BIOREMEDIATION OF TANNERY WASTEWATER USING *SACCHAROMYCES CEREVISIAE* (BAKERS' YEAST)

BY

J.O. JONAH
B.ENG(WATER RESOURCES AND ENVT'L ENG'G)

Submitted in Partial Fulfillment of the Requirements of the Degree of Master of Engineering (Environmental Engineering)

Department of Water Resources and Environmental Engineering,
Ahmadu Bello University,
Zaria.

DEDICATION

This work is dedicated to Almighty God. I also dedicate it to my father M.A.JONAH who has nurtured, guided and encouraged me all through my life.

DECLARATION

I hereby certify that the work embodied in the thesis entitled "Bioremediation Of Tannery Wastewater Using *SaccharomycesCerevisiae* (Baker's Yeast)" has been performed by me in the department of Water Resources and Environmental Engineering under the supervision of Prof. C. A Okuofu, Dr. J. A Otun and Dr. A Ismail.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any University.

J.O. JONAH

…….. …… ……..

Name of Student Signature Date

CERTIFICATION

This thesis entitled "BIOREMEDIATION OF TANNERY WASTEWATER USING *SACCHAROMYCES CEREVISIAE* (BAKER'S YEAST)" by Jonah, Joseph Omuya meets the regulations governing the award of the degree of Masters of Science of Ahmadu Bello University, Zaria and it is approved for its contribution to knowledge and literary presentation.

…… ……
Prof. C.A. Okuofu Date
Chairman, Supervisory Committee

…… ……
Dr. J. A. Otun Date
Member, Supervisory Committee

…… ……
Dr. J. A. Otun Date
Head of Department

…… ……
Dr. J. A. Otun Date
Dean, Postgraduate School

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ABBREVIATIONS AND SYMBOLS

BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
Cl	Chloride
d	Day
e	Exponential log base = 2.718
d	Depth of pond
ha	Hectare
k_1	Reaction rate (d^{-1})
S_o	Influent BOD (mg/l)
S_e	Effluent BOD (mg/l)
t	Time (day)
T	Temperature
SS	Suspended solids
TS	Total solids
δ	Diffusivity or disperse number
Θ	Temperature reaction coefficient
Q	Flow rate
λ_v	BOD Loading rate

ABSTRACT

This study investigates the performance of laboratory pond system containing immobilized cells of *Saccharomyces cerevisiae* in the treatment of raw tannery wastewater as compared to a pond system without cells. The performance of the pond system was evaluated by the measurements of BOD, COD, sulphate, TS, chloride, pH, chromium and cadmium. Also, the hydraulic characteristics of the pond configuration such as the average retention time and flow rate were estimated.

The results obtained indicated that the system containing the immobilized cells yielded better results in the reduction of some of the parameters when operated under the same hydraulic and organic loadings. For example, the overall BOD, COD and chloride removal were 73.5%, 62.96% and 69.56% respectively. Also, chromium and cadmium respectively attained a reduction of an average of 96.21% and 53.77%.

Using statistical tools in further analyzing the results showed that the immobilized cells of s. *cerevisiae* are solely responsible for the reduction of the parameters.

CHAPTER ONE

1.0 INTRODUCTION

Almost all industries discharge wastewaters at some stage of their manufacturing processes. The effects of different types of wastes when discharged for example to a stream are very complex. Leather industries are also one of the chief polluting industries that are responsible for the discharge of harmful and inorganic substances to the environment. The presence of tannery waste in water usually inhibits the development of such water for drinking and industrial use. Examples of such pollutants are suspended matter, which may kill fish and render water unsuitable for use as a domestic supply.

Tanneries have for many years, been associated with bad smells and the dumping of untreated waste effluent in rivers. In Nigeria, tanneries dispose their waste indiscriminately into sewer systems or nearby streams (Aliyu 1997).

There are difficulties in the treatment of tannery wastewaters due to the complexity of .the waste. Constituents that can cause difficulty at treatment works are suspended solids including hair and fleshing, lime (giving too high a pH value for effective treatment), sulphide and compounds of chromium (Ernest, 1980).

Due to the high cost of treatment of their wastes, most of the local tanners in Nigeria may not and are unable to adopt any form of treatment of their waste (Aliyu, 1997). However, some form of preliminary treatment is possible in the case of mechanized tanners who produce larger volumes of waste. Tanneries are heavy consumers of water (Ernest, 1980) and thus, large volume of waste waters are released daily. Most of the tanneries in Nigeria simply discharge their effluent without prior treatment into sewers or streams due to the high cost of treatment (Aliyu, 1997).

In view of the high cost of treating tannery effluent, this study therefore, focuses on the use of biotechnology which provides and environmentally accepted technique in treating tannery effluents.

1.2 TANNERY PROCESSES

Several processes are involved in the production of leather from raw hides and skins. Generally, a tannery is divided into three sections: the lime yard or beam house, the tan yard, and the finishing yard (Sarkar, 1981). The type of process is of more importance with respect to pollution control than the type of hide or skin processed (Tannery wastes, 1972).

1.2.1 THE LIME YARD

Hides and skins normally come into tanneries in wet-salted or dry-salted forms which preserve them from putrefaction and deterioration. They are

soaked in water in pits for 16 to 24 hours. When well soaked, the skins are then treated in a suspension of hydrated lime and sodium sulphide to swell the skin and to facilitate the removal of hair from the skin. They are un-haired, fleshed, washed and de-limed with ammonium sulphate or chloride (Sastry and Prasad, 1977). All the hairs, fleshing, waste liquors and washings make up the lime yard wastewater (Aliyu, 1997).

1.2.2 THE TAN YARD

Tanning is the process by which hide and skin molecules are structurally affected by substances known as tannins, such that the end product is a tender, non-putrefying material called leather. Tannins are applied to skins in water solutions. Two types of wastewater are normally produced in the tan yard: chrome tan wastewater is acidic, bluish green in colour and contains Cr^{3+} ions between 1,000 and 2,200 mg/l. This form of chromium, unlike Cr^{6+}, is non-toxic (Sastry, 1977). The vegetable tan liquor is acidic, brown and contains organic tannins.

1.2.3 THE FINISHING YARD

Finishing includes mainly dyeing, pigmentation and fat liquoring. The wastewater produced in the finishing yard is highly coloured and usually oily.

The combined wastewater, mainly from the lime yard, is called the composite wastewater. This wastewater is fairly neutral and has brownish or bluish colour depending on what type of tan wastewater predominates in its composition.

1.3 POLLUTIONAL STRENGTH OF TANNERY WASTEWATERS

Tanneries are heavy consumers of water (Ernest, 1980), and consequently large volumes of wastewaters are released daily to the surroundings. The volume of wastewater from the lime yard (soaking, liming, de-liming) varies from 1,600 to 2,200 litres per 100kg of hide or skin processed. The volume of the wastewater from the tan yard is between 400 and 500 litres per 100kg of hide or skin tanned. The quantity of wastewater from the finishing yard is comparatively small, and its discharge is intermittent. The composite wastewater has volumes between 3000 and 3500 litres per 100kg of skins processed (see table 1.1).

The objectionable constituents present in the tannery wastewaters are colour, high suspended solids, biochemical oxygen demand (BOD), chemical

oxygen demand (COD), chloride and chromium. Sastry and Prasad (1977) ranked the wastewater among the most polluting of all industrial wastewaters. Wastewaters from the lime yard and the finishing yard, or a combination of these, have varying characteristics with regard to their pollutional load.

The liming process wastewater which is highly alkaline has BOD_5 (20^0C) ranging from 6,000 to 9,000mg/l; COD from 10,000 to 20,000 mg/l: total solids from 16,000 to 45,000mg/l; and chloride content between 1,000 to 1,400mg/l (see table 1.1). The soak liquor which forms part of the lime yard liquor is the major source of chlorides, ranging between 10,000 and 15,000mg/l.

The BOD and COD of the chrome tan wastewater vary from 800 to 1,200mg/l and from 12,000 to 25,000mg/l respectively. The vegetable tan wastewater is the strongest waste stream of a tannery it has BOD values between 6,000 and 12,000mg/l and COD values ranging from 8,000 and 50,000mg/l, while the chloride content is from 1,200 to 2,000mg/l (see table 1.1).

The composite wastewater has BOD values between 2,000 and 3,000mg/l and COD values ranging from 1,400 to 4,000mg/l. the TS and chloride contents vary from 10,000 to 25,000mg/l and from 2,000 to 4,000mg/l

respectively. It is obvious that the liming liquor and the vegetable tan liquor are the most polluting waste streams from a tannery (Pithchai, 1977 and Tannery Wastes, 1972).

When compared with a typical raw domestic sewage with average BOD of 400mg/l, COD of 800mg/l, TS of 100mg/l (Sastry, 1977), the extreme level of pollution inherent in a tannery wastewater becomes evident.

When tannery wastewater is discharged into rivers and streams untreated, it may deplete the oxygen content in the river courses, thus depriving the animal and plant life therein of the vital elements, which may result in their complete extinction (Pitchai, 1977). Also, the wastes may hamper the development of water resources for drinking and industrial uses. When released unto lands, the wastewater may either percolate into the soil to pollute underground water or form of blanket on the land, which may prevent plant growth and thereby constitute a threat to agricultural production. Eventually, the wastewater blanket may be washed into rivers and streams by rain to pollute these bodies of water.

1.4 TREATMENT OF TANNERY WASTEWATER

Due to the high pollution strength of tannery wastewater, it is obvious that it must not be discharged into river courses and streams without adequate treatment.

Nearly all the sewage treatment processes can be employed for the treatment of tannery wastewater with varying degrees of performance (Kamal, 2001). These include screening for the removal of coarse materials and hair, the primary treatment by settling and the treatment methods include the activated sludge process and trickling filters (Ernest, 1980). The use of stabilization ponds for the treatment of wastewaters has generally been restricted to domestic sewage and to agricultural wastes from livestock, fruits, vegetables and food processing industries (Shuval *et. al.*, 1986). However, little information is obtainable in the literature on the use of stabilization ponds for the treatment of tannery wastewater.

TABLE 1.1 TYPICAL ANALYSES OF TANNERY EFFLUENTS (Pitchai & Guruswamy, 1977).

Effluent	Vol, lt/100kg of skin tanned	pH	TS (mg/l)	SS (mg/l)	BOD (mg/l)	COD (mg/l)	Alkalinity (mg/l)	Chloride (mg/l)
Soaking	250-400	7.5	8,000-28,000	2,500-4,000	500-5,000	2,000-6,000	800-1,200	10,000-15,000
Liming	650-1,000	10.0-12.5	16,000-45,000	4,500-6,500	6,000-9,000	10,000-20,000	1,200-1,800	1,000-1,400
De-liming	700-800	3.0-9.0	1,200-12,000	200-1,200	1,000-2,000	1,000-2,500	400-700	300-600
Vegetable tanning	200-400	5.0-6.8	8,000-50,000	5,000-20,000	6,000-12,000	8,000-20,000	-	1,200-2,000
Chrome tanning	400-500	2.6-3.2	2,400-12,000	300-1,000	800-1,200	12,000-25,000	-	-
Composite	3,000-5,000	7.5-10.5	10,000-25,000	1,250-6000	2,000-3,000	1,400-4,000	500-8,000	2,000-4,000
Pickling	-	3.0-4.0	-	400-650	500-900	500-800	-	2,000-2,500
Bating	-	8.5-9.5	-	400-600	100-200	200-400	-	500-600

1.5 AIM OF THE RESEARCH

The aim of this research herein is to treat tannery effluent using the fungi, *Saccharomyces cerevisiae* popularly known as the bakers yeast.

1.6 OBJECTIVES OF THE RESEARCH

The specific objectives of the research are:

1) To investigate the performance of bioremediation of raw tannery effluent using *S. cerevisiae.*
2) To determine the influence of immobilized *Saccharomyces cerevisiae* on other physico-chemical parameters as well as heavy metals.
3) To investigate the performance of stabilization ponds in the treatment of raw tannery wastewater.
4) To design a cost effective treatment plant for the treatment of tannery wastewater.

1.7 JUSTIFICATION

In his research on the treatment of tannery wastewater using waste stabilization ponds, Gabriel (1980) discovered that the method alone could

not degrade the effluent to acceptable standards. Furthermore, none of the tanneries in Nigeria provides any appreciable form of treatment of raw wastewaters before disposal (Aliyu, 2001). The commonest form of treatment provided is the sedimentation tank, for the sediment to settle before discharging the wastewaters into streams via a channel (Aliyu, 1997). Due to the high pollution strength, it is obvious that tannery effluents must not be discharged into streams and river courses without adequate treatment. This research therefore, hopes to use the fungus *Saccharomyces cerevisiae* to degrade effluent from leather industries.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DEFINITION AND IMPORTANCE OF BIOREMEDIATION

Bioremediation is pollution control technologies that use microorganisms to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. The acceptability of bioremediation a pollution control technology encompasses the practical applications of microorganisms (Paul, 1999). It is an ecologically sound, natural process that destroys organic contaminants and renders the residues obtained harmless (Madsen, 1991). Instead of merely transferring contaminants from one environment medium to another, bioremediation completely eliminates the target chemicals.

2.1.2 BIOREMEDIATION WITH FUNGI

Fungal treatment is a specialized bioremediation process in which fungi instead of bacteria destroy contaminants. The yeast cells as well as other microorganisms are grown under conditions to produce excessive cellular fat globules (Jeanne, 1977).

Any microorganism that synthesizes fat within itself, such as yeasts, molds or other fungi, is suitable for producing the cells into which the substances

to be encapsulated may be absorbed. This encapsulating mechanism of the yeast cells is employed in the degradation of contaminants (Turner, 1997).

Fungi are good in the accumulation of heavy metals such as cadmium, copper, mercury, lead and zinc (Bennet and Faison, 1997). The ability of fungi to transform a wide variety of hazardous chemicals has aroused interest in using them in bioremediation (Alexander, 1994).

Among other fungi used in bioremediation, the yeast e.g., *Candida tropiclis, Saccharomyces cerevisiae, S. carlbergensis* and *Candida utilis* are important in clearing industrial effluents of unwanted chemicals (Sullia, 2004).

2.2.0 BIOREMEDIATION AS BIOSORPTION OF HEAVY METALS

It is known that heavy metals are essential for the metabolism of many organisms as the constituents of metalloenzymes, but they can also be highly toxic above these trace concentrations (Kirk *et. al*., 1979). Certain species of bacteria and fungi have the ability to grow in media containing heavy metals (Venkateswerlu, *et. al*., 1989) and they can also absorb the metal ions if exposed to them in aqueous solutions over a period of time (Mullen*, et. al.,* 1989).

Bioremediation can be used to effectively reduce contaminant toxicity, mobility, or volume to levels that are protective to human health and the ecosystem (Nyer and Duffin, 1997). Biosorption can be defined as the gathering of a substance into a concentrated layer or cluster, performed by a biological system e.g. a bioreactor (Mullen *et. al*., 1989). In recent study, it was reported that *Saccharomyces cerevisiae* yeast biomass, when treated in hot alkali, exhibits an increase in its biosorption capacity for heavy metals (Wilhelmi B.S and Duncan, 1995).

2.3.0 BIOREACTORS

A bioreactor is a system which supports and may contain an organism during a desired process (Andersson, 1999). Bioreactors can be used to produce biomasses which in turn produce desired products. The bioreactors have defined and controllable environmental factors and are therefore an efficient way of exploiting capabilities of biological systems (Mueller, et. al., 1993). They have proven to be useful in both research and industry since the internal environment is usually manipulated and monitored.

In order for a process to be cost effective and feasible, certain criteria have to be met. The final goal needs to be reached in a reasonable time frame and production cost should be as low as possible (Paek *et. al*., 2005). Factors

affecting the efficiency of a process and a system therefore have to be defined and optimized. Factors may be temperature, nutrient availability, oxygen supply and demand, pH, mixing, agitation and mass transfer. In simple systems like shake flasks, the control of dissolved oxygen (DO) has proven difficult as the mass transfer is dependent on the surface to liquid ratio and the availability of gas in the flask and attempts to increase the surface area of the media and increase the flow of air proved to increase the DO in flasks (Paek, *et.al*., 2005). In more advanced bioreactors the DO is controlled by directly adding gases to the media.

Factors that may affect the biological system can be controlled by using cooling water jackets (temperature), acid/base addition (pH), feed lines (supplies nutrients) and baffles/agitators (mixing and shear stress).

2.3.1 TYPES AND CLASSIFICATION OF REACTORS

TYPES: There are numerous bioreactors with varying configurations to meet different requirements. The principal types of reactors used for the treatment of wastewater are:

I. The batch reactor ;

II. The plug flow reactor, also known as a tubular-flow reactor;

III. The continuous-flow stirred-tank reactor also known as the complete-mix reactor;

IV. The arbitrary flow reactor;

V. The packed-bed reactor and

VI. The fluidized-bed reactor. (Eddy and Metcalf, 2003)

CLASSIFICATION: Individual reactors can be classified in four different ways:

I. Based on the combination of the mode of substrate addition and the reactor geometry;

II. Based on the configuration of the biomass in the reactor (i.e. freely or immobilized);

III. The mode providing the mixing within the reactor (i.e. mechanical or gas agitation) and

IV. Based on the type of biocatalyst or enzyme used in the reactor whether aerobic or anaerobic (Atkinson, 1991).

2.4 DEFINITIONS AND PRINCIPLES OF OPERATION OF WASTE STABILIZATION PONDS

The WSP is widely recognized as one of the methods of biological treatment of wastewater. It is particularly suitable for developing tropical countries where land is available at low cost and where the prevailing climatic conditions ate favourable to its operations. Waste stabilization ponds include three major types: facultative, maturation and anaerobic ponds (Metcalf and Eddy 2003).

2.4.1 FACULTATIVE PONDS

A facultative stabilization pond is a complex biological system which calls for the symbiotic interaction between bacteria and algae in the presence of sunlight (Mara, 1986, and Arceivala *et. al.*, 1970). As far as it is presently known, the groups of organisms do not compete with each other for food within their environment. Rather, they depend of each other for their activities and survival. Dissolved oxygen (DO) in the pond is utilized by the aerobic bacteria to break down the complex organic matter. In the process, they release CO_2, H_2O, NO_3^-, PO_4^{3-} and other inorganic nutrients. The photosynthetic algae assimilate the end products of bacterial action and

liberate oxygen as an end product. The liberated oxygen and that derived from surface re-aeration supplement the oxygen content of the pond for bacterial metabolism. Figure 2.1 illustrates the process that occurs in a facultative pond.

2.4.2 MATURATION PONDS

The maturation pond is a polishing pond designed to achieve a high quality effluent, particularly with respect to the removal of bacteria and viruses. It is normally located in series at the tail end of other biological treatment systems. The pond, which is aerobic, brings about the efficient removal of bacteria and viruses through the combined effect of a time die-off mechanism, a toxic extermination from algal substances and consumption by other microscopic aquatic animals (Mills *et. al.*, 1992)**.** A maturation pond also gives some BOD and nutrient reductions (Mara, 1976).

2.4.3 ANAEROBIC PONDS

These are ponds specifically designed to receive and treat high organic loadings. Anaerobic ponds function in the absence of dissolved oxygen and degrade organic matter through two distinct types of bacteria (Juanico and Shelef, 1991). Facultative bacteria, the acid formers, first convert the complex organic matter by hydrolysis and fermentation to simple fatty acids mainly, and to some aldehydes and alcohols. The fatty acids are essentially

the acetic, propionic and butyric acids. Secondly, completely anaerobic bacteria (the methane formers) convert the acids to methane and carbon dioxide. It is at this stage that waste stabilization occurs and is directly proportional to the amount of methane produced. A suitable equilibrium under appropriate temperature and pH among other conditions has to obtain between acid formation and methane production before the system can perform satisfactorily.

2.5 DESIGN METHODS

Several methods and principles described herein are available for the design of various types of WSP.

2.5.1.0 FACULTATIVE PONDS

Various concepts have been used to describe the principles underlying the design of a facultative pond. These concepts include the completely mixed flow, the plug flow and the dispersed flow; other design methods are the empirical formulations.

2.5.1.1 COMPLETELY MIXED FLOW

This concept is based on the assumption that the contents of incoming waste are instantaneously and completely mixed with the pond contents and that the effluent is identical in composition with the contents of the pond. It also

assumes a first order process kinetics (Mara, 1976). The design equation based on these assumptions is given by the following expression:

$$\frac{Se}{So} = \frac{1}{1 + kt} \quad \text{.....} \quad (2.1)$$

Where,

So = influent BOD (mg/l)

Se = effluent BOD (mg/l)

k = reaction rate constant (d^{-1})

t = retention time (d)

The reaction rate constant k is reported to vary with temperature T, of the pond (Mara, 1976), and is related by the equation:

$$k_t = k_{20}\theta^{(t-20)} \quad \text{.....} \quad (2.2)$$

Where, k_{20} is the reaction rate constant at 20^0C, and θ is defined as temperature coefficient. Generally, θ and k have to be evaluated for each particular pond at given temperature ranges. Also, k varies with organic load, toxicity of waste and solar energy. Equation 2.1 is an ideal relationship that does not incorporate various factors like the difference between the break-down rates of the settleable and unsettlable solids (Mara, 1976). Moreover, complete mixing is usually unachievable without the aid of mechanical means which is normally not part of a facultative pond design.

Also, the equation assumes that only the food substrate is growth- limiting for the micro-organisms, and that all other conditions necessary for growth are present within the pond. Very often this is not the case.

2.5.1.2 PLUG FLOW

In plug flow it is assumed that all flow elements move with uniform velocity in the direction of flow and that there is no longitudinal mixing along the line of flow. However, there is mixing in directions perpendicular to the line of flow (Arceivala *et. al.*, 1970). The design equation for plug flow is as follows:

$$\frac{Se}{So} = e^{-kt} \quad \text{.....} \quad (2.3)$$

All the symbols are as previously defined. Although, the ideal plug flow assumption has been used in biological waste treatment designs (Metcalf and Eddy, 2003). Investigations have shown that hydraulic flow pattern cannot exist as plug flow in facultative ponds (Mara, 1976).

2.5.1.3 DISPERSED FLOW

The dispersed flow pattern lies between the plug flow and the completely mixed flow. In this flow pattern, there exists longitudinal dispersion in the line of flow as well as arbitrary inlet and outlet conditions that differ from conditions within the pond (Gloyna, 1971). Studies have shown that flow in

stabilization ponds represents a dispersed flow model given by Wehner and Wilhelm (Mara, 1976) as:

$$\frac{Se}{So} = \frac{4ae^{1/2\delta}}{(1+a)^2e^{a/2\delta} - (1-a)^2e^{-a/2\delta}} \quad \text{..... (2.4)}$$

Where $a = (1+4kt\delta)^{1/2}$

and $\delta = \frac{D'}{UL} = \frac{D't^2}{L^2}$

δ = Diffusivity constant or dispersion number

D'= Axial dispersion coefficient (m^2/hr)

U = Fluid velocity (m/hr)

L = Characteristic length of pond (m)

It has been suggested (Metcalf and Eddy, 2003) that for stabilization ponds, δ seldom exceeds 1.0. When δ is less than 2.0, equation (2.4) can be approximated to:

$$\frac{Se}{So} = \frac{4ae^{(1-a)/2\delta}}{(1+a)^2} \quad \text{..... (2.5)}$$

Equation (2.4) and (2.5) or charts prepared from them have been used for design purposes (Metcalf, 2003 and Thirumurthi, 1969).

2.5.2 DESIGN PARAMETERS OF WASTE STABILIZATION POND (WSP)

In the design of WSP, the four major parameters used are temperature, net evaporation, flow and BOD. Faecal coliform and helminth egg numbers are also important if the final effluent is to be used in agriculture or aquaculture.

2.5.2.1 TEMPERATURE AND NET EVAPORATION

The usual design temperature is the mean air temperature in the coolest month. This provides a small margin of safety as pond temperatures are 2-3^{o}C warmer than air temperatures in the cool season (the reverse is true in the hot season). Another design temperature commonly used is the air temperature in the coolest period of the irrigation season. Net evaporation (=evaporation – rainfall) has to be taken into account in the design of facultative and maturation ponds (Shaw, 1962), but not in that of anaerobic ponds as they generally nave a scum layer which effectively prevents significant evaporation.

2.5.2.2 FLOW

The mean daily flow should be measured if the wastewater exists. If it does not, it must be estimated very carefully since the size of the ponds and hence, their cost, is directly proportional to the flow.

2.5.2.3 BOD

The BOD may be measured using 24 hour flow-weighted composite samples. Although, in most tropical developing countries effluent standards do not exist (Mara, 1976). Even so design engineers need to ensure that the effluent produced in their treatment works will not pollute the receiving watercourse. As a general guide the minimum effluent standard of BOD < 25mg/l should be adopted (Mara, 1976).

2.6 SCALE – UP OF BIOPROCESS

The scaling up of biological processes for industrial use is accompanied by difficulties in reproducing the exact conditions used in the bench scale experiments (Andersson, 1999). The requirements of the organism for nutrients and inorganic substrates needed for metabolism have to be maintained within the system and have to be accounted for in the scaling up process. There may also be physical problems that may be addressed such as the difference in fluid motion between the bench scale and industrial reactors (Wheelwright, 1991).

CHAPTER THREE

3.0 MATERIALS AND METHODOLOGY

3.1 THE ORGANISM

Saccharomyces cereisiae is an inexpensive, readily available source of biomass for heavy metal removal from waste water (Wilhelmi and Duncan, 1995). Investigations conducted by several researchers demonstrated that *S. cerevisiae* is capable of accumulating heavy metals such as Cu^{2+}, Cd^{2+} ,Zn^{2+} , Cr^{2+} and Ni^{2+} (Volesky, 1995).

S. cerevisiae was chosen as the model organism for this study since it could readily be obtained locally as a paste. The organism is not known to be harmful to humans or animals and therefore is not considered a biohazard. *S. cerevisiae* is also known to have a high tolerance for a variety of heavy metals (Pim, *et. al.* 1998). Several studies show that the organism not only can withstand high concentrations of heavy metals but also reduces the pollution strength of waste water (Brendan and John, 1996).

3.2 PREPARATION OF MEDIA (POTASSIUM DEXTROSE AGAR, PDA)

The Irish potato (*solanum tuberosum*) was neatly peeled, chopped into smaller pieces and 250g was weighed out. This was washed

and put in a conical flask containing 500ml of distilled water. The content was left to boil and the extract was strained through a cheese cloth.

500ml of distilled water was used to dissolve 15g each of dextrose and agar. This was poured into a conical flask containing the potato extract. The mixture was left to boil until homogeny and distilled water was added to make the mixture up to 1000ml. The mixture was then sterilized in an autoclave at a temperature of 121^{o}C for 15 minutes after which it was left to cool. The sterilized PDA was poured into a sterile Petri dish and kept under aseptic conditions.

3.3 YEAST PROPAGATION

A sterile inoculating needle was used to pick the inoculum onto the Petri dish containing the sterile medium. This was carried out under aseptic conditions and the Petri dish was then incubated at 32^{o}C for 72 hours for propagation.

3.4 CELL IMMOBILIZATION

The method adopted for the cell immobilization is according to the one described by James (1992) with slight modification (Shide *et. al.*, 2004). The cells of the exponentially growing mycelia of the culture were harvested

aseptically using a sharp knife into a 250ml conical flask containing 50ml of distilled water and 3.063g of sodium alginate (Hopkin & Williams limited, England). The mixture was subsequently pumped through a sterilized silicon tube (1.6mm D) drop-wise into a beaker containing sterilized 500ml of 0.12M $CaCl_2$ (May & Baker Limited, England). The reaction which was almost instantaneous was allowed for retention time of 1hr for complete precipitation that formed spherical beads of diameter 3.75-4.5mm. The immobilized cells were removed and stored in distilled water at 4^0C.

3.5 LABORATORY SET-UP

The model treatment pond used consisted of a series combination of rectangular storage tank, an anaerobic pond and a facultative pond (figure 3.1). The systems were constructed of Perspex glass. The capacity of the storage tank was 70 litres**.** The internal dimension of the anaerobic pond was 65 by 40 by 25 cm with a working capacity of 60 litres. The side of the anaerobic pond was covered with a black polythene sheet to prevent light penetrations into the pond. The dimensions of the facultative pond were 36 by 15 by 9 cm with an operational capacity of 5 litres. Light was provided by 20-watts fluorescent bulb of light intensity 16,250 lumens/ m^2 (1,500 ft – candles) located at a distance of 20cm above the liquid surface of the

facultative pond. A 12 hour-on and 12 hour-off light cycles were maintained throughout the period of the investigation.

3.6 SAMPLE COLLECTION

The wastewater used in this study consists of raw composite tannery effluent from the Leather Research Institute, Zaria. The preliminary analysis of the wastewater showed that it was of very high strength in terms of BOD. The preliminary results showed that the BOD of the wastewater ranged from 1200mg/l to 2450mg/l with an average of 1825mg/l.

The Chemical Oxygen Demand (COD) ranges from 36,000 mg/l to 38,500 mg/l. The results from the table 3.1 showed that the effluent from the tannery is very strong and it undoubtedly became apparent that the designed facultative pond might be overloaded and consequently function as an anaerobic pond. As such, it was subjected to anaerobic pre-treatment.

Table 3.1 Initial Concentration of Raw Tannery Wastewater

Parameter	Range of Concentration (mg/l)
BOD	1,200 - 2,450
COD	36,000 - 38,500

Chloride	3,889 - 4,110
Sulphate	10,150 - 11,141
pH	8.5 - 9.7
Total Solids	7,120 - 7,450

The use of sulphate compounds in the process of for the treatment of hides and skins to facilitate the removal of hair compounds largely contributes in the high concentration of sulphate ions in the sample of raw wastewater.

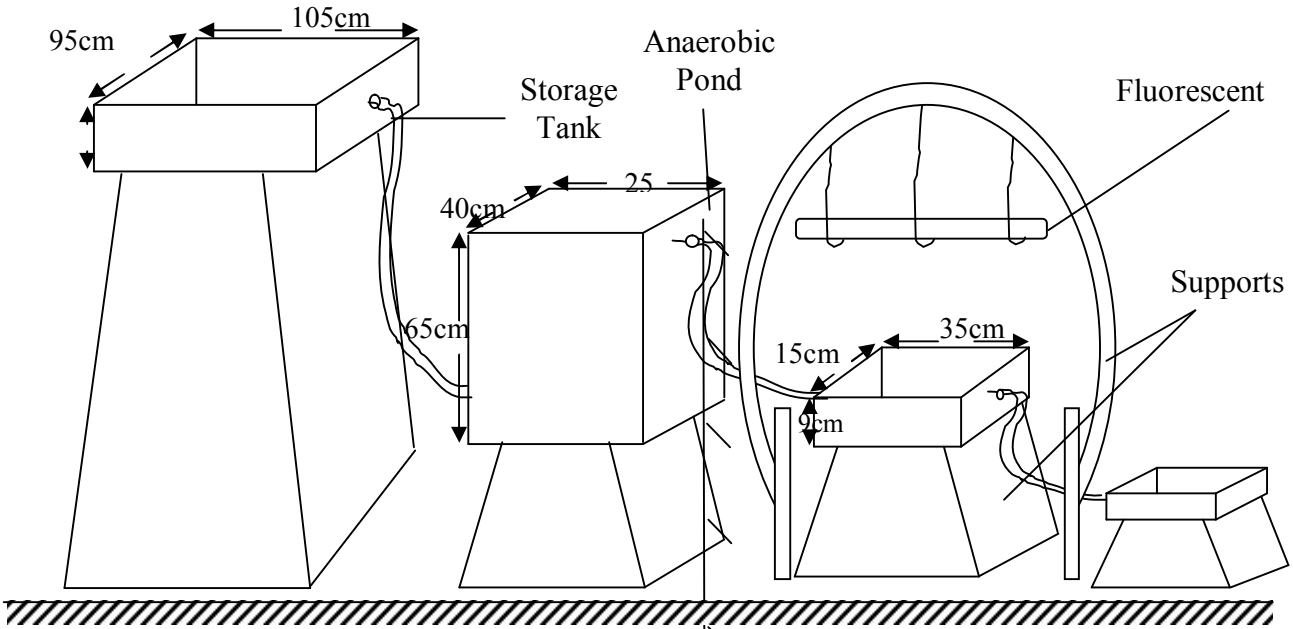


Fig. 3.1 ARRANGEMENT OF MODEL PONDS (Not to scale)

3.7 BIOREMEDIATION OF RAW WASTEWATER SAMPLE

The bioremediation of the tannery effluent collected was carried out in the 5 litres model pond and 100 beads of immobilized *S. cerevisiae* cells were inoculated into the pond. Samples were collected from the set-up at several intervals of 3hrs, 5hrs, 24hrs, 35hrs and 24hrs. Then, samples were collected for the next five days. These collected samples were analyzed for BOD_5, COD, TS, chloride, sulphate, pH, chromium, and cadmium in accordance with the Standard Methods for the Examination of Water and Wastewater (Apha, 1993). Another pond of same capacity was used to serve as a control experiment.

3.8 BIOREMEDIATION OF STERILIZED WASTEWATER SAMPLE

5 litres of the raw wastewater sample was sterilized and 100 beads of immobilized *S. cerevisiae* cells were inoculated and subjected to the same periods as the un-sterilized and the control. Samples were also analyzed for BOD_5, COD, TS, chloride, sulphate, pH, chromium and cadmium.

3.9 STATISTICAL ANALYSIS

The use of statistics was employed in analyzing the results to ascertain the performance of the immobilized cells of *Saccharomyces cerevisiae* on each parameter using the t-Test. Also, the statistical correlation was used to

determine the association between the immobilized yeast cells of *S. cerevisiae* and the decrease or increase of any of the parameter.

CHAPTER FOUR

RESULTS AND DISCUSSION

The analyses of the results obtained at the end of this research are shown in tables 4.1.0 to 4.8.2. The sample of tannery wastewater treated with the immobilized cells of *Saccharomyces cerevisae* was represented on the tables as WR while the sterilized sample of the wastewater treated with the immobilized cells was represented as WS. The control samples of the raw and the sterilized tannery wastewater are represented as CR and CS respectively.

The percentage of wastes removed from the wastewater of both the raw and sterilized samples are respectively shown in the table of each parameter.

The tables of each parameter that was analyzed include that obtained from the laboratory, statistical calculations of the student's t-test to ascertain the probable performance of the immobilized cells of *Saccharomyces cerevisiae* on each parameter as well as the statistical correlation to determine the likely association between the immobilized yeast cells and the decrease or increase of each of the parameter.

4.1 Results and Discussion for Biochemical Oxygen Demand (BOD)

Table 4.1.0 shows the Biochemical Oxygen Demand concentrations obtained for all the samples. There was a general decrease in the values of BOD with WR having 73.52% removal as compared to WS which had a reduction of 96.52%.

The results obtained for BOD showed that *Saccharomyces cerevisiae* has the potentials to reduce the strength of pollution of raw tannery wastewater as shown in table 4.1.0. There is the possibility that the sterilization of the sample destroyed other micro-organisms that could have interfered or be antagonistic to the activity of the yeast cells.

In a study on the treatment of raw tannery wastewater using waste stabilization pond by Gabriel (1980), the BOD concentration recorded after the process showed that the values were very high.

It was also observed that the tannery wastewater yielded an average of 25% BOD removal before 24 hours in the composite tannery wastewater. This compares with the BOD removal values of about 20 to 33% reported in literature by Eddy (1993) and Mara (1976).

Table 4.1.0 Results of Biochemical Oxygen Demand of the tannery wastewater during the study period.

PERIOD	WR SAMPLE	% REMOVED	CR SAMPLE	WS SAMPLE	% REMOVED	CS SAMPLE
0 HR	2,455	-	2,455	2,010	-	2,010
3 HRS	2,455	-	2,450	2,000	0.5	2,000
8 HRS	2,450	0.2	2,410	1,880	6.47	2,000
15 HRS	2,300	6.3	2,350	1,560	22.39	2,150
24 HRS	1,800	26.68	2,010	1,500	25.37	2,170
35 HRS	1,780	27.49	1,910	1,200	40.3	2,180
48 HRS	1,550	36.86	1,900	910	54.73	2,110
DAY 3	1,100	55.19	1,800	820	59.2	2,110
DAY 4	900	63.34	1,650	580	71.14	2,030
DAY 5	890	63.75	1,350	320	84.08	1,490
DAY 6	720	70.67	1,200	160	92.04	1,230
DAY 7	650	73.52	1,201	70	96.52	960

The t-test shows that t_{cal} is less than t_{val} at 0.05 level of probability (see table 4.1.1) this suggests that it is not likely that the means of WR and WS are significantly different.

It was also calculated that at 0.05 level of probability, the correlation analysis from table 4.1.2 suggests that there is the likelihood that an

association exists between the decrease in BOD concentration and the yeast cells as r_{cal} is greater than r critical.

Table 4.1.1 t-test for WR and WS results for BOD

Statistical Value	WR Sample	WS Sample
Mean	1587.58	1084.17
Sample Size	12	12
t_{cal} .	1.726	
$t_{val.}$ (0.05)	2.074	

Table 4.1.2 Correlation value for BOD of the tannery wastewater

Sample Size	r critical (0.05)	r cal.
12	0.576	0.9797

4.2 Results and Discussion for Chemical Oxygen Demand

For COD concentrations, WR had a removal of 62.96% and WS a removal of 86.67% (see table 4.2.0). This suggests that there is the likelihood of micro organisms to effectively reduce the level of organic pollution of tannery wastewater and efficiently reduce to oxygen demand exerted by chemicals in the waste water.

The results also showed that there was an abrupt reduction in the COD concentration after 2 days. The sudden drop in the concentration could not be explained. Thirumurthi (1969) similarly reported some erratic behaviour in the measurement of COD concentrations for reasons unknown. However, there was the presence of algal growth that covered the surface of the tank for about 24 hours but starts dying by the third day. It could be possible that the sudden reduction in the COD concentration is as a result of the algal growth because it coincides with the period when the algae start dying.

The removal at longer times may probably be as a result of the combining effects of settling and microbial degradation in the system.

There was a general decrease in the COD concentration in the samples with WR having a reduction of about 62% as compared to WS which had COD concentration removal of about 87%. There were no significant changes in CR and CS.

Table 4.2.0 Results of Chemical Oxygen Demand obtained from the tannery wastewater during the study period.

PERIOD	WR SAMPLE	% REMOVED	CR SAMPLE	WS SAMPLE	% REMOVED	CS SAMPLE
0 HR	27,000	-	27,000	25,500	-	15,000
3 HRS	27,000	-	27,000	12,400	16.67	15,000
8 HRS	26,500	1.85	27,000	25,500	26.67	14,500
15 HRS	26,800	0.74	26,000	20,500	40.00	14,000
24 HRS	26,800	5.56	25,000	18,800	48.00	14,500
35 HRS	27,000	25.93	24,000	16,600	56.00	13,500
48 HRS	17,500	35.19	24,500	11,200	58.67	14,000
DAY 3	15,000	44.44	24,000	5,300	64.67	13,000
DAY 4	12,500	53.70	23,000	3,500	76.67	12,000
DAY 5	11,500	57.41	23,500	3,000	80.00	11,500
DAY 6	11,500	57.41	24,000	2,000	86.67	11,500
DAY 7	10,000	62.96	22,000	2,000	86.67	11,500

The results obtained from the student's t-Test and the correlation of the COD concentrations is shown in tables 4.2.1 and 4.2.2. The statistical table suggests that there is the likelihood that there exists a relationship between the reduction in the COD concentrations and the immobilized cells of *S. cerevisiae.*

Table 4.2.1 The t-test measured for COD

Statistical Value	WR Sample	WS Sample
Mean	19237.5	6991.67
Sample Size	12	12
$t_{cal.}$	5.175	
$t_{val.}(0.05)$	2.074	

Table 4.2.2 Correlation Table for COD

SAMPLE SIZE	r critical (0.05)	r cal.
12	0.576	0.9235

4.3 Result s obtained for Sulphate Concentration during the Study Period.

Table 4.3.0 shows that sulphate concentrations in the wastewater samples were generally high during the study period. Sulphate ion occurs naturally in most industrial wastewaters and is required in the synthesis of proteins in tannery industries and is released in their degradation (Metcal f and Eddy, 2003).

The sulphate concentrations were increasing throughout the period of study (see table 4.3.0). From the results obtained, it is clear that the yeast cells did not have any effect on the removal of sulphate concentration. Isaiah (2000) for example, employed the use of carbonaceous filter media in the treatment of textile waste waters and experienced increasing values of sulphate concentrations during the secondary stage of treatment.

Table 4.3.0 Results of Sulphate concentration of the tannery wastewater obtained during the study period.

PERIOD	WR SAMPLE	% REMOVED	CR SAMPLE	WS SAMPLE	% REMOVED	CS SAMPLE
0 HR	8,000	-	8,000	7,250	-	7,250
3 HRS	8,250	3.12	8,000	7,250	-	7,350
8 HRS	8,290	3.62	8,100	8,500	17.24	7,450

15 HRS	9,250	15.62	8,500	8,700	20	7,500
24 HRS	10,000	25	8,800	9,200	26.9	7,450
35 HRS	11,790	47.38	8,900	9,350	28.96	7,700
48 HRS	12,100	51.25	9,000	9,850	35.86	7,750
DAY 3	13,000	62.5	9,100	11,100	53.1	7,900
DAY 4	13,650	70.62	9,000	11,250	55.17	8,300
DAY 5	13,700	71.25	9,000	11,270	55.45	8,550
DAY 6	13,700	71.25	9,250	12,000	65.52	8,500
DAY 7	13,750	71.87	9,300	12,200	68.27	8,900

After the study period, the value of WR ranged from 8,000mg/l to 13,750mg/l, while WS ranged from 7,250mg/l to 12,200mg/l. This increase in sulphate concentration is likely to be connected to the anaerobic process of treatment of the raw wastewater sample due to the generation of hydrogen sulphide gas that ended up as sulphate compound.

The increase in the sulphate concentration from the tannery wastewater could also be attributed to the lack of precipitation of sulphate compounds as reported in literature (Moshe, *et. al.* 1972) and the lack of conversion of

sulphated to sulphides (Shuval, *et.al* 1986), sulphur dioxide and other gases by bacteria present in the wastewaters (Mills, et.al. 1992).

After treatment during the study period, the value of WR ranged from 8,000mg/l to 13,750mg/l, while WS ranged from 7,250mg/l to 12,200mg/l.

Using the statistical tools to further analyze the results obtained, the t-Test in table 4.3.1 show that t_{cal} is less than t_{val}. This, therefore suggests that the likelihood that there could be no evidence that the means of WR and WS are significantly different. Consequently, using a 0.05 level of probability, the correlation analysis from table 4.3.2 shows that there is the probability that there is no association between the increase in sulphate concentration and the yeast cells as earlier stated.

Table 4.3.1 t-test for Sulphate

Statistical Value	WR Sample	WS Sample
Mean	23058.33	20366.67
Sample Size	12	12

t_{cal} .	0.467
$t_{val.}$ (0.05)	2.074

Table 4.3.2 Correlation Table for Sulphate

Sample Size	r critical (0.05)	r cal.
12	0.576	0.9835

4.4 Results Obtained and Discussion for Chloride Concentration

Chloride is another qualitative parameter of significance that occurs in tannery wastewater primarily as a result of the addition of ammonium chloride in the tanning process. From the results obtained in the analysis, it can be seen that the chloride concentration of WR had a decrease of 69.56% and WS had a reduction of 87.91%. Important information revealed by the result is that a mixture of microorganisms in the wastewater has the ability to efficiently breakdown chlorine compounds in the wastewater. Comparing CR to CS indicates that CR had an extremely low removal of chlorides due to the fact that the sample was not sterilized.

Table 4.4.0 Results obtained for Chloride Concentration of the tannery wastewater during the study period.

PERIOD	WR SAMPLE	% REMOVED	CR SAMPLE	WS SAMPLE	% REMOVED	CS SAMPLE
0 HR	3,450	-	3,450	3,299	-	3,299
3 HRS	3,400	1.45	3,400	2,679	18.79	3,270
8 HRS	3,250	5.8	3,260	2,199	33.34	3,250
15 HRS	3,299	4.38	3,100	2,049	37.89	3,250
24 HRS	2,699	21.77	2,870	2,000	39.38	3,220
35 HRS	2,349	31.91	2,740	1,600	51.5	3,219
48 HRS	1,899	44.96	2,450	1,450	56.05	3,219
DAY 3	1,450	57.97	2,440	1,250	62.11	3,219
DAY 4	1,390	59.71	2,220	999	69.72	3,200
DAY 5	1,300	62.32	2,110	749	77.96	3,200
DAY 6	1,199	65.25	2,210	525	84.09	3,200
DAY 7	1,050	69.56	2,040	399	87.91	3,200

The chloride content remaining after the study period showed that the sterilized sample of the wastewater containing the yeast cells was reduced beyond the limit set by WHO (see appendix 1). The other samples indicate

that the concentration of chloride in the wastewater is very high. *S.cerevisiae* therefore, is efficient in the removal of chloride from tannery wastewater.

The chloride content for WR had a reduction from 3,450mg/l to 1,050mg/l as compared to WS which had a reduction of 3,299mg/l to 399mg/l. WR, though had a total reduction rate of 69.56% did not met the World Health Organization standard for maximum allowable concentration of 600mg/l for discharged wastewater. FEPA gave a limit for the discharge of effluent into surface waters and land application of 600mg/l each and it can thus, be concluded from the results shown in table 4.4.0 that WS had a reduction rate of 399mg/l showing that it is an acceptable concentration for discharge wastewater.

Table 4.4.1 The t-test for Chloride

Statistical Value	WR Sample	WS Sample
Mean	2227.92	1599.83
Sample Size	12	12
t_{cal} .	1.672	
$t_{val.}$ (0.05)	2.074	

Table 4.4.2 Correlation Table for Chloride

Sample Size	r critical (0.05)	r cal.
12	0.576	0.9436

The t-Test result shown in table 4.4.1 gives that t_{cal} is less than t_{val}, denoting that there is no significant difference in the concentration of chloride at 0.05 level of probability of the raw wastewater containing yeast cells and the sterilized wastewater containing the cells.

From the chloride correlation analysis, r_{cal} is greater than r_{val} implying that there is the possibility of a strong association between the decrease in chloride concentration and yeast cells at 0.05 level of probability.

4.5 pH values obtained during the study period

The pH values indicate that the composite effluent tend to have a rising trend in pH values. Though, the results generally showed that the values remained about a neutral level.

Table 4.5.0 pH values of the tannery wastewater obtained during the study period.

PERIOD	WR SAMPLE	CR SAMPLE	WS SAMPLE	CS SAMPLE
0 HR	8.36	9.04	7.17	7.93
3 HRS	8.46	9.04	7.33	8.07
8 HRS	8.58	9.06	7.43	8.03
15 HRS	8.59	9.08	7.68	8.11
24 HRS	8.54	9.09	7.72	8.17
35 HRS	8.56	9.04	8.00	8.20
48 HRS	8.66	9.07	8.10	8.19
DAY 3	8.91	9.13	8.03	8.22
DAY 4	9.01	9.11	8.21	8.21
DAY 5	8.99	9.11	8.36	8.21

DAY 6	9.05	9.17	8.43	8.30
DAY 7	9.15	9.15	8.46	8.46

4.6 Result of Total Solids obtained during the Study Period

The results of the total solids obtained during the study period are given in table 4.6.0. The results showed that the reduction rate is relatively low as given in table 4.6.0. WR had a removal of 16.67% as compared to WS which had a removal of 40.45%.

Due to the slow rate of flow the settling process in the ponds is likely to affect the reduction on the amount of total solids. From the results in table 4.6.0, it could be seen that there is gradual rate in the settling process. Also, the high turbid nature of the tannery wastewater is a probable factor that could affect the low rate of reduction of the solids during the treatment process.

The values obtained from the control sample as well as the sterilized sample during the period of study showed that the rate of reduction is generally low. The variation observed from the two samples could possibly be as a result of the coupling effect of sterilization.

Table 4.6.0 Concentrations of the Total Solids obtained from the raw and treated tannery wastewater during the period of study.

PERIOD	WR SAMPLE	% REMOVED	CR SAMPLE	WS SAMPLE	% REMOVED	CS SAMPLE
0 HR	6,600	-	6,600	6,280	-	6,280
3 HRS	6,540	0.91	6,500	6,080	3.81	6,250
8 HRS	6,535	0.98	6,490	5,810	7.48	6,230
15 HRS	6,400	3.03	6,390	5,610	10.67	6,100
24 HRS	6,330	4.09	6,200	5,390	14.17	6,000
35 HRS	6,140	6.97	6,100	5,020	20.06	5,910
48 HRS	6,130	7.12	6,030	4,940	21.34	5,820
DAY 3	6,060	8.18	5,900	4,740	24.52	5,750
DAY 4	5,980	9.39	5,890	4,400	29.94	5,610

DAY 5	5,840	11.52	5,820	4,200	33.12	5,400
DAY 6	5,770	12.58	5,712	3,900	37.9	5,210
DAY 7	5,500	16.67	5,701	3,740	40.45	5,040

The student's t-Test shown in table 4.6.1 compares the variation of the treatment of WR and WS while the correlation table gives the association between the decrease in the concentration of total solids and the yeast cells during the study period.

Table 4.6.1 The t-test of the Total Solids

Statistical Value	WR Sample	WS Sample
Mean	6125.08	5009.17
Sample Size	12	12
tcal .	4.238	
t val. (0.05)	2.074	

Table 4.6.2 Correlation Table for Total Solids

Sample Size	r critical (0.05)	r cal.
12	0.576	0.9843

4.7 Chromium Concentration during the Study Period

Traces of many metals such as cadmium and chromium are important constituents of most wastewaters. The presence of any of these metals in excessive quantities will interfere with many beneficial uses of the water because of their toxicity.

From the analysis for chromium and cadmium given in tables 4.7.0 and 4.8.0 it showed that for chromium concentrations, WR and WS respectively attained a reduction of 50.35% and 99.53%. This showed that fungus has the ability to effectively absorb chromium from tannery wastewater. Table 4.8.0 also showed that cadmium was effectively absorbed by the fungus. WR had a reduction of 53.77% and WS a reduction of 99.56%.

Table 4.7.0 Concentrations of Chromium obtained for the raw and treated tannery wastewater during the period of study period.

PERIOD	WR SAMPLE	% REMOVED	CR SAMPLE	WS SAMPLE	% REMOVED	CS SAMPLE

0 HR	4.798	-	4.798	4.72	-	4.72
3 HRS	4.711	1.81	4.798	4.637	1.76	4.72
8 HRS	4.6	4.13	4.798	4.2655	9.63	4.72
15 HRS	4.5368	5.44	4.798	3.9162	17.03	4.72
24 HRS	4.0195	16.23	4.798	3.0192	36.03	4.72
35 HRS	3.9886	16.87	4.798	2.194	53.52	4.72
48 HRS	3.5515	25.98	4.798	1.1011	76.67	4.72
DAY 3	3.0192	37.07	4.798	0.1381	97.07	4.72
DAY 4	2.65	44.77	4.798	0.0925	98.04	4.72
DAY 5	1.4013	70.79	4.798	0.0791	98.32	4.72
DAY 6	0.611	87.27	4.798	0.0421	99.11	4.72
DAY 7	0.182	96.21	4.798	0.0222	99.53	4.72

Appendix 3 showed that chromium and cadmium were both reduced to their respective maximum permitted concentrations of toxic substances in industrial effluents in Nigeria for the protection of groundwater. The parameters also reduced significantly below the limits set by WHO as shown in appendix 1.

There was no significant reduction shown in the control samples of both the sterilized and un-sterilized wastewater. This suggests that *S.cerevisiae* is an efficient microorganism in the removal of heavy metals from wastewater.

Table 4.7.0 showed the chromium concentration during the study period. It showed that there was a general decrease in the samples containing the yeast cells as compared to the samples without the cells. The table showed that WR had a reduction rate of 96.21% as compared to WS which had a reduction of 99.53%. The student's t-Test and the correlation tables for the chromium concentrations are given in tables 4.7.1 and 4.7.2

Table 4.7.1 t-Test for Chromium Concentration

Statistical Value	WR Sample	WS Sample
Mean	3.1987	2.0189
Sample Size	12	12
tcal .	1.589	
t val. (0.05)	2.074	

Table 4.7.2 Correlation Table for Chromium

Sample Size	r critical (0.05)	r cal.
12	0.576	0.833

4.8 Concentration of Cadmium obtained During the Study Period

The cadmium concentrations are given in table 4.8.0. It showed that WR had a reduction of 53.77% as compared to WS that had a reduction of 99.56% during the study period.

Though the maximum permitted concentrations for cadmium concentration substances from Nigeria industrial effluents for the protection of groundwater is less than 0.01mg/l (see appendix 2), the values obtained from this research showed that the yeast cells did not reduced the cadmium concentrations to this acceptable limits.

However, it meets with the limits set as guidelines for interim uniform effluent limits in Nigeria for all categories of industries for wastewater effluents discharged on the land surface (see appendix 3).

Table 4.8.0 Cadmium concentrations of the raw and treated tannery wastewater obtained during the period of study.

PERIOD	WR SAMPLE	% REMOVED	CR SAMPLE	WS SAMPLE	% REMOVED	CS SAMPLE
0 HR	1.9512		1.9512	1.6		1.6
3 HRS	1.9099	2.12	1.9512	1.5767	1.46	1.58
8 HRS	1.8837	3.46	1.9512	1.098	31.38	1.58
15 HRS	1.8315	6.13	1.9512	0.844	47.25	1.58
24 HRS	1.7271	11.49	1.9512	0.5312	66.8	1.58
35 HRS	1.701	12.82	1.9512	0.3202	79.99	1.58
48 HRS	1.6767	14.07	1.9512	0.1821	88.62	1.58
DAY 3	1.4998	23.13	1.9512	0.0948	94.08	1.58
DAY 4	1.3316	31.75	1.9512	0.0411	97.43	1.58
DAY 5	1.1137	42.92	1.9512	0.0303	98.11	1.58
DAY 6	0.9998	48.76	1.9512	0.011	99.31	1.58
DAY 7	0.9021	53.77	1.9512	0.007	99.56	1.58

Tables 4.8.1 and 4.8.2 shows the result of the student's t-Test comparing the variation of the treatment of WR and WS and the correlation between the relationship of cadmium decrease and the yeast cells.

Table 4.8.1 t-test for Cadmium concentration

Statistical Value	WR Sample	WS Sample
Mean	1.544	0.536
Sample Size	12	12
tcal .	4.817	
t val. (0.05)	2.074	

Table 4.8.2 Correlation Table for Cadmium

Sample Size	r critical (0.05)	r cal
12	0.576	0.7934

CHAPTER FIVE

5.0 STRATEGIC ACTION PLAN

5.1 QUANTITY OF WATER REQUIRED PER DAY

Tanning industry is a large consumer of water and hence a large producer of wastewater. The amount of water used per process depends largely on the weight of the skin. The processes of tanning and water consumption are shown in table 5.1.

Table 5.1 Amount of water used of skin weight for different processes in tanning.

PROCESS	AMOUNT OF WATER USED AS % OF SKIN WEIGHT
Soaking	600-700
Hair Pulping	200-270
Liming	300-400
De-liming	100-120
Bating	20% of De-liming
Washing/Rinsing	Running water (quantity unknown)

Pickle	100
Chromium	60-70

5.2 WEIGHT OF SKINS/HIDES PROCESSED PER DAY

The weight of salted goat and sheepskin from abattoir fall in ranges of heavy, medium and light but the ranges normally used in tannery industries is from:

Goat: 700-820 grams

Sheep: 900-1300 grams (Kamal, 2002)

Considering industries that have the capacity to treat 10,000 skins per day using an average weight of 1,300grams, it therefore implies that:

Total weight processed per day = 10000 x 1300g

= 13tonnes per day.

5.3 AMOUNT OF WASTEWATER GENERATED PER DAY

The amount of wastewater generated per day can be estimated per process using the average percentages from table 5.1. For each process it can be estimated as follows:

Soaking = 350 x 13000/100 = 84500 kg

Hair Pulping = 210 x 13000/100 = 27300 kg

Liming = 350 x 13000/100 = 45500 kg

De-liming = 110 x 13000/100 = 14300 kg

Bating = 20 x 13000/100 = 2860 kg

Pickle = 100 x 13000/100 = 13000 kg

Chromium = 65 x 13000/100 = 8450 kg

Therefore, total amount of wastewater estimated = 190190 kg; approximately 200,000 kg. But 1000 kg of water occupies 1 cubic meter by volume and the volume of water used per day = $200m^3$

Multiplying by a factor of 1.5,

The volume of water used per day = 1.5 x 200 m^3

= 300 m^3 per day.

5.4 AMOUNT OF YEAST REQUIRED

From the research, it was discovered that using the Baker's Yeast as the fungi for the treatment of the wastewater, 1.5g of *S.cerevisiae* was used to degrade 5 litres of wastewater. Since it is readily cheap, available in the market and does not contain pollutants, it can be estimated to treat 300 m^3 of wastewater generated per day.

For 300 m^3 per day of wastewater, it implies that:

(300 m^3 x 1.5g) / 0.005 m^3 = 90 kg of yeast is required per day for

the treatment of 300 m^3 of wastewater.

5.5 DESIGN OF TREATMENT PLANT

The implementation of a WSP system is the best solution for treating the wastewater generated by tannery industries. The method is inexpensive, not requiring heavy machinery or electricity and having need of about two or three workers to oversee the facility.

Two ponds, an anaerobic and a facultative pond are required for this system. The anaerobic pond is designed to process 300m^3 of the tannery influent per day. The dimensions of the anaerobic pond will include a length of 14.2m, width 28.3m and a depth of 3m. The length and width follow a ratio 2:1 as recommended by Mara (2003) to avoid formation of sludge banks near the inlet to the pond, as well as a depth that will ensure that the pond remains thoroughly mixed. The total area of the anaerobic pond is 400m^2.

From table 3.1, average influent BOD, $l_{i,}$ is about 1200mg/l, and it is estimated that 84% removal of BOD will be achieved from the anaerobic pond. Remaining effluent of 190g/m^3 per day BOD is high to be discharged and will require a secondary facultative pond for further treatment.

The facultative pond designed will be adjoined to the anaerobic pond along its length. A weir will allow influent BOD at a flow rate of 300m^3 per day to

enter the pond to begin disinfection process. The dimensions of the pond will include a length of 28.3m and width 56.6m, following the 2:1 width to length ratio recommended by Mara (2003). The depth of the pond is assumed to be 1.5m. The surface area of the pond is 1600m^2 with a retention time of 8 days. The details of the calculations and equations used to determine the specifications of the pond for both the facultative pond and anaerobic pond are as shown in appendix 1.

5.6 COSTING OF THE FACILITIES

Of major significance in the selection and design of alternative wastewater treatment facilities, is the question of costs –not only the initial costs of construction but as well as its operation and maintenance costs (Eddy and Metcal f, 2003).

The costing of the tannery treatment processes will be categorized into three as follows:

i. Average cost of the treatment plant
ii. Average operation and maintenance cost
iii. Cost of yeast required per day

5.6.1 AVERAGE COST OF TREATMENT PLANT

The cost of treatment plants and facilities depend on the specific site conditions and vary within a very wide range (Juanico, 1991). The individual tannery share in the cost of designing of the treatment plant is often determined on the basis of actual effluent volume and pollution load that is generated from the industry (Kamal, 2001).

Appendix 2 shows the estimated cost involved for the designing of a treatment plant to handle effluent with an average flow rate of $300m^3$.

Using a concrete strength of $25m/mm^2$ material schedule with prescribed mix 1:2:4, the respective factors for cement, sand and aggregates are 6, 0.1386 and 0.2197 (Devdas, 2003).

Based on the calculated dimensions of the design of the treatment facilities (anaerobic and facultative ponds) in appendix 1, the total cost of treatment plant was estimated to be N3, 000,000.

5.6.2 OPERATION AND MAINTENANCE COST

After the anaerobic pre-treatment of the wastewater, it is expected that there will be huge accumulation of sludge. Therefore, the cost of de-sludging is an important parameter in estimating operation cost of a treatment plant.

The disposal of the disposal of the sludge will be anaerobic digestion because of its usefulness as biogas generation and the high fertilizer value (Hammer, 1975).

Sludge from wastewater treatment is usually in form of liquid or semi-liquid and typically contains from 0.25 to 12 percent solids (Mara, 1976).

Estimated wastewater generated per day = 300 m^3

Assume volume of sludge generated per day = 300 x 12% = 36 m^3

Assume capital cost per cubic meter/day = N5

Capital cost per day = N5 x 300 = N1, 500

5.6.3 AVERAGE COST OF YEAST REQUIRED PER DAY

Bakers Yeast is readily cheap and available in the market and about 15g of yeast is estimated to cost N650. Since 90 kg of yeast is estimated per day for the treatment of 300m^3 of wastewater, it therefore implies that approximately N3, 900 is required for daily treatment of wastewater from a tannery effluent of 300m^3.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

From the results obtained at the end of the study it was observed the fungus has the potential to degrade to relatively low levels, raw tannery wastewater if provided with favourable conditions.

When comparing the levels of reduction in the pollution strength for the samples containing the cells, it is clear that fungus would meet at least some of the standards set by WHO and FEPA.

In terms of BOD and COD measurements, raw tannery wastewaters may be described as an extremely strong industrial wastewater. The anaerobic pre-treatment of the wastewater became necessary as the average initial BOD and COD were 3,825mg/l and 36,250mg/l respectively. The pre-treatment also results in the saving of retention time (Mara, 1976).

With results obtained for BOD concentration, it could be seen that fungus has the potentials to further reduce the strength of the raw wastewater to the acceptable limits set by FEPA (see appendix 3).

The results showed a reduction pattern in the chloride concentrations. This was not the case for sulphate. It is therefore deduced that the anaerobic pre-

treatment of raw tannery wastewater cannot be devoid of high concentrations of sulphates due to the production of hydrogen sulphide.

The reduction in the heavy metals concentrations clearly suggests that *Saccharomyces cerevisiae* has high adsorption capacities. It can then be concluded that for metal-contaminated effluents, *S.cerevisiae* have the capability of reducing the level of toxicity of metal-contaminated tannery effluents.

Though, at a very slow rate and higher detention time, the modeled pond revealed that it can withstand and naturally reduce the pollution strength of raw tannery wastewater. This was deduced from the results obtained from the respective control samples of BOD and COD.

The fungus can reduce pollutants of raw tannery wastewater at high concentrations and can therefore be valuable in tannery wastewater treatment applications.

6.2 RECOMMENDATIONS

From the study, the following were recommended: -

I. Although, large amounts of TS and COD were removed by both anaerobic pre-treatment and inoculation with yeast cells, the final effluent from the system is still left with a substantial amount of these parameters. The quality of the effluent should therefore be improved by additional treatment such as

provision of facultative ponds in series before discharging it into the receiving stream.

II. The final effluent after treatment should undergo a carbonaceous filtration process to reduce the concentrations of sulphate that was observed to be increasing in value during the period of the research.

III. Furthermore, this study revealed that more research are required in the following areas :-

i. The relationship between COD concentration and the algal growth that covered the surface of the tank;

ii. How tolerant and effective the fungus is at removing metal ions from wastewater;

iii. Focussing in scaling up the bio-process to handle large volumes of industrial wastewater each day and at low cost.

IV. Also, further research is recommended to determine the optimal number of immobilized beads of *S.cerevisiae* is required for the treatment of 5 litres of wastewater sample of tannery wastewater to serve as standard for extrapolation for the treatment of larger volumes of wastewater.

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APPENDIX 1:

CALCULATIONS

1. **Anaerobic Pond:**

$$A_a = \frac{Qt_a}{D_a} = \frac{l_i Q}{\lambda_v D_a}$$

$Q = 300m^3$ per day
lowest temp = 20^0C
For air temp of 20^0C, loading = $\frac{300gBOD}{m^3 day} = \lambda_v$

i. Pond Volume

$\lambda_v = \frac{l_i Q}{V_a}$ A_{vg} BOD,

$$l_i = \frac{1200mg}{l} \times \frac{1l}{0 \cdot 001m^3} \times \frac{1g}{1000mg} = 1200g/m^3$$

$$V_a = \frac{l_i Q}{\lambda_v} = \frac{\left(1200\,{}^{g}\!/_{m^3}\right)\left(300\,{}^{m^3}\!/_{day}\right)}{\left(300\,\frac{gBOD}{m^3 \cdot day}\right)} = 1200m^3$$

ii. Pond Area

Assuming a working depth of 3m

$$A_a = \frac{l_i Q}{\lambda_v D_a} = \frac{\left(1200\,{}^{g}\!/_{m^3}\right)\left(300\,{}^{m^3}\!/_{day}\right)}{\left(300\,\frac{gBOD}{m^3 day}\right)\left(3m\right)} = 400m^2$$

Pond Dimensions

Ratio 2:1, length to width ratio

$$A = lw = 400 \qquad \therefore l = \frac{400}{w}$$

$$2l = w$$

$$2\left(\frac{400}{w}\right) = w \qquad \therefore w = 28 \cdot 3m \qquad l = 14.2m$$

iii. Retention Time

$$t_a = \frac{A_a D_a}{Q} = \frac{(400m^2)(3m)}{300\,\frac{m^3}{day}} = 4days$$

iv. BOD Removal

From Mara, amount of BOD remaining, $L_{4days} = L_0 e^{-k_t * 4}$

$$= 1200\,\frac{mg}{l} \times \ell^{-\left(\frac{1 \cdot 06}{2 \cdot 3}\right) \times 4}$$

= 190mg/l i.e. 84% removed ⟶ GO TO FACULTATIVE POND

2. **Facultative Pond:**

Effluent BOD = 190mg/l = 190g/m^3

Assume, depth $D_f = 1 \cdot 5m$

Loading Rate $\lambda_f = 350kg / ha \cdot day @ 25°C$

$$= 350\,\frac{kg}{ha \cdot day} \times \frac{1000g}{1kg} \times \frac{1ha}{10000m^2} = 35g / m^2 \cdot day$$

i. Pond Area

$$\lambda_f = \frac{l_i Q}{A_f}$$

$$\therefore A_f = \frac{l_i Q}{\lambda_f} = \frac{\left(190\,g/m^3\right)\left(300\,m^3/day\right)}{\left(35\,g/m^2 \cdot day\right)} = 1629m^2$$

ii. Pond Volume

$$V_f = A_f D_f = 1629 \times 1 \cdot 5 = 2444 m^3$$

iii. Retention Time

$$t_f = \frac{V_f}{Q_f} = \frac{2444 m^3}{300 \, m^3 / day} = 8 days$$

iv. Calculated Pond Area (Due to new retention time)

$$A_f = \frac{Qt_f}{D_f} = \frac{\left(300 \, m^3 / day\right)\left(8 days\right)}{1 \cdot 5 m} = 1600 m^2$$

Pond Dimensions

$$A = lw \rightarrow l = \frac{A}{w} = \frac{1600}{w}$$

$2l = w$ 2:1 length to width ratio

$$2\left(\frac{1600}{w}\right) = w$$

$$\therefore w = 56 \cdot 6 m$$

$$l = 28.3 m$$

v. BOD Removal

$$\frac{l_e}{l_i} = \frac{1}{1 + k_i t_f}$$

$$k_{25°C} = k_{(20°C)} \theta^{(25-20)°C}$$

25^0C

where k_{25} and k_{20} are the values for k_i at and 20^0C respectively.

$$k_{25°C} = (0 \cdot 5)(1 \cdot 06)^{25-20}$$

$$= 0 \cdot 67 days^{-1}$$

$$\therefore l_e = \frac{l_i}{1 + k_1 t_f} = \frac{\left(190 \, g/m^3\right)}{1 + \left(\frac{0 \cdot 67}{days}\right)\left(8 days\right)} = 29.9 g/m^3$$

Filtered BOD:

l_e (filtered) = $0 \cdot 3$ (l_e unfiltered) = $0 \cdot 3 \times 29 \cdot 9$

= $8 \cdot 97 g/m^3 \rightarrow$ suitable for surface water discharge.

APPENDIX 2:

VOLUME AND COST OF MATERIALS

2a: VOLUME OF MATERIALS

Anaerobic Pond

Volume = $1200m^3$

Assume thickness, t = 0.15m

i. Top of Rectangular tank:

$V = 3m \times 0 \cdot 15 \times 28 \cdot 3m = 12 \cdot 7m^3$
for 4 columns of the tank
$V = 4 \times 12 \cdot 7m^3 = 50 \cdot 8m^3$

ii. Bottom of tank:

$V = 0 \cdot 15 \times 14 \cdot 2m \times 28 \cdot 3m = 60 \cdot 3m^3$

Facultative Pond

i. Top of tank:

$V = 28 \cdot 3m \times 0 \cdot 15m \times 1 \cdot 5m = 6 \cdot 4m^3$
for 4 columns
$V = 6 \cdot 4 \times 4 = 25 \cdot 6m^3$

ii. Bottom of tank:

$V = 56 \cdot 6m \times 0 \cdot 15m \times 28 \cdot 3m = 240 \cdot 3m^3$

2b: MATERIAL SCHEDULE @ PRESCRIBED MIX 1:2:4 AND COST

Anaerobic Pond

i. **Top:**

Cement	=	$6 \times 50 \cdot 8 = 304 \cdot 8$ bags
Sand	=	$0 \cdot 1386 \times 50 \cdot 8 = 7 \cdot 04$ tonnes
Aggregate	=	$0 \cdot 2197 \times 50 \cdot 8 = 11 \cdot 16$ tonnes

ii. **Bottom:**

Cement	=	$6\times 60\cdot 3 = 361\cdot 8$ bags
Sand	=	$0\cdot 1386\times 60\cdot 3 = 8\cdot 36$ tonnes
Aggregate	=	$0\cdot 2197\times 60\cdot 3 = 13\cdot 25$ tonnes

Facultative Pond:

i. Top:

$C = 6\times 25\cdot 6 = 153\cdot 6$ bags

$S = 0\cdot 1386\times 25\cdot 6 = 3\cdot 55$ tonnes

$A = 0\cdot 2197\times 25\cdot 6 = 5\cdot 62$ tonnes

ii. Bottom:

$C = 6\times 240\cdot 3 = 1441\cdot 8$ bags

$S = 0\cdot 1386\times 240\cdot 3 = 33\cdot 30$ tonnes

$A = 0\cdot 2197\times 240\cdot 3 = 52\cdot 79$ tonnes

The total material schedule is:

$C = 304\cdot 8 + 361\cdot 8 + 153\cdot 6 + 1441\cdot 8 = 2{,}262$ bags

$S = 7\cdot 04 + 8\cdot 36 + 3\cdot 55 + 33\cdot 30 = 52\cdot 25 \approx 53$ tonnes

$A = 11\cdot 16 + 13\cdot 25 + 5\cdot 62 + 52\cdot 76 = 82\cdot 79 \approx 83$ tonnes

$\therefore$ Total cost of material $\Rightarrow$

Cement	=	2262	@	₦1,100 per bag	=	₦2,488,200
Sand	=	53	@	₦2,000 per tonne	=	₦ 10,000
Aggregate	=	83	@	₦3,200 per tonne	=	₦ 265,600
					Total:	₦ 2,859,800
						≈ ₦3,000,000

APPENDIX 3

Maximum permitted concentrations of toxic substances in industrial effluent in Nigeria for the protection of groundwater.

Variable	Maximum Concentration (mg/l)
Arsenic	0.05
Barium	1.00
Cadmium	0.01
Chromium	0.05
Lead	0.05
Mercury	0.002
Selenium	0.01
Silver	0.05

Source:FEPA 1991

APPENDIX 4

stated Guidelines for interim uniform effluent limits in Nigeria for all categories of industries (mg/l unless otherwise)

Variables	Discharge to Surface Water	Land Application
Temperature	< 40^0C within 15m of outfall	< 40^0C
Colour (Lovibond Units)	7	-
pH Units	6.0-9.0	6.0-9.0
BOD_5 at 20^0C	50	500
Total Suspended Solids	30	-
Total Dissolved Solids	2,000	2,000
Chloride	600	600
Sulphate	500	1,000
Sulphide	-	-
Cyanide	-	-
Oil and Grease	10	30
Nitrate	20	-
Phosphate	5	10

Variables	Discharge to Surface Water	Land Application
Arsenic	-	-
Barium	5	5
Tin	10	10
Iron	20	-
Manganese	5	-
Phenolic Compounds	-	-
Chlorine	1	-
Cadmium	<1	-
Chromium	<1	-
Copper	<1	-
Lead	<1	-
Mercury	-	-
Nickel	<1	-
Selenium	<1	-
Silver	-	-
Zinc	<1	-
Total Metals	3	-
Calcium	200	-

Magnesium	200	-
Boron	5	5

Source: FEPA 1991