TDS | 6000mg/L | 7.8 |
| COD | 4325.9Mg$O_2$/L | 4182.5mg$O_2$/L |
| $BOD_5$ @20°C | 823mg/L | 820mg/L |
| Sulphide | 5mg/L | 4.1mg/L |
| EC | 16.04mS/cm | 8.55mS/cm |
| Cr(VI) | 0.5mg/L | 0.009mg/L |
| $Cl^-$ | 7047.5mg/L | 7012mg/L |
| $SO^{4-}$ | 710.0mg/L | 500mg/L |
| Salinity | 930ppm | 875ppm |

As seen from **Table 3** the characteristic parameters value of the tannery wastewater discharged from the point of spent chrome is high compared to the permissible discharge limit set by

Ethiopian Environmental Impact Assessment guidelines' shown in **Table 1** including Cr(VI) concentration that needs special attention because of its hazardous property to the environment .

Generally, the processing of hides and skins into leather is carried out in an aqueous medium and hence the discharged water from pits, drums or paddles containing several soluble and insoluble constituents of effluents from the tannery. In the present study, investigation of results from characterization of the tannery wastewater from spent chrome discharge point showed that the tannery wastewater from chrome tanning is with a highly disagreeable pH, salinity, total solids, total dissolved solids, suspended solid, chemical oxygen demand, biochemical oxygen demand, Cr (VI) concentration ,chlorides and sulfides. The results of the analysis indicate that the wastewaters from chrome tanning unit of the tannery do not satisfy the legal ranges of selected parameters and the designed treatment technology reduces the Cr(VI) from 0.5mg/L to 0.009mg/L.

## 4.2. Calibration curve used for analysis

The calibration curve for $Cr^{+6}$ analyses was prepared from 0,4,6,8,10,20,40.80 and 100µg/ml of $K_2Cr_2O_7$ standards. The linear calibration curve with equation, absorbance=0.0048Conc+0.016, where absorbance units miliabsorbance-$min^{-1}$and concentration is measured in µg/ml, gave a correlation coefficient $R^2$>0.9837.

Table 4: Absorbance vs. concentration

| Cr(VI)conc.(ug/ml) | Absorbance |
|---|---|
| 0 | 0 |
| 4 | 0.025 |
| 8 | 0.07 |
| 10 | 0.088 |
| 20 | 0.13 |
| 40 | 0.16 |
| 60 | 0.32 |
| 80 | 0.4 |
| 100 | 0.5 |
| ∑b= | 0.0048 |
| $R^2$= | 0.9837 |

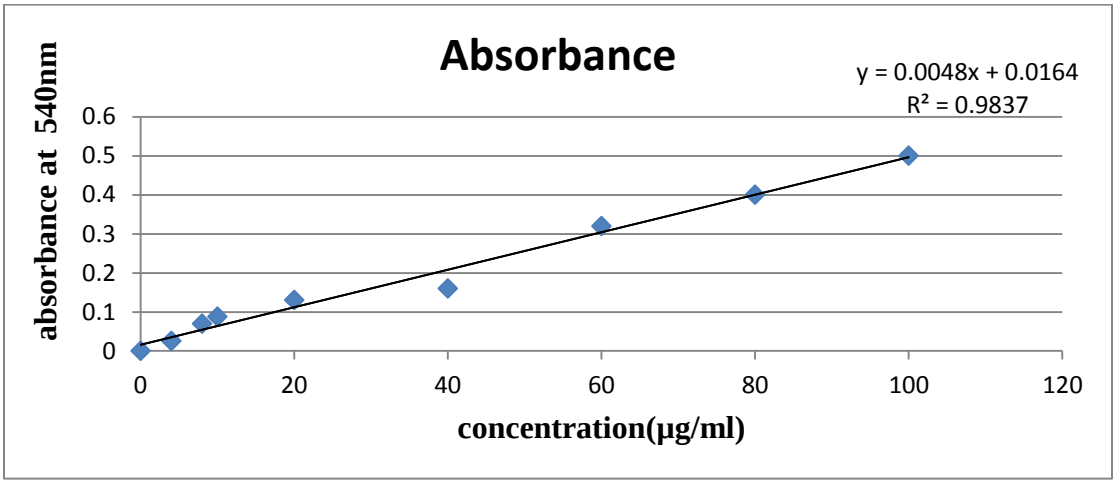



Figure 5: Concentration vs. absorbance standard calibration curve

Figure 6: Standard solution and measurement of its absorbance using UV-spectrophotometer

### 4.3. UV-Vis spectroscopy analysis of Cr (VI)

A calibration equation ($y$ = 0.0048$x$ + 0.0164, $R2$ = 0.9837, where $y$ is absorbance and $x$ is concentration in µg/ml) derived from a calibration curve is used for the quantitation of Cr(VI) in wastewater samples after treatment.

Figure 7: Color developed by the filtrates of Cr(VI) solution left after bioaccumulation

- For samples with absorbance values greater than the working range of the standard curve must be diluted with a reagent blank (distilled water) and reanalyzed and the dilution factor (DF) must be recorded according to the standards of hexavalent determination.
- Then,

$$\textbf{Total amount of Cr(VI) in a sample} = \textbf{amount of Cr(VI)}\left(\frac{\mu g}{ml}\right)\textbf{left in the sample} * \textbf{dilution factor(DF)} \quad (2)$$

Final concentration of Cr(VI)=$C_f$*DF

The actual experimental data including the percentage uptake of Cr(VI) at different bioaccumulation parameters by using UV-Vis spectroscopy was calculated from the absorbance curve and is given in **Appendix B**.

### 4.4. Development of empirical model

Empirical models for the output response of Cr(VI) uptake efficiency in terms of the bioaccumulation parameters in actual and coded factors were developed by using general factorial methodology. The sequential model sum of squares, Lack of Fit Tests and Model Summary Statistics are given at **Appendix C** and was found that the model was suitable for the

present study; because quadratic model had high R-squared, adjusted R-squared and predicted R-squared for response of Cr(VI) uptake efficiency .

## 4.5. Model adequacy check

It is essential to confirm whether the fitted model gives an adequate approximation of actual value or not. The model was tested for adequacy by analysis of variance. The regression model was found to be significant with the correlation coefficients of R-squared(R), adjusted R-squared and predicted R-squared having values of 0.9219, 0.9059 and 0.8732 respectively as indicated in **Appendix C**. The quality of the model developed could be evaluated from their coefficients of correlation. The value of R-squared for the developed correlation is 0.9219 implies that 92.19% of the total variation in the Cr (VI) uptake efficiency is attributed to the studied experimental variables.

The adequacy of the model was further checked with analysis of variance (ANOVA) as shown at the end of **Appendix D** and the graph of the predicted values obtained using the developed correlation versus actual value is also shown in **Figure 9**.

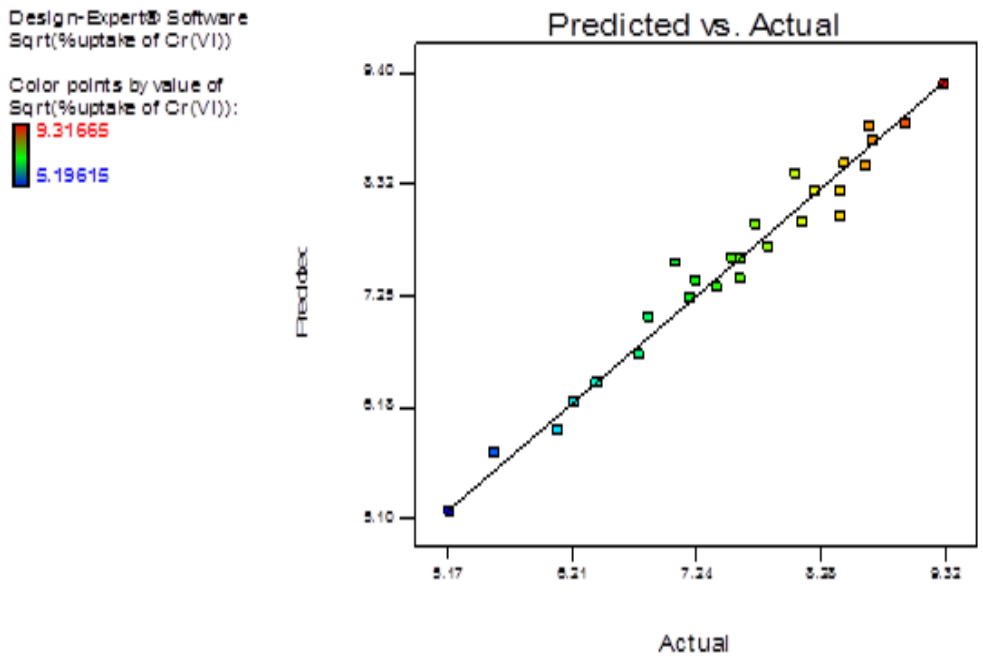



Figure 8: Predicted vs. actual Cr (VI) uptake efficiency

This plot represents the line of perfect fit with the points corresponding to zero error between predicted values and actual values and demonstrated that the regression model equation provided accurate description of the experimental data, in which all the points are close to the line of perfect fit. This result indicates that it was successful in capturing the correlation between the three bioaccumulation variables to the Cr(VI) uptake efficiency.

As shown by **Table 5** Values of "Prob > F" less than 0.0500 indicate model terms are significant.In this case A, B, C, AB, $A^2$, $B^2$are significant model terms. This shows that individual factors such as incubation time, pH and initial Cr (VI) concentration and interaction between incubation time and pH affects the response of Cr(VI) uptake efficiency much significantly. Each bioaccumulation variable effects and interaction effects of incubation time and pH on Cr (VI) are discussed with a help of figures in the subsequent section (**ref. section 4.8**)

Table 5: Analysis of variance (ANOVA) for the Regression Model Equation and Coefficient

| Source | Sum of Squares | DF | Mean Square | F Value | p-value Prob > F | remark |
|---|---|---|---|---|---|---|
| Model | 11414.20 | 6 | 1902.37 | 86.14 | < 0.0001 | significant |
| A-time | 911.47 | 1 | 911.47 | 41.27 | < 0.0001 | |
| B-pH | 4596.73 | 1 | 4596.73 | 208.15 | < 0.0001 | |
| C-initial Cr(VI) conc. | 1504.79 | 1 | 1504.79 | 68.14 | < 0.0001 | |
| AB | 306.72 | 1 | 306.72 | 13.89 | 0.0005 | |
| $A^2$ | 1351.88 | 1 | 1351.88 | 61.22 | < 0.0001 | |
| $B^2$ | 2023.39 | 1 | 2023.39 | 91.62 | < 0.0001 | |
| Residual | 1037.92 | 47 | 22.08 | | | |
| Lack of Fit | 647.06 | 20 | 32.35 | 1.2 | 0.3284 | Not significant |
| Pure Error | 390.86 | 27 | 14.48 | | | |
| Cor Total | 12452.12 | 53 | | | | |

Since values greater than 0.100 indicate the model terms are not significant, then the interaction between incubation time and initial Cr(VI) concentration and interaction between pH and initial Cr(VI)concentration do not have effect on Cr(VI).The "Lack of Fit F-value" of 1.2 implies the Lack of Fit is not significant.There is a 32.84% chance that a "Lack of Fit F-value" could occur due to noise. Not significant lack of fit is good -- we want the model to fit.

## 4.6. Development of regression model equation

The regression coefficients of the developed model are determined from the regression analysis. From the **Appendix C** the second order quadratic model is suggested, as the p-value of this model is smaller than that of other models. The model equation that correlates the response Cr(VI) uptake efficiency to the bioaccumulation process variables in terms of actual value after excluding insignificant terms is given below. As indicated in **Appendix D** the predicted model for Cr (VI) in terms of the actual factors is given by **Equation 3.**

- **Cr(VI) uptake efficiency = -31.05699+27.91727* A+22.99582* B-0.21345*C**
  **-2.65349* $A^2$ -2.17858* $B^2$**
  **-0.71027* A*B (3)**

Where, A=time, B=pH, C=initial Cr(VI)concentration.

## 4.7. Effects of bioaccumulation process variables on Cr (VI) uptake efficiency

Full general factorial design methodology was used to estimate the effect of three bioaccumulation variables on Cr (VI) uptake efficiency. Perturbation and contour surface plots were drawn by using full factorial to investigate the effect of all the factors on the responses. Based on the analysis of variance, the bioaccumulation process was significantly affected by interactions between the process variables. On the other hand, individual process variables that affect the Cr (VI) uptake efficiency significantly are incubation time (A), pH( B), and initial Cr(VI) concentration (C).

### 4.7.1. Direct effects of process variables on Cr (VI) uptake efficiency

**Effect of contact time**

Incubation time (contact time) is one of the factors that affect uptake efficiency of Cr (VI) from the contaminated environment. Duration of contact time (exposure) of the yeast cell to the contaminant significantly affects the capacity of Cr (VI) bioaccumulation in a way that as contact time increases the cell growth increases so that the bioaccumulation since bioaccumulation of Cr(VI) is both metabolic and non-metabolic pathway dependent. When time increase, growth and age of cell also increase but nutrition decreases(Gadd, 2009). When the cell grows its surface area to volume ratio of the yeast cell increases which makes it favorable for surface bioaccumulation of Cr(VI). Incubation time has significant effect on Cr (VI) uptake efficency as shown in **Figure 10**. Incubation time has both positive and negative effects on Cr(VI) bioaccumulation due to increasing of growth, age and depletion of nutrient with time and as time increase so that the Cr(VI) bioaccumulation but up to day 5 which implies that there may be depletion of nutrient and increase of age of the yeast cell. As seen from the following figure at actual factors of pH of 3, initial Cr(VI) concentration of 5mg/ml, the Cr(VI)uptake efficiency increase from 71.2% at incubation time of 3 days to 76% at incubation time of 5days and then decrease to 61.7% at time of 7 days may be due to the depletion of nutrient, increased age of yeast cell and occupation of the binding sites of hexavalent chromium.

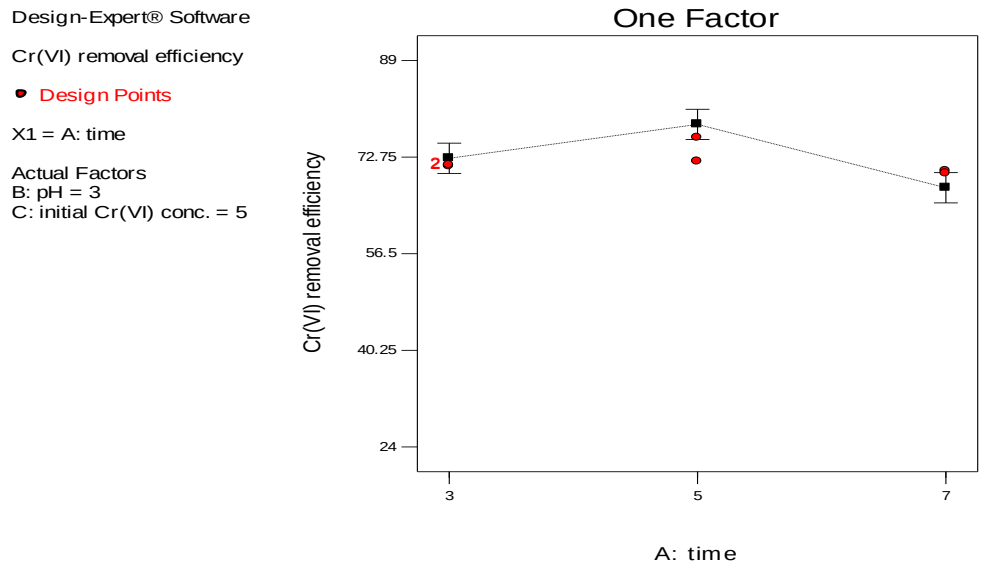



Figure 9: Effect of incubation time on Cr (VI) uptake efficiency

**Effect of pH**

PH is the most important parameter in the bioaccumulation process; it affects metal ion speciation in solution, surface charge of the biomass, and chemistry of biomass binding site(Amirnia, 2015). At low pH, the increase of protonation of yeast cell wall ligands decrease metals adsorption. On the other hand, the increase of pH can result in the precipitation of metals hydroxides, reducing metal accumulation by the biomass(Fomina & Michael, 2014) and is proved in the **Figure 11** shown below. The optimum pH values for bioaccumulation is in the range of 3-5(Juan, 2010).Cr(VI) uptake efficiency increases as the pH of the solution increases from 3-5 and after pH value of 5 uptake efficiency decreases due to increased protons can compete with Cr(VI) for the active site of the biosorbent which decreased the removal/bioaccumulation of Cr(VI) by yeast *S.cerevisiae* cell. As shown by **Figure 11** at actual factors of incubation time of 3days, initial Cr(VI) concentration of 5mg/ml, the Cr(VI)uptake efficiency increase from 71.2% at pH of 3 to 73.712% at pH value of 5 because uptake efficiency increase as pH increases to a certain point and then decrease to 55% at pH value of 8 because of an increase in anions ($-OH^-$) and makes competition of $Cr^{+6}$ with the active sites of biosorbent and produces chromium hydroxides instead of bioaccumulation which confirms with most literatures regarding effect of pH on bioaccumulation and implies bioaccumulation of Cr(VI) is best at acidic medium as indicated by the work of(S. Papirio et al, 2017).

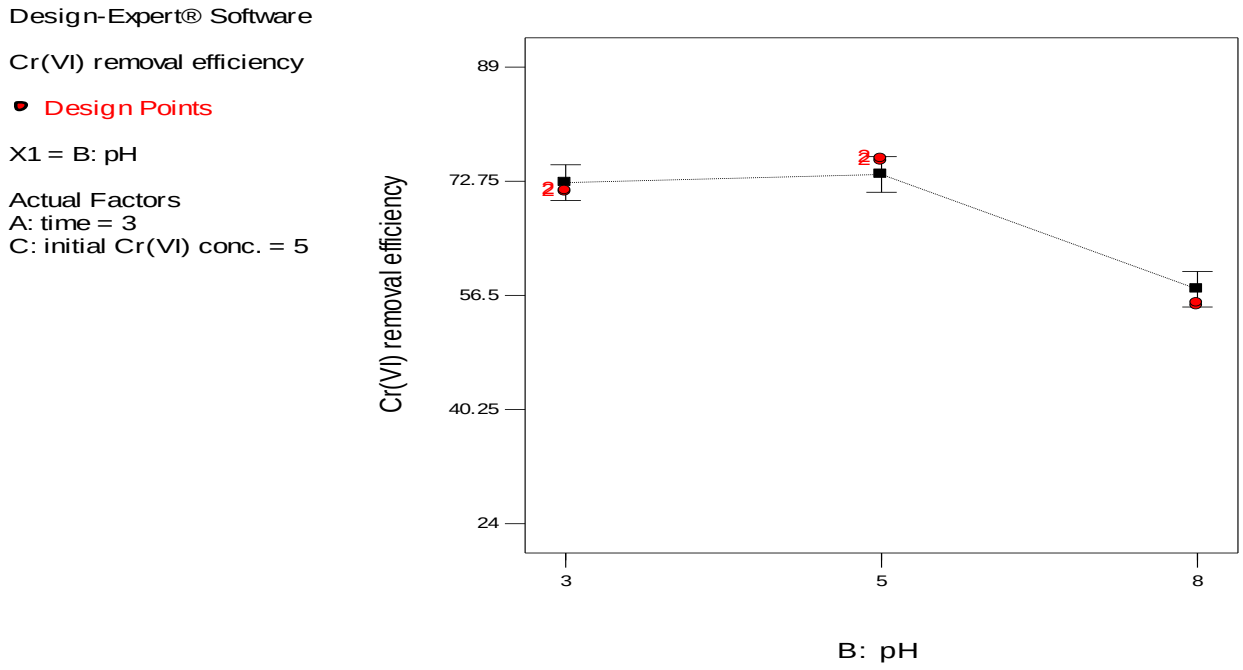



Figure 10: Effect of pH on Cr(VI) uptake efficiency

***Effect of initial concentration of Cr (VI)***

Initial pollutant concentration is another major factor of bioaccumulation process, which when increased so that the quantity of biosorbed pollutant is also increased to the optimum point(Saurav & Czech, 2011) but after the optimum point as it increases the binding sites are occupied and the decreases. In contrary for the case of this work since the initial pollutant concentration is high, as the initial concentration increases, the Cr (VI) uptake efficiency of yeast *Saccharomyces cerevisiae* decreases at actual factors of incubation time of 3 days, pH value of 3 and initial Cr (VI) of 5mg/ml, 50 mg/ml and 100mg/ml the Cr(VI) uptake efficiency is 72.55%.,64.5% and 59.7 % respectively which is decreasing down as shown by **Figure12**. The reason behind may be the high concentration of pollutant makes the binding site of the biosorbent occupied and as observed from the characteristics of the tannery effluent the concentration of anions such as $S^{2}$-,$SO4^{2-}$ and $Cl^{-}$ is high which competes active site for $Cr^{6+}$ makes the uptake efficiency to decrease.

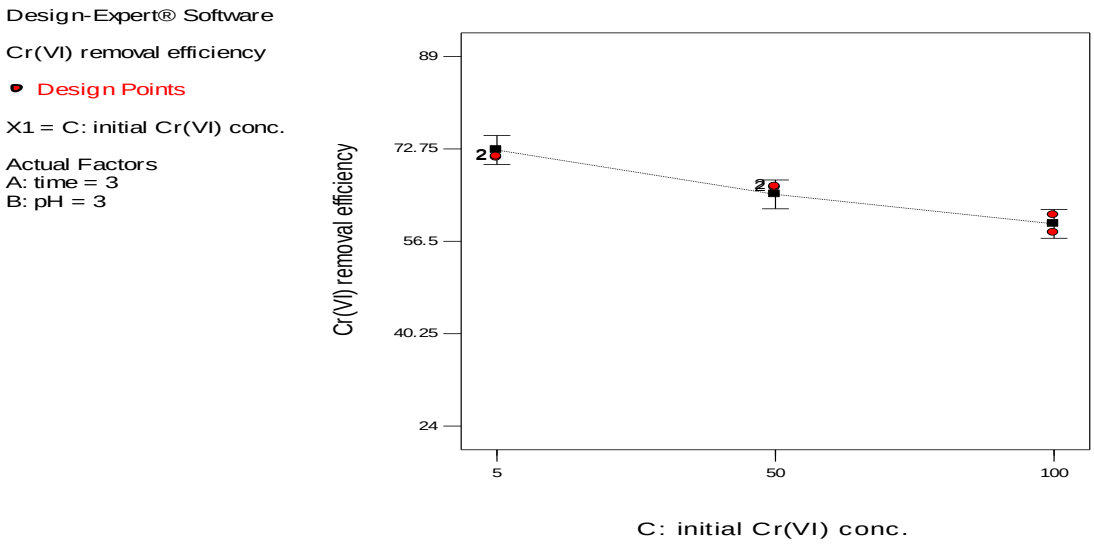



Figure 11: Effect of initial Cr(VI) concentration on Cr(VI) uptake efficiency

### 4.7.2. Effects due to interaction between variables of bioaccumulation

Bioaccumulation variables were found to have significant interaction effects. Figure 4-5 and 4-6 shows the interaction effects between incubation time and pH. Generally, an increase in bioaccumulation time is found to increase the Cr (VI) uptake efficiency up to optimum incubation time, but beyond optimum incubation time Cr (VI) uptake efficiency decreased. This is due to similar explanation given in the previous section. At actual factor of initial Cr (VI) concentration of 5mg/ml, Cr (VI) uptake efficiency increases with increasing of both incubation time and pH of solution to a certain point call optimum point but then decreased after the optimum point even both time and pH increases. In the following figures (**Figure10**, **11** and **12**) from time of 3-5 days and pH of 3-5, Cr (VI) uptake efficiency increased from 72.2% to 88.5% and then decreased to 39% as time and pH of solution increased to 7days and 8 respectively. The notable observation of figures shown below shows that at lower and middle range of incubation time and pH value of solution(3days, 3 and 5day,5) always resulted higher Cr(VI) uptake efficiency 72.55% and 88.5% respectively than using higher range of incubation time and pH (7days,8 ), which is 38.5%. Therefore, higher range of incubation time and pH (7days, 8) always resulted in lower uptake efficiency38.5% than using lower and middle range of time and pH

72.55% and 88.5% respectively which indicates that the interaction effect between incubation time and pH of the solution greatly affects the uptake efficiency of Cr(VI) by the yeast cell *Saccharomyces cerevisiae.*

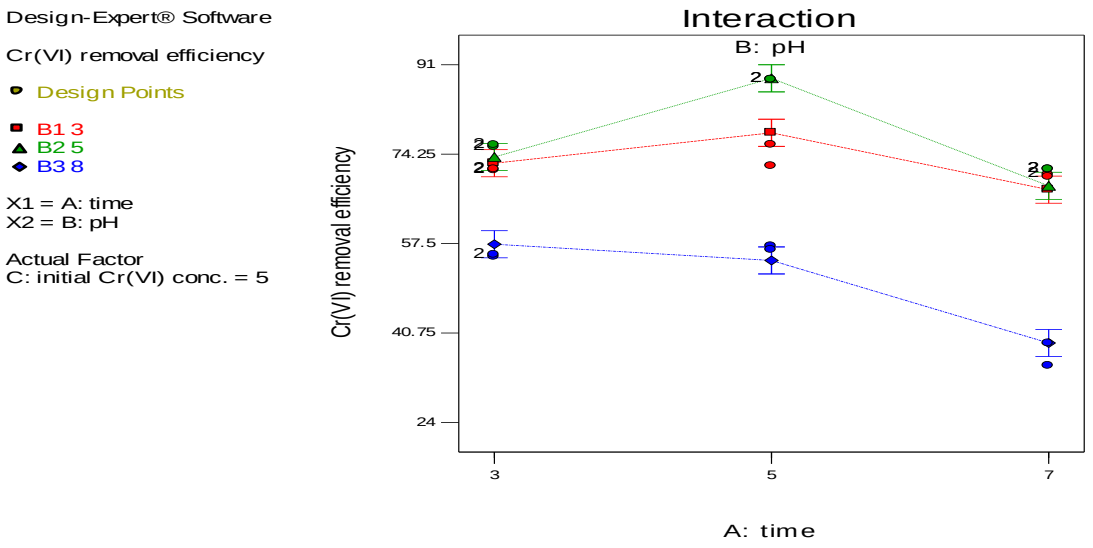



Figure 12: Effect of incubation time on Cr(VI) uptake efficiency

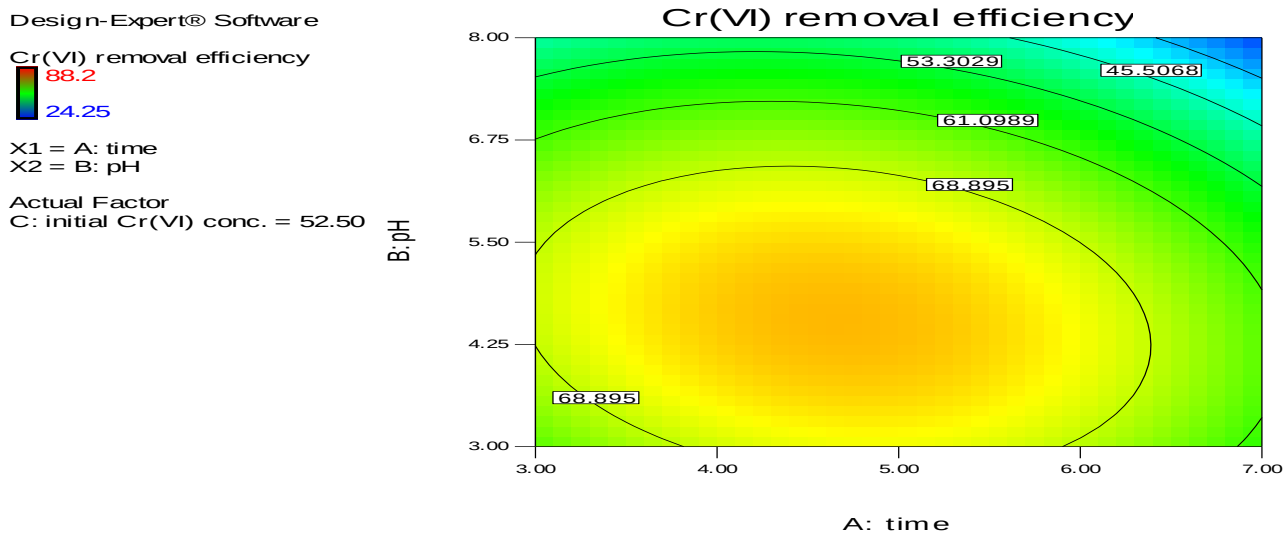


Figure 13: Contour plot of interaction effect of time and pH on Cr(VI) uptake efficiency

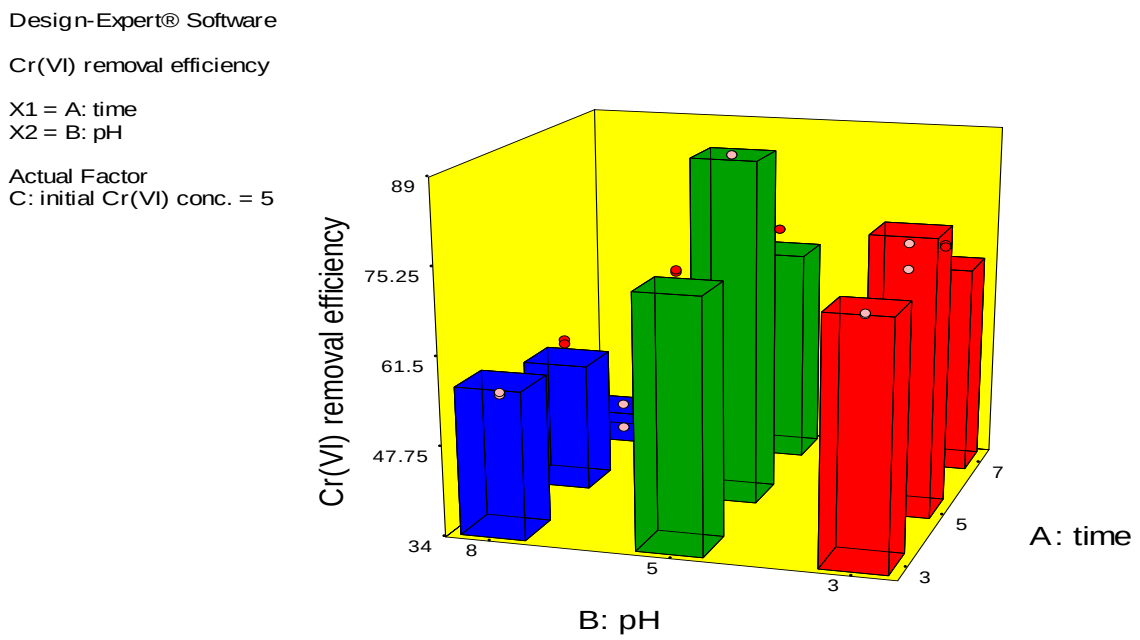



Figure 14:3D surface plot of interaction effect of time and pH on Cr(VI) uptake efficiency

## 4.8. Numerical Optimization of bioaccumulation process

The results above have shown that the three fermentation process variables and the interaction among the parameters affect the Cr(VI)uptake efficiency. Therefore, the next step is optimizing the process variables in order to obtain the highest uptake efficiency using the developed model regression. In optimizing the Cr(VI) bioaccumulation process the incubation time, pH and initial Cr(VI) concentration are a set of process variables held to be "in range" while Cr(VI) uptake efficiency is set of responses that need to be "maximized". **Table 6** shows the constraints of factors and responses, goals of optimization and selected solution for maximum Cr (VI) uptake efficiency.

Table 6: Constraint and solution for optimization of Cr(VI) bioaccumulation

| Criteria | Response | Goal | Lower limit | Upper limit | Selected solutions | Desirability |
|---|---|---|---|---|---|---|
| Incubation time | --- | In range | 3 | 7 | 4.65day | 1 |
| PH | --- | In range | 3 | 8 | 4.52 | 1 |
| Initial con of Cr | ---- | In range | 5 | 100 | 5mg/ml | 1 |
| --- | Cr(VI) uptake efficiency | Maximum | 24.25 | 88.2 | 84.26% | 0.938 |

Numerical optimization was used to provide highest Cr (VI) uptake efficiency in the given constraints and it was predicted at the following conditions; 5days of incubation time, pH of 4.5 and initial Cr (VI) concentration of 5mg/ml, that an optimum Cr (VI) of 84.26%canbeobtained

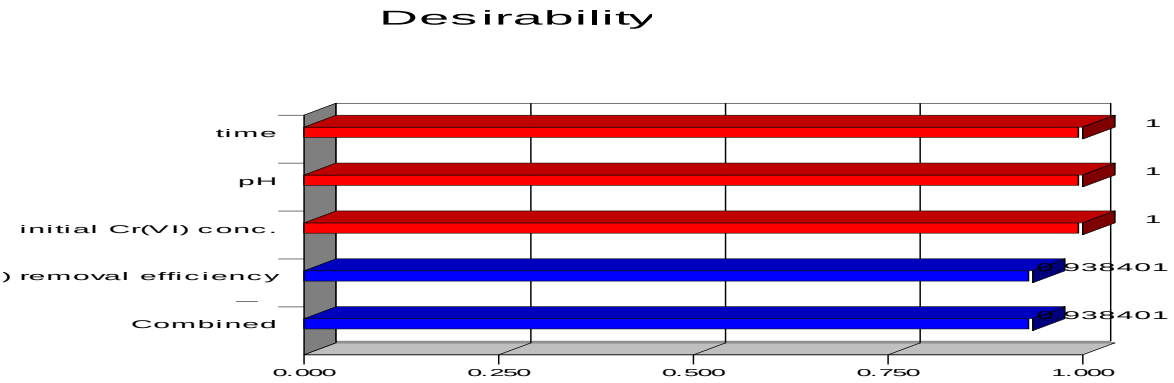



Figure 15: Desirability plot of optimization solution of the response

Desirability function was used to identify the optimum levels of factors and to get the maximum desirable responses. The optimized condition was selected with maximum combined desirability value of 0.938.

This value is in the range of findings of different researchers even though their works were at low concentration. Work of (Benazir et al., 2010) proved that *S.cerevisiae* had an efficiency of 95.6% in remedying chromium with a reduction rate of 1.829mg/L per hour and (Gavrilescu,

2009) showed that yeast *S. cerevisiae* has 99.5 % uptake efficiency of bioaccumulation Cr (VI) at the pH range of 4.5-5.5 and 25°C temperature with 120 mg/g uptake capacity. Another study by (Sujita, 2014) indicated that in a medium containing dichromate 20mg/ml more than 87% of dichromate ions was achieved within 72hrs using yeast *Saccharomyces cerevisiae*.

## 4.9. Validation of the developed model

To confirm the developed model, validation experiment was carried out at optimum incubation time, initial pH and initial Cr (VI) concentration, which are 5days, 5 and 5mg/ml respectively. For validation, actual experimental value, predicted value and the percentage of error is required and listed in **Table 7**.

As observed from confirmation result, the percentage accuracy is higher than predicted R-Squared value, which is 0.9219 (92.19%) as indicated in appendix C and based on this, it is possible to conclude that the developed model is much accurate.

Table 7: Actual, predicted and percentage of error

| Incubation time | pH | Initial Cr(VI)concentration | Actual value (%) | Predicted value (%) | Error (%) | Accuracy (%) |
|---|---|---|---|---|---|---|
| 5 | 4.5 | 5 | 78.35% | 84.26 | 5.91 | 94.09% |

## 4.10. Colony forming unit count result

Viability was determined by culturing method of CFU. After serial dilution of cell suspensions from control, 5, 50 and 100mg/ml Cr(VI) of solution, it was grown on to solid YPD agar for 48hr at 37 °C and counted by microscope, the following results of **Table 8** are obtained.

- 1ml of the diluted (1/10) of cell suspension were used for plate count and 10^4 dilution factor and number of colonies formed by control, 5, 50 and 100mg/ml were 357,124, 68 and 29 respectively.

Table 8: Results of colony forming unit count of tolerance test assay

| Concentration(mg/ml) | Total number of cell (cfu/ml) |
|---|---|
| Control | 3.57*10^7 |
| 5 | 1.24*10^7 |
| 50 | 68*10^5 |
| 100 | 29*10^5 |

The colony forming units count result of **Table 8** showed that there is a decrease in number of colony forming units( number of living cells) and its decreasing order is control >5mg/ml>50mg/ml>100mg/ml. This implies that there is an inhibition effect of hexavalent chromium on the growth of yeast cell *saccharomyces cerevisiae* and more specifically as seen also from the bioaccumulation experiment, at higher concentration of Cr(VI) such as at 50mg/ml and 100mg/ml was low proving that higher concentration of chromium ion have toxification effect on the biosorbent living yeast cell.

## 4.11. FTIR Test Result

FTIR spectroscopy was used to determine the functional groups on the yeast cells involved in the metal binding process. FTIR analysis is used as technique for bioaccumulation characterization, as well as to explore the possible mechanism involved in the removal of Cr(VI) by *S. cerevisiae*. Cell wall of yeast contains polysaccharides and glycoproteins in which they mainly possess functional group such as COOH, -OH, C=O, C-O, C≡N-NH, P=O, CH and their IR spectra wave number is shown in **Table 9** and the FTIR spectrum of *S. cerevisiae* before and after bioaccumulation of Cr(VI) is shown in **Figure 16** and compared to identify the changes in the frequency of the above functional groups.

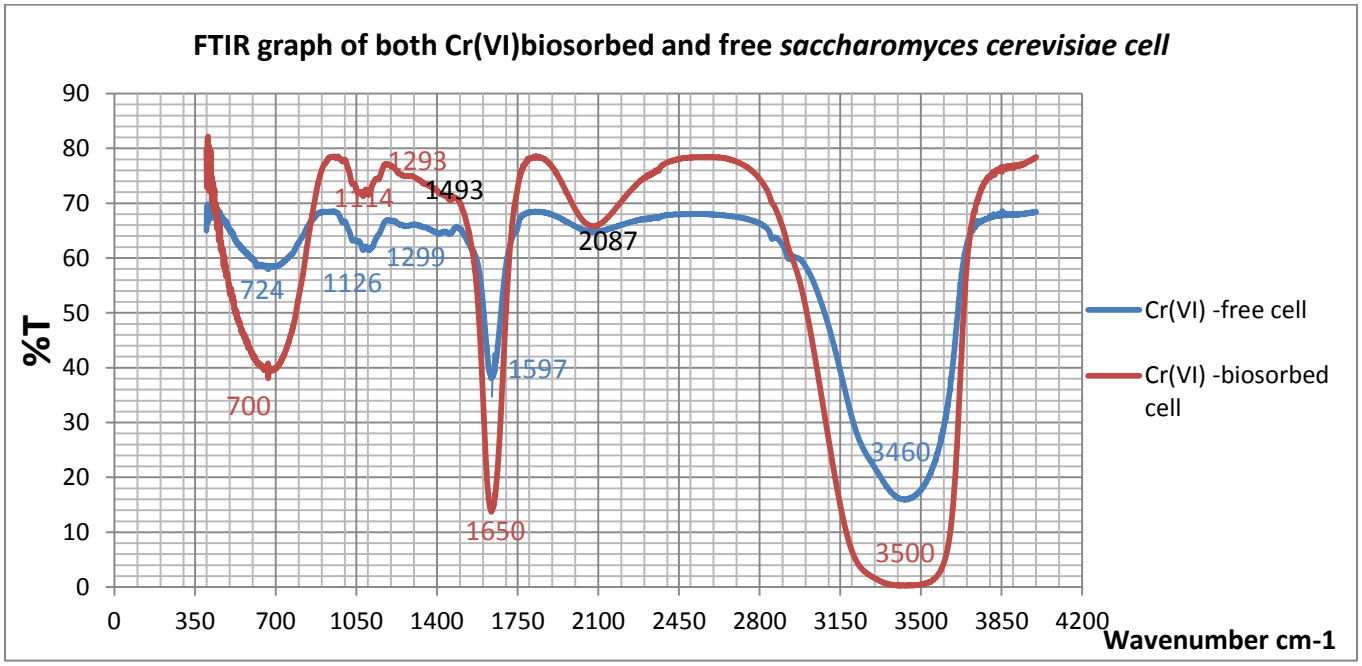



Figure 16: FTIR spectra of *S.cerevisiae* cell before and after Cr(VI) loading

**Figure 16** depicts the FT-IR spectrum of Cr(VI) loaded and unloaded yeast cells in the range of 400– 4000 $cm^{-1}$. The comparison of the spectra has given some valuable information which supports the formation of Cr-functional group complex. The IR spectrum of pure cell shows a peak at 3460 $cm^{-1}$ indicating the existence of hydroxyl (–OH) and (–NH) groups. This peak has been changed to a broad peak of 3500$cm^{-1}$ in the Cr(VI)loaded cell, which demonstrates the binding of O-H group with Cr ion via deprotonation. The peak in the IR spectrum of Cr(VI) free cell is a C=O stretching sharp peak which appeared around 1597 $cm^{-1}$ and the peak is shifted to 1650$cm^{-1}$ in the Cr(VI)loaded cell IR spectrum confirming the binding of Cr(VI) with carboxylic acid. Further, the characteristics peaks due to C-C C-O,-CN groups shifted from 1126 to 1114 $cm^{-1}$ and 724 to 700 $cm^{-1}$ respectively proving that there is an interaction of Cr(VI) and the cell wall functional groups and produce a complex product of chromium.

The IR spectrum peak at3460$cm^{-1}$ is considered as –OH and –NH groups, Peak of 2087 $cm^{-1}$ is assigned for C≡N(nitrile), while peak at 1597 $cm^{-1}$ can be considered as C=O stretching vibration of a carboxylic acid. Peak at 1493cm-1 represents amide II bond, 1299 $cm^{-1}$ can be assigned to P=O bond, the absorption band at 1114 cm-1 can be due to —C–O stretching vibrations and peak 724$cm^{-1}$ is assigned to -C-N (D. Park et al, 2005;Benazir et al., 2010 and Gualtar, 2013).

Table 9: FTIR adsorption bands and suggested corresponding functional groups of control and Cr(VI) loaded yeast cell

| Functional group | Spectra wave number($cm^{-1}$) | |
|---|---|---|
| | Cr(VI) free cell | Cr(VI) loaded |
| ➢ (-OH and –NH groups) | 3460 | 3500 |
| ➢ COOH(C=O) | 1597 | 1650 |
| ➢ P=O | 1299 | 1293 |
| ➢ –C-O | 1126 | 1114 |
| ➢ -CN | 724 | 700 |
| - | | |

Source:(Hlihor, 2009;Amirnia, 2015;D. Park et al, 2005)

In conclusion, the observed changes in the spectrum of the Cr(VI)-loaded cells with respect to unloaded cells are the changes in intensity and shift in the peaks position or shifts in wavenumbers, and shows that Chromium ions have strong interactions with functional groups –COOH,–OH, –NH and P=O stretching bonds on the cell walls of yeast(**Table 9**). Similar changes have been observed for copper loaded *Saccharomyces cerevisiae* by Amirna (2015); and Cr(VI) loaded heat inactivated *Saccharomyces cerevisiae* by Hlihor (2009).

## 4.12. Modeling of bioaccumulation of Cr(VI) by *S.cerevisiae*

In bioaccumulation, like any adsorption processes, binding of sorbate species from liquid to biosorbent continues until equilibrium is established between them. Kinetics study of bioaccumulation process is necessary to determine the time required to achieve equilibrium.

**The experimental data**

In this study six sets of bioaccumulation data were used for modeling. These bioaccumulation data were experimentally obtained by a series of six batch tests of Cr (VI) bioaccumulation for an initial Cr (VI) of 5mg/ml at pH value of 5 which is the optimum bioaccumulation point in 100 ml volume flask with 0.6 gram of biosorbent at time interval of 2 hours. The procedure of determining the Cr (VI) concentration in the solution was the same as previous Cr (VI) determination procedure.

Bioaccumulation capacity (qe) is calculated as follows: $\boldsymbol{Qe} = \left[\frac{(\boldsymbol{Ci}-\boldsymbol{Cf})}{\boldsymbol{m}} * \boldsymbol{V}\right]$ (**4**)

Where $C_i$ (mg/ml) and $C_f$ (mg/ml) are initial and final Cr (vi) concentrations in the solution, V is the volume of the solution (V=5ml of 5mg/ml $K_2CR_2O_7$ solution=5ml) and m(g) mass of biosorbent used.

Table 10: Recorded data for modeling of bioaccumulation of equilibrium isotherm and kinetics

| Ci(mg/ml) | Cf=Ce(mg/ml) | v(ml) | W(g) | qe(mg/g) | t(min) |
|---|---|---|---|---|---|
| 5 | 5 | 5 | 0.6 | 0 | 0 |
| 5 | 4.7 | 5 | 0.6 | 2.5 | 120 |
| 5 | 4.2 | 5 | 0.6 | 6.666667 | 240 |
| 5 | 4 | 5 | 0.6 | 8.333333 | 360 |
| 5 | 3.5 | 5 | 0.6 | 12.5 | 480 |
| 5 | 2.5 | 5 | 0.6 | 20.83333 | 600 |

**Equilibrium model**

The profile obtained from the study of concentration at different time was used to obtain Langmuir and Freundlich bioaccumulation isotherm by using well known adsorption equations.

$$\frac{1}{\text{Qeq}} = \left(\frac{1}{\text{qmax}*\text{b}}\right)\frac{1}{\text{Ceq}} + \frac{1}{\text{qmax}} \quad (5)$$

$$lnQe = lnk + \frac{1}{n} * lnCe \quad (6)$$

where, Qeq and Ceq are the amount of Cr(VI)accumulated per unit weight of bioaccumulate at equilibrium(mg/g) and un accumulated concentration of Cr(VI) in solution at equilibrium( mg/L) respectively while qmax is the maximum amount of metal per unit weight of biomass to form a complete monolayer on the surface bound (mg/g), b is Langmuir constant, K is Freundlich constant, 1/n is the intensity of accumulation.

The values of qmax, b, k, n and nonlinear regression correlation ($R^2$) are shown in the following table and graphs of Langmuir and Freundlich model respectively.

Table 11: Langmuir model data

| Ce(mg/ml) | Qe(mg/g) | 1/Ce (ml/mg) | 1/Qeq (g/mg) | B | qmax | $R^2$ |
|---|---|---|---|---|---|---|
| 5 | 0 | 0.2 | ∞ | -0.4 | 1.9 | 0.4785 |
| 4.7 | 2.5 | 0.212766 | 0.4 | | | |
| 4.2 | 6.666667 | 0.238095 | 0.15 | | | |
| 4 | 8.333333 | 0.25 | 0.12 | | | |
| 3.5 | 12.5 | 0.285714 | 0.08 | | | |
| 2.5 | 20.83333 | 0.4 | 0.048 | | | |

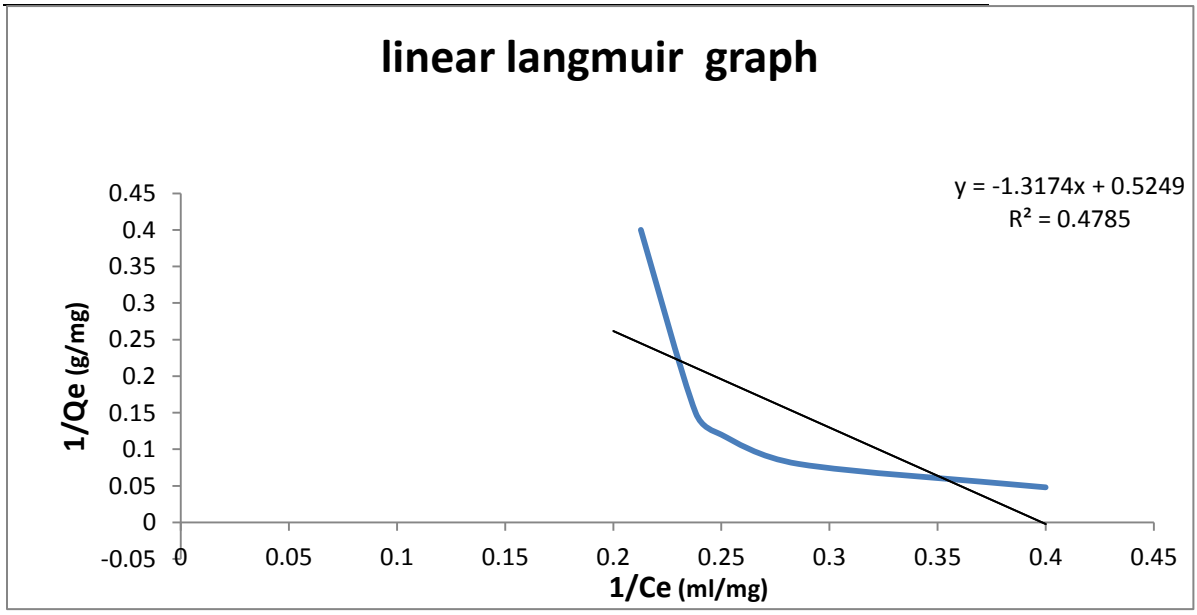



Figure 17: 1/Ce vs. 1/Qe Linear Langmuir graph

Table 12: Freundlich model data

| lnCe | lnQe | k | n | $R^2$ |
|---|---|---|---|---|
| 1.609438 | #NUM! | 2450 | -0.255 | 0.7804 |
| 1.547563 | 0.916291 | | | |
| 1.435085 | 1.89712 | | | |
| 1.386294 | 2.120264 | | | |

| | |
|---|---|
| 1.252763 | 2.525729 |
| 0.916291 | 3.036554 |

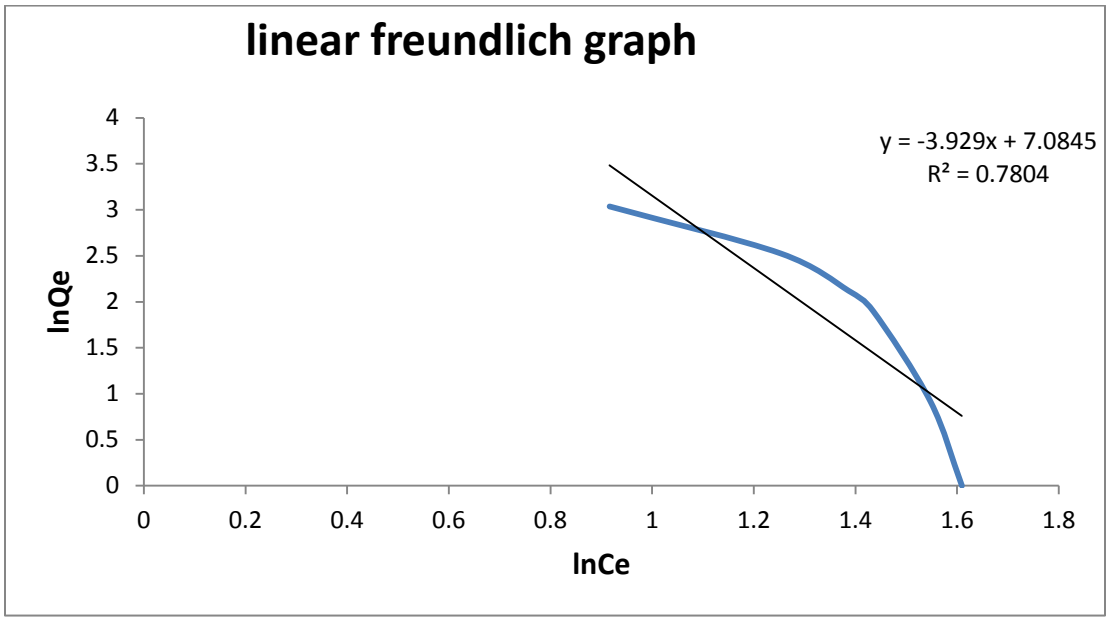



Figure 18: lnCe vs. lnQe linear Freundlich graph

The conformity between experimental data and the model predicted values was expressed by the correlation coefficients ($R^2$ values close or equal to 1). A relatively high $R^2$ value indicates that the model successfully describes the kinetics of Cr (VI) bioaccumulation. Therefore, based on their $R^2$ value freundlich bioaccumulation model is the isotherm model to describe the bioaccumulation of Cr(VI) by yeast cell *Saccharomyces cerevisiae* since its $R^2$ value 0.7804 is greater than that of the Langmuir model $R^2$ value which is 0.4785.

**Bioaccumulation Kinetics model**

According to Amirnia, (2015) metal bioaccumulation process took place in two steps: 1) rapid stage which represented a surface adsorption/accumulation mechanism; 2) slow stage until the biomass saturation was achieved, which was controlled by an intracellular diffusion process.

Kinetics of bioaccumulation of Cr (VI) on yeast cells was tested by the first-order and Second-order kinetic models. The conformity between experimental data and the model predicted values was expressed by the correlation coefficients ($R^2$ values close or equal to 1).

First-order and second-order kinetic equations based on the capacity of bioaccumulation are presented in the following form:

First pseudo order kinetic equation: $\ln(qe - qt) = -k1 * t + \ln(q_e)$ (7)

And pseudo second order kinetic equation: $\frac{1}{q_t} = \frac{1}{q_e} + \frac{1}{k2{q_e}^2} * \left(\frac{1}{t}\right)$ (8)

And qt is calculated using equation (4)

Table 13: recorded kinetic data and values of parameters

| Conc. (mg/ml) | Qt (mg/g) | Time (min) | Pseudo second order | | | Pseudo first order | | |
|---|---|---|---|---|---|---|---|---|
| | | | K2 | Qeq (calc.) | $R^2$ | K1 | Qeq (calc.) | $R^2$ |
| 5 | 0 | 0 | $2.5 * 10^{-5}$ | 27.7 | 0.99 | 0.0041 | 22 | 0.95 |
| 4.7 | 2.5 | 120 | | | | | | |
| 4.2 | 6.67 | 240 | | | | | | |
| 4 | 8.33 | 360 | | | | | | |
| 3.5 | 12.5 | 480 | | | | | | |
| 2.5 | 20.83 | 600 | | | | | | |

Using Equation 7 and 8, *ln(qe-qt)* versus *t* and *1/qt* versus 1/*t* were plotted as shown in **Figure 19** and **Figure 20**. The pseudo-first-order and second order rate constant (*k1*, k2) determined from the model are presented. Results are presented in **Table 13** for the removal of Cr(VI) *saccharomyces cerevisiae* at 27°C.

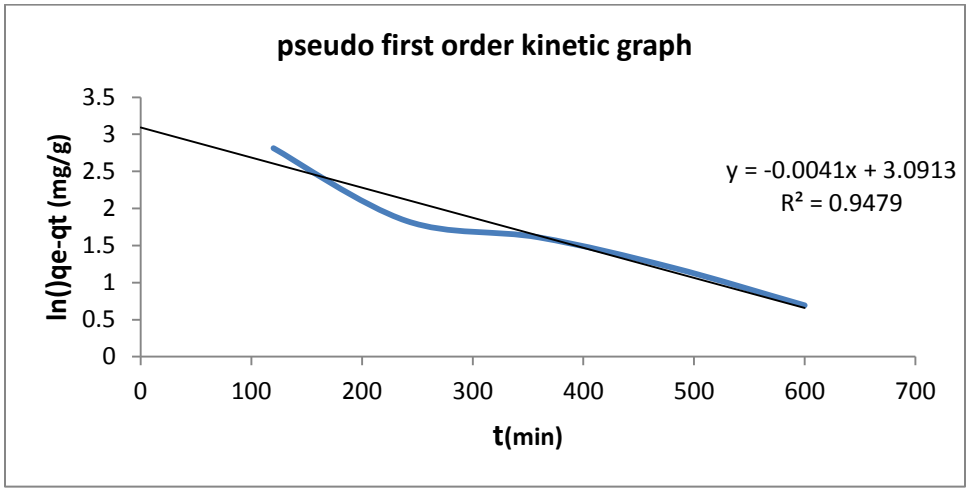



Figure 19: t vs. ln(qe-qt) graph of pseudo first order kinetics model

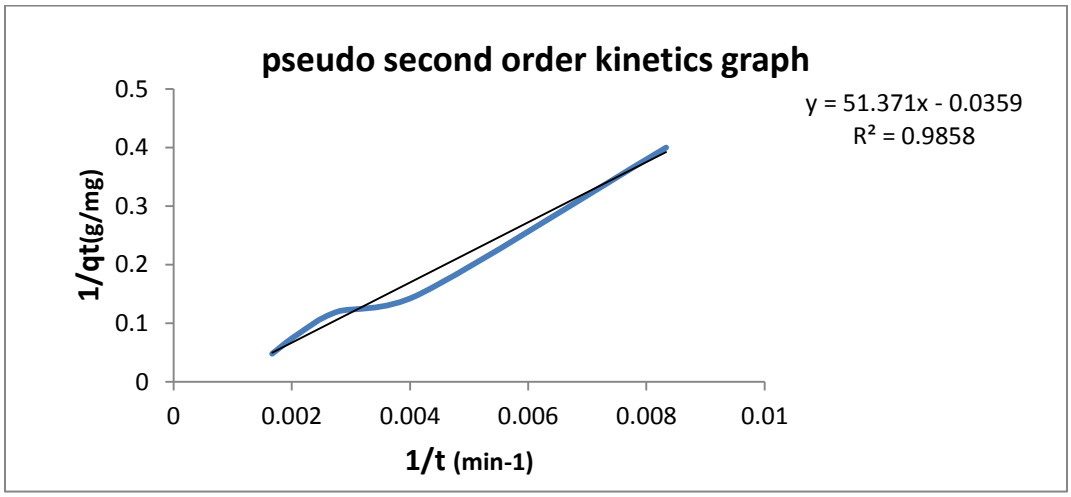



Figure 20:1/t vs. 1/qt graph of pseudo second order kinetics model

From above kinetic graphs of **Figure 19** and **Figure 20** correlation coefficients obtained from the first order and second order kinetic model are 0.95 and 0.99 respectively. A relatively high $R^2$ value indicates that the model successfully describes the kinetics of Cr (VI) bioaccumulation and based on this the pseudo second order kinetic model gives the higher $R^2$ value which indicates that the bioaccumulation kinetics of Cr(VI) by *S.cerevisiae* can be expressed using second order kinetic model.

# CHAPTER FIVE

# 5. Conclusion and Recommendation

## 5.1. Conclusion

Tanning industries are the source of most pollutants that causes Sevier health problems of most societies in the world. The characterization of collected tannery wastewater proves that pollutants discharged to the environment are above their standard limit due to lack of efficient or no treatment of the wastes. *S.cerevisiae* has showed its capability of bioaccumulation of Cr (VI) from Cr(VI) contaminated wastewater at different pH values, Cr(VI) concentration and incubation time. Cr(VI)uptake efficiency of *S.cerevisiae* can be affected by individual and by interaction of parameters such as pH, Cr(VI) and time. Its efficiency decreases as the pH value, Cr (VI) concentration and incubation time increase but increase at low pH value, Cr(VI)concentration and incubation time as shown by **Figure 9**,**Figure 10**,**Figure 11** and **Figure 12**. The optimum condition for bioaccumulation of Cr(VI) are pH value of 4.5, Cr(VI)concentration of 5mg/ml and incubation time of 5days and its maximum efficiency at these operating conditions is 84.2%.

Several research activities are performed regarding bioaccumulation of Cr(VI) at its low concentration but not any at its high concentration and in this work bioaccumulation Cr(VI) at its high concentration by *S.cerevisiae* is possible though the efficiency decreases as its concentration increases and it is also proved by the tolerance test assay result.

Freundlich equilibrium model and pseudo-second order kinetic model are used based on their high correlation coefficient ($R^2$) to mathematically model the extracellular bioaccumulation of Cr(VI) by *S.cerevisiae* and the intracellular precipitation and accumulation of Cr(VI) is analyzed by FTIR. From the FTIR there is an observable shift in position of peak and these observations favor the formation of Cr complex (precipitate) with hydroxyl, carboxyl, amide and phosphate groups of the yeast *Saccharomyces cerevisiae* cell wall.

## 5.2. Recommendation

Though the FTIR result showed that there is Cr ion complexation with the functional groups, the complex/precipitated products are not well identified and it is recommended to use LC-MS to analyze the metabolites present in the treated waste water. Batch systems are mostly reported for Cr(VI) removal in the literature. However, the effluents are generated by the industries in very large quantities, treatment of which in the batch systems can no longer be applied. Therefore, a suitable operational strategy needs to be developed for continuous removal of Cr(VI) from the industrial effluents. Most of the studies in reported literature have been conducted using synthetic Cr(VI) containing solution prepared in the laboratory and scanty information is available on treatment of actual industrial effluents. Effect of Parameters such as temperature and dose of biomass on Cr(VI) uptake efficiency should be analyzed.

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# APPENDIX

## Appendix A: cell determination, preparation of standards and reagents

### A.1. Determination of the cell used for treatment

The quantity of cell used for treatment can be expressed by either cell number or dry weight.

Colony count method

Serial dilution is done for yeast cell count up to 10^6 where this is the dilution factor better for yeast cell colony count and incubated at 37 ˚C for 48 hr on nutrient agar to develop colonies and counted using a microscope.

- *The total colony count (CFU/mL)=No. of colonies*DF/volume of culture plate*

  Where CFU is colony forming unit, DF is dilution factor

- 5ml of cell suspension was used and from this 1ml of cell suspension were used for plate count and 10^6 dilution factor.
- Therefore, total colony count(CFU/mL)=157*10^6/1ml=1.57*10^8CFU/mL

*Dry weight method*

In order to quantify the dry mass of the 5ml of cell suspension used in treatment, the 5ml of cell suspension were centrifuged at5000rpm for 10 minutes and again washed with distilled water twice and dried at 105°C oven for 30 minutes and taken to desiccator to cool.

- $dry\ weight\ of\ cell(g) = (weight\ of\ dish + biomass) - weight\ of\ empty\ dish = 10.8 - 10.64 = 0.16g$

Therefore the dry weight of the cell used for treatment is 0.16g for 50ml YPD media

### *A.2.* Preparation of standard solutions of hexavalent chromium

The two types of working solutions to draw a standard curve are stock solution and working solution. Stock solutions are solutions which are concentrated and are used for working solutions preparation.

- Standard solution preparation: Analytical reagent grade 2.829g $K_2Cr_2O_7$ has been dried at 105˚C for an hour and dissolved in 1000ml distilled water in conical flask according to the American Public Health Association(APHA,1999) and the solution contains 1000ug$Cr^{+6}$/ml
- Working solution: 1ml of the chromium stock(standard) solution is pipetted into a 100ml of volumetric flask and diluted to the mark with distilled water and the solution contains 10ug$Cr^{+6}$/ml
- 0.25% 1,5-Diphenylcarbizide solution preparation: 0.5g of 1,5-diphenylcarbazide is dissolved in 100ml of acetone first in 250 ml beaker and then the solution is diluted to 200ml with distilled water
- 6N $H_2SO_4$ solution: 6N of analytical grade $H_2SO_4$ were prepared by diluting 166ml of 98%stock $H_2SO_4$ to 1000ml distilled water (APHA, 1999) for adjustment of pH.

✓ All the above prepared solutions are stored at 4°C until work has been done.

## Appendix B: Actual experimental data for Cr(VI)optimization

| St.no | Incubation time(day) | pH | Initial Cr(VI) conc.(mg/ml) | Absorbance | Calibrated conc. (µg/ml) | Dilution factor(DF) | Final Cr(VI) conc.(mg/ml) | Cr(VI)uptake efficiency Cr(VI)=$\frac{CO-C1}{C0}*100$ |
|---|---|---|---|---|---|---|---|---|
| 1 | 3 | 3 | 5 | 0.36 | 71.58 | 20 | 1.43 | 71.4 |
| 2 | 3 | 3 | 5 | 0.361 | 71.79 | 20 | 1.44 | 71.2 |
| 3 | 3 | 3 | 50 | 0.37 | 73.67 | 230 | 16.94 | 66.12 |
| 4 | 3 | 3 | 50 | 0.372 | 74.1 | 230 | 17.04 | 65.9 |
| 5 | 3 | 3 | 100 | 0.42 | 84.08 | 500 | 42 | 58 |
| 6 | 3 | 3 | 100 | 0.39 | 77.8 | 500 | 38.9 | 61.1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 7 | 3 | 5 | 5 | 0.31 | 61.16 | 20 | 1.22 | 75.6 |
| 8 | 3 | 5 | 5 | 0.305 | 60.125 | 20 | 1.2025 | 75.95 |
| 9 | 3 | 5 | 50 | 0.38 | 75.75 | 230 | 17.42 | 65.2 |
| 10 | 3 | 5 | 50 | 0.382 | 76.167 | 230 | 17.52 | 64.96 |
| 11 | 3 | 5 | 100 | 0.4 | 80 | 500 | 40 | 60 |
| 12 | 3 | 5 | 100 | 0.41 | 82 | 500 | 41 | 59 |
| 13 | 3 | 8 | 5 | 0.375 | 75 | 30 | 2.25 | 55 |
| 14 | 3 | 8 | 5 | 0.373 | 74.29 | 30 | 2.23 | 55.4 |
| 15 | 3 | 8 | 50 | 0.388 | 77.42 | 350 | 27.1 | 45.8 |
| 16 | 3 | 8 | 50 | 0.384 | 76.58 | 350 | 26.8 | 46.4 |
| 17 | 3 | 8 | 100 | 0.48 | 96.6 | 650 | 62.8 | 37.2 |
| 18 | 3 | 8 | 100 | 0.481 | 96.79 | 650 | 62.9 | 37.1 |
| 19 | 5 | 3 | 5 | 0.3 | 59.08 | 20 | 1.2 | 76 |
| 20 | 5 | 3 | 5 | 0.32 | 63.25 | 20 | 1.265 | 74.47 |
| 21 | 5 | 3 | 50 | 0.354 | 70.3 | 200 | 14 | 72 |
| 22 | 5 | 3 | 50 | 0.351 | 69.7 | 200 | 13.94 | 72.12 |
| 23 | 5 | 3 | 100 | 0.36 | 71.6 | 450 | 32.2 | 67.8 |
| 24 | 5 | 3 | 100 | | 71.2 | 450 | 32 | 68 |
| 25 | 5 | 5 | 5 | 0.32 | 65.75 | 10 | 0.66 | 86.8 |
| 26 | 5 | 5 | 5 | 0.3 | 59.08 | 10 | 0.59 | 88.2 |
| 27 | 5 | 5 | 50 | 0.4 | 80 | 120 | 9.6 | 80.8 |
| 28 | 5 | 5 | 50 | 0.38 | 75.75 | 120 | 9.09 | 81.82 |
| 29 | 5 | 5 | 100 | 0.36 | 71.58 | 350 | 25.05 | 75 |
| 30 | 5 | 5 | 100 | 0.357 | 70.96 | 350 | 24.836 | 75.164 |
| 31 | 5 | 8 | 5 | 0.36 | 71.58 | 30 | 2.15 | 57 |
| 32 | 5 | 8 | 5 | 0.366 | 72.8 | 30 | 2.184 | 56.32 |
| 33 | 5 | 8 | 50 | 0.38 | 75.75 | 350 | 26.5 | 47 |
| 34 | 5 | 8 | 50 | 0.385 | 76.92 | 350 | 26.92 | 46.16 |
| 35 | 5 | 8 | 100 | 0.42 | 84 | 700 | 58.8 | 41.2 |
| 36 | 5 | 8 | 100 | 0.45 | 90.3 | 700 | 63.21 | 36.79 |
| 37 | 7 | 3 | 5 | 0.375 | 74.7 | 20 | 1.5 | 70 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 38 | 7 | 3 | 5 | 0.371 | 73.88 | 20 | 1.48 | 70.4 |
| 39 | 7 | 3 | 50 | 0.42 | 84 | 250 | 21 | 58 |
| 40 | 7 | 3 | 50 | 0.42 | 84 | 250 | 21 | 58 |
| 41 | 7 | 3 | 100 | 0.48 | 96.58 | 500 | 48.3 | 51.7 |
| 42 | 7 | 3 | 100 | 0.44 | 88.25 | 500 | 44.125 | 55.875 |
| 43 | 7 | 5 | 5 | 0.36 | 71.58 | 20 | 1.43 | 71.4 |
| 44 | 7 | 5 | 5 | 0.36 | 71.58 | 20 | 1.43 | 71.4 |
| 45 | 7 | 5 | 50 | 0.4 | 80 | 240 | 19.2 | 61.6 |
| 46 | 7 | 5 | 50 | 0.42 | 84 | 240 | 20.16 | 59.68 |
| 47 | 7 | 5 | 100 | 0.472 | 95 | 500 | 47.5 | 52.5 |
| 48 | 7 | 5 | 100 | 0.48 | 96.58 | 500 | 48.29 | 51.71 |
| 49 | 7 | 8 | 5 | 0.31 | 61.16 | 50 | 3.06 | 38.8 |
| 50 | 7 | 8 | 5 | 0.33 | 65.33 | 50 | 3.27 | 34.6 |
| 51 | 7 | 8 | 50 | 0.43 | 86.167 | 400 | 34.47 | 31 |
| 52 | 7 | 8 | 50 | 0.40 | 80 | 400 | 32 | 36 |
| 53 | 7 | 8 | 100 | 0.37 | 73 | 1000 | 73 | 27 |
| 54 | 7 | 8 | 100 | 0.38 | 75.75 | 1000 | 75.75 | 24.25 |

## APPENDIX C:Model fit summary for response of Cr(VI) uptake efficiency bioaccumulation efficency

**Effect analysis**

| | Term | DOF | SumSqr | MeanSqr | Prob>F | F Value | % Contribtn | Require |
|---|---|---|---|---|---|---|---|---|
| Model | A-time | 2 | 2185.31 | 1092.65 | < 0.0001 | 75.4778 | | |
| Model | B-pH | 2 | 7417.38 | 3708.69 | < 0.0001 | 256.187 | | |
| Model | C-initial Cr(VI) conc.2 | | 1537.26 | 768.628 | < 0.0001 | 53.095 | 12.3453 | |
| Model | AB | 4 | 643.44 | 160.86 | < 0.0001 | 11.1118 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Error | AC | 4 | 48.1866 | 12.0467 | 0.5166 | 0.832153 |
| Error | BC | 4 | 49.6594 | 12.4149 | 0.5017 | 0.857588 |
| Error | ABC | 8 | 180.036 | 22.5045 | 0.1854 | 1.55456 |
| Error | Lack Of Fit | 0 | 0 | | | 0 |
| Error | Pure Error | 27 | 390.865 | | | 3.13894 |
| Residuals | 27 | 390.865 | 14.4765 | | | |

**TableC1: Sequential Model Sum of Squares**

**Response 1 Cr(VI) Transform: None**

***** WARNING: The Cubic Model is Aliased! *****

TableC1: **Sequential Model Sum of Squares**

| Source | Sum of Squares | df | Mean Square | F Value | Prob > F | p-value |
|---|---|---|---|---|---|---|
| Mean vs Total | 1.923E+005 | 1 | 1.923E+005 | | | |
| Linear vs Mean | 7732.21 | 3 | 2577.40 | 27.30 | < 0.0001 | |
| 2FI vs Linear | 339.45 | 3 | 113.15 | 1.21 | 0.3150 | |
| Quadratic vs 2FI | 3407.73 | 3 | 1135.91 | 51.38 | < 0.0001 | Suggested |
| Cubic vs Quadratic | 128.52 | 7 | 18.36 | 0.80 | 0.5888 | Aliased |
| Residual | 844.22 | 37 | 22.82 | | | |
| Total | 2.048E+005 | 54 | 3792.07 | | | |

**TableC.2:Lack of FitTests**

| Source | Sum of Squares | df | Mean Square | F Value | Prob > F | | |
|---|---|---|---|---|---|---|---|
| Linear | 4329.05 | 23 | 188.22 | 13.00 | < 0.0001 | | |
| 2FI | 3989.61 | 20 | 199.48 | 13.78 | < 0.0001 | | |
| Quadratic | 581.88 | 17 | 34.23 | 2.36 | | 0.0222 | Suggested |
| Cubic | 453.35 | 10 | 45.34 | 3.13 | | 0.0088 | Aliased |
| Pure Error | 390.86 | 27 | 14.48 | | | | |

**TableC.3: Model Summary Statistics**

| Source | Std. Dev. | R-Squared | Adjusted R-Squared | Predicted R-Squared | PRESS | |
|---|---|---|---|---|---|---|
| Linear | 9.72 | 0.6210 | 0.598 | 20.5667 | 5395.83 | |
| 2FI | 9.65 | 0.648 | 20.6033 | 0.5563 | 5524.59 | |
| Quadratic | 4.70 | 0.9219 | 0.9059 | 0.8732 | 1578.7 | Suggested |
| Cubic | 4.78 | 0.9322 | 0.9029 | 0.8361 | 2041.48 | Aliased |

A model with highest order polynomial but not aliased, insignificant lack of fit, low press and high Adj.$\mathbf{R}^2$ and pred.$\mathbf{R}^2$ is a good model.

## APPENDIX D:ANOVA FOR Cr(VI)

**Response**: **Cr(VI)**

**ANOVA for Response Surface Reduced Quadratic Model**

**D.1. Analysis of variance table [Partial sum of squares]**

| Source | Sum of Squares | df | Mean Square | F Value | Prob > F | |
|---|---|---|---|---|---|---|
| Model | 11414.20 | 6 | 1902.37 | 86.14 | < 0.0001 | significant |
| *A-time* | *911.47* | *1* | *911.47* | *41.27* | *< 0.0001* | |
| *B-pH4596.73* | | *1* | *4596.73* | *208.15* | *< 0.0001* | |
| *C-initial Cr(VI) conc.1504.79* | | *1* | *1504.79* | *68.14* | *< 0.0001* | |
| *AB306.72* | | *1* | *306.72* | *13.89* | *0.0005* | |
| *$A^2$1351.88* | | *1* | *1351.88* | *61.22* | *< 0.0001* | |
| *$B^2$2023.39* | | *1* | *2023.39* | *91.62* | *< 0.0001* | |
| Residual | 1037.92 | 47 | 22.08 | | | |
| *Lack of Fit* | *647.06* | *20* | *32.35* | *1.2* | *0.384* | not*significant* |
| *Pure Error* | *390.86* | *27* | *14.48* | | | |
| Cor Total | 12452.12 | 53 | | | | |

The Model F-value of 86.14 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, $A^2$, $B^2$ are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The "Lack of Fit F-value" of 1.2 implies the Lack of Fit is not significant. There is a 32.84% chance that a "Lack of Fit F-value" this large could occur due to noise.Significant lack of fit is bad -- we want the model to fit.

| | | | |
|---|---|---|---|
| Std. Dev. | 4.70 | R-Squared | 0.9166 |
| Mean | 59.68 | Adj R-Squared | 0.9060 |
| C.V. % | 7.87 | Pred R-Squared | 0.8866 |
| PRESS | 1412.19 | Adeq Precision | 34.735 |

The "Pred R-Squared" of 0.8866 is in reasonable agreement with the "Adj R-Squared" of 0.9060.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 34.735 indicates an adequate signal. This model can be used to navigate the design space.

| Factor | Coefficient Estimate | df | Standard Error | 95% CI Low | 95% CI High | VIF |
|---|---|---|---|---|---|---|
| Intercept | 75.15 | 1 | 1.48 | 72.18 | 78.11 | |
| A-time | -5.05 | 1 | 0.79 | -6.63 | -3.47 | 1.01 |
| B-pH | -11.30 | 1 | 0.78 | -12.88 | -9.72 | 1.01 |
| C-initial Cr(VI) conc. | -6.46 | 1 | 0.78 | -8.04 | -4.89 | 1.00 |
| AB | -3.55 | 1 | 0.95 | -5.47 | -1.63 | 1.01 |
| $A^2$ | -10.61 | 1 | 1.36 | -13.34 | -7.88 | 1.00 |
| $B^2$ | -13.62 | 1 | 1.42 | -16.48 | -10.75 | 1.01 |

**Final Equation in Terms of Coded Factors:**

Cr(VI) =

+75.15

-5.05 * A

-11.30 * B

-6.46 * C

-3.55 * A * B

-10.61 * $A^2$

-13.62 * $B^2$

**Final Equation in Terms of Actual Factors:**

Cr(VI) =

-32.00252

+27.91727 * time

+22.99582 * pH

-0.13605 * initial Cr(VI) conc.

-0.71027 * time * pH

-2.65349 * $time^2$

-2.17858 * $pH^2$

## .Diagnostics Case Statistics

❖ **T**ableD.2:experimental and predictedvalues of Cr(VI) removal efficience

| St order | Actual value | Predicted value | residuals |
|---|---|---|---|
| 1 | 71.2 | 69.81 | 1.39 |
| 2 | 71.4 | 69.81 | 1.59 |
| 3 | 76 | 80.05 | -4.05 |
| 4 | 72 | 80.05 | -8.05 |
| 5 | 70.4 | 69.07 | 1.33 |
| 6 | 70 | 69.07 | 0.93 |

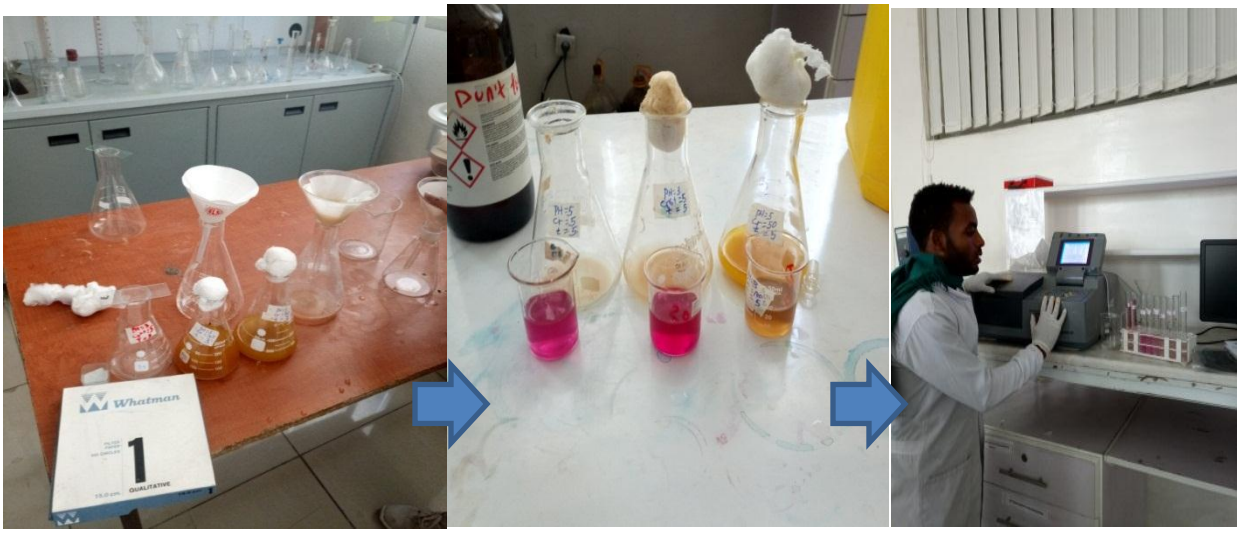
| | | | |
|---|---|---|---|
| 7 | 75.6 | 76.52 | -0.92 |
| 8 | 75.95 | 76.52 | -0.57 |
| 9 | 88.2 | 83.93 | 4.27 |
| 10 | 88.2 | 83.93 | 4.27 |
| 11 | 71.4 | 70.1 | 1.3 |
| 12 | 71.44 | 70.1 | 1.3 |
| 13 | 55 | 53.92 | 1.08 |
| 14 | 55.4 | 53.92 | 1.48 |
| 15 | 57 | 57.06 | -0.06 |
| 16 | 56.32 | 57.06 | -0.74 |
| 17 | 34.6 | 38.98 | -4.38 |
| 18 | 38.8 | 38.98 | -0.18 |
| 19 | 65.9 | 62.92 | 2.98 |
| 20 | 66.12 | 62.92 | 3.2 |
| 21 | 72 | 72.08 | -0.081 |
| 22 | 72.12 | 72.08 | 0.0039 |
| 23 | 58 | 60.01 | -2.01 |
| 24 | 58 | 60.01 | -2.01 |
| 25 | 64.96 | 69.79 | -4.83 |
| 26 | 65.2 | 69.79 | -4.59 |
| 27 | 80.8 | 76.11 | 4.69 |
| 28 | 81.82 | 76.11 | 5.71 |
| 29 | 59.68 | 61.2 | -1.52 |
| 30 | 61.6 | 61.2 | 0.4 |
| 31 | 46.4 | 47.41 | -1.01 |
| 32 | 45.8 | 47.41 | -1.61 |
| 33 | 46.16 | 49.47 | -3.31 |
| 34 | 47 | 49.47 | -2.47 |

| | | | |
|---|---|---|---|
| 35 | 31 | 30.29 | 0.71 |
| 36 | 36 | 30.29 | 5.71 |
| 37 | 61.1 | 58.75 | 2.35 |
| 38 | 58 | 58.75 | -0.75 |
| 39 | 68 | 66.7 | 1.1 |
| 40 | 67.8 | 66.7 | 2.46 |
| 41 | 55.88 | 53.42 | 2. |
| 42 | 51.7 | 53.42 | -1.72 |
| 43 | 60 | 65.76 | -5.79 |
| 45 | 75.16 | 70.89 | -6.79 |
| 46 | 75 | 70.89 | 4.27 |
| 47 | 51.71 | 54.77 | 4.11 |
| 48 | 52.5 | 54.77 | -3.06 |
| 49 | 37.2 | 43.66 | -2.27 |
| 50 | 62.9 | 43.66 | -6.46 |
| 51 | 36.79 | 44.5 | 19.24 |
| 52 | 41.2 | 44.5 | -3.3 |
| 53 | 24.25 | 24.12 | 0.13 |
| 54 | 27 | 24.12 | 2.88 |

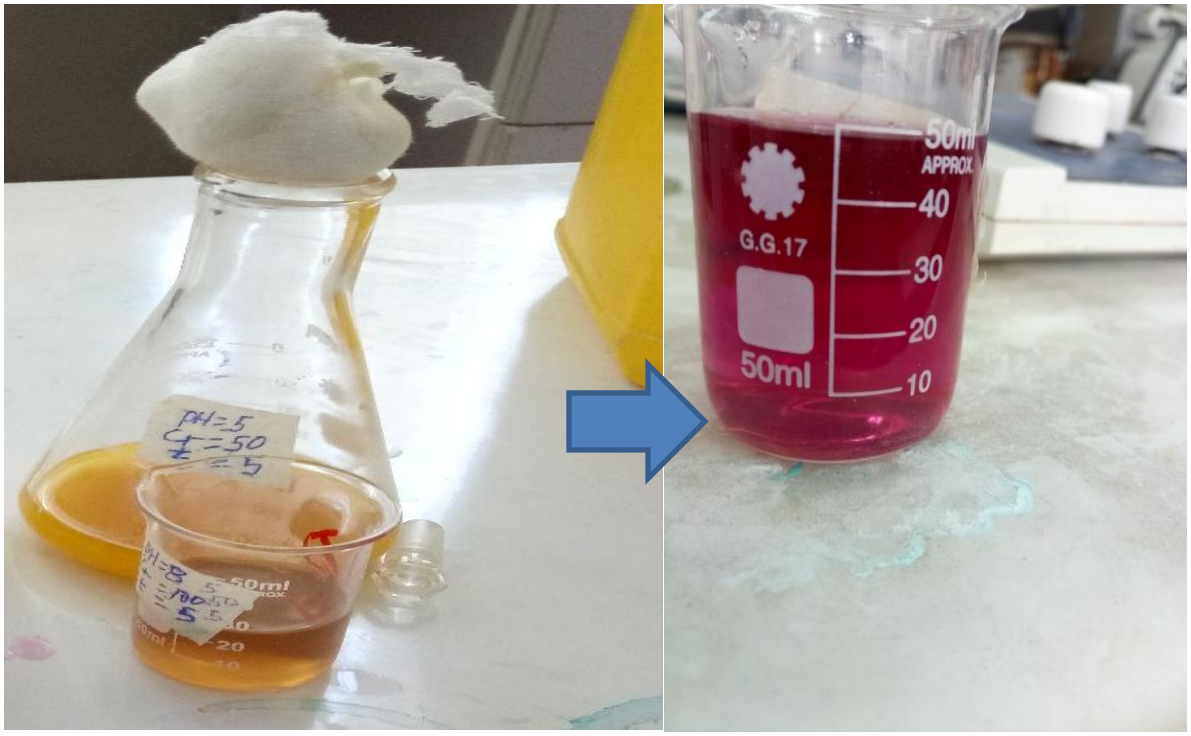
## Appendix E: pictures taken during the laboratory work


Standard solution preparation and absorbance reading

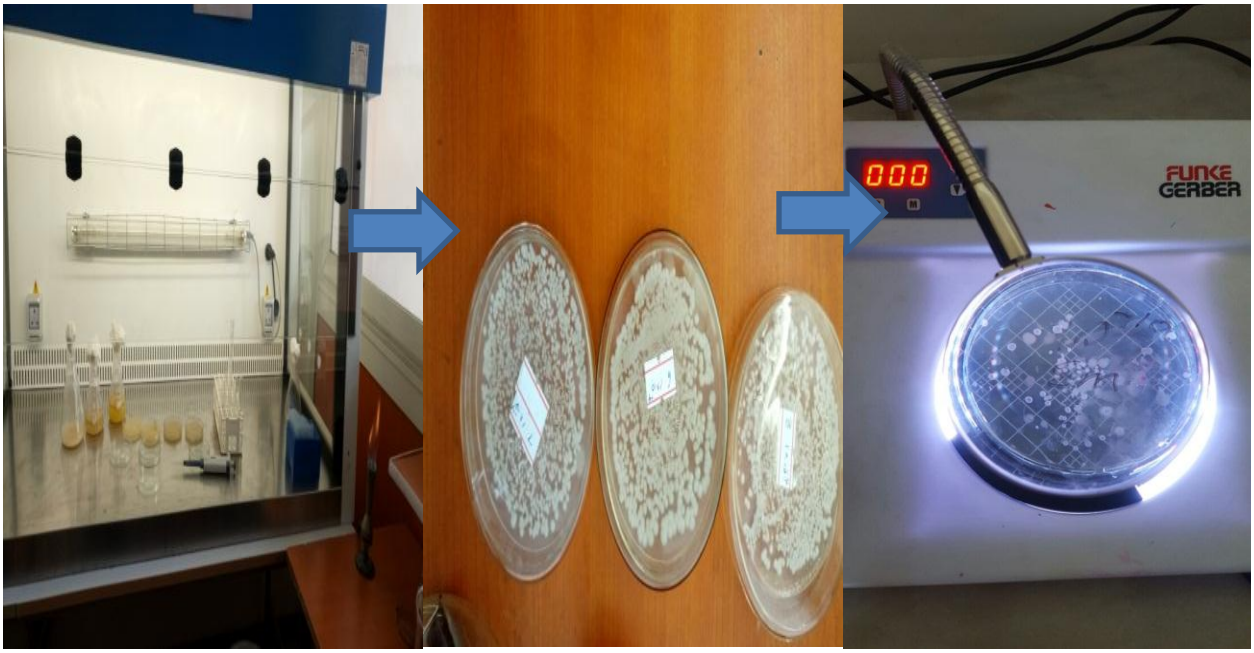

Media preparation and incubation



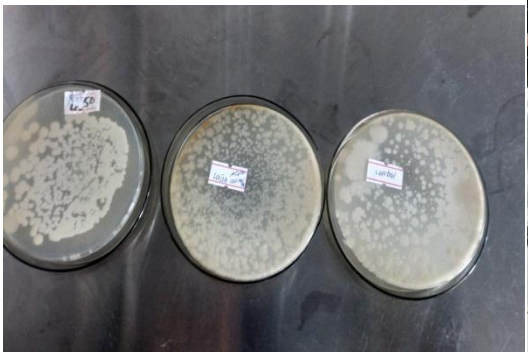
Filtration, color development and absorbance measurement for Cr(VI)determination




Color developed after dilution of high Cr(VI) (100mg/ml)concentration in the solution

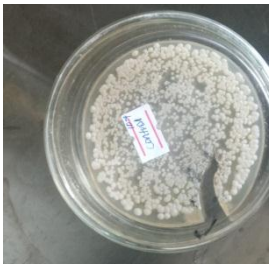



Serial dilution and colony forming unit count using microscope



Contaminated colony

tannery wastewater sample



Colony formed by pure *S.cerevisiae* yeast cell