



Addis Ababa University

Addis Ababa institute of technology (AAiT)

School of Chemical and Bio Engineering

**Characterization and Treatment of Beseka Lake Water
Quality Using Microbes to reduce Total Dissolved Solid
(TDS) and Alkalinity**

A Thesis submitted to Addis Ababa Institute of Technology, In Partial
Fulfillment of the requirement of degree of Master of Science in
chemical Engineering (biochemical Engineering Stream)

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DECLARATION

I declare that thesis work entitled —: Characterization and Treatment of Beseka Lake Water Quality Using Microbes to reduce Total Dissolved Solid (TDS) and Alkalinity submitted for master degree at Addis Ababa university is my original work and has not previously been submitted for degree at this or any other university, and that all resources of materials used for this thesis has been dully acknowledged /referred.

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ABSTRACT

Water is one of the natural resource that used for irrigation, industrial, sanitation and other purpose that facilitate human activity. Lake Beseka is volcanically dammed, endorheic lake, which is located to the East direction, situated around 200 km from Addis Ababa, the capital city of Ethiopia. The Lake water is saline and alkaline with high concentration of fluoride so that the water could not be used for drinking and irrigation purpose. Different infrastructures such as a health center, high school, youth center, offices and others were affected by the lake flooding. So, this lake needs to be treatment.

In present study, biological neutralization of saline- alkaline beseka lake water in the presence of glucose (carbohydrate) as carbon source was studied. To do so, determination of physiochemical characteristics of the lake water, which was pH, EC, TDS, HCO_3^- , CO_3^{2-} , total alkalinity, chloride and fluoride were measured and also isolation of bacteria were carried out. In this study *Bacillus alkalophilus* bacteria was also used to compare its treatment performance with isolate (T₁). Treatments of lake water sample at different pH, incubation time and inoculum size were monitored. Maximum removal of total alkalinity and TDS (Total Dissolved Solid) were observed at pH, incubation time and inoculum size of 9,72hr and 32ml respectively. Which is resulted in removal of total alkalinity; 774.669mg/l and TDS;1939.92mg/l for isolated (T₁) and total alkalinity;779.631mg/l and TDS;1944.97mg/l for *Bacillus alkalophilus* from original value. Based on these findings, isolated (T₁) bacteria were shown to be have good performance for biological treatment of beseka lake.

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ACRONYMS

ANOVA	Analysis of Variance
ASTM	American society for testing and materials
APHA	American Public Health Association
BBD	Box Behnken Design
CFU	Colony Forming Unit
ED	Electrodialysis
EPA	Environmental Public Agency
EC	Electrical Conductivity
EBI	Ethiopian Biodiversity Institute
ETB	Ethiopian Birr
EEPA	Ethiopian Environmental Protection Authority
FAO	Food and Agriculture Organization
ISE	Ion Selective Electrode
MER	Main Ethiopian Rift
MSE	Metahara Sugar Estate
MEB	Multi-Effect Boiling
MSF	Multi- Stage Flash
NE	North East
RO	Reverse Osmosis
SW	South West
TFE	Tetrafluoroethylene
TDS	Total Dissolved Solid
TBT	Top Brine Temperature
VC	Vapor Compressor
WWDSE	Water Work Design and Supervision Enterprise

1. INTRODUCTION

1.1 Background

Water is one of the natural resource that used for irrigation, industrial, sanitation and other purpose that facilitate human activity. This resource directly or indirectly affected by human activates. Now day, most of our water bodies are seriously polluted due to rapid population growth, industrial proliferation, urbanizations, increasing living standards and wide spheres of human activities. Ethiopia is one of African country rich in ground and surface water that used for different purpose. However, some of these water bodies polluted naturally due to its saline property like Lake Beseka and other polluted by human activities.

East Africa is a region of extremely complex meteorological and climatologically phenomena and one of the most extensive rift systems on earth. The topography of the rift exerts a strong influence on microclimate, drainage systems, and local ecosystems (Gissila et al., 2004; Grant, 2006). The Ethiopian rift valley is part of the East African rift system, which extends from the Kenyan border up to the Red Sea and contains lakes of different hydrological and morphometric characteristics. One of the lakes in the rift valley which are situated in the awash basin is Lake Beseka, which is located in the northern Main Ethiopian Rift. Lake Beseka is volcanically dammed, endorheic lake, which is located to the East direction, situated around 200 km from Addis Ababa, the capital city of Ethiopia. The lake has become habitat of a variety of birds, fishes, crocodiles and other aquatic species and plays an important role in the wildlife ecology, as it is located in the northern part of Awash National Park.

Recent news and discussions organized by Ministry of Water, Irrigation and Energy indicated that the Lake has created several problems instead of giving advantage to the community. The lake is expanding as opposed to the other rift valley lakes in Ethiopia, which are shrinking (Alemayehu et al., 2006). Studies done by Gulilat (2000) and Alemayehu et al. (2006) indicated that the total surface area of the lake is estimated to be 42 km², which was about 3 km² in the 1960's (Gulilat, 2000; Alemayehu et al., 2006). According to Simon and Michele (2007), the Beseka Lake depth has increased by >3 m from 1976 to 1997, and continues to rise 15 cm/year, requiring Ethiopia's main highway and only railroad to the Gulf of Aden to be raised on an embankment. Additionally, the lake water cannot be used for irrigation and drinking purpose due to its high salinity, alkalinity and mineral contents (Olumana and Loiskandl, 2015).

Several studies have done to investigate the potential contributors to lake level change and chemical composition, and several reasons were highlighted such as due to an increase in groundwater level resulting from excessive irrigation downstream (Zemedagegnehu et al., 1999), increased recharge from irrigation runoff and Koka Dam construction (Ayenew, 2004), flow of tectonic spring (hot and cold spring) from ground water (Berihu, 2007), and due to increase in recharge from submerged hot springs (Anna et al., 2008). All the aforementioned studies agreed and indicated that the volume and the height level of the lake are drastically increased; as a result, the lake had created several problems in the surrounding area and is a severe threat to the wellbeing of the indigenous people and the economic welfare of the nation. However, most of the studies conducted in the area concentrated on the geological evolution, hydrological and identifying the cause of lake expansion and assessing the resulting damage. Yet, none of these studies adequately address treatment methods on the water and its watershed, and techniques in the utilization of the lake water so as to reduce its expansion, level and volume.

The expansion of Lake Beseka is alarming and has had detrimental effect on the surrounding biological, physical, hydrological and infra-structural environment. The lake growth has affected the highway and railway structures which run along its northern shore, and lake has flooded the highway, one of the import-export lines to the port of Djibouti, several times. The nearby Awash National Park has been affected indirectly, as displaced nomads encroached into the park, looking for a settlement area, water and grazing land. The expanding lake is in less than 3 km from the River Awash, which is the source of drinking water and irrigation for millions of people downstream. If the lake continues to expand at current rate and other influencing factors remain the same, the lake will cross the natural water divide and invade the town of Addis Ketema and join the River Awash. This would be disastrous, as the quality of the river water will be deteriorated such that Ethiopia's oldest state-owned sugar factory and agricultural development downstream would be at risk. Megersa et al. (2009) indicated that the expansion of the lake is affecting both the groundwater dynamics and soil salinization of the nearby agricultural field and sugarcane plantation and, if it continuous, the sustainability of the farmlands and plantation itself is under great risk. Currently, the lake water is saline and alkaline with high concentration of fluoride so that the water could not be used for drinking and irrigation purpose. Studies showed that the rift valley region of Ethiopia was plagued by water scarcity and poor water quality (Gizaw, 1996; Reimann et al., 2002). These indicated that it is serious threat to the surrounding community health

including animal health if they used without treatment and reduce crop productivity and cultivated crop diversity in the surrounding areas. The aforementioned studies indicated that there was lack of enough information on the methods to treat the lake.

1.2 Statement of the Problem

Lake Beseka is located south of Fentale Volcano in the main Ethiopian rift. Time to time the expansion of lake becomes the big challenges for Metahara town and Metahara sugar state in socio-economic and environmental. According to Megersa (2017b) studies, the expansion of the lake has already started affecting Metahara Sugar Estate (MSE) in terms of groundwater dynamics, soil salinization, yield decline, etc. and alarmed us before it brings total devastation.

The extension of Lake Beseka resulted salinization of Abadir farm land and forced the government to change the affected routes (road and railway) and if it is not take any remedial measures still threaten routes (road and railway) from Addis Ababa to Djibouti. The lake flooded fully and partially about 150 ha of the sugar cane plantation and over 2000ha of grazing land. The Lake level rise and flooding created problems on the factory activities. Recent data done by WWDSE in November 2010 shows that flooded and damaged area from sugar factory estimated to be 178.4 hectares (Dershaye, 2017).

Different infrastructures such as a health center, high school, youth center, offices and others were affected by the lake flooding. From these, more than 3,500ha of valuable rural farmlands, about 36.45km² valuable woodlands were lost by over flooded during the last three decades. As a result of Lake Beseka level rise and expansion, 5 households with about 25 members were completely displaced and 9 households in Kebele 01 of Metahara town administration were slightly affected. According to the Metahara town land administration office, at present time totally 422 households were displaced due to the lake water over flooding.

The Metahara town administration has faced serious challenges on investment attraction due to the beseka lake expansion and flooding that discouraging the investment activities of the town and different social services. According to the administration of Metahara town, the socioeconomic and development activities of the town was severely affected and depressed due to expansion and flooding of the lake which affect the social and companies invested in and around the town. For instance, 122.68 hectares of investment areas were covered by the lake water, as well as Dandi

Gudina tumsa foundation (Gudina Tumsa Foundation Office, student's dormitory and high school) and public health center with surface area of 20ha and 15ha respectively were lost due to unlimited expansion and flooding of beseka lake. Moreover, companies that have invested earlier in and around Metahara town such as Anino Trading, Mofti Nuredin, Saf Trading, animal fattening and Adey Abeba textile have left the area, which can be good sources of job opportunity to the town community, and created negative image on other investors (Dershay, 2017).

The lake level rise and expansion also created health impacts on communities. It can aggravate bone and teeth decay problems. It also aggravates problems on safe water provision as the saline lake water imposes pressure on water treatment. The lake has a potential to join Awash River in the near future, thereby impacting all downstream irrigation developments in the Awash Basin and affecting the livelihood of the people depending on the water resources of the basin (Megersa, 2017a). Currently, daily the communities shocked since they are assuming that one day the town submerged by Lake Besaka water.

To overcome the problem of the lake, the former Ministry of Water and Energy of Ethiopia spent 35 million ETB to make drainage lake water into Awash River (2% of Awash river water by volume) starting from 2004(Abate et al, 2016). However, the draining of Beseka lake water into the Awash River might be causes the deposition of salinity and other minerals within soil of the middle Awash farm through the time. After long period of time, in order to leach the deposited minerals from the affected soil take another cost. Considering the fact that up to now only limited research has been performed by various organizations, a wide knowledge gap exists regarding the mechanisms to reduce the expansion through biological treatment by utilizing appropriate microbial species so that the lake water can be used for construction, watering of tree seedlings and irrigation.

1.3.1 Main Objectives

The main objective of the present research is characterization and treatment of Beseka Lake water quality using microbes to reduce Total Dissolved Solid (TDS) and Alkalinity

1.3.2 Specific Objectives

- To characterize the physic-chemical composition of Lake Beseka water.
- To isolate bacteria from Beseka lake.
- To treat the lake water using microbes at different concentrations, pH and incubation time.
- To optimize the biological treatment process for reduction of salinity (TDS) and alkalinity.

1.4 Significance of the study

Water plays a significant role in every organism on earth. Today, this natural resource polluted due to nature of its property, industrial plants, chemicals, household activities and other uses of water in the community.

It is known that, Lake Beseka cover large surface area in Metahara town and becoming a challenge for Metahara community and Metahara sugar factory because of expansion of lake level and not has any function due to saline property of lake. Up to now, no visible treatment means applied rather than diversion of it to Awash River. Since the biological treatment employed in this study is natural treatment process, it is economic and environmental friendly alternatives to chemical treatment methods. The study can also provide information about natural resource management through water treatment.

1.5 Scope of the Study

To achieve the objectives of the research study, biological treatment of Beseka Lake water experiments was carried out with generate series of lab work by using Design of Experiment software. This thesis generally covers isolation of bacteria from Beseka Lake and uses it for treatment purpose and investigation of the effects of pH, incubation time and dosage of inoculum during treatment. And evaluate the treatment performance of both the isolated bacteria and, provided bacteria from Ethiopian biodiversity institute.

2. LITERATURE REVIEW

2.1 Brief Description of Lake Beseka and related lakes in Ethiopia

Ethiopia is gifted with many Lakes and rivers that comprise diverse aquatic ecosystems of great scientific interest and economic importance. The Ethiopian Rift Valley, as part of the Great East African Rift Valley, splits the Ethiopian highlands into northern and southern halves, forming a floor in between occupied with the chain of lakes characterized by varied size, hydrological and hydrogeological settings. With the total area of 1.3 million ha, it encompasses three major water basins from NE to SW. The Awash basin with Koka, Beseka, Gemari and Abe, the Central Ethiopian Rift Valley with Lake Ziway, Langano, Abiyata and Shalla and the Southern basin with Awassa, Abaya, Chamo and Chew-Bahir as the most important lakes (Tigist, 2009).

Lake Beseka is one of the Awash Basin situated Lake, located in the Fentalle Woreda, East Showa Zone of Oromia regional state at about 200 km southeast of Addis Ababa, the capital city of Ethiopia. It is volcanically dammed, endorheic lake located in the northern part of the Main Ethiopian Rift (MER). It situated on the main road from Addis Ababa to the port of Djibouti. It is located at the southern end of the Awash National Park, one of Ethiopia's major parks. In contrast to East African lakes, the size of Lake Beseka has increased dramatically since 1960. The total surface water catchment of the lake is about 500 km² (Olumana, 2017a, 2017b). The total surface area of the lake is estimated to be about 50 km² in the year 2015, which were about 3 km² in the 1960's. As the area is situated in the upper most part of MER, Lake Beseka is vulnerable to the occurrences of different tectonic and volcanic activities. It has saline-alkaline properties with high fluoride contents. The lake expansion affects the surface- and ground-water dynamics and soil properties of the region and the condition is specifically dangerous for the sustainability of MSE and Metahara town (Olumana, 2017b). Moreover, Beseka Lake is instilling terror to local and international investors and also communities due to its expansion and saline -alkaline properties of it.

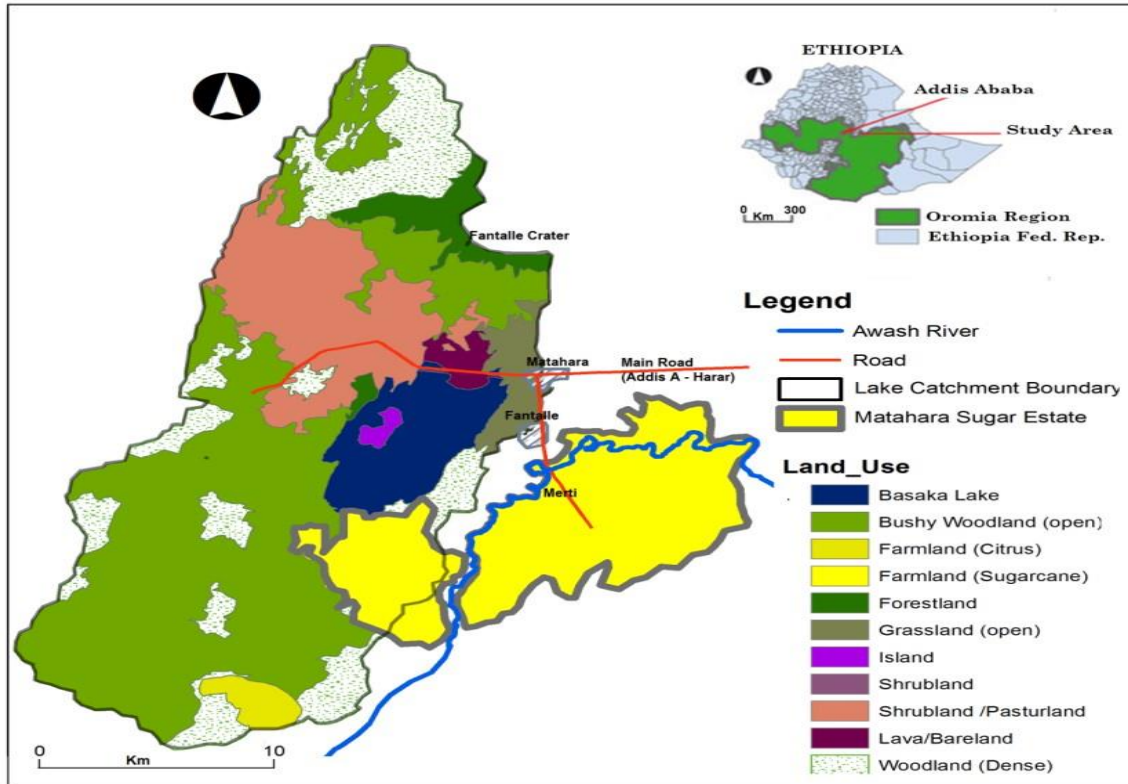


Figure 1.1 Geographical location of Lake Beseka (source: Olumana, 2017a)

Like Lake Beseka, Chew Bahir, Abiyata and Shalla Lakes are found in Ethiopian Rift Valley Lake and have saline properties. Lake Abiyata is a shallow, highly productive, saline-alkaline lake, whose muddy shoreline supports one of the most notable collections of bird life (flamingo, white pelicans, etc.) in Africa. The maximum reduction in the water level of Lake Abiyata occurs at same time with the period of large-scale water abstraction for soda ash production, and for irrigation with water from Lake Ziway after the 1980s (Ayenew and Legesse, 2007). Lake shall also have alkaline nature, which discourages its use for irrigation purpose (Belete et al., 2016).

Chew Bahir is at the southern end of the Ethiopian section of the Great Rift Valley. It lies across the border of South Omo Zone of Southern Nations, Nationalities and Peoples' Region (SNNP) to the west, and the Borena Zone of Oromia Region to the East. The water of Chew Bahir is so highly saline that it cannot be used for either irrigation or domestic purposes (Daniel, 2014). Lake Afdera is another saline lake located in the afar region, Ethiopia. Since the lake is highly saline, only used for extraction of salt. According to 1988 studies, the pH of the lake is pH 6.55. Unlike the other

saline lake in Ethiopia (lakes shala, beseka, chitu, chew Bahir and abijata) the pH is low that is acidic in nature (Getahun, 2001).

2.2 Source of saline- alkaline of water

Water is one of the most abundant resources on earth, covering three fourths of the planet's surface. About 97% of the world's water resources is in the oceans and seas and is too salty for most productive uses (Hailu et al., 2016; Méridien, 2013). The sources of such saline and alkaline can be due to the levels of hard-water minerals and release of basic industrial and agricultural effluents, dissolution of carbonate rocks, the high anthropogenic activities and geological weathering conditions (Olumana and Loiskandl, 2015). High saline wastewater is mainly discharged from industry like chemical, pharmaceutical, petroleum industries, fish caning, seafood processing, meatpacking and tannery (Lefebvre and Moletta, 2016; Méridien, 2013), and natural saline lake.

2.3 Salinity and alkalinity hazards on human, plants and crops

Salinity is a measure of the amount of salts in the water. The most important criteria for evaluating salinity hazards are the total concentration of salts. The quantity of salts dissolved in water is expressed in terms of Total Dissolved Solids (TDS), mg/l (ppm) (Siosemarde et al., 2010). Mostly cations like Na^+ , Ca^{2+} and Mg^{2+} and anions like Cl^- , SO_4^{2-} , HCO_3^- and CO_3^{2-} are the major constituents contained in saline waters.

Salinity and alkalinity hazards on human: Using saline water causes the problems like stomach and laxative effects. Drinking alkaline water lead to a condition called metabolic alkalosis, which may cause confusion, nausea, vomiting, hand tremors and so on (Méridien, 2013),.

Salinity and alkalinity hazards on plants and crops: Plants growth is adversely affected with saline water, primarily through the effectives of excessive salts on osmotic pressure of the soil solution resulting in reduced availability of water. The first reaction of plants to application of saline water is reduction in germination (Verma, 2012). Some waters, when used for irrigation of crops, have a tendency to produce alkalinity hazards depending upon the absolute and relative concentrations of specific cations and anions. Salinity may cause nutrient deficiencies or imbalances of the plant, due to the competition of Na^+ and Cl^- with nutrients such as K^+ , Ca^{2+} , and NO_3^- (Hu & Schmidhalter, 2005). Irrigation with sodic waters contaminated from Na^+ relative to Ca^{2+} and Mg^{2+} and high carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) leads to an increase in

alkalinity and sodium saturation in the soil. The increase in exchangeable sodium percentage adversely affected soil physical properties including infiltration and aeration (Verma, 2012).

2.4 Remediation measurement taken on Lake Beseka

To overcome the beseka lake problems and challenges, government and other stakeholders have taken limited treatment mechanisms such as diverting 2% of the Beseka lake water to Awash River in daily basis (Abate et al, 2016). However, such method might have negative effect on the downstream through the accumulation of salt and fluoride in the long-term period, and so it is better to search treatment mechanism rather than diverting.

2.5 Desalination technologies

Water is polluted due to municipal, agricultural, industrial or natural activity. The conditions of the polluted water without treatment will be deleterious to the organism and plants in the river, lake and human health. Desalination is a separation process that produces two streams, one that has a low concentration of salt (treated water or product water), and the other with a much higher concentration than the original feed water(brine). The two major types of technologies that are used around the world for desalination are thermal and membrane technology. Both technologies need energy to operate and produce fresh water.

2.5.1 Thermal processes

Thermal technologies, as the name implies, involve the heating of saline water and collecting the condensed vapor (distillate) to produce pure water. These processes include Multi-Stage Flash (MSF), Multi-Effect Boiling (MEB), and Vapor Compression (VC) Distillation. In all these processes, condensing steam is used to supply the latent heat needed to vaporize the water.

A. Multi-stage flash desalination (MSF)

Multi-stage flash desalination (MSF) is a thermal distillation process that involves evaporation and condensation of water. The evaporation and condensation steps are coupled to each other in several stages so that the latent heat of evaporation is recovered for reuse by preheating incoming water. In the so-called brine heater, the incoming feed water is heated to its maximum temperature (top brine temperature) by condensing saturated steam from the cold end of a steam cycle power plant or from another heat source. The hot seawater then flows into the first evaporation stage where the

pressure is set lower. The sudden introduction of hot water into the chamber with lower pressure causes it to boil very quickly, almost flashing into steam. The vapor generated by flashing is condensed on tubes of heat exchangers that run through the upper part of each stage. The tubes are cooled by the incoming feed water going to the brine heater, thus pre-heating that water and recovering part of the thermal energy used for evaporation in the first stage(Méridien, 2013). The top brine temperature (TBT) range is usually within 90 to 120°C. Although higher efficiency is observed by increasing TBT beyond 120°C, scaling and corrosion at high temperature affects the process significantly. To accelerate flashing in each stage, the pressure is maintained at a lower value than that in the previous stage. Hence, the entrance of heated seawater into the flash chamber causes vigorous boiling caused by flashing at low pressure (Bahar et al., 2013). Multi-stage flash (MSF) units are widely used in the Middle East (particularly in Saudi Arabia, the United Arab Emirates, and Kuwait) and they account for 34% of the world's seawater desalination. The MSF process requires a considerable amount of steam for the evaporation process and also significant amounts of electricity to pump the large liquid streams(Méridien, 2013).

B. The multiple-effect boiling (MEB)

The MEB process is composed of a number of elements, which are called effects to desalinate saline water. In this process, the feed water is sprayed or otherwise distributed onto the evaporator surface (usually tubes) of different chambers (effects) in a thin film to promote evaporation after it has been preheated in the upper section of each chamber. The evaporator tubes in the first effect are heated by steam extracted from a power cycle or from a boiler. The steam from one effect is used as heating fluid in another effect which while condensing, causes evaporation of a part of the salty solution. The produced steam goes through the following effect, while condensing; it makes some of the other solution evaporate and so on.

In principle, MEB plants can be configured for high temperature or low temperature operation. At present, they operate at top brine temperatures below 70°C to limit scale formation and corrosion. The top brine temperature can be as low as 55°C which helps to reduce corrosion and scaling and allows the use of low-grade waste heat. If MEB coupled to a steam cycle, the power losses will be much lower than those obtained when coupling a MSF plant , and even standard condensing turbines may be used instead of back-pressure turbines(Méridien, 2013).

C. The vapor-compression (VC)

In a VC plant, heat recovery is based on raising the pressure of the steam from a stage by means of a compressor. The condensation temperature is thus increased and the steam can be used to provide energy to the same stage it came from or to other stages. As the conventional MEB system, the vapor produced in the first stage is used as the heat input to the second effect which is at a lower temperature. The vapor produced in the last effect is then passed to the vapor compressor where it is compressed until its saturation temperature is raised before it is returned to the first effect. The compressor represents the major energy input to the system and since the latent heat is effectively recycled around the plant.

2.5.2 Membrane processes

Membrane processes include Reverse Osmosis (RO) and Electrodialysis (ED). Whereas ED is suitable for brackish water, RO can be used for both brackish and seawater.

A. Reverse osmosis (RO)

Osmosis is a natural phenomenon by which water from a low salt concentration passes into a more concentrated solution through a semi-permeable membrane. When pressure is applied to the solution with the higher salt concentration solution, the water will flow in a reverse direction through the semi-permeable membrane, leaving the salt behind. This is known as the Reverse Osmosis (RO) process. The pressure needed for separation ranges within 50 bars (seawater) to 20 bars (brackish water) (Bahar et al., 2013). RO membranes are sensitive to pH, oxidizers, and a wide range of organics, algae, bacteria, and deposition of particulates and fouling. Therefore, pre-treatment of the feed water is an important process step and can have a significant impact on the cost and energy consumption of RO. Recently, micro-, ultra- and nano-filtration has been proposed as an alternative to the chemical pre-treatment of raw water in order to avoid contamination of the seawater by the additives in the surrounding of the plants. RO post-treatment includes removing dissolved gases (CO₂), and stabilizing the pH via the addition of calcium or sodium salts, and the removal of dangerous substances from the brine.

B. Electrodialysis (ED)

Electrodialysis is an electrochemical process in which the salts pass through the cation and anion membranes, leaving the water behind. It is a process typically used for brackish water. Because

most dissolved salts are ionic (either positively or negatively charged) and the ions are attracted to electrodes with an opposite electric charge, membranes that allow selective passage of either positively or negatively charged ions accomplish the desalting (Subashini, 2015)

2.6 Effects of desalination technology

By the year 2025, two out of every three persons in the world could be living in water-stressed regions. One of the solutions to increase potable water availability is desalination of brackish water and seawater. It is estimated that worldwide desalination capacity will increase by 2015 to 97.5 million m³ of water per day, with energy accounting for 40% of the total water cost. Energy demands for water desalination range from 650 $\frac{kWh}{m^3}$ for energy intensive single-stage evaporation of seawater to 68 $\frac{kWh}{m^3}$ for multistage flash evaporation and 3.7 $\frac{kWh}{m^3}$ for reverse osmosis (RO) (Mehanna et al., 2010).

Approximately 78% of the desalinated water in Saudi Arabia is produced from seawater, and the main technologies used by the Saline Water Conversion Corporation (SWCC) are multi-stage flash distillation (MSF), reverse osmosis (RO), and multi-effect distillation (MED). The unit capital cost in 2010 for seawater desalination plants in the Arab region has been estimated to be between \$1000-\$2000 per m³/day, while energy costs are both highly variable and can represent 50-77% of the total operating costs (\$0.46-\$0.60/m³) in cogeneration plants (Kajenthira et al., 2012). The majorities of existing desalination plants are powered by fossil fuels and result in substantial greenhouse gas emissions. Desalination also results in the production of concentrated brine which may affect coastal water quality and marine life due to its discharge temperature and exceedingly low concentrations of dissolved oxygen if not disposed in good manner (Kajenthira et al., 2012; Méridien, 2013).

2.7 Other desalination processes

I. Freezing

During the process of freezing, dissolved salts are excluded during the formation of ice crystals. Under controlled conditions seawater can be desalinated by freezing it to form the ice crystals. Before the entire mass of water has been frozen, the mixture is usually washed and rinsed to remove the salts in the remaining water or adhering to the ice.

The ice is then melted to produce fresh water. Therefore, the freezing process is made up of cooling of the seawater feed, partial crystallization of ice, separation of ice from seawater, melting of ice, refrigeration, and heat rejection. The advantages of freezing include a lower theoretical energy requirement, minimal potential corrosion, and little scaling or precipitation. The disadvantage of freezing involves handling ice and water mixtures which are mechanically complicated to move and process (Khawaji et al., 2008).

II. Solar evaporation

Solar desalination process is similar to a part of the natural hydrologic cycle in which the seawater is heated by the sun's rays to produce water vapor. The water vapor is then condensed on a cool surface, and the condensate collected as product water. An example of this type of process is the green house solar still, in which the saline water is heated in a basin on the floor and the water vapor condenses on the sloping glass roof that covers the basin.

III. Using microbial desalination cells to reduce water salinity prior to reverse osmosis

A new concept for water desalination was recently demonstrated based on using electrical power generated by bacteria in devices called microbial desalination cells (MDCs). The concept is similar to water Electrodialysis in the fact that there is a voltage applied between the chambers in order to drive the ions out of the desalination compartment, but instead of using an external source of electrical energy desalination is achieved with the current and potential generated by bacteria in the MDC. When a lower salinity wastewater stream is used in the anode chamber to desalinate the water in the middle chamber, the MDC also has characteristics of a reverse Electrodialysis (RED) process as the salinity gradient between the two chambers can contribute to current generation (Mehanna et al., 2010).

2.8 Chemical ways for neutralization of alkaline polluted water

2.8.1 Neutralization with Sulfuric Acid

Sulfuric Acid (H_2SO_4) is the most widely used and produced chemical in the world. Available in concentrations ranging from 0% to 98%, sulfuric acid is most economical of all and used universally for neutralization reactions. It is easier and safer to use than HCl or HNO_3 and is more potent than all of the other acids except for phosphoric acid. Sulfuric acid is typically used in concentrations ranging from 25% to 96%. However, 30% to 50% concentrations of Sulfuric acid

are generally recommended (Kumar et al., 2007). Reaction times of 15-30 minutes are needed. The formation of calcium sulfate is possible when calcium containing polluted waters are neutralized (Eckenfelder et al., 1989).

2.8.2 Neutralization with Hydrochloric

Hydrochloric Acid (HCl), also known as muriatic acid, is the second most commonly used acid in industry, Sulfuric acid being the primary choice since it is more effective and relatively inexpensive. Reaction times of 15-20 minutes are needed. At a maximum available concentration of 37%, HCl is about 1/3 as potent as Sulfuric acid, thus making it relatively more expensive to use. Depending on temperature and agitation, HCl at concentrations above 10% evolves hydrogen chloride vapors which combine with the water vapors present in the air. The gas thus formed, is highly corrosive and attacks all metallic objects including building structures, sprinkler heads, copper wiring, stainless steel, etc. Therefore, it must be properly vented or used outdoors where the gases can easily dissipate (Eckenfelder et al., 1989; Kumar et al., 2007).

2.8.3 Neutralization with Nitric Acid

Nitric Acid (HNO₃) though a widely used chemical in many industries it does not enjoy the popularity of hydrochloric or Sulfuric acid, as it is more expensive to use than either of them. Nitric acid evolves noxious gas which combines with water vapors present in the air. The gas is highly corrosive and attacks all metallic objects including building structures, sprinkler heads, copper wiring, stainless steel, etc. Therefore, it must be properly vented or used outdoors where the gases can easily dissipate (Kumar et al., 2007).

2.8.4 Neutralization with Carbon Dioxide

Carbon Dioxide (CO₂), the third most concentrated gas found in earth's atmosphere, CO₂ is itself not an acid. It forms carbonic acid (H₂CO₃) when dissolved in water; and it is this carbonic acid that brings about the neutralization of alkalinity in solution. The most appealing feature of CO₂ is that it will not lower the pH of water below 7.0 for all practical purposes. Additionally, CO₂ is non-corrosive. However, as it is heavier than air asphyxiation is a hazard. Carbon dioxide is difficult to use and its use is limited because the gas must be dissolved into solution to be used. This requires the use of a carbonator, or some method to dissolve the gas into solution. Significant out-gassing also occurs, which does not hold a problem unless the process also requires the settling of Solids. In cement pouring operations large amounts of alkaline wastewaters are generated. It is

an excellent choice for Such applications as the site is temporary, the gas is nonhazardous, can be used in-line assuming retention and mixing is considered and is self-buffering so regardless of dosage it will not lower the pH below 7.5-7.0 (Verma, 2012).

2.8.5 Neutralization with Phosphoric Acid

Phosphoric Acid (H_3PO_4), very widely used in the production of agricultural fertilizers and detergent products it is a relatively inexpensive acid. However, it still does not compete well with sulfuric and hydrochloric acid as it is a weak acid and does not fully disassociate in water at normal concentrations. This renders it safer to use compared to sulfuric or hydrochloric acid and the evolution of gasses is rare. It tends to buffer neutralization reactions and this makes for a slower reaction that is easier to control. Due to its cost (as compared to Sulfuric) and availability, phosphoric acid is not commonly used in neutralization system (Kumar et al., 2007)

2.8.6 Neutralization with flue gas

Flue gas also contains a considerable amount of carbonic acid (7.5 to 12%), which can be used for neutralization of alkaline wastewaters. It is produced in heating plants and power stations and is therefore available free of charge. Neutralization with flue gas requires a large amount of flue gas; this gas is not injected into the air suction pipe, but distributed over the pipe cross section via a branch line. This means that only flue gas or only air can be injected. The injection is controlled by a multi way a valve. If the pH value exceeds the legally admissible discharges limits, a pH control and measuring devices activates the multi way valve and the flue gas injection starts (Verma, 2012).

2.9 Halophilic and alkalophilic Microorganisms

Halophilic microorganisms comprise a heterogeneous group of microorganisms and require salts for optimal growth. They have been isolated from diverse salinity environments, varying from natural brines, hyper saline lakes to saturation salinities. Microorganisms able to grow in the presence of salt are found in all three domains of life: Archaea, Bacteria, and Eukarya (Margesin and Schinner, 2001; Waditee-sirisattha et al, 2016). Non-halophilic microorganisms, able to grow in the absence as well as in the presence of salt, are designated halotolerant; those halotolerants that are able to grow above approximately 15% (w/v) NaCl (2.5 M) are considered extremely halotolerant. Microorganisms requiring salt for growth are referred to as halophiles. Halophiles

can be distinguished as Slight halophiles (0.2-0.5 M NaCl), moderate halophiles (0.5-2.5M NaCl) and extreme halophiles (2.5-5.5M NaCl)(Margesin and Schinner, 2001; Zhuang et al, 2010).

The term “alkaliphile” is used for microorganisms that grow optimally or very well at pH values above 9 but cannot grow or grow only slowly at the near-neutral pH value of 6.5. Alkaliphiles include prokaryotes, eukaryotes, and archaea. The cell surface may play a key role in keeping the intracellular pH value in the range between 7 and 8.5, allowing alkaliphiles to thrive in alkaline environments. Alkaliphiles can be classified as obligatory and facultatively alkaliphilic organisms. Obligatory alkaliphilic microorganisms cannot grow below pH 9. Facultatively alkaliphilic organisms show optimal growth at pH 10.0 or above but they also have the ability to grow at neutral pH(Coban, 2004). Alkaliphiles consist of two main physiological groups of microorganisms; alkaliphiles and haloalkaliphiles. Alkaliphiles require an alkaline pH of 9 or more for their growth and have an optimal growth pH of around 10, whereas haloalkaliphiles require both an alkaline pH (>pH 9) and high salinity(Mudidi, 2014).

2.10 Alkalophilus reaction mechanism

In order to survive at alkaline environment and high salt concentrations, alkaliphilic microorganisms have to maintain an osmotic balance with their external environment. The following point shows the withstanding ability of the alkaliphilic microorganisms at alkaline environment and high salt concentrations:

Internal pH: The cell surface is a key feature in maintaining the intracellular neutral environment separate from the extracellular alkaline environment. Alkaliphiles use proton pumps to maintain a neutral pH internally, and so the intracellular enzymes from these microorganisms need not to be adapted to extreme growth conditions. The Na^+/H^+ antiporter protein present on the plasma membrane enables cells to adapt to a sudden upward shift in pH and to maintain a cytoplasmic pH that is 2 to 2.3 units below the external pH in the most alkaline range of pH for growth. These mechanisms of these membrane proteins play a key role in keeping the intracellular pH value in the range between 7 and 8.5(Mudidi, 2014).

Cell Walls: Alkaliphiles have a peptidoglycan cell wall, although in addition to this they have negatively charged cell wall polymers. *Alkaliphilic Bacillus* species contain certain acidic polymers, such as galacturonic acid, gluconic acid, glutamic acid, aspartic acid and phosphoric acid. These function as a negatively charged matrix and reduce the pH values at the cell surface

because the cell membrane is very unstable at alkaline pH values below the optimum pH for growth and therefore must be kept below 9. Therefore, it helps to stabilize the cell membrane(Horikoshi, 1999). They may give the cell its ability to adsorb sodium and hydronium ions and repulse hydroxide ions and, therefore, may assist cells to grow in alkaline environments.

Na⁺ ions and membrane transport: Alkaliphiles require Na⁺ ions for growth and they have exhibited vigorous growth at pH range of pH 9 to 11. In Na⁺ ion–dependent transport systems, the H⁺ ion is exchanged with Na⁺ by Na⁺/H⁺ antiporter systems, thus generating a sodium motive force, which drives substrates accompanied by Na⁺ into the cells. Incorporation of substrates at pH 9 is observed to greatly increase and the presence of Na⁺ significantly enhances the incorporation. Plasma membranes play a role in maintaining pH homeostasis by using Na⁺/H⁺ antiporter systems, K⁺/H⁺ antiporter and ATPase drives H⁺ expulsion(Mudidi, 2014).

2.11 Biological Neutralization of alkaline polluted water

Compare to chemical methods, biological treatment method is a cost effective, sustainable, nontoxic downstream process, and environmental friendly methods.

2.11.1 Neutralization of alkaline industrial wastewaters using *Exiguobacterium* sp

A facultatively alkaliphilic bacterium capable of lowering the pH of highly alkaline wastewater from 12 to 7.5 within just 2hr. The bacterium was isolated from industrial wastewater drained sludge of a beverage industry. The metabolic product which the bacterium produces in order to neutralize alkaline wastewater was found to be carboxylic acid in nature by Fourier Transfer-Infrared (FT-IR) spectroscopy. The isolate belongs to genus *Exiguobacterium* as determined by 16S rRNA gene sequencing and biochemical characterization. It was found to be gram positive, no spore-forming, nonmotile, rod shaped, growing aerobically and anaerobically at a wide range of temperatures (5-40°C), pH (6.5-12.0) and NaCl concentrations (0-9.5%). The strain was able to hydrolyze starch and was catalase positive but lacked oxidase or amino peptidase activity. Acid was produced from glycerol, cellobiose, D-mannose, Mannitol, methyl α –D-glucoside, amygladin and arbutin. From the 16S rRNA gene sequence analysis, the strain appeared to be most similar (99%) to the sequence from *Exiguobacterium aurantiacum* strain DSM6208. Neutralization of highly alkaline waste water without addition of any external carbon source highlights the potential use of isolate as an alternative to the conventional acid neutralization method for treatment of alkaline wastewater (Verma, 2012).

2.11.2 Biotechnological process for neutralizing alkaline beverage industrial wastewater

A biotechnologically pure culture of the strain *Exiguobacterium* sp., identification number MTCC 5183, said strain being capable of growth in a medium with a pH in the range of 10-12 and being capable of lowering a pH of 12 to 11.5 of beverage industrial waste water to a neutral pH of 7.5 to 7.00 within a period of 1-1.5 hours, said strain being gram positive, non-motile, rod shaped and oxidase negative, and being capable of hydrolyzing starch and producing acids from glycerol, cellobiose, D-mannose, Mannitol, methyl α -D-glucoside, amygdalin and arbutin (Kumar et al., 2008).

2.11.3 Biological neutralization of highly alkaline textile industrial wastewater

Bacterium, *Kurthia* sp., is aerobic in nature, is gram positive, shows optimal growth at a temperature in the range of 25-42°C., is capable of growth in a high pH environment (pH= 12), is capable of hydrolyzing starch, is motile, catalase positive and reduces nitrate. The bacterium *Kurthia* sp., as screened above, was inoculated in 200-220ml basal carbonate medium. The culture was incubated at 35-37°C for 8 hours under shaking conditions (100-120 rpm). After observing the heavy bacterial growth (optical density (O. D)=1.5), the culture was centrifuged at 5,000-7000 rpm at about 4°C. The culture pellet was added in a flask containing 200ml textile industrial waste water (pH 12-11.5). The bacterial pellet is used in a ratio in the range of 1:5 to 1:10 to the wastewater. The flask was kept at shaking conditions (100-120 rpm). Decrease in pH was been capable to bring the pH from 12- 11.5 to 7.5-7.0 with a short period of time 1.5-2 hours, and pH was monitored by a pH meter. (Kumar et al., 2007).

2.11.4 Biological neutralization of chlor-alkali industry wastewater

The present work reports biological neutralization of chlor-alkali industrial effluent by an alkaliphilic bacterium, isolated from the Gujarat coast, which was identified as *Enterococcus faecium* strain R-5 on the basis of morphological, biochemical and partial 16S rRNA gene sequencing. The isolate was capable of bringing down the pH of waste water from 12 to 7.0 within 3h in the presence of carbon and nitrogen sources, with simultaneous reduction in total dissolved solutes (TDS) up to 19-22%. This bacterium produces carboxylic acid, as revealed by FT-IR analysis, which facilitated neutralization of alkaline effluent. The presence of unconventional raw materials viz. *Madhuca indica* flowers or sugar cane bagasse as carbon and nitrogen sources could effectively neutralize alkaline effluent and thus making the bioremediation process economically

viable. The time required for neutralization decreased with an increase in inoculum size (from 5ml to 20ml of bacterial suspension with specified cell density per 100 ml of medium) inoculated in 200ml of chlor-alkali industry wastewater supplemented with carbon and nitrogen sources. This is first report on biological neutralization of a chlor-alkali industrial effluent (Jain et al., 2011).

2.11.5 Neutralization of alkaline wastewater from pulp and paper industry by Alkaliphiles

Two alkaliphilic bacterial strains T₁ and T₂ capable of lowering pH and alkalinity of alkaline wastewater from pulp and paper industry were isolated. Bacterial strain T₁ reduces the pH from 12 to 8.1 and alkalinity from 1040mg/l to 480mg/l whereas strain T₂ reduces the pH from 12 to 7.95 and alkalinity from 1080mg/l to 460mg/l. Both alkaliphilic bacterial strains were gram positive and spore forming. Strain T₁ has regular margin, glistening, convex and oval round in shape whereas strain T₂ has regular margin, flat and irregular in shape. Both the strains can grow at wide range of temperatures (10-44°C), pH (6-12), and NaCl concentrations (0-20%). Strains T₁ and T₂ can utilize all the different types of carbohydrates. Strain T₁ can hydrolyze starch and show positive results for lipase screening, pectinase screening and gelatin liquification test. Neutralization of highly alkaline wastewater without addition of any external carbon source highlights the potential use of the isolate as an alternative to the conventional acid neutralization method for treatment of alkaline wastewater (Verma, 2012).

2.11.6 Neutralization of Alkaline Waste-Waters using a Blend of Microorganisms

The pre-treatment of alkaline waste-waters, generated from various industries like textile, paper & pulp, potato-processing industries, etc., having a pH of 10 or higher, is essential. The pre-treatment, i.e., neutralization of such alkaline waste-waters can be achieved by chemical as well as biological means. However, the biological pretreatment offers better package over the chemical means by being safe and economical. The biological pre-treatment can be accomplished by using a blend of microorganisms able to withstand such harsh alkaline conditions. In the present work, for the proper pre-treatment of alkaline waste-waters, the blend of *B. alkalophilus* and *B. sp.* are capable of lowering the pH from 9-10.5 to pH of 5.26-8.26 after 5 days' incubation in the presence of carbohydrates (sucrose and glucose) (Kumar et al., 2011).

3 MATERIALS AND METHODS

The experimental works were conducted at Metahara Research and Development Center, Ethiopian Public Health Institute (EPHI) and Addis Ababa Institute of Technology(AAiT) Laboratories.

3.1 Materials and reagents

Beseka lake water was used as raw material. The reagents used in this research were hydrogen peroxide (30%), H₂O₂, Phenolphthalein indicator solution, Potassium chromate indicator solution, silver nitrate solution (0.025N), sodium chloride solution (0.025N), sodium hydroxide solution (0.25N), sulfuric acid solution (0.02N), methyl orange indicator solution, Sodium Carbonate (anhydrous) and basal agar medium. The apparatus and equipment's which will be used for this research are conductivity meter, analytical balance, standard laboratory titrimetric equipment, including 1 mL or 5 mL micro burette with 0.01 mL gradations, and 25 mL burette, beakers, pipettes (volumetric), volumetric flasks (1000mL, 500ml, 200mL), UV-Visible Spectrophotometer, biological safety cabinet, autoclave and incubator.

3.2 Methods

3.2.1 Water Sampling method

The water samples were collected to analyze the selected physic-chemical properties of Beseka Lake water. Accordingly, water samples were collected from Lake Beseka, Metahara, Fentalle Woreda, Oromia region by systematic random sampling method from the surface at different sites of Lake using clean poly ethylene plastic bottles (0.5L). Before taking the sample; the bottles first were rinsed by sample water. The sample bottles then were labeled with an identification number. Water sample used for isolation was collected by sterilized glass bottle. All water samples were collected on the same day (morning) and transported to Metahara Research and Development Center laboratories in ice box at 4°C before being prepared for analysis.

3.2.2 Sample Preparation and analysis

The alkalinity and chloride contents of collected samples were analyzed by titration while fluoride was analyzed by UV-Visible Spectrophotometer method.

The water pH and electrical conductivity (EC) were measured by pH meter and EC meters respectively while salinity was determined by TDS dried at 180°C. pH, alkalinity, EC and TDS were done with three replicates while chloride and fluoride with two replicates. The water sampling and analysis were done as per the APHA standards (APHA,1999) and described as follows:

I. pH

The pH value of water sample was determined by electrometric method according to APHA-4500-H⁺ B. In this procedure, in a clear dry small beaker 50ml water sample was taken and stirred well. The electrode was then placed in well mixed water sample and checked for the reading in pH meter. Finally, the electrode was waited until stable reading appeared and recorded.

II. Electrical conductivity (EC)

Electrical conductivity of water was measured by Electrical conductivity (EC) meter (APHA2520 B). In this procedure, first the meter was calibrated by immersing into standard potassium chloride solution which has conductivity of 1413µS/cm. Then, the electrode was placed in 50ml water sample and records the conductivity of water with the corresponding temperature.

III. Salinity (TDS)

The well-mixed water sample was filtered under vacuum pump through Gooch Crucible filter. 100mL of filtered water sample was then transferred in a weighed evaporating dish and then evaporated to dryness on steam bath. The evaporated sample was dried to constant weight in an oven either at 103-105°C or 179-181°C. The dried sample was cooled in a desiccator and then weighed. The drying procedure was repeated until constant weigh was obtained. The result will be then analyzed as follows (APHA2540 C):

$$Total\ filterable\ residue\ (mg/L)\ at\ 180^{\circ}C = \frac{(A - B) \times 10^6}{C} \dots\dots\dots (3.1)$$

Where: A = weight of dried residue + dish, B = weight of dish, C = mL of filtrate used, 10⁶ = conversion factor.

IV. Alkalinity test

Alkalinity of water was determined by titration following APHA2320-B standard. In this procedure, 50mL of unfiltered water sample was taken in a conical flask and 2-3 drops of methyl orange indicator solution was added to the flask. The sample was then titrated with 0.02 N H₂SO₄, constantly swirling the flask content above a white surface until just after the colour of the flask content change from yellow to red.

V. Chloride (Cl⁻) test

50 mL of water sample was poured into a white porcelain container. The end point of the silver nitrate for titration of chloride was indicated by potassium chromate. The method number used was APHA4500- Cl⁻ B.

VI. Fluoride (F⁻) test

50ml of water sample was used to analyze fluoride ion. The concentration of fluoride in water was determined using UV-Visible Spectrophotometer (APHA4500- F⁻ D).

3.3 Media Composition and Isolation of Bacteria

3.3.1 Media Composition

The isolation of bacterial was done on basal media. The media contained ingredients were given in the table 3.1 as g/l of distilled water.

Table 3.1: media composition (Dhundale, 2012; Joshi & Kanekar, 2008)

Ingredient	composition (g/l)
Glucose	10
Yeast extract	5
Peptone	5
KH ₂ PO ₄	1
MgSO ₄ .7H ₂ O	0.2
Na ₂ CO ₃ (anhydrous)	10
Agar (for solid medium)	20

3.3.2 Isolation of Bacteria

Sample was collected from Beseka Lake, Metahara, Oromia region, in order to isolate potent microbes for biological treatment. Isolation of bacteria was done by the streak plate method. In this method, first the 1ml of sample water was transferred in 9ml of sterilized distilled water and mixed by vortex shaker for 20 seconds. The suspension was then diluted up to 10^{-6} dilutions. From each dilution, 0.1 ml of the diluted sample were then plated onto sterilized surface plate of nutrient agar. Then, the plate was streaked following quadrant streak method. Finally, the plate was incubated at 35°C for 3days. Then, the pure colonies were distinguished by size and color. Individual bacterial isolates were further sub cultured on the basal agar medium in order to obtain pure culture. Out of these the best grown isolate on basal agar was selected on the basis of their growth and, maintained at 4°C in refrigerator for beseka lake water treatment and further studies (Kumar et al., 2007; Demissie, 2014).

3.4 Experimental procedures

3.4.1 Inoculum preparation

Frozen cultures of *Bacillus alkalophilus* kindly given by Ethiopian Biodiversity Institute and the isolated bacteria from Lake Beseka (good growth on basal agar medium) stored in vial and agar slant respectively were inoculated separately on sterilized a basal broth of flask composed of 10g glucose, 5g yeast extract, 5g peptone, 1g KH_2PO_4 , 0.2g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 10g Na_2CO_3 at aseptic conditions Then, the inoculated broth of flasks were covered with cotton and incubated on rotary shaker at 35°C, 150rpm and 72 h(Sivaprakasam et al, 2008). Finally, the grown culture suspensions were used as sources of inoculum for beseka lake water treatment experiments.

3.4.2 Viable Cell Count

The number of living cells was counted by spread plate methods. Doing spread plate by making serial dilution from 10^{-1} to 10^{-6} then 0.1 mL of bacterial suspension broth was spread over nutrient agar plates. Plates were incubated in incubator at 35°C for 48hours. After 48hours of incubation, colonies were counted. The calculation of the number of cells per mL was described as follow:

$$\text{number of cells (cfu/ml)} = \frac{\text{number of colonies} \cdot \text{dilution factor}}{\text{volume of inoculum (ml)}} \dots\dots\dots (3.2)$$

3.5 Beseka lake water treatment procedures

Treatments of beseka lake water sample were done using *Bacillus alkalophilus* and isolated bacteria at different inoculum dosage, pH and incubation time. The 500ml of volumetric flasks were filled with 400ml of beseka lake water sample enriched with 1% glucose (Kumar et al., 2011), autoclaved and 24ml, 28ml and 32 mL of inoculated broth of *Bacillus alkaliphilic* and isolated bacteria were added to the flask with sample separately. Before inoculation of the inoculum, the water samples were adjusted to the pH of the lake (pH of 8.5, 9 and 9.5). Then the flasks were putted on rotary shaker at 35°C and 150rpm. In addition, the treatment processes were set at 24hr,48hr and 72hr to examine the effect of exposures time.

3.6 Experimental design for Beseka lake water treatment

Response surface methodology (RSM) was adopted in the design of experimental combinations. The main advantage of RSM is to reduce the number of experimental runs needed to provide sufficient information for statistically acceptable results. Box-Behnken Design (BBD) of response surface methodology is based on the construction of balanced incompleted block designs and requires at least three levels for each factor. In Box-Behnken experimental design, the level of one of the factors is fixed at the center level while combinations of all levels of the other factors are applied(Tekindal1 et al., 2012). Therefore, in this study Box- Behnken design (BBD) with three levels for three factors was employed.

The first task before conducting the experiments was selection of potential parameters to be varied. The three main factors selected in this study were dosage of inoculum, exposure time, and pH. The levels of the selected factors were determined from reviewing several research literatures. The factors among with their levels are described in Table 3.2. The experiment performed as a completely randomized design with three main factors at three levels and two response variables. The processing variables (dosage of inoculum, exposure time, and pH.) were optimized using RSM of BBD to study their effect on the biological treatment of Beseka Lake. The responses that were considered during the optimization of the Processing variables are total alkalinity and salinity (TDS).

Table 3.2: Independent Variables and Levels Used in the BBD for the Beseka Lake water treatment

Variable	Unit	Coded symbol	Coded Levels		
			-1	0	+1
PH value	-	A	8.5	9.0	9.5
Incubation time	hr.	B	24	48	72
Inoculum size	ml	C	24	28	32

3.7 Data analysis

Data analysis has performed by DESIGN EXPERT ® 7 software using Response surface methodology design method and randomize the runs. Randomization ensures that the conditions in one run neither depend on the conditions of the previous runs nor predict the conditions in the subsequent runs. Randomization is essential for drawing conclusions from the experiment, in correct, unambiguous and defensible manner. Box- Behnken design (BBD) for a three factor (dosage of inoculum exposure time, and pH) and three level combinations with a total number of 17 runs for each bacterium were used to conduct this study. Significance of the result was performed using analysis of variance (ANOVA). Analysis of variance, ANOVA, is a statistical decision making tool used for detecting any differences in average performances of tested parameters(Manohar et al., 2013). It employs sum of squares and F statistics to find out relative importance of the analyzed processing parameters, measurement errors and uncontrolled parameters. It was used to check the adequacy of the model for the responses in the experimentation.

4 RESULTS AND DISCUSSION

4.1 Chemical compositions of Lake Beseka water

The chemical compositions of beseka lake water were determined and presented in table 4.1

Table 4.1 characteristics of Lake Beseka water

Parameters	unit	Value
pH	-	9.0
EC (electrical conductivity)	$\mu\text{S/cm}$	3906
TDS (total dissolved solid)	mg/l	3178
Cl^- (chloride)	mg/l	349.89
F^- (fluoride)	mg/l	16.13
HCO_3^- (bicarbonate ion)	mg/l	1016.80
CO_3^{2-} (carbonate ion)	mg/l	216.0

4.1.1 pH

In this study, the pH of beseka lake water was found to be with the range of (8.5- 9.5) with average value of 9. This values show that the lake is basic (alkaline) in nature ($\text{pH}>7$), and to a small extent above the recommended range for fresh lake water (7.3–9.2) (Mali et al., 2012). The value of the present study agrees with the former research result done by Ayenew and Legesse (2007) with average value of pH (9.08). In the opposite, the pH values of Lake Beseka water was close to the most irrigated lake (lake Ziway), which has a pH value of 8.4-.8.9 (Masresha et al., 2011). The pH of the Ziway lake is within the permissible limits of (WHO,1993; EPA,2003) for drinking, recreation, agricultural and aquatic life water use (6.5-8.5/9). Thus, the reasons for rising of pH are due to the presence of ions (HCO_3^- and CO_3^{2-}) while absence of dissolved CO_2 . The other sources of alkaline pH can be the levels of hard-water minerals and release of basic industrial and agricultural effluents. The presence of bicarbonate ions, HCO_3^- , in the lake is resulting from limestone deposits react with water.

4.1.2 Electrical conductivity (EC)

As indicated in Table 4.1, the measured value of electrical conductivity (EC) of Beseka Lake was between 3032 to 4780 $\mu\text{S}/\text{cm}$, with average value of 3906 $\mu\text{S}/\text{cm}$, such that the present value was far above the WHO (WHO, 1993) guideline value prescribed for drinking purpose (1500 $\mu\text{S}/\text{cm}$) and EPA guideline (1000 $\mu\text{S}/\text{cm}$) and for livestock and poultry watering purposes (<1000 $\mu\text{S}/\text{cm}$). Even, the value of EC in the lake was higher than lake Ziway(479-530 $\mu\text{S}/\text{cm}$) (Masresha et al., 2011). Higher the EC, less amount of water will be available to plants, even though the soil may appear wet. This is because plants can only transpire "pure" water as the usable plant water in the soil solution decreases dramatically with an increase in EC (Abduro and W/michael, 2017). Therefore, irrigation water with high EC reduces yield potential. This higher amount of EC from beseka lake was due to the presence of higher value of dissolved salts ions in the water. The large variations in the EC and TDS may be attributed to variation in geochemical processes and anthropogenic activities(Mondal et al., 2008). Generally, there are anthropogenic activities like discharge of effluents from municipals of MER, agrochemical run off, effluents discharged nearby MER factories without treatment, and the natural processes like precipitation inputs, erosion, weathering of crustal materials are contributors of higher values EC and TDS in Lake Beseka water (Abduro and W/michael, 2017; Olumana and Loiskandl, 2015).

4.1.3 Total dissolved solid (TDS)

Salinity or Total Dissolved Solids (TDS) is a measure of the total ionic concentration of dissolved minerals in water. There is a positive correlation between EC and TDS. The TDS of the Lake Beseka (2425 to 3930 mg/L which has average value of 3178 mg/l) were greater than the Lake Ziway which ranges from 200 to 400 mg/L (Worako, 2015). The value of TDS decreased in the lake in comparing with the previous researches done by Ayenew & Legesse (2007) (TDS=4936mg/l) and Olumana and Loiskandl (2015) (5000-12,000mg/l). The possible reason for reduction of TDS was probably due to the excessively high amount of flood discharged into the Lake from Koka dam and irrigation runoff, and microbial degradation of dissolved solid. TDS is composed of the following principal cations (or positively charged ions): Sodium (Na^+), Calcium (Ca^{2+}), Potassium (K^+), Magnesium (Mg^{2+}), and anions (or negatively charged ions): Chloride (Cl^-), Sulfate (SO_4^{2-}), Carbonate (CO_3^{2-}), Bicarbonate (HCO_3^-), and, to a lesser extent by Nitrate (NO_3^-), Boron (B^{3+}), Iron (Fe^{3+}), Manganese (Mn^{2+}) and Fluoride (F^-) (Siosemarde et al., 2010). Therefore, in lake beseka, cations like Sodium (Na^+), Potassium (K^+), and anions like Chloride

(Cl⁻), Sulfate (SO₄²⁻), Bicarbonate (HCO₃⁻), and Fluoride (F⁻) are major sources for rise of TDS (Olumana & Loiskandl, 2015). High concentration of dissolved ions can damage the organism's cells and also reduced the photosynthesis activity and increases the water turbidity and water temperature.) (Mondal et al, 2008).

4.1.4 Chloride (Cl⁻)

The existence of chloride and fluoride in water in excess amounts is not desirable. In the present investigation, the concentration of Cl⁻ was reached to 349.89mg /l, which was in far above the prescribed limits set by WHO (1993), 250 mg/L for drinking water, higher concentration of Cl in drinking water causes a salty taste and has a laxative effect in people not accustomed to it (Mondal et al, 2008). The value obtained in the present study decreased in the lake in comparing with the previous researches done by Olumana & Loiskandl (2015) (937mg/l) and Ayenew & Legesse (2007) (525mg/l). The decrease in measured value of Cl⁻ could be associated with the sampling period and a dilution effect due to overflow of Koka Dam and irrigation runoff. Among the halogens (Cl, Br, I, F), chloride is the most abundant. Cl ion usually exists in the form of chlorine salts (NaCl, CaCl and MgCl) and is extremely soluble in water. High chloride content in the Rift Valley lakes is primarily contributed from the alkaline/saline soils in the drainage area and due to the pollution from chloride rich effluents of sewage and municipal waste(Njenga, 2004).

4.1.5 Fluoride (F⁻)

Fluoride is an essential element for maintaining normal development of teeth and bones. A fluoride value in the present study was recorded as 16.13mg/l, which was far apart from the recommended limit (WHO,1993; EPA, 2003) of F⁻ concentration for the drinking water (1.5mg/l). Concentrations in drinking water above the permissible limit (1.5 mg/L) causes dental fluorosis. In addition, continuous intake of 3 mg/L to 6mg/L fluoride content water for a long period may leads to skeletal fluorosis, if these concentrations exceeded, crippling skeletal fluorosis occur(Worako, 2015). Fluorine is a common element that does not occur in the elemental state in nature because of its high reactivity. It is present in the soil and rock formation in the form sellaite (MgF₂), fluorspar (CaF₂), cryolite (Na₃AlF₆) and fluorapatite [3Ca₃(PO₄)₂Ca(F,Cl₂)], and due to anthropogenic sources (aluminum industries, phosphate industries, coal plants, cosmetics, etc.) (Mondal et al, 2008). Furthermore, the volcanic activity and weathering processes let fluorine to be introduced

into groundwater and thus results an increase in the levels of fluoride. The possible sources of fluoride in Beseka Lake may be due to chemical weathering and volcanic activity

4.1.6 Alkalinity

Alkalinity of water is described as the presence of all those substances in water which can resist the change in pH when an acid is added to water. Total alkalinity of water is due to CO_3^{2-} (carbonate ion) and HCO_3^- (bicarbonate ion) anions (Ali et al., 2014). The concentration of HCO_3^- and CO_3^{2-} are 1016.80mg/l and 216mg/l in study area respectively. According to this study, the concentration of HCO_3^- compared to CO_3^{2-} is negligible. The value of total alkalinity (carbonate and bicarbonate) obtained in the present study decreased in the lake (1232.80mg/l) in comparing with the previous researches done by Olumana and Loiskandl (2015) (1500mg/l) and Ayenew and Legesse (2007) (2838mg/l). The microbes are the possible reasons for neutralization of alkaline waste water. HCO_3^- value in the present study higher than Lake Ziway (255mg/l) (Masresha et al., 2011). Based on this reality the Lake Beseka water is not suitable for the drinking, irrigation, & livestock watering purposes. The major source of bicarbonate and carbonate are the dissolution of carbonate rocks containing calcite (CaCO_3) and dolomite [$\text{CaMg}(\text{CO}_3)_2$] and the remainder from silicate minerals like albite ($2\text{NaAlSi}_3\text{O}_8$) (Njenga, 2004; Mondal et al, 2008). The presences of high values of alkalinity in water have leded unpleasant taste and may be deleterious to human health with high pH, TDS, and total hardness (Ogunbode et al., 2016; Olumuyiwa et al., 2012).

4.2 Isolation and viable cell count

4.2.1 Isolation and selection of bacteria

In the present work, three different types of colonies were observed on agar plates that were transferred on basal agar medium. Out of which one strain named T₁ showed good growth. This isolate strain has red color and rod shape form in agar plate. This strain was selected for beseka lake water treatment study.

4.2.2 Viable cells count for isolate (T₁) and *Bacillus alkalophilus*

The plate spread technique were used to count the nubmer of viable cells present in a sample and the result was tabulated in Table 4.2. As indicated in Table 4.2, the isolate have higher colonies than *B.alkalophilus*. This implies that, basal medium, temperature and incubation time are good conditions for replication of isolate than *B.alkalophilus*.

Table 4.2 Populations of isolate (T₁) and *Bacillus alkalophilus*

Strain	Dilution	Number of colonies	Plate count
<i>B. alkalophilus</i>	10 ⁻⁶	110	1.1*10 ⁹ cfu/ml
Isolate (T ₁)	10 ⁻⁶	142	1.42* 10 ⁹ cfu/ml

4.3 Statistical analysis of the experimental result

Effect of pH, incubation time and inoculum size on the treatments of Beseka Lake water were studied and presented in APPENDIX A (Table A-1 and A-2). A total of 34 experimental data for isolated and *bacillus alkalophilus* bacteria were collected for treatments of Beseka Lake water sample. These data were obtained according to experimental design developed by response surface methodology (RSM) using box Behnken design (BBD). These results are an input for further analysis. Since the isolate (T₁) have good removal efficiencies of total alkalinity and total dissolved solid (TDS), throughout this study, only experimental observation for isolated bacteria was discussed.

4.3.1 Statistical analysis for isolate (T₁)

Usually, it is essential to confirm first whether the fitted model provides an adequate approximation of the actual values or not. The adequacy of the model was checked by analysis of variance (ANOVA) and some diagnostic plots. Analysis of variance (ANOVA) is employed to test the significance of the developed models. Table 4-3 and Table 4-4 are show the summary of the analysis of variance (ANOVA) of the two responses total alkalinity and TDS respectively for the isolated bacteria. The F-value is measure of variation of the data about the mean. The high F-value and a very low probability indicate that the present models are in a good prediction of the experimental results. The p-value serves as a tool for checking the significance of each of the coefficients. The variables with low probability levels contribute to the model, whereas the others can be neglected and eliminated from the model. Values of Prob> F less than 0.0500 indicate that model terms are significant. In this study A, B, C, AB, AC, BC, A², B² and C² for the response of total alkalinity and A, B, C, AC, BC, A² and B² for the response of TDS are significant model's terms.

Table 4.3 Analysis of variance for Total alkalinity

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	Remark
Model	1.336E+005	9	14839.04	636.44	< 0.0001	Significant
A	3666.68	1	3666.68	157.26	< 0.0001	
B	2984.55	1	2984.55	128.01	< 0.0001	
C	1.166E+005	1	1.166E+005	4999.63	< 0.0001	
AB	1651.20	1	1651.20	70.82	< 0.0001	
AC	923.55	1	923.55	39.61	0.0004	
BC	1509.71	1	1509.71	64.75	< 0.0001	
A ²	344.70	1	344.70	14.78	0.0063	
B ²	795.42	1	795.42	34.12	0.0006	
C ²	5051.46	1	5051.46	216.66	< 0.0001	
Residual	163.21	7	23.32			
Lack of Fit	135.46	3	45.15	6.51	0.0510	not significant
Pure Error	27.75	4	6.94			
Cor Total	1.337E+005	16				

Table 4.4 Analysis of variance for TDS

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	Remark
Model	1.860E+005	9	20668.48	101.90	< 0.0001	significant
A-pH	6160.50	1	6160.50	30.37	0.0009	
B- Incubation time	17112.50	1	17112.50	84.37	< 0.0001	
C- Inoculum size	1.405E+005	1	1.405E+005	692.46	< 0.0001	
AB	529.00	1	529.00	2.61	0.1504	
AC	2025.00	1	2025.00	9.98	0.0159	
BC	6084.00	1	6084.00	30.00	0.0009	
A ²	12096.67	1	12096.67	59.64	0.0001	
B ²	1928.25	1	1928.25	9.51	0.0177	
C ²	40.46	1	40.46	0.20	0.6686	
Residual	1419.80	7	202.83			
Lack of Fit	1181.00	3	393.67	6.59	0.0500	not significant
Pure Error	238.80	4	59.70			
Cor Total	1.874E+005	16				

The model adequacy was further investigated using R^2 for the two responses, which were found to be 0.9988 and 0.9924 for total alkalinity and TDS respectively. For a good statistical model, the R^2 value should be close to one. The results ensured a satisfactory adjustment of the quadratic model to the experimental data. It was indicated that approximately 99.88 % and 99.24 % of the variability in the dependent variables of total alkalinity and TDS respectively could be explained by these models. The value of R^2 (correlation coefficient) for all responses is very high and close to the one which indicates a good agreement between experimental and predicted values.

‘Predicted R-squared’ values are in reasonable agreement with the ‘Adjusted R-Squared’ values. "Adeq Precision" measures the signal to noise ratio. A ratio of adequate precision greater than 4 is

desirable. In case of this study the ratio for the two responses were greater than 4, which is 83.483 for total alkalinity and 43.532 for TDS. This indicates an adequate signal.

Table 4.5 Model adequacy measures for total alkalinity

Std. Dev.	4.83	R-Squared	0.9988
Mean	964.18	Adj R-Squared	0.9972
C.V. %	0.50	Pred R-Squared	0.9835
PRESS	2210.71	Adeq Precision	83.483

Table 4.6 Model adequacy measures for TDS

Std. Dev.	14.24	R-Squared	0.9924
Mean	2192.41	Adj R-Squared	0.9827
C.V. %	0.65	Pred R-Squared	0.8972
PRESS	19269.13	Adeq Precision	43.532

The response total alkalinity equation was obtained for the Beseka Lake treatment:

Final Equation in Terms of Coded Factors:

$$\text{Total alkalinity} = +982.69 + 21.41 \times A - 19.31 \times B - 120.71 \times C - 20.32 \times A \times B + 15.19 \times A \times C - 19.43 \times B \times C + 9.05 \times A^2 - 13.74 \times B^2 - 34.64 \times C^2$$

Final Equation in Terms of Actual Factors:

$$\text{Total alkalinity} = +3571.46125 - 740.09850 \times \text{pH} + 22.39044 \times \text{Incubation time} + 32.38794 \times \text{Inoculum size} - 1.69313 \times \text{pH} \times \text{Incubation time} + 7.59750 \times \text{pH} \times \text{Inoculum size} - 0.20237 \times \text{Incubation time} \times \text{Inoculum size} + 36.19200 \times \text{pH}^2 - 0.023862 \times \text{Incubation time}^2 - 2.16481 \times \text{Inoculum size}^2$$

The response TDS equation was obtained for the Beseka Lake treatment:

Final Equation in Terms of Coded Factors:

$$\text{TDS} = +2175.80 + 27.75 \times A - 46.25 \times B - 132.50 \times C - 11.50 \times A \times B - 22.50 \times A \times C - 39.00 \times B \times C + 53.60 \times A^2 - 21.40 \times B^2 + 3.10 \times C^2$$

Final Equation in Terms of Actual Factors:

$$\text{TDS} = +16334.00000 - 3442.70000 \times \text{pH} + 21.63958 \times \text{Incubation time} + 76.77500 \times \text{Inoculum size} - 0.95833 \times \text{pH} \times \text{Incubation time} - 11.25000 \times \text{pH} \times \text{Inoculum size} - 0.40625 \times \text{Incubation time} \times \text{Inoculum size} + 214.40000 \times \text{pH}^2 - 0.037153 \times \text{Incubation time}^2$$

$$+0.19375 \times \text{Inoculum size}^2$$

4.3.2 Effect of Process Parameters on the treatments of beseka lake water sample

Here, direct effect, interaction effect or a comparison between any two input parameters have been discussed and the third parameter would be on its central level. 3D surface plots were drawn by using BBD methods to investigate the effect of all the factors on the responses. The data obtained from the software were discussed below.

A. Direct Effect

Effect of pH

The selection of optimum pH is essential for removal of total alkalinity and TDS from wastewater. The effect of pH on removal of total alkalinity and total dissolved solid (TDS) for isolate (T_1) are shown in figure 4.1. It is evident from figure 4.1 that increasing pH value to 9 results in increasing removal of total alkalinity and TDS. However, increasing pH after 9 decrease removal of total alkalinity and TDS from original value, which mean that, increasing in pH value beyond 9 has negative effect on metabolic activity of microbes. The microbes can't withstand the pH beyond 9.

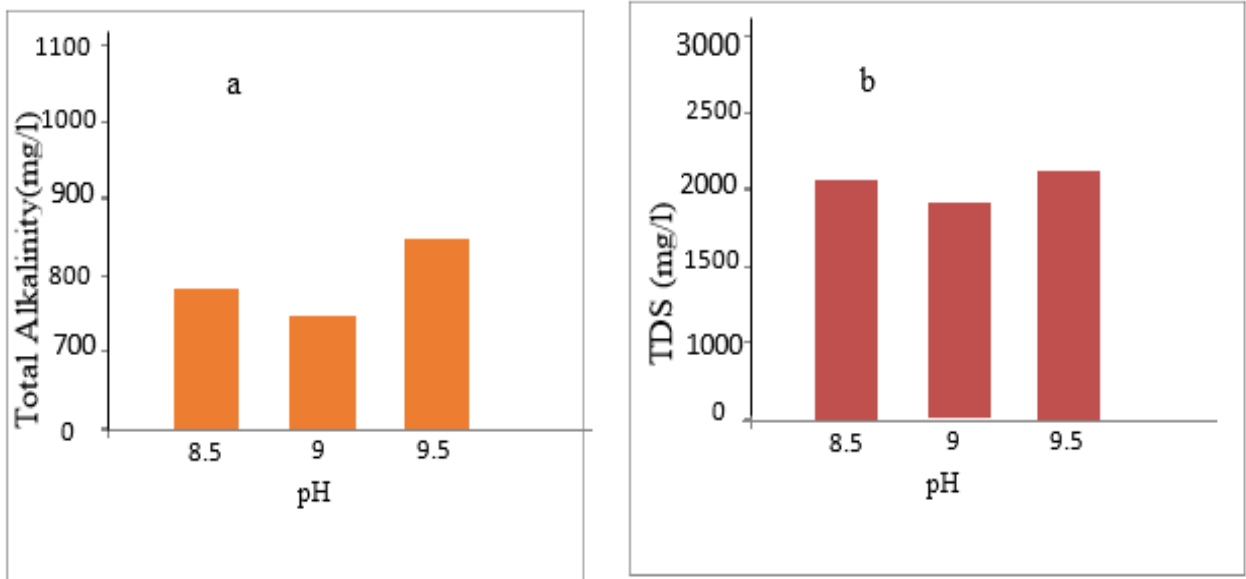


Figure 4.1 Effect of pH on (a)Total alkalinity (b) TDS

Effect of incubation time

Figure 4.2 shows effects of incubation time on reductions of total alkalinity and TDS. Total alkalinity removal at 24hr was 857.74mg/l, 868.81mg/l at 48hr and 774.76mg/l at 72hr, and TDS removal at 24 was 2100, 2120 at 48hr and 1940 at 72hr. As shown from Figure 4.2 increasing an incubation time have a fluctuation on total alkalinity and TDS removal. However, the removal of total alkalinity and TDS at high level of the incubation time might be due to the fact that the organism grew slowly and the time period for the bacteria to reach in the stationary phase was increased. The declines in growth of microbial populations is due to either the exhaustion of available nutrients or by the accumulation of toxic products of metabolism(Lee, 2002). Production of this toxic matter (acid in this case) contribute for neutralization of alkalinity of water.

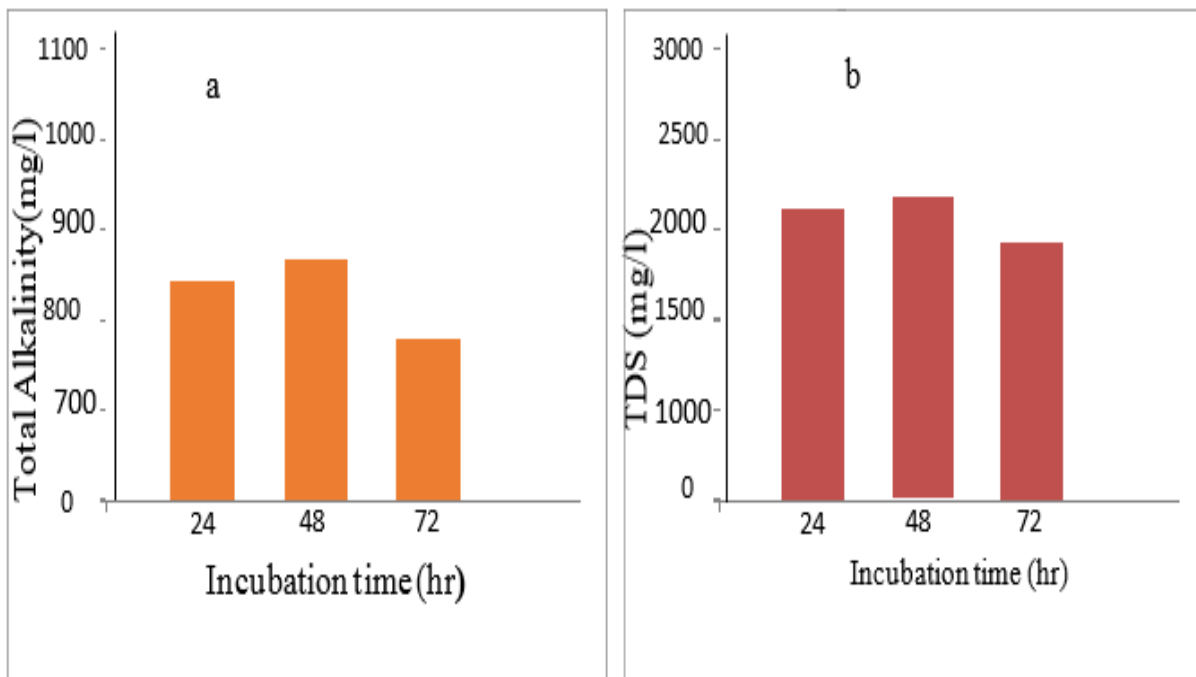


Figure 4.2 Effect of incubation time on (a)Total alkalinity (b) TDS

Effect of inoculum size

Removal of total alkalinity and TDS were investigated using different inoculum dosage. Figure 4.3 illustrates the effects of inoculum size on reduction of total alkalinity and TDS. As observed from the Figure, the inoculum size had a positive effect on the reductions of total alkalinity and TDS of water sample.

The removal of total alkalinity and TDS were increased with increase in the size of inoculum. As, the inoculum level was further increased, the removal of total alkalinity and TDS were gradually increased. However, the removal of total alkalinity and TDS at low level of the inoculum might be due to the fact that the organism grew slowly and the time period for the bacteria to reach in the stationary phase was increased. This is agreeing with the study done by Jain et al., (2011). This behavior was due to the fact that, as the inoculum size increase, much amount of acid produced by the bacteria colonies, which facilitated neutralization of alkaline water, and also the presence of more inoculum increases degradation of TDS. The maximum reductions of total alkalinity and TDS were found at inoculum size of 32ml.

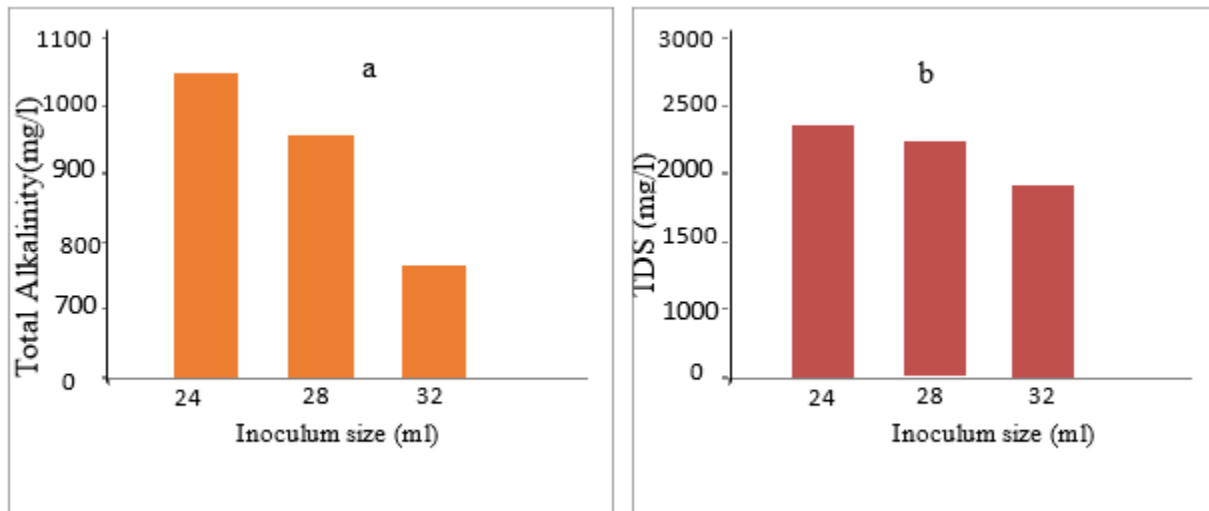


Figure 4.3 Effect of inoculum size on (a)Total alkalinity (b) TDS

B. Interaction Effect

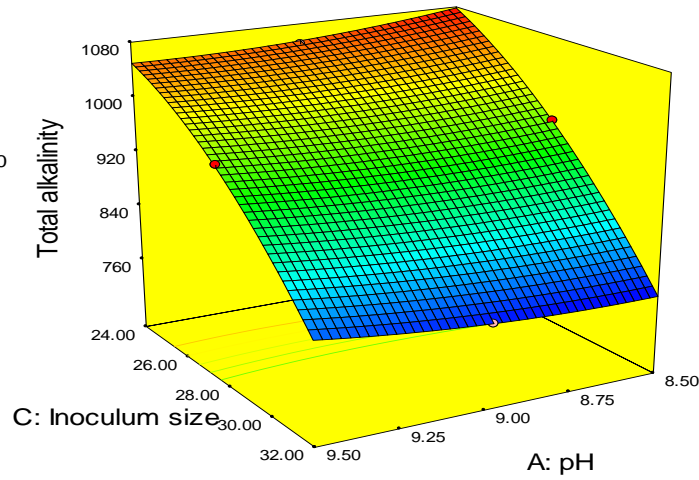
In this section only, the interaction effect of inoculum size and pH was plotted, while the plot of the other interaction effects for all responses was given at APPENDIX B (Table B -1 and B -2). The interaction effects of these variables on the biological treatments of beseka lake water were studied by plotting 3D surface against the two independent variables. The three-dimensional surface for the responses as a function of inoculum size and pH was shown in Figure 4-4 using the design expert statistical software, 7.0.0 trial version.

Design-Expert® Software

Total alkalinity
1085.14
774.76

X1 = A: pH
X2 = C: Inoculum size

Actual Factor
B: Incubation time = 72.00



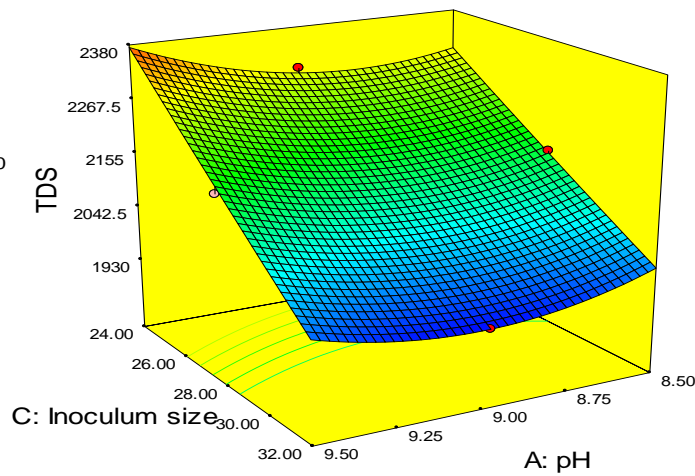
(a)

Design-Expert® Software

TDS
2420
1940

X1 = A: pH
X2 = C: Inoculum size

Actual Factor
B: Incubation time = 72.00



(b)

Figure 4-4 Interaction effects of pH and inoculum size on reduction of (a) total alkalinity (b) TDS

From the response plot in Figures 4-4 (a) and (b) observed that, Interaction effects of inoculum size and pH have positive effect on the removal of total alkalinity and TDS. Removal of total alkalinity and TDS increases as increasing inoculum size and pH. The maximum reduction of total

alkalinity and TDS obtained at maximum inoculum size (32ml) and medium pH (9). This is due that high inoculum size can be neutralize the alkaline Beseka Lake water in short time, and the microbials activity decreases beyond pH 9. Even if the isolated bacteria had high reduction of total alkalinity and TDS than provided one (*bacillus alkalophilus*), not only the interaction effects of inoculum size and pH but also interaction effects (pH & incubation time and incubation time & inoculum size for total alkalinity) and (incubation time & inoculum size for TDS) have the same trained for *bacillus alkalophilus*.

4.4 Optimization of process factors and response variables for isolate

One of the specific objectives of the present study was to find the optimum process parameters to get the highest reduction of total alkalinity and TDS. In order to determine the optimum processing conditions, viewed by response surface plot, pH, incubation time, inoculum size, total alkalinity and salinity (TDS) were considered to be in range, in range, in range, minimum and minimum respectively. Table 4-7 shows the summary of factors/responses and goals for isolated bacteria. Table 4-7 exhibits the desired combinations of process parameters that would provide the highest responses.

Table 4-7: Constrains applied for optimization

Lower	Upper	Lower	Upper			
Name	Goal	Limit	Limit	Weight	Weight	Importance
pH	is in range	8.5	9.5	1	1	3
Incubation time	is in range	24	72	1	1	3
Inoculum size	is in range	24	32	1	1	3
Total alkalinity	minimize	774.76	1085.14	1	1	3
TDS	minimize	1940	2420	1	1	3

Numerical optimization was used to find the optimum value of the process parameters and for the responses. Since the developed model was capable of predicting the maximum value, therefore, from Figure 4-5 the ramp plot shows that the highest reduction of total alkalinity and TDS were

found at a pH of 8.99, incubation time of 71.99 and inoculation size of 32ml and which were resulted 774.669mg/l in total alkalinity and 1939.92mg/l in TDS.

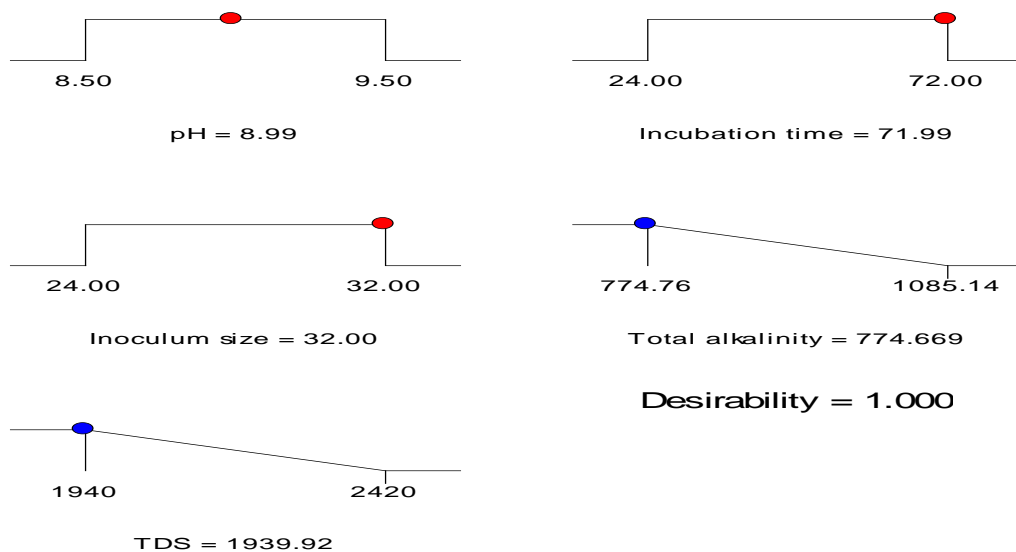


Figure 4-5: Ramp display of desirability

4. 5 Comparison of alkalinity and TDS removal from wastewater

The optimized value of biological beseka lake water treatment is indicated in the Table 4.5. Table 4.5 illustrated that the highest removal of total alkalinity and TDS were found to be 774.669mg/l and 1939.92mg/l respectively. This means that 37% of Alkalinity and 20% of TDS were removed from original value of 1229.31mg/l for alkalinity and 2422mg/l for TDS. In the previous work as Reported in the literature, alkalinity removal percentage of 57.4% was found by Verma (2012) using pulp and paper wastewater effluent. Compared to my study, this value was high. This is due to the absence of reasonable nutrient or organic matter in beseka lake that enhances populations of microorganism. The other reason is may be due to experimental procedures. 22% of TDS removal was obtained by Jaint (2011) from chlor-alkali industry wastewaters as reported in literature and this value is high compared to my work. This is due to using glucose and peptone as carbon and nitrogen sources in addition to industry waste. The other reason is may be due to the absence of enough transfer of oxygen in flask(reactor). However, treatment with acid (nitric acid, hydrochloric acid and sulfuric acid) increase the TDS content (Jaint (2011)).

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study result indicated that the use of Lake Besaka for irrigation and drinking purpose is difficult. The lake water has high TDS, alkalinity and fluoride. It is possible to say that the quality of such lake leading in damaging water resources and soil quality of the region groundwater quality. This is a reason for the abstains of investors from investing in Metahara area. This research was designed to utilize microorganism for treatment of beseka lake water. The Present study concluded that isolate (T_1) and *Bacillus alkalophilus* are able to neutralize alkaline beseka lake water with concomitant reduction in TDS, in the presence of glucose as carbon source, and the isolated bacteria (T_1) have good neutralizing ability of the lake than *Bacillus alkalophilus*.

It is possible to conclude from the results obtained that beseka lake water treatment using the isolate (T_1) and *Bacillus alkalophilus* are influenced by the pH, incubation time and dosage of the inoculum. Design Expert software was used to develop design of experiment and analyzed the results. Box Behnken design in Response surface method was used to treat beseka lake water. And for this particular study the quadratic model was the best fit model for all responses as the p-value of this model was smaller than the other models and had the highest p-value for Lack of Fit Tests. Among the three parameters studied, ANOVA showed that, inoculum size was found to be the most important parameter influencing the treatment of the lake water followed by pH, while incubation time had the least effect relative to the two factors. As the inoculum size increased from 24ml to 32ml the neutralizing efficiencies of bacteria also increased.

The optimal treatment of the lake for isolated (T_1) and *Bacillus alkalophilus* were resulted at a pH of 8.99, incubation time of 71.99hr and inoculum size of 32 ml. And the highest removal of total alkalinity and TDS value were 774.669mg/l and 1939.92mg/l for isolate and 779.631mg/l and 1944.97mg/l for *Bacillus alkalophilus* respectively. The data revealed that, the values of the total alkalinity and TDS doesn't meet National Environmental Quality Standard (EEPA, 2003) and WHO guidelines (1993). However, compared to untreated, treated values of those parameters through biological treatment method reduces their effects on downstream irrigation system.

5.2 Recommendation

In this study, biological treatment of beseka lake was done under different pH, incubation time and inoculum size. From the knowledge and insight gained during the course of this work, the following future work is recommended in the treatment of beseka lake water:

- The literature and the present study demonstrated the success to employ microbes for alkaline wastewater treatment; however, further investigation is recommended for biological treatments of Beseka Lake water.
- Even the lake water was treated biologically, integration of physical and chemical with biological treatment could be needed for enhancement of treatment efficiency.
- Due to limitation of time only isolation of bacteria was done. For future work, investigations on morphological and biochemical tests of the isolate is also recommended.
- In this study, pH, incubation time and inoculum size considered as operating parameters. One can also investigate the effects of salt concentration as other operating parameters for future work.
- It is recommended to consider saline-alkaline beseka lake as serious problem and taking remediation technique by government and supporters of government.
- In Ethiopia, there are a factory that generate alkaline wastewaters. Those alkaline wastewaters are either chemically treated or discharged to environment without treatment. It is better to treat those wastes with biological method, which is environmental friendly and cost-effective method than that of chemical one.

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APPENDIX

Appendix A: Experimental Results for the isolated and *bacillus alkalophilus* bacteria

Table A1: Experimental observation for isolated bacteria

Standard order	Run	Factor 1 A:pH	Factor 2 B: Incubation Time (hr)	Factor 3 C:Inoculum Size (ml)	Response 1 Total alkalinity (mg/l)	Response 2 TDS (mg/l)
1	2	8.50	24.00	28.00	951.3	2230
2	7	9.50	24.00	28.00	1037.83	2289
3	13	8.50	72.00	28.00	958.8	2150
4	17	9.50	72.00	28.00	964.06	2163
5	4	8.50	48.00	24.00	1075.79	2300
6	15	9.50	48.00	24.00	1085.14	2420
7	9	8.50	48.00	32.00	798.68	2090
8	16	9.50	48.00	32.00	868.81	2120
9	5	9.00	24.00	24.00	1055.01	2297
10	6	9.00	72.00	24.00	1049.74	2293
11	10	9.00	24.00	32.00	857.74	2100
12	3	9.00	72.00	32.00	774.76	1940
13	12	9.00	48.00	28.00	981	2166
14	11	9.00	48.00	28.00	983.23	2180
15	8	9.00	48.00	28.00	979.07	2169
16	14	9.00	48.00	28.00	984.97	2181
17	1	9.00	48.00	28.00	985.2	2183

Table A2: Experimental observation for *Bacillus alkalophilus*

Standard order	Run	Factor 1 A:pH	Factor 2 B:Incubation time (hr)	Factor 3 C:Inoculum size (ml)	Response 1 Total alkalinity (mg/l)	Response 2 TDS (mg/l)
1	10	8.50	24.00	28.00	955.3	2235
2	13	9.50	24.00	28.00	1042.74	2294
3	17	8.50	72.00	28.00	963.8	2155
4	16	9.50	72.00	28.00	969.06	2168
5	1	8.50	48.00	24.00	1080.65	2305
6	4	9.50	48.00	24.00	1090.1	2425
7	11	8.50	48.00	32.00	803.68	2095
8	2	9.50	48.00	32.00	873.91	2125
9	9	9.00	24.00	24.00	1060.01	2302
10	3	9.00	72.00	24.00	1054.74	2298
11	7	9.00	24.00	32.00	862.7	2105
12	14	9.00	72.00	32.00	779.76	1945
13	12	9.00	48.00	28.00	985.57	2171
14	15	9.00	48.00	28.00	988.23	2185
15	5	9.00	48.00	28.00	984.07	2174
16	8	9.00	48.00	28.00	989.84	2186
17	6	9.00	48.00	28.00	990.2	2188

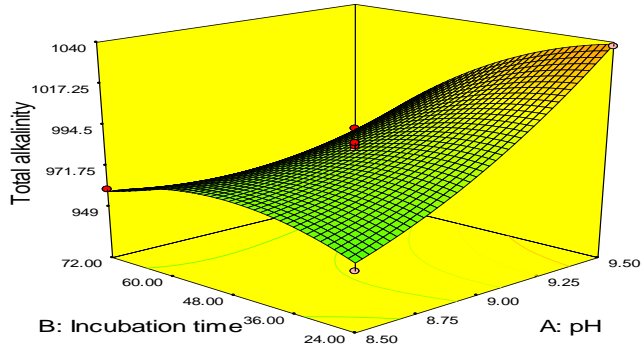
APPENDIX B: 3D plots for the responses: for isolated and *bacillus alkalophilus* bacteria

Design-Expert® Software

Total alkalinity
1085.14
774.76

X1 = A: pH
X2 = B: Incubation time

Actual Factor
C: Inoculum size = 28.00

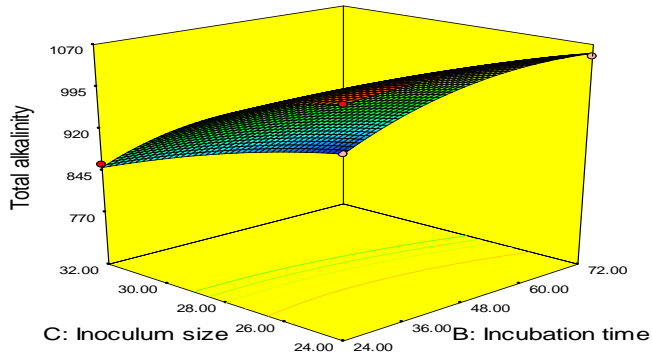


Design-Expert® Software

Total alkalinity
1085.14
774.76

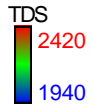
X1 = B: Incubation time
X2 = C: Inoculum size

Actual Factor
A: pH = 9.00



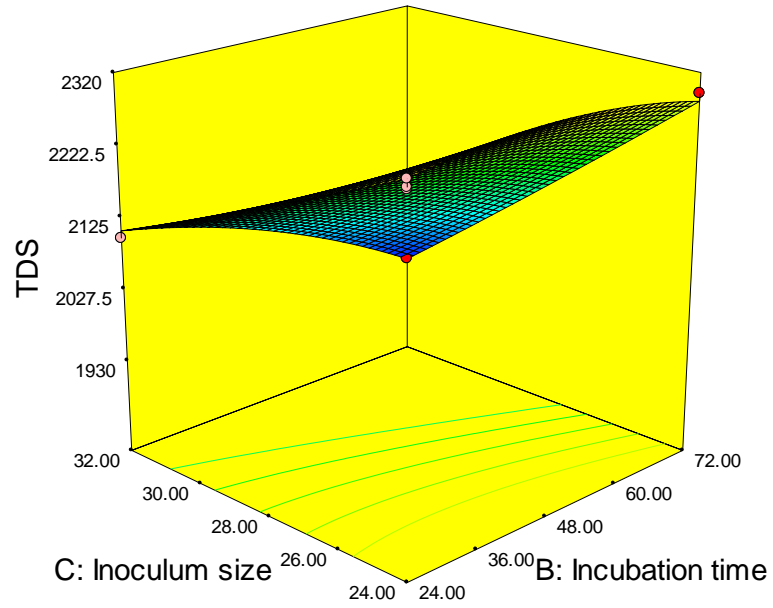
(a)

Design-Expert® Software



X1 = B: Incubation time
X2 = C: Inoculum size

Actual Factor
A: pH = 9.00



(b)

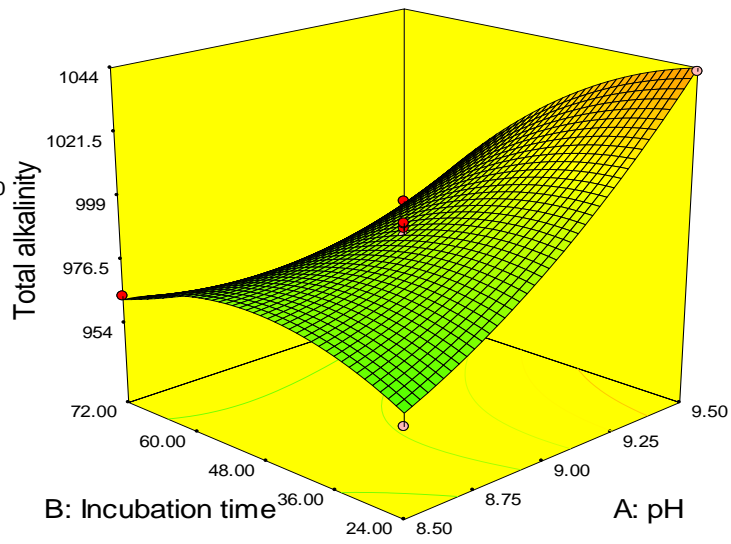
Figure B₁: 3D plots for isolated bacteria (a) Total alkalinity (b) TDS

Design-Expert® Software

Total alkalinity
1090.1
779.76

X1 = A: pH
X2 = B: Incubation time

Actual Factor
C: Inoculation size = 28.00

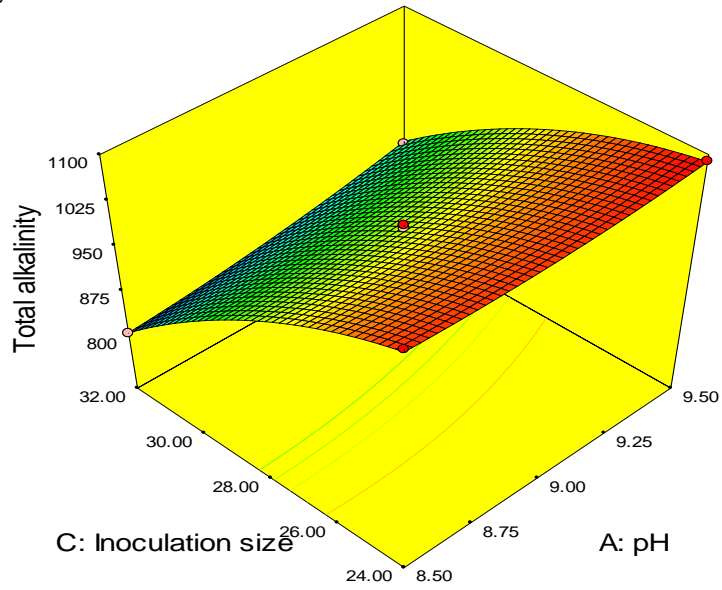


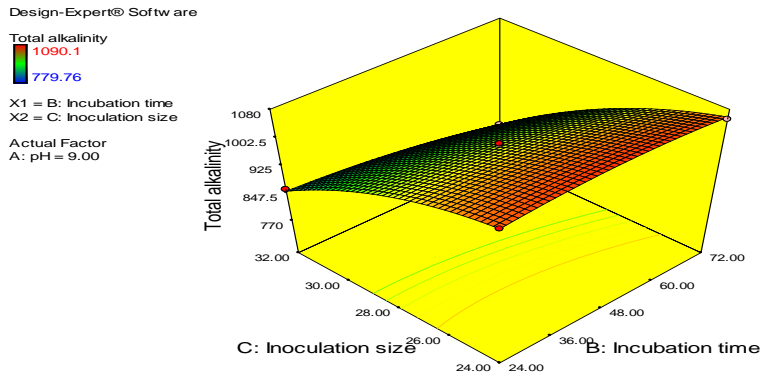
Design-Expert® Software

Total alkalinity
1090.1
779.76

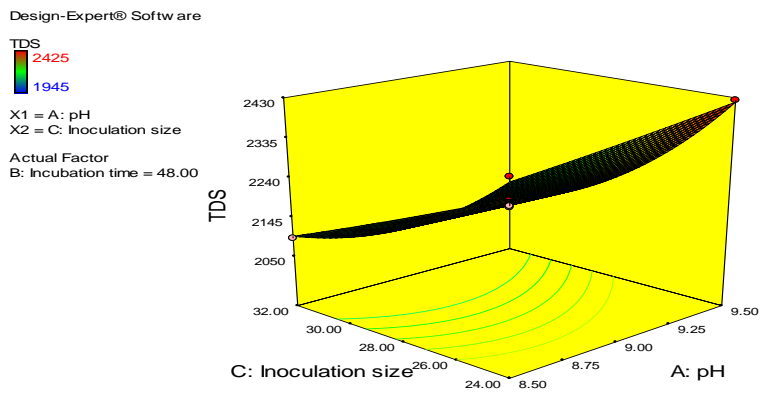
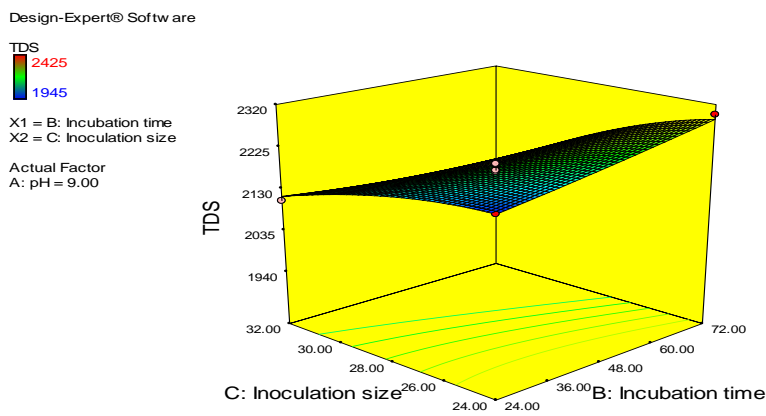
X1 = A: pH
X2 = C: Inoculation size

Actual Factor
B: Incubation time = 48.00





(a)



(b)

Figure B2: 3D plots for *Bacillus alkalophilus* bacteria (a) Total alkalinity (b) TDS

APPENDIX C: Figures Taken during Laboratory Work

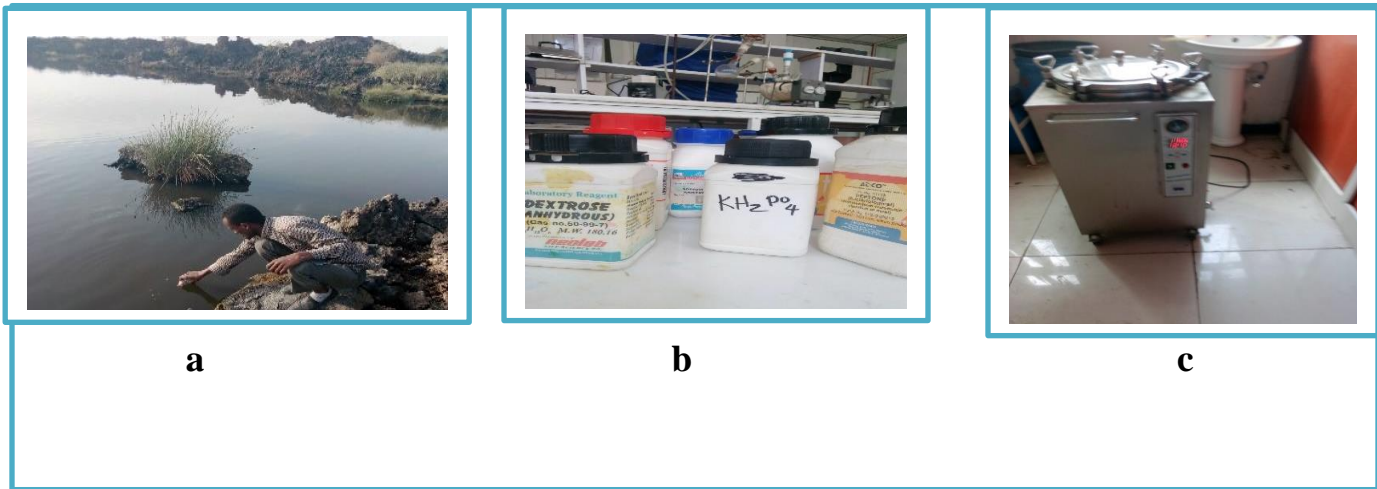


Figure C1: water sampling from beseka lake (a), media(b)Autoclave (c)

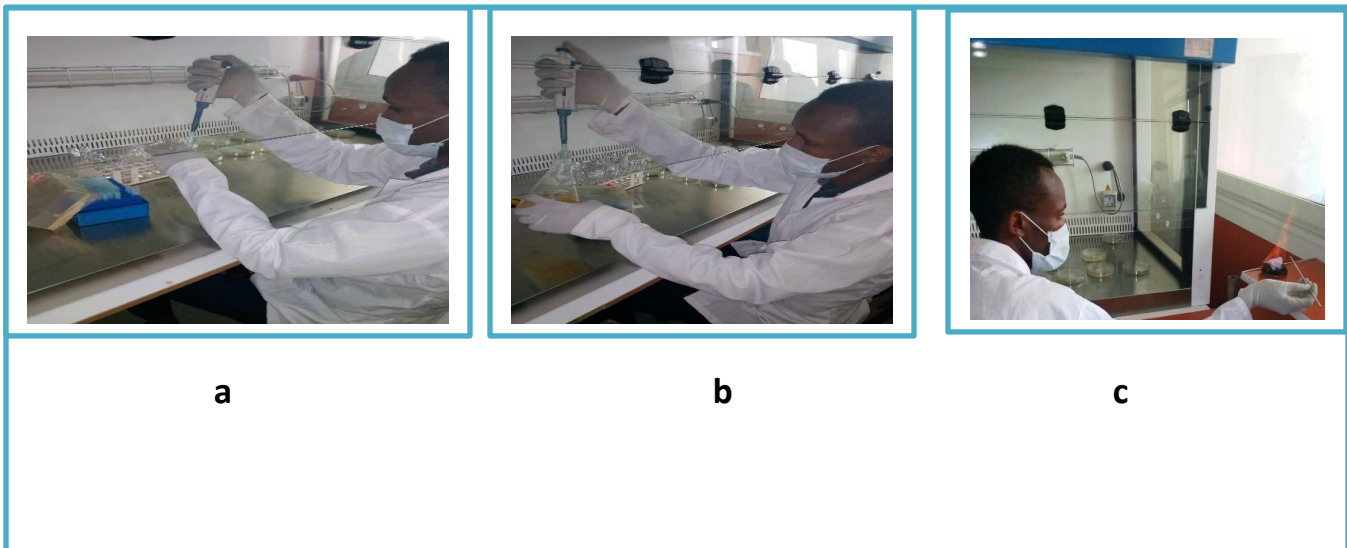


Figure C2: serial dilution for isolation (a)Serial dilution for counting (b), sterilization (c)

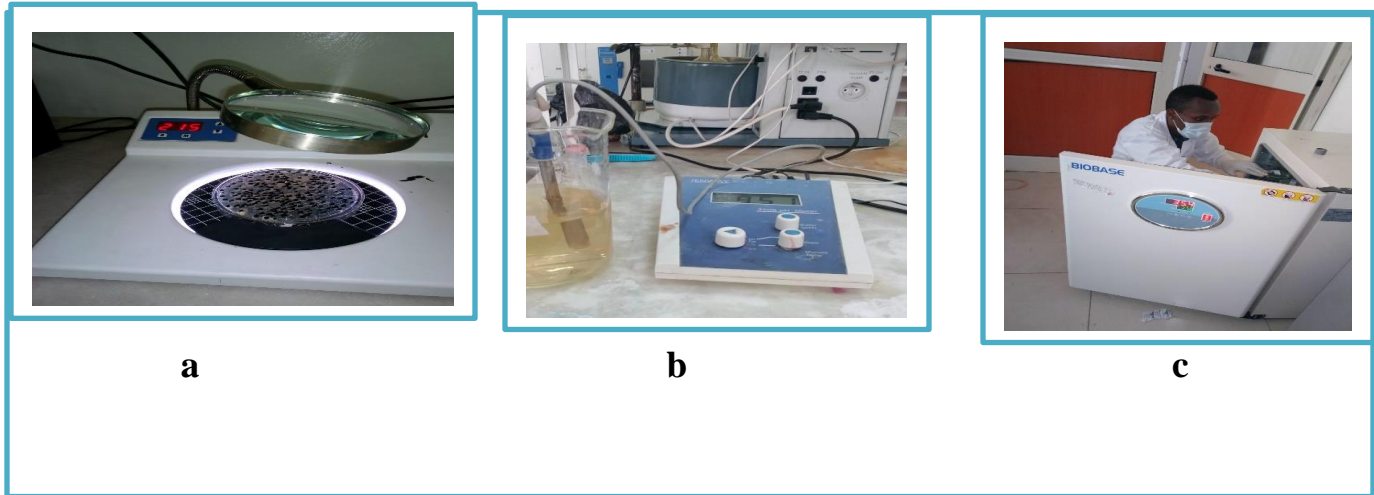


Figure C3: viable cell (a), pH meter (b) incubation (c)

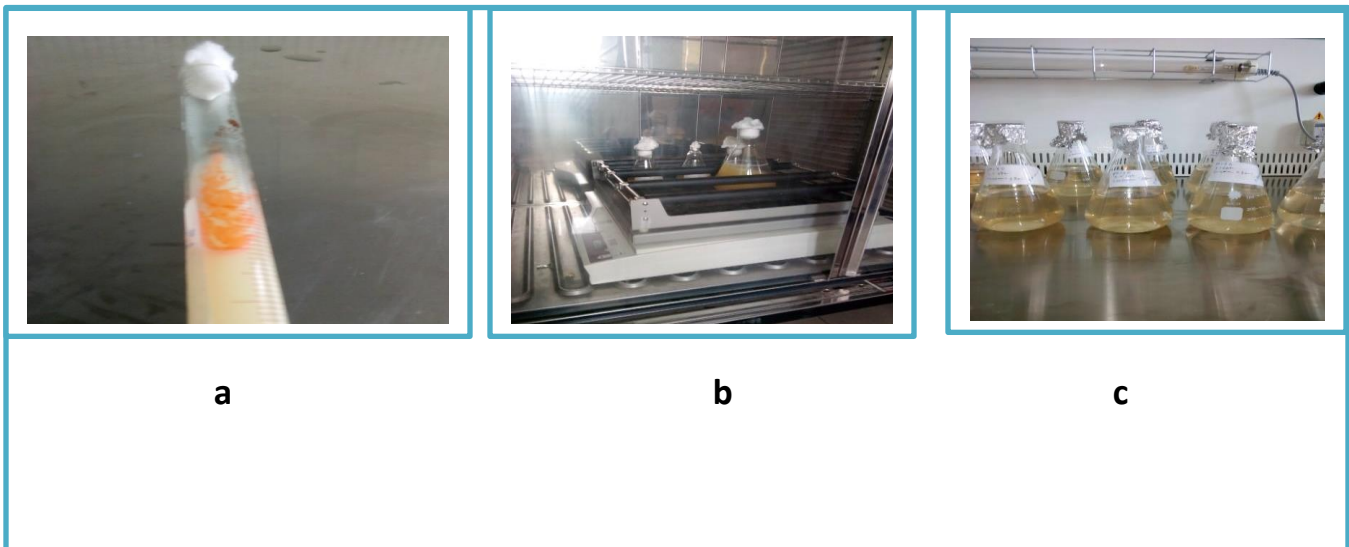


Figure C4: Slant culture (a) Broth culture (b), untreated water before inoculation (c)

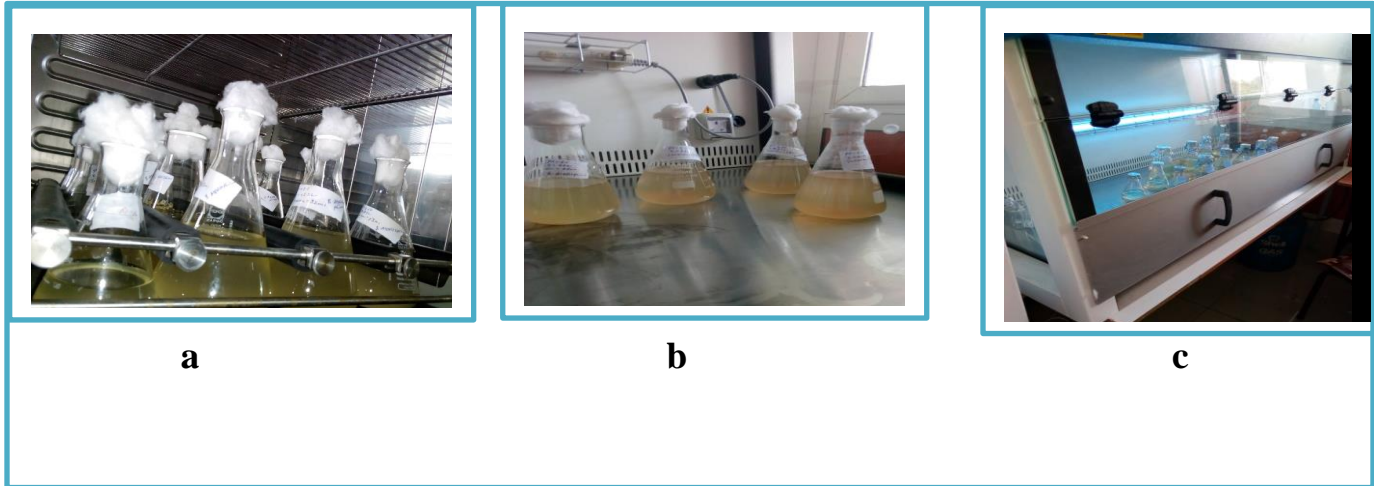


Figure C5: Untreated water after inoculation (a) treated water (b), Biological safety cabinet(c)

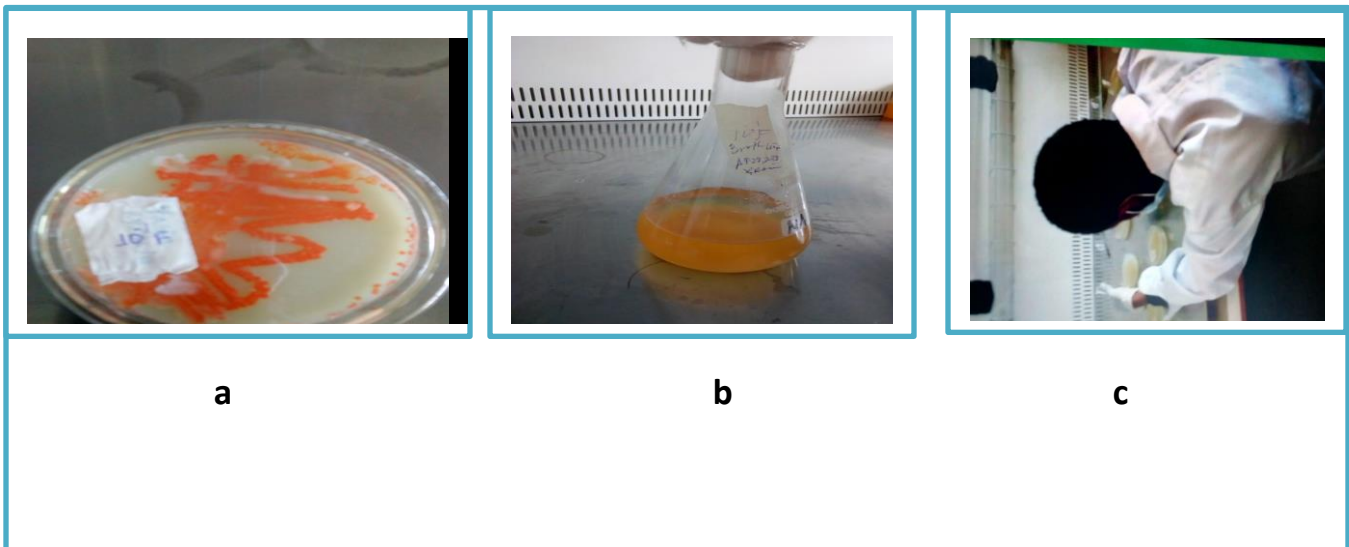


Figure C6: Isolate (a) broth culture (b) spread plating(c)